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Macro-moths as possible assessment endpoints for non-target effects of Bt-maize pollen: a faunistic study in three Italian protected areas

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Abstract

Lepidoptera are the phytophagous group most at risk of impact by insect-resistant genetically modified (GM) plants since most of these crops have been developed to express toxins to specifically target lepidopteran pests. In the case of Bt-maize cultivation, pollen grains released by GM plants could dust larval host plants growing inside or nearby maize fields, thus leading to the exposure of caterpillars to the Cry toxins. This way of exposure has been studied mainly for butterflies, whereas moths, in spite of their great diversity and abundance, were rarely considered. To evaluate the potential exposure of moth species to maize pollen in absence of Cry-expressing plants, macro-moths were sampled by means of light traps in three protected areas of Northern, Central and Southern Italy where conventional maize is among the main crops. Light traps were activated in two consecutive years (2010-11) from the beginning of June to the end of July, a period that encompasses the anthesis of most maize cultivars grown in Italy. Overall, 11446 individuals belonging to 201 species and nine families were collected. Given that larvae of several sampled species are likely to match the criteria of potential exposure to maize pollen and susceptibility to Cry toxins, macro-moths might be considered among the potential assessment endpoints for environmental risk assessment of GM plants, especially in the vicinity of protected areas.

Key words: Lepidoptera, light traps, host plants, non-target organisms, genetically modified organisms, Cry toxins.

Introduction

The need to implement measures to protect flora, fauna and biological diversity is a recurring theme in European regulations defining policy for the sustainable management of natural resources. The Habitats Directive (92/43/EC) together with the Birds Directive (09/147/EC) form the cornerstones of Europe's nature conservation policy (EC, 1992; 2009). In this framework, the implementation of protected areas represents one of the fundamental pillars of this legislation. In 2011, the protected areas included in the Natura 2000 network covered 19% of the Italian territory (http://www.minambiente.it/pagina/rete-natura-2000).

Many protected areas are adjacent to or include cultivated fields. Therefore, the possible cultivation of genetically modified (GM) plants in such areas could cause potential risks to biodiversity (Lang *et al*., 2015). The risks need to be assessed in the light of the specific protection goals of the areas (EC, 2001). In some cases, mitigation measures, including buffer zones, are recommended by the European Food Safety Authority (EFSA) for situations where protected areas with highly endangered species would rest in close proximity (EFSA, 2010; 2011).

The most common GM events worldwide are insect-resistant and herbicide-tolerant plants (James, 2015). Currently, the only GM event grown in the European Union (EU) is Bt-maize MON 810, expressing lepidopteran-specific Cry1Ab toxin of *Bacillus thuringiensis* (Berliner). At present, Bt-maize is commercially cultivated in Europe mostly in Spain (James, 2015), but in the future it may be cropped also in countries where it is presently banned (e.g. France, Germany, Italy), and several other GM events are applied for future cultivation (see http://registerofquestions.efsa.europa.eu/roqFrontend/). The possible negative effects of GM crops can be classified into ecological effects, economic effects, effects on agriculture, and effects on other anthropocentric values (Arpaia, 2010). Possible effects of genetically modified organisms (GMOs) must be monitored due to EU legislation (EC, 2002), which can be done by selecting a set of appropriate indicators out of a larger group of non-target organisms (NTOs).

Butterflies and moths are the non-target herbivorous groups most at risk from current Bt-maize events as these produce Cry toxins active on Lepidoptera (Lang and Otto, 2010). In maize MON 810 Cry toxins are expressed also in pollen, albeit at a lower level than in leaves or grains (Nguyen and Jehle, 2007). However, other Bt-maize events such as event 1507 produce much higher Cry1Ab amounts (EFSA, 2005). The pollen can be transported by wind and lepidopteran larvae occurring in natural habitats nearby maize fields may be exposed to the toxins while feeding on their host plants (e.g. Lang *et al*., 2015).

