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Diversity of Coccinellidae in Ecological Compensation Areas of Italy and overlap with maize pollen shedding period

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Abstract

Ladybird beetles (Coleoptera Coccinellidae) are important predators of aphids and other crop pests, and there is great interest in their conservation in agroecosystems. Bt-maize, genetically engineered to express insecticidal Cry proteins, is regarded as a taxon-specific way of controlling pests, but some concerns have been raised about the possibility of unintended negative effects on non-target organisms, including coccinellids. One of the possible routes of exposure of ladybird beetles to Cry toxins is by feeding on maize pollen, as for many species pollen is an important integrative food source. In this study, coccinellid adults were sampled by sweep netting in Ecological Compensation Areas (ECAs) in three sites of Northern and Central Italy, where conventional maize cultivars are grown (Bt-maize is currently forbidden for commercial cultivation in Italy). The coccinellid communities were sampled during and around the typical flowering periods of maize in order to check their diversity and their overlap with pollen shedding. A total of 11 species were recorded. *Hippodamia variegata* (Goeze), *Coccinella septempunctata* L. and the exotic *Harmonia axyridis* (Pallas) were the most abundant species in Northern Italy, whereas *Tytthaspis sedecimpunctata* (L.) and *Coccinula quatuordecimpustulata* (L.) were dominant in Central Italy. The potential exposure to maize pollen was different in the two areas, since in Northern Italy the maize flowering coincided with a period of high coccinellid activity in the field, whereas in Central Italy the ladybird population peaks occurred roughly one month earlier than the anthesis. The collected data might be useful for exposure characterization of ladybird beetles in a possible future scenario of Bt-maize cropping in the studied areas.

Key words: Bt-maize, marginal areas, agroecosystems, functional biodiversity, exposure pathways.

Introduction

Coccinellids also known as ladybird beetles (Coleoptera Coccinellidae) are widely regarded as beneficial insects, because many species prey on aphids (Rhynchota Aphididae), scale insects (Rhynchota Coccoidea) and other agricultural pests (Honek *et al*., 2014; Michaud, 2012). For this reason, coccinellids have often been considered for biological control and several species were also introduced in new countries as part of classical control programs (Michaud, 2012). A recent example is *Harmonia axyridis* (Pallas), an Asian species that was introduced in Europe and North America (Katsanis *et al*., 2012; Koch, 2003). Moreover, there is also a great interest in the conservation and the augmentation of wild populations of coccinellids, for example through the proper management of Ecological Compensation Areas (ECAs), such as hedgerows, green lanes and woody patches nearby cropped fields (Burgio, 2007; Burgio *et al*., 2004), as well as through landscape management at a larger scale (Diepenbrock and Finke, 2013; Woltz and Landis, 2014). Beneficial coccinellids are considered annual cyclical colonizers of both crops and ECAs (Burgio, 2007) and many ladybird species occurring in Italian agro-ecosystems are multivoltine. Wheat is the key crop for the development of the first generation, whose adults can then move to maize fields and other spring-sown crops to complete further generations during the summer (Burgio *et al*., 2001).

While currently forbidden in Italy and most of other European countries in the framework of EU Directive 2015/412, genetically modified plants (GMPs) are largely cultivated worldwide and insect resistance characters are among the most common traits introduced in such plants (James, 2014). For example, several maize varieties that express different Cry proteins from *Bacillus thuringiensis* Berliner (Bacillales Bacillaceae) are used to prevent damages caused by the corn borers - *Ostrinia nubilalis* (Hubner) (Lepidoptera Crambidae) and *Sesamia nonagrioides* (Lefebvre) (Lepidoptera Noctuidae) - and the corn rootworms - *Diabrotica* spp. (Coleoptera Chrysomelidae) (e.g. Farinòs *et al*., 2011; Sansinenea, 2012; Stephens *et al*., 2012). Cry toxins act as taxon-specific insecticides, as they bind to specific receptors in the midgut of the target insects as the first step of events leading to a toxic effect (Adang *et al*., 2014; Sansinenea, 2012), and thus they are generally considered less detrimental to the biocenosis than broad-spectrum chemicals (Romeis *et al*., 2006; Sansinenea, 2012; Wolfenbarger *et al*., 2008). However, some concerns have been raised about potential unintended negative effects on non-target arthropods, including coccinellids (e.g. EFSA Panel on Genetically Modified Organisms, 2010). For this reason, the taxon specificities of different Cry toxins are the subject of continuous studies (van Frankenhuyzen, 2009).

Environmental Risk Assessment (ERA) is an important step in the process of GMP regulation (Lener *et al*., 2013), as well as in exploring the possibility of new Integrated Pest Management (IPM) strategies incorporating also GMPs (Arpaia *et al*., 2014; Lundgren *et al*., 2009). The direct and indirect impacts of Cry proteins and Bt-plants have been deeply investigated in the last two decades by means of laboratory and field experiments (Arpaia, 2010). With few exceptions (e. g. Schmidt *et al*., 2009; Stephens *et al*., 2012) most authors agree that Bt-plants do not show significant effects on ladybird beetles and do not negatively affect their populations in the field (e. g. Alvarez-Alfageme *et al*., 2011; Eckert *et al*., 2006; Li *et al*., 2011; 2015; Lundgren Lundgren and Wiedenmann, 2002; Porcar *et al*., 2010; Tian *et al*., 2012; Zhang *et al*., 2014). However, the fauna of many European areas is still relatively understudied from this point of view, as GMPs are not widely cultivated in Europe, with the exception of Spain (de la Poza *et al*., 2005; Eizaguirre *et al*., 2006). Identifying local species that are likely to be exposed to Bt-plant material and Cry toxins and focusing research effort on those species might help to better characterize and manage possible risk scenarios for European ecosystems (Romeis *et al*., 2014).

Predatory ladybird beetles can be exposed to Cry toxins and GMP material both through consumption of prey that has fed on Bt-plants (tritrophic exposure) and through ingestion of Bt-plant tissue (direct or bitrophic exposure) (Andow *et al*., 2006; Harwood *et al*., 2005; 2007; Obrist *et al*., 2006). Several prey taxa of coccinellids such as spider mites (Trombidiformes Tetranychidae) (Alvarez-Alfageme *et al*., 2008; 2011) and, to a lesser extent, aphids (Burgio *et al*., 2011; Zhang *et al*., 2006) are known to uptake Cry proteins. Direct ingestion of plant material may include seedling leaf tissue (Moser *et al*., 2008) and pollen (Li *et al*., 2015; Lundgren and Wiedenmann, 2002; Zhang *et al*., 2014). Even though the content of Cry toxins is much lower in pollen than in leaves (88-115 times lower for Europe approved MON810 maize - see Székàcs *et al*., 2010), pollinivory is considered the most relevant pathway of direct exposure, as pollen is an important component of the diet of many predatory ladybird beetles that are useful in biological control (Giorgi *et al*., 2009; Lundgren, 2009a; 2009b). Pollen has been recognized as the most important food when aphids and other prey arthropods are scarce or of low quality (Lundgren, 2009a; 2009b). In some species such as the Nearctic *Coleomegilla maculata* De Geer maize pollen is an essential food source (Hodek and Evans, 2012). European species known to feed on pollen include *Coccinella septempunctata* L., *Adalia bipunctata* (L.), *Hippodamia variegata* (Goeze), *Propylea quatuordecimpunctata* (L.), *Tytthaspis sedecimpunctata* (L.) as well as the exotic *H. axyridis* (Berkvens *et al*., 2010; Hodek and Evans, 2012; Lundgren, 2009a; 2009b; Lundgren *et al*., 2005; Triltsch, 1999).

The present study is aimed at describing the coccinellid fauna associated with maize-neighboring ECAs in two distinct geographic areas in Italy where maize is a major crop (according to Census of Agricultural Holdings by ISTAT - Istituto Nazionale di Statistica). Particular emphasis is given to predatory species that can be valuable in biological control and thus should be considered among primary protection goals in agroecosystem management. The other main goal of the study is to evaluate the overlap of the presence of coccinellids with maize pollen shedding. This is a first step to assess potential exposure and to develop a protocol of risk assessment for species that could interact with Bt-maize in a possible future scenario of large-scale GMP cultivation in Italy.

Materials and methods

Sampling sites

Transects were established in two Italian protected areas: SPA/pSCI "Biotopi e ripristini ambientali di Bentivoglio, San Pietro in Casale, Malalbergo e Baricella" (Natura 2000 code: IT4050024), located in the province of Bologna, Northern Italy, and pSCI "Macchia di Sant'Angelo Romano" (Natura 2000 code: IT6030015), near Rome, Central Italy. Both sites are of great naturalistic interest and belong to different biogeographical regions (table 1). The protected area in Northern Italy includes a large portion of farmland (59.1% of the total surface) and the natural habitats are highly fragmented. A rich fauna of marsh birds is the main reason for protection. Wheat, sugar beet and sorghum are the dominant crops besides maize. Given the extension of the protected area (3224 ha), two different sites (denominated "La Rizza" and "Casone") were sampled with one transect each (table 1). "Macchia di Sant'Angelo Romano" is a 798 ha nature reserve, about half of which is cropped. The fragmented natural vegetation areas consist mainly of turkey oak (*Quercus cerris* L.) woods and thermophilic scrubs. Arable and vegetable crops cover most of the cultivated areas. A single site, denominated "Tor Mancina", was selected for the monitoring (table 1).

Ladybird sampling

The sites in Northern Italy were sampled weekly from June to July of 2011 and 2012. Monitoring effort was increased between the 20th of June and the 20th of July at two samplings per week because in this area the flowering of typical maize cultivars usually occur in that period.

The samplings in "Tor Mancina" were performed from the end of April to the beginning of August in 2011 and from the end of May to the beginning of September in 2012. Samplings were carried out once every 10-15 days, except for the period between the 20th of July and the 10th of August, when weekly samplings were performed because of maize flowering in that area.

Transects (100 m long) were established in field margins, adjacent to cropped fields (table 1). No Bt-maize was used in the study, as GM crops are currently forbidden for commercial cultivation in Italy.

The insects were collected using a sweep-net. 40 sweeps were performed on herbaceous plants (table 1) and shrubs per sampling date in each site. The back-and-forth movement of the net over roughly the same vegetation spot was considered as one single sweep. In all sites, the samplings were carried out between 10:00 AM and 1:00 PM.

Table 1. Main features of the sampling areas.

SCI	Bio-geographical region	Habitat directive biotopes (code)	Area (ha)	Cultivated area	Typical maize sowing period	Sampling site	Transect features	Altitude (m asl)
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% (main crops: maize, wheat, sugar beet, sorghum)	Last decade of March	Casone N44°40'37" E11°26'23"	mature hedgerow at field margins (main herbaceous plants: *Urtica dioica* L., *Galega officinalis* L., *Cirsium arvense* (L.) Scop., *Cerastium glomeratum* Thuill.)	7
						La Rizza N44°38'58" E11°25'13"	young hedgerow between fields and marsh areas (main herbaceous plants: *Urtica dioica* L., *Sorghum halepense* (L.), *Calystegia sepium* (L.) R. Br., *Dipsacus fullonum* L.)	4
SCI IT6030015	Mediterranean	Thermo-Mediterranean and pre-desert scrub (5330) Pseudo-steppe with grasses and annuals of the Thero-Brachypodietea (6220)	798	50% (main crops: arable and vegetable crops)	Last decade of April	Tor Mancina N42°05'37" E12°37'50"	roadside with sparse scrubs connecting cropped and set-aside fields (main herbaceous plants: *Convolvulus arvensis* L., *Equisetum telmateja* Ehrh., *Sylibum marianum* (L.), *Urtica dioica* L.)	234

Table 2. Number of individuals and percent relative abundance (in brackets) for each ladybird species, site and year.