Most of the laboratory and field studies dealing with the toxic effects of Bt-maize pollen focused on butterflies (Papilionoidea and Hesperiidae) (Lang and Otto, 2010), with the exception of two pest species *Plutella xylostella* (L.) (Lepidoptera Plutellidae), and *Galleria mellonella* (L.) (Lepidoptera Pyralidae) and two further species not occurring in Europe, i.e. *Euchaetes egle* (Drury) (Lepidoptera Arctiidae) (Jesse and Obrycki, 2002) and *Antheraea pernyi* (Guérin-Méneville) (Lepidoptera Saturniidae) (Li *et al*., 2005). Nocturnal Lepidoptera have been less studied than butterflies, but moth communities include many taxa of ecological and faunistic interest (New, 2014; Merckx and Mcdonald, 2015). Host plants for several moth larvae may grow in ditches, nearby hedgerows and in field margins, and they can be dusted by pollen from adjacent Bt-maize, thus leading to an exposure of larval populations to Cry toxins. For these reasons moths should also be considered in a species selection process during environmental risk assessment for GMOs, especially nearby protected areas.

The goals of our study were:

(i) to provide faunistic lists of the moth species occurring in protected areas close to maize fields in Italy;
(ii) to assess the possible spatial and temporal exposure of selected moth species to Bt-maize pollen;
(iii) to evaluate the potential usefulness of light trap sampling of moths from the standpoint of GMO monitoring.

Materials and methods

Sampling sites

A two-year study (2010-2011) was carried out in three Italian nature conservation areas (figure 1): Biotopi e Ripristini Ambientali di Bentivoglio, San Pietro in Casale, Malalbergo e Baricella (SCI IT4050024), Macchia di Sant'Angelo Romano (SCI IT6030015) and Costa Ionica-Foce Bradano (SCI IT9220090).

The first site in Northern Italy (SCI IT4050024) is located in the plain rural 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 reclaimed and cropped. The fragmented protected area (3224 ha) encompasses a high proportion of cultivated fields (59.1% of the total surface), and arable crops are grown in most of them. The main reason of conservation interest is the marsh bird fauna, which comprises 49 European protected species (SPP-AAPB, 2008).

Macchia di Sant'Angelo Romano (SCI IT6030015) is a large protected area (798 ha) located in the province of Rome, Central Italy. Woods of Turkey oaks (*Quercus cerris* L.) and thermophilic scrubs cover most of the natural areas. Natural vegetation is fragmented by human land use, and about a half of the total area is cropped with olive orchards and forage crops. Moreover, grazing of cattle and sheep is allowed.

The SCI Costa Ionica-Foce Bradano (SCI IT9220090) is a 470 ha large protected area located in the province of Matera on the coast of the Ionian Sea near the border between Apulia and Basilicata. The prevailing habitats are characterized by dunes, scrubland and forests (mainly conifers as a result of reforestation). The agricultural land represents approximately 30% of the protected area.

Moth collection methods

Because of the large extension of the protected area, two sampling sites were established in Northern Italy (SCI IT4050024), whereas samplings were performed at only one site both in Macchia di Sant'Angelo Romano (SCI IT6030015) and Costa Ionica-Foce Bradano (SCI IT9220090) (table 1). In each site, one standard light trap (Pennsylvanian model, Southwood and Henderson 2000) was installed at approximately 1.5 m above the ground. The light source was provided by a mixed light lamp (Philips ML 160 W). Loosely arranged egg cartons were put in the collecting bucket to provide resting places for the captured individuals. Ethyl acetate, poured in a 250 mL bottle inside the collecting bucket, was used as killing agent. A timer switch automatically turned the light on at 20:30 and off at 06:30. Traps were checked twice a week to recover moths and to refill ethyl acetate. Light trap surveys were carried out continuously from the beginning of June to the end of July, a period that encompasses the flowering period of most maize cultivars grown in Italy. Besides light trap, additional data were obtained in Costa Ionica-Foce Bradano

Figure 1. Geographic position of the sampling sites: Biotopi e Ripristini Ambientali di Bentivoglio, San Pietro in Casale, Malalbergo e Baricella (SCI IT4050024), Macchia di Sant'Angelo Romano (SCI IT6030015) and Costa Ionica-Foce Bradano (SCI IT9220090).