Species	Northern Italy				Central Italy		Total
	Casone		La Rizza		Tor Mancina		
	2011	2012	2011	2012	2011	2012	
Coccinella septempunctata	16 (38.1)	38 (40.4)	23 (10.2)	6 (5.1)	3 (1.7)	7 (8.0)	93
Harmonia axyridis	3 (7.1)	7 (7.4)	3 (1.3)	67 (57.3)			80
Adalia bipunctata			5 (2.2)	2 (1.7)			7
Hippodamia variegata	1 (2.4)	9 (9.6)	166 (73.8)	21 (17.9)	11 (6.4)	8 (9.1)	216
Ceratomegilla undecimnotata					1 (0.6)		1
Subcoccinella vigintiquatuorpunctata	4 (9.5)	1 (1.1)			1 (0.6)		6
Calvia quatuordecimguttata				1 (0.9)			1
Propylea quatuordecimpunctata	18 (42.9)	17 (18.1)	23 (10.2)	13 (11.1)	9 (5.2)	2 (2.3)	82
Psyllobora vigintiduopunctata		22 (23.4)	4 (1.8)	7 (6.0)	2 (1.2)	1 (1.1)	36
Tytthaspis sedecimpunctata			1 (0.4)		82 (47.4)	40 (45.5)	123
Coccinula quatuordecimpustulata					64 (37.0)	30 (34.1)	94
Total	42	94	225	117	173	88	739

The study focused on large and medium-sized coccinellids (Coccinellinae, Epilachninae, Chilocorinae), as they are often rather easy to identify by morphological traits. This allowed to efficiently identify most specimens directly in the field and then immediately release them every 2-3 sweeps. Only a few difficult specimens (e.g. some infrequent colour morphs) were collected and later identified in the laboratory of the Department of Agricultural Sciences at the University of Bologna. Given that larvae and pupae are not reliably identifiable in the field, coccinellid immature stages were not included in this study.

Data analysis

Individual-based rarefaction curves were calculated using the formula of Hurlbert (1971) for each site by pooling the samplings of the two years. Data analysis was performed using EstimateS ver. 9.1.0 (Caldwell, 2013).

Correspondence Analysis (CA) was performed to ordinate sites on the basis of species presence and abundances (sampling years were considered separately), and to describe the association between sites and species. Data were analysed using the software STATISTICA version 10 (StatSoft, Inc.).

Results

Overall, 739 individuals belonging to 11 coccinellid species were sampled in this study. A total of 8 species (342 individuals) were found in "La Rizza", 6 species (136 individuals) in "Casone" and 8 species (261 individuals) in "Tor Mancina" (table 2). All recorded species belong to the subfamily Coccinellinae, except for the phytophagous *Subcoccinella vigintiquatuorpunctata* (L.), which belongs to Epilachninae. All the rarefaction curves, which show the estimated number of species as a function of the number of sampled individuals (figure 1), tend to level off, but the asymptote is reached only in "Casone".

H. variegata, *C. septempunctata* and *P. quatuordecimpunctata* were found in all the sites and in both years (table 2). *H. variegata* was the dominant species in "La Rizza" in 2011 (166 individuals) and *P. quatuordecimpunctata* was the most abundant species in "Casone" in 2011 (18 individuals), whereas in 2012 it was exceeded by *C. septempunctata* (38 individuals). *H. variegata* was the most abundant species of the whole study, with a total of 216 individuals. *H. axyridis* was recorded in both the sites near Bologna, and it was the most abundant species in "La Rizza" in 2012 (67 individuals), while it was not found in "Tor Mancina". In both years this site was characterized by the dominance of *T. sedecimpunctata* and *Coccinula quatuordecimpustulata* (L.); these species were almost absent from the sites in Northern Italy (i.e: a single *T. sedecimpunctata* specimen was found in "La Rizza" in 2011). *T. sedecimpunctata* was the most abundant species in both years, accounting for 82 individuals in 2011 and 40 in 2012.

The CA chart shows that coccinellid communities in each geographic area group together and are clearly separated from each other (figure 2). *C. septempunctata*, *P. quatuordecimpunctata* and *Psyllobora vigintiduopunctata* (L.) are correlated with "Casone", whereas *H. variegata* and *H. axyridis* are correlated with "La Rizza" (figure 2). *C. quatuordecimpustulata* and *T. sedecimpunctata* were sampled almost exclusively in "Tor Mancina", and consequently are strongly associated with that site.

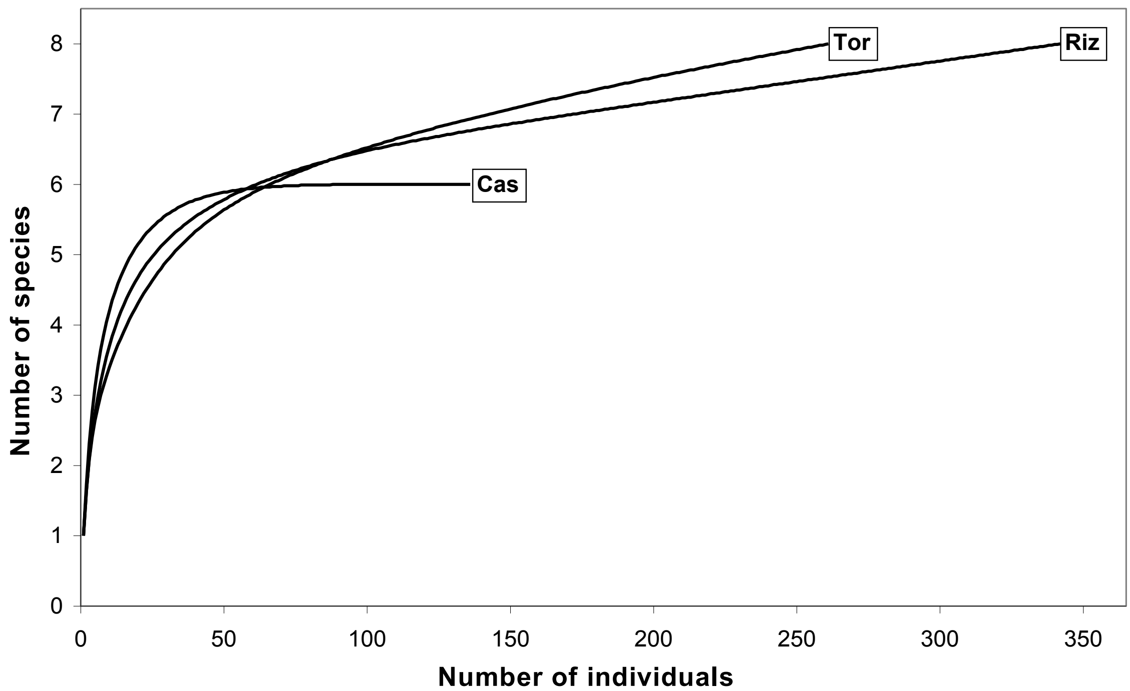

Figure 1. Individual-based rarefaction curves showing the estimated number of coccinellid species as a function of the number of individuals in the sampled sites (Cas = Casone; Riz = La Rizza; Tor = Tor Mancina).

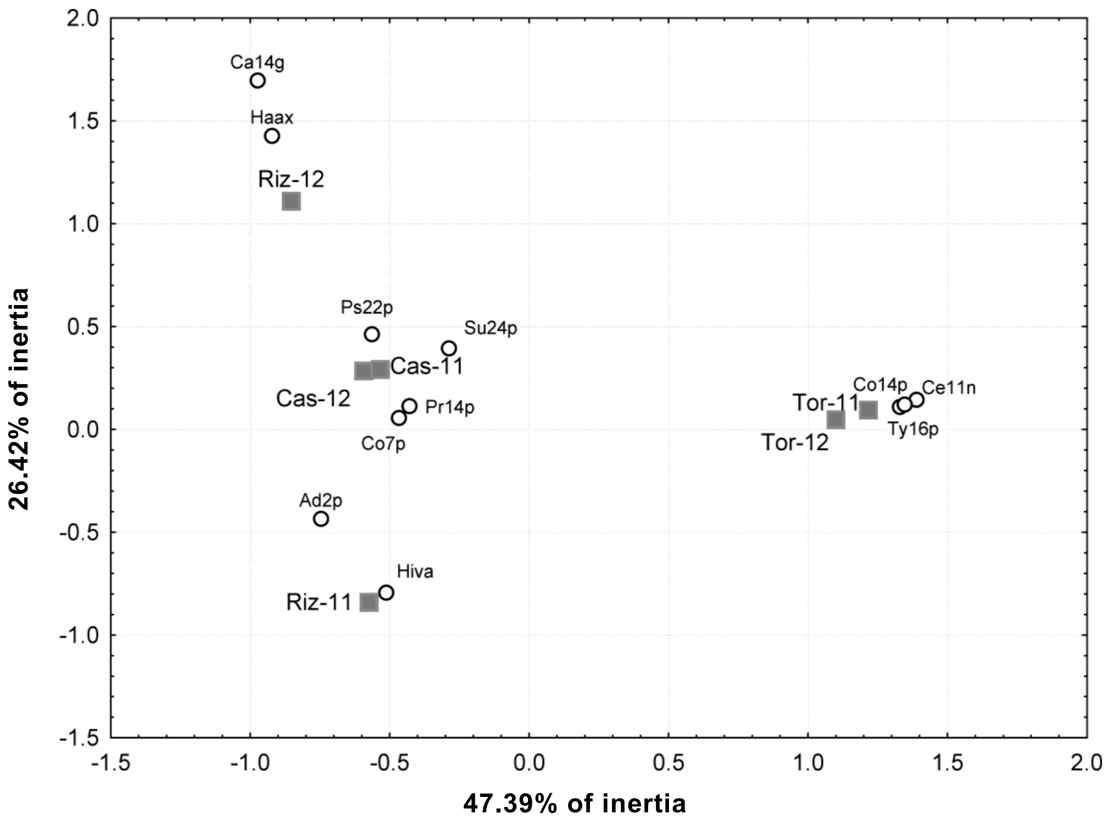

Figure 2. Biplot of the correspondence analysis relating coccinellids and sites. Site (grey squares) denominations: Cas-11 = Casone 2011; Cas-12 = Casone 2012; Riz-11 = La Rizza 2011; Riz-12 = La Rizza 2012; Tor-11 = Tor Mancina 2011; Tor-12 = Tor Mancina 2012. Species (open circles) denominations: Ad2p = *Adalia bipunctata*; Ca14g = *Calvia quatuordecimguttata*; Ce11n = *Ceratomegilla undecimnotata*; Co7p = *Coccinella septempunctata*; Co14p = *Coccinula quatuordecimpustulata*; Haax = *Harmonia axyridis*; Hiva = *Hippodamia variegata*; Pr14p = *Propylea quatuordecimpunctata*; Ps22p = *Psyllobora vigintiduopunctata*; Su24p = *Subcoccinella vigintiquatuor-punctata*; Ty16p = *Tytthaspis sedecimpunctata.*

In both sites near Bologna the maize flowering was recorded from June 24th to July 12th in 2011 and from June 29th to July 17th in 2012, and it overlapped partially with a period of high coccinellid abundance in the field (figure 3). Predatory species *H. variegata*, *H. axyridis*, *C. septempunctata* and *P. quatuordecimpunctata*, which occasionally feed on pollen, were abundant during maize flowering in this area (supplemental material). In "Tor Mancina" maize flowering spanned from July 28th to August 5th in 2011 and from July 26th to August 3rd in 2012. In these periods few coccinellid individuals, mainly belonging to the species *C. quatuordecimpustu-lata* and *T. sedecimpunctata*, were sampled (figure 2; see also supplemental material).

Discussion

In this study 10 species of Coccinellinae, representing 25% of the 40 Italian species, were recorded, as well as one out of the four Italian species of Epilachninae (data source: http://www.faunaitalia.it/checklist/, where only 39 species of Coccinellinae are listed, as the web site is updated to 2003 and *H. axyridis*is was still not recorded). This relevant fraction of the Italian species, along with the levelling trends of the rarefaction curves (figure 1), is a reliable indication that coccinellid fauna was sampled rather exhaustively.

Many of the most abundant species (*C. septempunc-tata*, *H. variegata*, *P. quatuordecimpunctata*, *C. qua-tuordecimpustulata* and *H. axyridis*) are important predators of pest arthropods, and are considered key-species for the control of many aphid pests (Bertolaccini *et al*., 2008; Burgio *et al*., 2004; Hodek and Evans, 2012; Kalushkov and Hodek, 2005; Koch, 2003; Obry-cki and Kring, 1998; Sørensen *et al*., 2013; Wo-jciechowicz-Żytko, 2011). The dominant species in the Central Italy site, *T. sedecimpunctata*, is considered mainly mycetophagous and palinophagous, but it is also known to feed on thrips (Thysanoptera Thripidae), mites and aphids (Hodek and Evans, 2012; Sutherland and Parrella, 2009), and it is thus potentially useful in bio-logical control.