Table 1. Characteristics of the sites sampled during the 2010-2011 field study.

Site Bio-geographical region	Habitat directive biotopes	Area (ha)	Cultivated area (%)	Sampling site coordinates
Biotopi e Ripristini Ambientali di Bentivoglio, San Pietro in Casale, Malalbergo e Baricella (SCI IT4050024) Continental	Natural eutrophic lakes with *Magnopotamion* or *Hydrocharition* - type vegetation (3150); Rivers with muddy banks with *Chenopodion rubri* p.p. and *Bidention* p.p. vegetation (3270); Mediterranean deciduous forests *Salix alba* and *Populus alba* galleries (92A0)	3224	59	N44°40'49.16" E11°26'27.13" 7 m asl N44°40'31.65" E11°34'4.24" 4 m asl
Macchia di Sant'Angelo Romano (SCI IT6030015) Mediterranean	Thermo-Mediterranean and pre-desert scrub (5330); Pseudo-steppe with grasses and annuals of the Thero-Brachypodietea (6220)	798	50	N42° 2'44.08" E12°42'31.99" 234 m asl
Costa Ionica-Foce Bradano (SCI IT9220090) Mediterranean	Humid dune slacks (2190); Coastal dunes with *Juniperus* spp. (2250); *Salicornia* and other annuals colonizing mud and sand (1310); *Brachypodietalia* dune grasslands with annuals (2240); Mediterranean and thermo-Atlantic halophilous scrubs (*Sarcocornetea fruticosi*) (1420); Mediterranean salt meadows (*Juncetalia maritimi*) (1410); Shifting dunes along the shoreline with *Ammophila arenaria* ('white dunes') (2120); *Malcolmietalia* dune grasslands (2230); *Crucianellion maritimae* fixed beach dunes (2210)	470	30	N40°22'37.67" E16°50'52.34" 2 m asl

(SCI IT9220090) by hand-sampling of moths attracted by a lighted sheet, which was performed once per year on 22 July 2010 and 27 July 2011.

Only well preserved macro-moth specimens, allowing identification at the species level were considered in the study. We have adopted the binomial nomenclature following Fauna Europaea (https://fauna-eu.org), while the traditional and largely widespread family classification was chosen in order to simplify comparative analysis of our data with previous records. Voucher specimens were deposited at the Dipartimento di Scienze Agrarie, Università di Bologna.

Results

Macro-moth fauna

In total, 11446 individuals belonging to 201 species and nine families (Arctiidae, Cossidae, Drepanidae, Geometridae, Lasiocampidae, Lymantriidae, Noctuidae, Notodontidae and Sphingidae) were collected in June-July 2010 and 2011 and examined for taxonomical identification (supplemental material table S1). The 201 collected species represent 12.4% of the Italian macro-moth fauna recorded for the sampled families, which overall consists of 1623 species (http://www.faunaitalia.it). The family Noctuidae, represented by a total of 8115 individuals and 118 species, was the most abundant and species-rich family. Geometridae (1734 individuals) accounted for 15.1% of the moths and overall 30 species were recorded. The 941 individuals (16 species) belonging to Arctiidae represented the 8.2% of the catches. In total, the remaining six families accounted for 5.7% of the individuals and for 37 species (figure 2).

Abundant species

Only 22 species had a relative abundance higher than 0.5% and in total these abundant species accounted for 82.4% of the collected individuals. Most of the abundant species, whose larvae feed on common weeds in the maize field or in the field edges, are likely to be sampled in sufficient number for meaningful studies on population trends in most of the Italian maize growing areas.