The exotic *H. axyridis*, which was found only in the sites near Bologna, is considered an effective natural enemy of many pests but also a potential threat to native coccinellid species in some countries (Koch, 2003). *H. axyridis* was first observed in Italy in the urban area of Turin in 2006, and it was recorded in Emilia-Romagna in 2008 (Burgio *et al*., 2008). *H. axyridis* should be monitored to assess its population trend and evaluate potential impacts of this species on native coccinellid

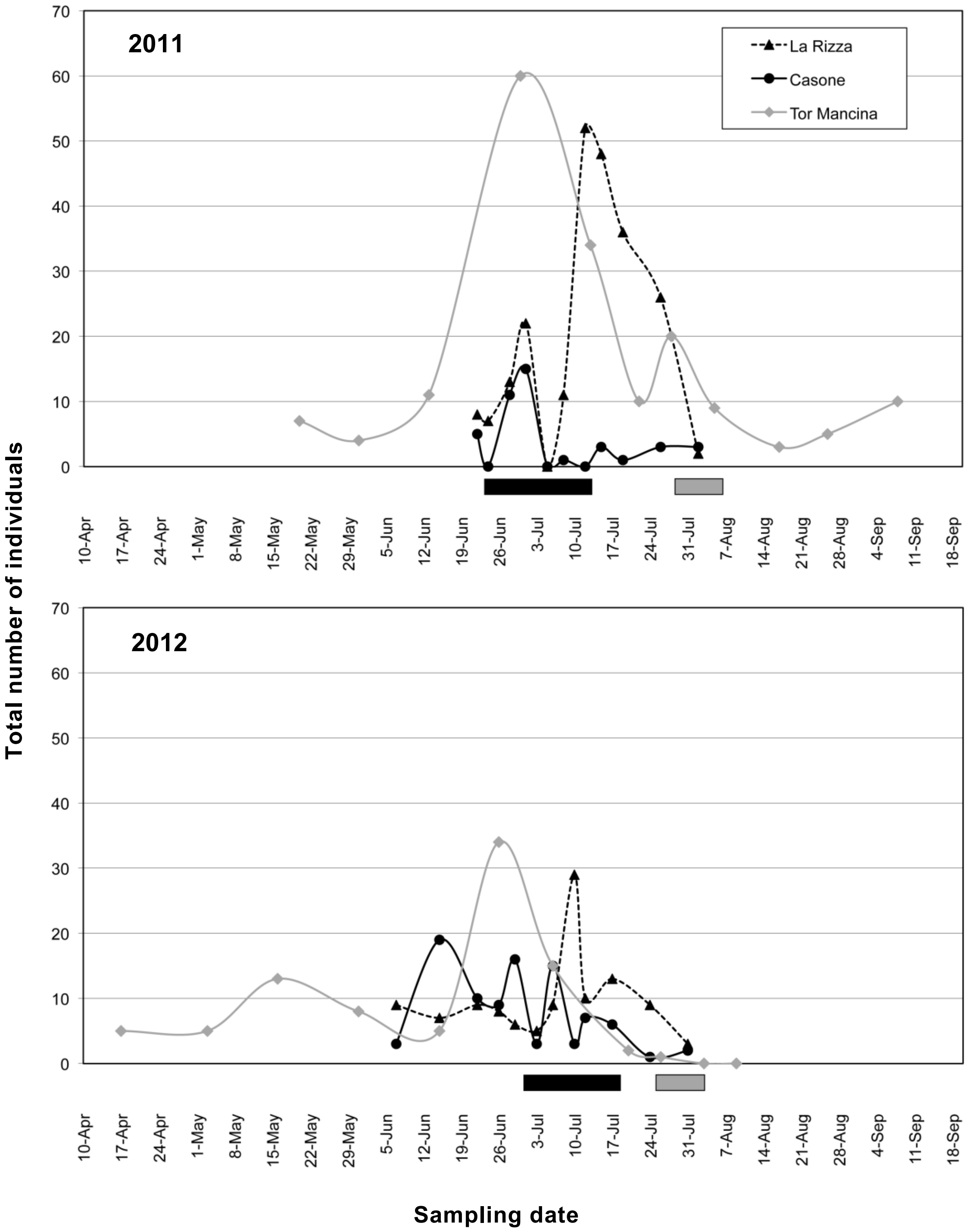

Figure 3. Coccinellid trends in the three sites. The boxes represent maize flowering periods in Bologna (black) and in Rome (grey).

assemblages, either through competition or intra-guild predation (Bazzocchi *et al*., 2004; Brown *et al*., 2011; Burgio *et al*., 2005; Katsanis *et al*., 2012; Koch, 2003).

The coccinellid community in Northern Italy was quite different in comparison with that of Central Italy (table 2 and figure 2). Some species (*T. sedecimpunctata* and *C. quatuordecimpustulata* in particular) were found almost exclusively in "Tor Mancina", whereas others (such as *H. axyridis*) were found only in the sites near Bologna. Furthermore, even the species found in both areas and in all three sites showed consistently different relative abundances (table 2). The structure of coccinellid communities in "Tor Mancina" and "Casone" kept rather stable over the two years of study, whereas in "La Rizza" *H. axyridis* and *H. variegata* showed wide fluctuations across the years.

Given the differences in the composition and phenology of coccinellid communities and in maize sowing and flowering periods between Northern and Central Italy, the expected exposure of ladybird beetles to maize pollen varies between the two areas (figure 3). Likely owing to differences in micro- and macro-habitat as well as in community composition, the periods of coccinellid population peaks varied across the sites, even between the two geographically close sites in Northern Italy. Moreover, in the northern sites maize flowering occurred earlier (from the end of June to the first half of July) than in "Tor Mancina", where maize was sown later (table 1) and pollen shed covered a short period from the end of July to the first days of August. The final outcome of these differences is that in Northern Italy sites maize flowering coincided with a period of high presence of coccinellids in the field (potential high-exposure scenario), whereas in "Tor Mancina" coccinellids were scarce during the pollen shed, as their population peak had occurred roughly one month earlier than the maize anthesis. Therefore, in a scenario of Bt-maize cultivation, the chance of exposure to Cry toxins through maize pollen would be much higher in Northern than in Central Italy sites, with maize sowing and flowering periods probably being the leading factors. Similar differences were noted when other non-target organisms (i.e. Lepidoptera) were sampled in different maize growing areas (Masetti *et al*., 2013) (MAN-GMP-ITA project report, http://www.man-gmp-ita.sinanet.isprambiente.it/documenti/output-finali/laymans-report/view).