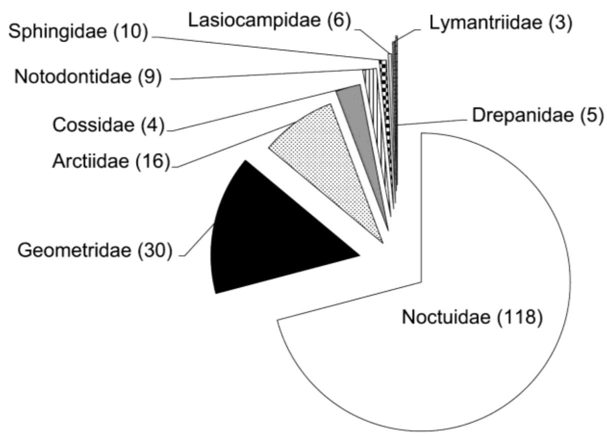

Figure 2. Pie chart of the individuals sampled for each family of macro-moths. The number of species for each family is reported in parentheses.

The marsh moth, *Athetis pallustris* (Hubner) (Noctuidae), was the most abundant species in both sampling years (33.79% of the total catches), but it showed high discrepancy in total number of sampled individuals across the years. As stated by its common name, this species is typical of wetlands and almost all individuals were sampled in Northern Italy (SCI IT4050024). Larvae might be exposed to maize pollen shed spatially, because the main host-plants are plantains (*Plantago* spp.), which commonly grow in field margins. However, given that larvae occur from August on, only a marginal temporal overlap with the anthesis of a few late flowering maize cultivars occur. For these reasons, the marsh moth does not seem a relevant and suitable indicator for Bt-maize monitoring even in wetland habitats were high numbers of individuals are likely to be sampled.

The spotted sulphur, *Acontia* (*Emmelia*) *trabealis* (Scopoli), was second for relative abundance (10.28% of the total catches), and it was sampled in all the sites. Bindweed (*Convolvolus arvensis* L.), its main foodplant, may grow in field margins but also within maize fields, where it is considered a weed of some concern (Meissle *et al*., 2010). Therefore, populations of this species could be influenced by Bt-maize cropping. Tóth *et al*. (2004) have suggested that larvae of *A. trabealis* may provide some ecosystem services by reducing the seed production of bindweed. The field bindweed moth, *Tyta luctuosa* (Denis et Schiffermuller), a species with comparable ecology to the spotted sulphur, occurs also on *Convolvolus*, and was recorded in all three sites albeit at lower abundance in comparison with *A. trabealis*.

The Geometridae *Isturgia arenacearia* (Denis et Schiffermuller) and the latticed heath, *Chiasmia clathrata* (L.), were also abundant (7.85% and 3.00% of the total catches). Larvae of both species feed on Fabaceae and may occasionally reach pest status in alfalfa cropping systems (Atanasova *et al*., 2010). The survey of the larvae of *A. trabealis*, *T. luctuosa* and *C. clathrata* has already been suggested as part of a GMO monitoring scheme (VDI, 2010).

Two of the species with high relative abundance, The marbled clover *Heliothis viriplaca* (Hufnagel) (2.21%) and the beet armyworm *Spodoptera exigua* (Hubner) (0.98% of the total catches), are considered pests. The first one causes occasional outbreaks on leguminous crops, and the second may damage various plants, in particular vegetables (van Emden, 2013). The use of pest species as indicators would be problematic because the availability of suitable crops and the pest management practices may strongly influence their populations thus masking potential effects of Bt pollen.

Among the Arctiidae, the ruby tiger *Phragmatobia fuliginosa* (L.) and the four-spotted footman *Lithosia quadra* (L.) showed the highest number of individuals (3.86% and 1.55% of the total catches). The ruby tiger, a polyphagous species recorded both in Northern and Central Italy, is considered common all over Europe (Leraut, 2006). The four-spotted footman was recorded in all three sites; its larvae feed on lichens (Luciano and Roversi, 2001). So far, maize pollen deposition on lichens was not documented, and such information would be paramount in order to assess the potential exposure of caterpillars of *L. quadra*.