Laboratory research to assess the effect of Bt-maize pollen using *C. septempunctata*, *H. variegata* and *P. quatuordecimpunctata* as non-target model organisms might be useful, as these important native predators were among the most abundant (and thus potentially exposed) species in Northern Italy sites during the maize flowering (supplemental material). However, it must be pointed out that the overlap with the flowering period represents only a part of the exposure assessment. Further research is needed to better identify and prioritize the species that are more likely to actually ingest significant amounts of maize pollen in field conditions, which is a very important information given the limited amount of toxin expressed in the pollen of widely used Bt-maize cultivars such as MON810 (Székàcs *et al*., 2010). For this reason, even though in Central Italy chances of coccinellid exposure to maize pollen seem rather low, the locally common *T. sedecimpunctata* should be considered in such research too. This species, being highly palinophagous (Hodek and Evans, 2012; Sutherland and Parrella, 2009), might indeed have higher chances of ingesting maize pollen than most of the other coccinellids.

Conclusions

This study provides information on the species of ladybird beetles occurring during maize flowering in Northern and Central Italy, where differences in their assemblages were found. Faunistic research is pivotal to plan conservation actions aimed at preserving these important predators and the ecosystem services they provide; it is also a key knowledge to assess the potential of coccinellids as bioindicators, which is still relatively unexplored. Their status as widespread and ecologically diversified insect predators and the relative ease of identification makes ladybird beetles promising candidates for the role of bioindicators, a possibility that has been stressed by various authors (Burgio, 2007; Iperti, 1999; Woltz and Landis, 2014). Additional field studies are needed to assess whether coccinellid communities tend to be stable over time and whether fluctuations in their composition can be related to specific environmental variables. The stability of ladybird communities across long time periods was recently reported and discussed by Honek *et al*. (2013).

Faunistic and phenological data presented in this study might be useful in a possible future scenario of Bt-maize cropping in the sampled areas, which are among the most important productive ones in Italy, as the peculiarities of the receiving environments need to be specifically considered during ERA before the commercial release of GMPs (EFSA Panel on Genetically Modified Organisms, 2010). However, it must be remarked that information about the actual interactions between coccinellids and maize plants in the field is still scarce, especially regarding the Italian ecosystems. Further research will thus be necessary to assess the role played by maize pollen in the diet of ladybird species in the studied areas, and to complete the characterization of this exposure pathway.

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References

ADANG M. J., CRICKMORE N., JURAT-FUENTES J. L., 2014.- Diversity of *Bacillus thuringiensis* crystal toxins and mechanism of action.- *Advances in Insect Physiology*, 47: 39-87.

ALVAREZ-ALFAGEME F., FERRY N., CASTANERA P., ORTEGO F., GATEHOUSE A. M., 2008.- Prey mediated effects of Bt maize on fitness and digestive physiology of the red spider mite predator *Stethorus punctillum* Weise (Coleoptera: Coccinellidae).- *Transgenic research*, 17 (5): 943-954.

ALVAREZ-ALFAGEME F., BIGLER F., ROMEIS J., 2011.- Laboratory toxicity studies demonstrate no adverse effects of Cry1Ab and Cry3Bb1 to larvae of *Adalia bipunctata* (Coleoptera: Coccinellidae): the importance of study design.- *Transgenic Research*, 20 (3): 467-479.

ANDOW D. A., LOVEI G. L., ARPAIA S., 2006. - Ecological risk assessment for Bt crops.- *Nature Biotechnology*, 24 (7): 749-751.

ARPAIA S., 2010.- Genetically modified plants and "non-target" organisms: analysing the functioning of the agro-ecosystem.- *Collection of Biosafety Reviews*, 5: 12-80.

ARPAIA S., MESSEAN A., BIRCH N. A., HOKANNEN H., HARTEL S., VAN LOON J., LOVEI G., PARK J., SPREAFICO H., SQUIRE G. R., STEFFAN-DEWENTER I., TEBBE C., VAN DER VOET H., 2014.- Assessing and monitoring impacts of genetically modified plants on agro-ecosystems: the approach of AMIGA project.- *Entomologia*, 2 (1): 79-84.

BAZZOCCHI G., LANZONI A., ACCINELLI G., BURGIO G., 2004.- Overwintering, phenology and fecundity of *Harmonia axyridis* in comparison with native coccinellid species in Italy.- *BioControl*, 49: 245-260.

BERKEVENS N., LANDUYT C., DEFORCE K., BERKEVENS D., TIRRY L., DE CLERCQ P., 2010.- Alternative foods for the multicoloured Asian lady beetle *Harmonia axyridis* (Coleoptera: Coccinellidae).- *European Journal of Entomology*, 107: 189-195.

BERTOLACCINI I., NUNEZ-PEREZ E., TIZADO E. J., 2008.- Effect of wild flowers on oviposition of *Hippodamia variegata* (Coleoptera: Coccinellidae) in the laboratory.- *Journal of Economic Entomology*, 101 (6): 1792-1797.

BROWN P. M. J., FROST R., DOBERSKI J., SPARKS T., HARRINGTON R., ROY H., 2011.- Decline in native ladybirds in response to the arrival of *Harmonia axyridis*: early evidence from England.- *Ecological Entomology*, 36: 231-240.

BURGIO G., 2007.- The role of ecological compensation areas in conservation biological control. 154 pp., *PhD Thesis*, Wageningen University, The Netherlands.

BURGIO G., VAN LENTEREN J., FERRARI R., 2001.- Indagine sui parassitoidi e predatori di afidi su colture erbacee dell'Emilia-Romagna, pp. 635-642. In: *5° Convegno nazionale sulla biodiversità*, 9-10 September 1999, Caserta, Italy.

BURGIO G., FERRARI R., POZZATI M., BORIANI L., 2004.- The role of ecological compensation areas on predator populations: an analysis on biodiversity and phenology of Coccinellidae (Coleoptera) on non-crop plants within hedgerows in Northern Italy.- *Bulletin of Insectology*, 57 (1): 1-10.

BURGIO G., SANTI F., MAINI S., 2005.- Intra-guild predation and cannibalism between *Harmonia axyridis* and *Adalia bipunctata* adults and larvae: laboratory experiments.- *Bulletin of Insectology*, 58 (2): 135-140.

BURGIO G., SANTI F., LANZONI A., MASETTI A., DE LUIGI V., MELANDRI M., REGGIANI A., RICCI C., LOOMANS A. J. M., MAINI S., 2008.- *Harmonia axyridis* recordings in northern Italy.- *Bulletin of Insectology*, 61 (2): 361-364.

BURGIO G., DINELLI G., MAROTTI I., ZURLA M., BOSI S., LANZONI A., 2011.- Bt-toxin uptake by the non-target herbivore, *Myzus persicae* (Hemiptera: Aphididae), feeding on transgenic oilseed rape in laboratory conditions.- *Bulletin of Entomological Research*, 101 (2): 241-247.

COLWELL R. K., 2013.- *EstimateS: statistical estimation of species richness and shared species from samples*, Version 9.1.- [online] URL: http:purl.oclc.org/estimates

DE LA POZA M., PONS X., FARINOS G. P., LOPEZ C., ORTEGO F., EIZAGUIRRE M., CASTANERA P., ALBAJES R., 2005.- Impact of farm-scale Bt maize on abundance of predatory arthropods in Spain.- *Crop Protection*, 24 (7): 677-684.

DIEPENBROCK L. M., FINKE D. L., 2013.- Refuge for native lady beetles (Coccinellidae) in perennial grassland habitats.- *Insect Conservation and Diversity*, 6 (6): 671-679.

DUAN J. J., HEAD G., MCKEE M. J., NICKSON T. E., MARTIN J. W., SAYEGH F. S., 2002.- Evaluation of dietary effects of transgenic corn pollen expressing Cry3Bb1 protein on a non-target ladybird beetle, *Coleomegilla maculata*.- *Entomologia Experimentalis et Applicata*, 104: 271-280.

ECKERT J., SCHUPHAN I., HOTHORN L. A., GATHMANN A., 2006.- Arthropods on maize ears for detecting impacts of *Bt* maize on nontarget organisms.- *Environmental Entomology*, 35 (2): 554-560.

EFSA PANEL ON GENETICALLY MODIFIED ORGANISMS, 2010.- Scientific opinion on the assessment of potential impacts of genetically modified plants on non-target organisms.- *EFSA Journal*, 8 (11): 1877. doi:10.2903/j.efsa.2010.1877.

EIZAGUIRRE M., ALBAJES R., LOPEZ C., ERAS J., LUMBIERRES B., PONS X., 2006.- Six years after the commercial introduction of Bt maize in Spain: field evaluation, impact and future prospects.- *Transgenic Research*, 15: 1-12.

FARINÓS G. P., ANDREADIS S. S., DE LA POZA M., MIRONIDIS G. K., ORTEGO F., SAVOPOULOU-SOULTANI M., CASTAÑERA P., 2011.- Comparative assessment of the field-susceptibility of *Sesamia nonagrioides* to the Cry1Ab toxin in areas with different adoption rates of Bt maize and in Bt-free areas.- *Crop Protection*, 30 (7): 902-906.

GIORGI J. A., VANDENBERG N. J., MCHUGH J. V., FORRESTER J. A., SLIPINSKI S. A., MILLER K. B., SHAPIRO L. R., WHITING M. F., 2009.- The evolution of food preferences in Coccinellidae.- *Biological Control*, 51: 215-231.

HARWOOD J. D., WALLIN W. G., OBRYCKI J. J., 2005.- Uptake of Bt endotoxins by nontarget herbivores and higher order arthropod predators: molecular evidence from a transgenic corn agroecosystem.- *Molecular Ecology*, 14 (9): 2815-2823.

HARWOOD J. D., SAMSON R. A., OBRYCKI J. J., 2007.- Temporal detection of Cry1Ab-endotoxins in coccinellid predators from fields of *Bacillus thuringiensis* corn.- *Bulletin of Entomological Research*, 97 (6): 643-648.

HODEK I., EVANS E. W., 2012.- Food relationships, pp. 141-274. In: *Ecology and behaviour of ladybird beetles (Coccinellidae)* (HODEK I., VAN EMDEN H. F., HONĚK A., Eds).- Wiley, Chichester, UK.

HONEK A., MARTINKOVA Z., KINDLMANN P., AMEIXA O. M. C. C., DIXON A. F. G., 2013.- Long-term trends in the composition of aphidophagous coccinellid communities in Central Europe.- *Insect Conservation and Diversity*, 7: 55-63.

HURLBERT S. H., 1971.- The nonconcept of species diversity: a critique and alternative parameters.- *Ecology*, 52: 577-586.

IPERTI G., 1999.- Biodiversity of predaceous coccinellidae in relation to bioindication and economic importance.- *Agriculture, ecosystems & environment*, 74 (1): 323-342.

JAMES C., 2014.- Global status of commercialized biotech/GM crops: 2014.- ISAAA Brief No 49, ISAAA, Ithaca, NY, USA.

KALUSHKOV P., HODEK I., 2005. - The effects of six species of aphids on some life history parameters of the ladybird *Propylea quatuordecimpunctata* (Coleoptera: Coccinellidae).- *European Journal of Entomology*, 102 (3): 449-452.

KATSANIS A., BABENDREIER D., NENTWIG W., KENIS M., 2012.- Intraguild predation between the invasive ladybird *Harmonia axyridis* and non-target European coccinellid species.- *BioControl*, 58: 73-83.

KOCH R. L., 2003.- The multicolored Asian lady beetle *Harmonia axyridis*: a review of its biology, uses in biological control, and non-target impacts. - *Journal of Insect Science*, 3: 32.

LENER M., GIOVANNELLI V., ARPAIA S., BALDACCHINO F., BENEDETTI A., BURGIO G., CANFORA L., DINELLI G., MANACHINI B., MAROTTI I., MASETTI A., SBRANA C., RASTELLI V., STAIANO G., 2013.- Applying an operating model for the environmental risk assessment in Italian Sites of Community Importance (SCI) of the European Commission Habitats Directive (92/34/EEC).- *Bulletin of Insectology*, 66 (2): 257-267.

LI Y., ROMEIS J., WANG P., PENG Y., SHELTON A. M., 2011.- A comprehensive assessment of the effects of Bt cotton on *Coleomegilla maculata* demonstrates no detrimental effects by Cry1Ac and Cry2Ab.- *PLoS ONE*, 6 (7): e22185

LI Y., ZHANG X., CHEN X., ROMEIS J., YIN X., PENG Y., 2015.- Consumption of Bt rice pollen containing Cry1C or Cry2A does not pose a risk to *Propylea japonica* (Thunberg) (Coleoptera: Coccinellidae).- *Scientific reports*, 5: 7679.

LUNDGREN J. G., 2009a.- Nutritional aspects of non-prey foods in the life histories of predaceous Coccinellidae.- *Biological Control*, 51 (2): 294-305.

LUNDGREN J. G., 2009b.- *Relationships of natural enemies and non-prey food*.- Progress in Biological Control 7, Springer, The Netherlands.