Species with conservation or aesthetic value

All the species of conservation interest or aesthetic value were caught rarely and showed relative abundance below 0.05% (at most five specimens in a site pooling the two years). These species are caught too rarely to be included in routine monitoring schemes. Nevertheless, as rare species are often species of conservation concern and represent protection goals, information about their occurrence is very useful in the preliminary definition of the potential environmental impacts. Before starting the commercial cultivation of Bt-maize within or nearby protected areas, surveys of the moth fauna should be suggested. If endangered species are recorded, the cultivation of Bt-maize in the nearby areas may necessitate mitigation measures (e.g. isolation distances) (EFSA, 2012).

In terms of conservation value the most interesting record in our surveys was the willowherb hawkmoth, *Proserpinus proserpina* (Pallas) (Sphingidae), a species included in Annex IV of the Habitats Directive (92/43/EEC). Two specimens were found in 2011 at the sampling sites located in Northern Italy (SCI IT4050024). The likely larval host plants in this area were *Epilobium* (= *Chamaenerion*) *hirsutum* L. and *Lythrum salicaria* L., which frequently occur in the banks of ditches. The willowherb hawkmoth is a monovoltine species, which overwinters in the pupal stage, and the adult flying period spans from May to June. The larval stage lasts approximately three weeks, thus the caterpillars will feed on their host plants in June-July (Leraut, 2006) and might be exposed to maize pollen for most of their lifespan.

The lappet, *Gastropacha quercifolia* (L.) (Lasiocampidae), is another species of potential conservation interest found in Northern and Central Italy (one and four individuals respectively). The larvae feed mainly on blackthorn (*Prunus spinosa* L.), a shrubby tree commonly found in hedgerows at field margins. As these habitats are commonly disrupted by a number of agricultural activities, the lappet already disappeared from many localities of the Po valley (Marini, unpublished data). *G. quercifolia* has two flight-periods a year: May-June and August-October. Caterpillars of the second brood feeding in June-July (Luciano and Roversi, 2001) could overlap with maize anthesis.

Two individuals of the cream-spot tiger, *Arctia villica* (L.) (Arctiidae) were caught in Macchia di Sant'Angelo Romano (SCI IT6030015). This is considered a quite common species in Italy, though it is declining in rural landscapes due to the disappearing of natural vegetation and habitats (Marini, unpublished data). The night-flying males are strongly attracted by artificial lights; the females instead are day-fliers. Larvae of *A. villica* feed on *Taraxacum*, *Plantago*, *Achillea* and other widespread herbaceous plants. The adults are active in May-June. In June-July the newly hatched caterpillars may be exposed to maize pollen grains in their early larval instars.

One specimen of the eastern lappet, *Pachypasa otus* (Drury), was found in 2010 in the Southern Italy site. This species is the largest European Lasiocampidae and it is considered rare in Italian pinewoods. The adults fly in summertime and particularly in August, and lay eggs on the plants such as *Cupressus sempervirens* L., *Pinus pinea* L., *Pinus halepensis* Mill., and *Quercus* spp. (Bertaccini *et al*., 1994). The caterpillars grow slowly and overwinter hidden deeply in bark crevices of their food plants. In the subsequent spring they reach the maturity when maize is producing pollen, and then pupate in a white cocoon at the base of the trunk (Parenzan and Porcelli, 1985), unfortunately exposed to fire for weed control. *P. otus* is an Eastern-Mediterranean species; in Italy it is spread along Southeastern coasts, but its populations are quite localized and scarce (Parenzan and Porcelli, 2006). Some populations once living on *C. sempervirens* trees in cemeteries, such as that of Castellaneta (Taranto) have been recently eliminated by man, owing to the unjustified bad reputation of their large and hairy larvae. Other populations living on trees (*P. pinea* and *C. sempervirens*) along some roads near Foggia and Taranto (Apulia) have been destroyed by fire for weed control along the roads (Marini, unpublished data). For these reasons *P. otus* can be considered vulnerable.

Discussion

The criteria to guide selection of indicator species for potential impact of GM crops, in particular GM maize, on NTOs have been discussed over the last years (Schmitz *et al*., 2003; Hilbeck *et al*., 2014). EFSA (2010) has suggested a stepwise method based on the identification of the functional groups of interest, the listing of species in the functional groups presumably occurring in the receiving environments, and the ranking of these species by means of ecological criteria and conservation concerns. Macro-moths match these ecological criteria proposed by EFSA.

Exposure

Light trapping has been carried out in areas where conventional maize cultivars are usually cropped. If the cultivation of Bt-maize will be allowed in these areas, larvae of the sampled moths could potentially feed on host plants dusted by pollen expressing Cry toxins.

Moth larvae are potentially exposed only if their presence overlaps both temporally and spatially with maize pollen (Lang *et al*., 2015). The temporal coincidence depends on the phenology of each species, which is additionally affected by latitude and climate. The phenologies as well as the number of generations per year in the studied areas are not known exactly for many of the species. However, the larval stages of the sampled moth species have a certain probability to temporally overlap with the period of maize pollen shed, because the light traps were activated in synchrony with the flowering periods of the common maize cultivars in each study area. In fact, most of adult moths caught before maize anthesis were likely to be near oviposition phase and newly hatched larvae may ingest pollen deposited on their host plants. On the other hand, adult moths sampled just after maize flowering may have developed from larvae that fed during anthesis.

The spatial overlap is a result of the occurrence of host plants within or nearby the maize fields and of the use of these plants by larvae. A frequently reported concern about light trapping is the supposed large attraction range, allowing the collection of adult moths whose larvae may have not developed in areas close to the traps (Beck and Linsenmair, 2006). This may hinder the establishment of a causal relationship between stressors in the local habitats and trends of sampled species. Nevertheless, several studies have demonstrated that even for strong light sources the attraction radius is usually less than 30 m for most species (Baker and Sadovy, 1978; Truxa and Fiedler, 2012). Although the capture of some individuals of migratory species traveling from long distance cannot be ruled out, the vast majority of moths collected by light trap are likely local species that completed their preimaginal development in the areas nearby traps, especially if the light source is not placed too high above the surrounding vegetation and combined with low power and a certain spectrum of the light source (Wirooks, 2005; Lang *et al*., 2013). A concurrent vegetation survey aimed at determining the abundance of the most important host plants in the area nearby light traps can aid to decide which moth species are likely to establish populations in the vicinity of the cultivated field and which ones are not.

Also the larval feeding behaviour is of key importance. Only moths whose larvae feed ectophytically are likely to be affected the most by pollen from Bt-maize, whereas endophytic larvae have negligible chances to ingest maize pollen. The larvae of 11 sampled species (marked with asterisk in supplemental material table S1) feed as borers within tissues of a variety of plants. Some of these plants, such as the common reed *Phragmites australis* (Cav.) and *Typha* spp., may occur frequently in ditches at maize field edges. Yet, all these moth species cannot be considered relevant target for risk assessment, because they will not be exposed to Bt maize pollen. The Cossidae *Phragmataecia castaneae* (Hubner), whose larvae bore into the stems of the common reed, was the most abundant among the plant-borers and it was the seventh most abundant species overall.

Finally, leaf shape and size of host plants are other relevant aspects in order to estimate the likelihood of pollen accumulation on leaves where larvae feed.

Susceptibility

The susceptibility of Lepidoptera to Cry toxins is well demonstrated and for some butterflies also the relationships between density of Bt-maize pollen grains on host plants and larval mortality was determined in laboratory studies (Felke *et al*., 2002; 2010; Lang and Vojtech, 2006; Schuppener *et al*., 2012). No data are available on the relationship between density of maize pollen grains on host plant and mortality for European macro-moth larvae. However, the toxicity of Bt sprays has been demonstrated for several moth species (Johnson *et al*., 1995; Peacock *et al*., 1998).