LUNDGREN J. G., WIEDENMANN R. N., 2002.- Coleopteran-specific Cry3Bb toxin from transgenic corn pollen does not affect the fitness of a nontarget species, *Coleomegilla maculata* DeGeer (Coleoptera: Coccinellidae).- *Environmental Entomology*, 31 (6): 1213-1218.

LUNDGREN J. G., HUBER A., WIENDENMANN R. N., 2005.- Quantification of consumption of corn pollen by the predator *Coleomegilla maculata* (Coleoptera: Coccinellidae) during anthesis in an Illinois cornfield.- *Agricultural and Forest Entomology*, 7: 53-60.

LUNDGREN J. G., GASSMANN A. J., BERNAL J., DUAN J. J., RUBERSON J., 2009.- Ecological compatibility of GM crops and biological control.- *Crop Protection*, 28 (12): 1017-1030.

MASETTI A., PERRY J. N., DINELLI G., 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.

MICHAUD J. P., 2012.- Coccinellids in biological control, pp. 488-519. In: *Ecology and behaviour of ladybird beetles (Coccinellidae)* (HODEK I., VAN EMDEN H. F., HONĚK A., Eds).- Wiley, Chichester, UK.

MOSER S. E., HARDWOOD J. D., OBRYCKI J. J., 2008.- Larval feeding on *Bt* hybrid and non-*Bt* corn seedlings by *Harmonia axyridis* (Coleoptera: Coccinellidae) and *Coleomegilla maculata* (Coleoptera: Coccinellidae).- *Environmental Entomology*, 37 (2): 525-533.

OBRIST L. B., DUTTON A., ALBAJES R., BIGLER F., 2006.- Exposure of arthropod predators to Cry1Ab toxin in Bt maize fields.- *Ecological Entomology*, 31 (2): 143-154.

OBRYCKI J. J., KRING T. J., 1998.- Predaceous Coccinellidae in biological control.- *Annual Review of Entomology*, 43: 295-321.

PORCAR M., GARCIA-ROBLES I., DOMINGUEZ-ESCRIBA L., LATORRE A., 2010.- Effects of *Bacillus thuringiensis* Cry1Ab and Cry3Aa endotoxins on predatory Coleoptera tested through artificial diet-incorporation bioassays.- *Bulletin of Entomological Research*, 100 (3): 297-302.

ROMEIS J., MEISSLE M., BIGLER F., 2006.- Transgenic crops expressing *Bacillus thuringiensis* toxins and biological control.- *Nature Biotechnology*, 24: 63-71.

ROMEIS J., MEISSLE M., ÁLVAREZ-ALFAGEME F., BIGLER F., BOHAN D. A., DEVOS Y., MALONE L. A., PONS X., RAUSCHEN S., 2014.- Potential use of an arthropod database to support the non-target risk assessment and monitoring of transgenic plants.- *Transgenic research*, 23 (6): 995-1013.

SANSINENEA E., 2012.- Bacillus thuringiensis *biotechnology*.- Springer, Dordrecht, The Netherlands.

SCHMIDT J. E. U., BRAUN C. U., WHITEHOUSE L. P., HILBECK A., 2009.- Effects of activated Bt transgene products (Cry1Ab, Cry3Bb) on immature stages of the ladybird *Adalia bipunctata* in laboratory ecotoxicity testing.- *Archives of Environmental Contamination and Toxicology*, 56: 221-228.

SØRENSEN C. H., TOFT S., KRISTENSEN T. N., 2013.- Cold-acclimation increases the predatory efficiency of the aphidophagous coccinellid *Adalia bipunctata*.- *Biological Control*, 65 (1): 87-94.

STEPHENS E. J., LOSEY J. E., ALLEE L. L., DITOMMASO A., BODNER C., BREYRE A., 2012.- The impact of Cry3Bb Bt-maize on two guilds of beneficial beetles.- *Agriculture, Ecosystems and Environment*, 156: 72-81.

SUTHERLAND A. M., PARRELLA M. P., 2009.- Mycophagy in Coccinellidae: review and synthesis.- *Biological Control*, 51: 284-293.

SZÉKÁCS A., LAUBER É., TAKÁCS E., DARVAS B., 2010.- Detection of Cry1Ab toxin in the leaves of MON 810 transgenic maize.- *Analytical and bioanalytical chemistry*, 396 (6): 2203-2211.

TIAN J. C., COLLINS H. L., ROMEIS J., NARANJO S. E., HELLMICH R. L., SHELTON A. M., 2012.- Using field-evolved resistance to Cry1F maize in a lepidopteran pest to demonstrate no adverse effects of Cry1F on one of its major predators.- *Transgenic research*, 21 (6): 1303-1310.

TRILTSCH H., 1999.- Food remains in the guts of *Coccinella septempunctata* (Coleoptera: Coccinellidae) adults and larvae.- *European Journal of Entomology*, 96: 355-364.

VAN FRANKENHUYZEN K., 2009.- Insecticidal activity of *Bacillus thuringiensis* crystal proteins.- *Journal of Invertebrate Pathology*, 101 (1): 1-16.

WOJCIECHOWICZ-ŻYTKO E., 2011.- Syrphids (Diptera, Syrphidae) and coccinellids (Coleoptera, Coccinellidae) occurring in *Myzus cerasi* (F.) (Hemiptera) colonies on *Prunus avium* L.- *Folia Horticulturae*, 23 (1): 37-42.

WOLFENBARGER L. L., NARANJO S. E., LUNDGREN J. G., BITZER R. J., WATRUD L. S., 2008.- Bt crop effects on functional guilds of non-target arthropods: a meta-analysis.- *PLoS One*, 3 (5): e2118.

WOLTZ J. M., LANDIS D. A., 2014.- Coccinellid response to landscape composition and configuration.- *Agricultural and Forest Entomology*, 16 (4): 341-349.

ZHANG G., WAN F., LOVEI G. L., LIU W., GUO J., 2006.- Transmission of *Bt* toxin to the predator *Propylaea japonica* (Coleoptera: Coccinellidae) through its aphid prey feeding on transgenic *Bt* cotton.- *Environmental Entomology*, 35 (1): 143-150.

ZHANG X., LI Y., ROMEIS J., YIN X., WU K., PENG Y., 2014.- Use of a pollen-based diet to expose the ladybird beetle *Propylea japonica* to insecticidal proteins.- *PloS ONE*, 9 (1): e85395.

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