Practicability

The macro-moths match also several criteria of practicability:

i) light-trapping is a standardized sampling technique that allows the collection of many specimens generally in good shape for identification;
ii) taxonomy is quite stable and identification to species level is relatively easier than for many other insect groups;
iii) macro-moths represent a great part of Lepidoptera fauna and high species richness and diversity has been described worldwide;
iv) in comparison with most insect taxa, moths are well-studied, larval host plants and habitat requirements are known for many species;
v) a sufficient number of experts of macro-moth taxonomy are available;
vi) Lepidoptera represent a well-recognized and accepted protection goal both in public and in many legislative frameworks.

All these aspects have been already recognized and the survey of moth communities has been suggested for GMO monitoring purpose (Schmitz *et al*., 2003; Lang *et al*., 2015; VDI, 2010).

Species selection for environmental risk assessment, to identify potential focal species as assessment endpoints can be done by database analysis only in areas where monitoring networks exist or extensive surveys have been run for several years (for example in the UK and in the Netherlands). Despite the general existing knowledge about European moth species, moths are less extensively studied than diurnal Lepidoptera, and information is sometimes rare for some regions in Europe, in particular so for farmland. In Italy, the state of knowledge of insect biodiversity in many protected areas is still incomplete and scattered or it is limited to flagship taxa like butterflies. Even in the few protected areas where arthropod fauna has been extensively studied (for instance Bosco della Fontana, Lombardy) (Cerretti *et al*., 2003; 2004), the faunistic inventories available for macro-moths include only few families such as Geometridae, Noctuidae and Sphingidae (Mason *et al*., 2002).

Microlepidoptera

Micro-moths represent a large part of lepidopteran diversity (Pogue, 2009), and also in our samplings many individuals belonging to microlepidoptera were caught by light traps. However, the use of microlepidoptera as ecological indicators implies several problems with regard to species identification, and the lack of knowledge on ecology of most species (New, 2004a; 2004b). Moreover, the particular feeding habits of several taxa, whose larvae are endophytic or feed on non-exposed tissues (e.g. in leafrolls) (Parenti, 2000), decreases the potential exposure to Bt pollen. For these reasons, a general survey of microlepidoptera assemblages cannot be suggested as a routine technique to monitor side effects of Bt-maize cultivation. Nevertheless, some specific taxa (like Crambidae) may be useful and practical indicators (Lang *et al*., 2011).

Conclusions

Albeit being often considered of minor importance than butterflies, moths are of greater functional significance in ecosystems (New, 2004a; Merckx and Mcdonald, 2015). Because of their abundance and diversity, moths are important prey organisms and play a substantial role in pollination and other ecosystem services. Therefore, moths represent a valuable protection goal of agroecosystems, and should be included as assessment endpoints in environmental risk assessment and monitoring regarding GM crop cultivation.

The field data collected in this study demonstrated that a high number of individuals belonging to several species could be sampled by means of light-traps during maize flowering periods. Moreover, moths are generally considered suitable for ecological indication (Pogue, 2009) and proved to be reliable indicators for restoration measures in semi-natural habitats (Rakosy and Schmitt, 2011). All these considerations lead to the conclusion that the sampling of nocturnal Lepidoptera could provide significant information in the evaluation of the possible environmental impacts of GM crops, in particular for Bt-maize.

This study provides data useful to plan a detailed exposure analysis, which is a fundamental component of any environmental risk assessment. The next necessary step will then be a hazard evaluation in order to assess which measurement endpoints of moth assemblages could be linked causally to the potential impact of Bt-maize. In monitoring lepidopteran populations, the detection of trends requires long-time series to discriminate between natural year-to-year fluctuations and actual tendencies (Roy *et al*., 2001), and a great challenge of monitoring programmes is to relate recorded effects to the causal factors. With respect to GM crops, this may be tackled by comparing GMO with non-GMO sites or by analysing a pre-GMO situation with a post-GMO situation, in order to differentiate between natural fluctuations, the influences of other stressors and the GMO effect itself, and to finally identify the drivers of fluctuation patterns of moths.

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