Response of spiders (*Araneae*) to the introduction of an exotic pest (*Diabrotica virgifera* LeConte,1868 - Coleoptera, Chrysomelidae) in corn cultivations in Northern Italy

Giuseppe Camerini[1]*, Luca Franzini[2], Riccardo Groppali[2]

1 Istituto di Istruzione "Taramelli-Foscolo" – Via Mascheroni, 53 - 27100 - Pavia - Italy
2 Dipartimento di Scienze della Terra e dell'Ambiente - Università degli Studi, Via S. Epifanio, 14 – 27100 - Pavia - Italy

* Corresponding author: E-mail: giuseppe_camerini@libero.it

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Abstract

The response of spiders (Araneae) to the introduction of the corn rootworm (*Diabrotica virgifera*, Coleoptera Chrysomelidae, LeConte, 1868) was studied in 2010 and 2011 in two study areas in the Padana Plain (Northern Italy). The research was aimed at identifying the community of spiders which can prey on *D. virgifera* in corn fields, the rate of predation and the influence of landscape on the natural enemies' response to the invader. The two study areas were different in terms of both land use and agricultural patterns. In the area south of the Po River corn is cultivated together with many other crops, according to a crop rotation pattern, while in the area north of the Po River (Po) the agricultural landscape is mostly dedicated to rice monoculture. Predation was observed only in the area south of the Po River, ranging from 9.9% to 40.3%, where *Phylloneta impressa* (Araneae Theridiidae, Koch,1881) was the most important predator of corn rootworm adults. In the area north of the Po River no predation was recorded. Those results are one more example of how the richness and abundance of potential natural enemies can be crucially affected by structure and composition of the landscape surrounding crop fields. Predation by spiders could potentially support an integrated pest management of *D. virgifera* in agricultural districts where the pest is limited by crop rotation. The rate of predation was inversely dependent on victims' density, therefore predation by spiders tended to be of minor importance when *D. virgifera* was at epidemic levels.

Abbreviations

OP (Oltrepo' Pavese); P (Pavese)

Introduction

Expanding global trade has increased the number of exotic organisms responsible for significant damage to crops (Hulme, 2009; Pimentel *et al.*, 2001). Several factors can affect the probability of an exotic pest establishment and further spread: number of individual invaders, climate conditions, competitor occurrence, resource availability, natural enemies response, diversity of native communities, landscape structure (Hagley & Allen, 1990; Byers & Noonburg, 2003; Torchin *et al.*, 2003; Von Holle & Simberloff, 2005; Yang *et al.*, 2008).

With regard to natural enemies, the ability of predators in limiting an exotic pest depends on several variables: palatability of the victim, synchronization of life cycles, avoidance of predation behaviours, prey density, landscape structure (Symondson *et al.*, 2002; Borges *et al.*, 2006; Gardiner *et al.*, 2009).

Landscape composition is recognized as a major factor in structuring animal communities (Schmidt *et al.* 2004; Isaia *et al.*, 2006; Picchi *et al.*, 2016). In addition, landscape structure can affect exotic species' invasion patterns especially because it can modulate the biological resistance coming from natural enemies (Batáry *et al.*, 2008; Gardiner *et al.*, 2009; Schmidt *et al.*, 2008; Thorbek & Topping, 2005).

Here the results of a research on the response of spiders (*Araneae*) to the introduction of the exotic western corn rootworm (*Diabrotica virgifera virgifera*, Coleoptera Chrysomelidae, LeConte, 1868) in the Po flood plain (Northern Italy) are reported. *D. virgifera* is an endemic and noxious pest in North America (Krysan & Miller, 1986; Lundgren *et al.*, 2009, 2015; Wangila *et al.*, 2015) and it is expected to spread worldwide, as an exotic, thus becoming one of the major threats to corn production (Kriticos *et al.*, 2012; Marchioro and Krechemer, 2018). Since 1992 (first record in Serbia) *D.virgifera* has colonized several eastern European Countries as a result of multiple invasions (Aragón *et al.*, 2010; Kiss *et al.*,2005; Ciosi *et al.*, 2008).

D. virgifera was recorded for the first time in Italy in the eastern Po flood plain in 1998 (Furlan, 2007), but thanks to an eradication program its diffusion was temporarily delayed, though not interrupted. In 2006 the pest colonized most part of the Po flood plain, where Italy's corn is mostly produced.

Most of the crop yield loss caused by *D. virgifera* is due to larvae, which feed on roots (Hill, 1975; Branson & Krysan, 1981; Toth *et al.*, 2020). In addition to that, adults reduce both fertility and production by feeding primarily on corn silk (Lanzanova *et al.*, 2014; Marton *et al.*, 2009; Tollefson, 2007). In the Po flood plain *D.virgifera* develops a single generation; the overwintering eggs start to hatch in late spring and the larvae feed for 3 - 4 weeks on maize roots, finally turning into pupae. Adult beetles emerge from the second half of June to September and their maximum density is usually recorded between 20th July and 15th August (Furlan, 2006).

The establishment of this pest in European agro-ecosystems was favoured by suitable climatic conditions, plenty of food and lack of natural enemies. One more factor supporting *D.virgifera* populations was corn monoculture, which tends to increase the risk of pest outbreaks (Toepfer *et al.*, 2005).

Since the first colonization, in Hungary and Romania it has been observed that some spider species belonging to Theridiidae and Agelenidae can feed on *D. virgifera* adults (Tóth *et al.*, 2002; Grozea, 2010; Grozea *et al.*, 2016); the same kind of predation was recorded in Northern Italy, in the Po flood Plain (Boriani *et al.*, 2005). Actually, the predation of *D. virgifera* adults by spiders was regularly recorded between 2009 and 2016 all over Western and Central Po flood Plain (Piedmont, Lombardy, Emilia Romagna - unpublished data).

Even if generalist predators are usually considered poor biocontrol agents (Snyder &Wise, 1999; Polis and Holt,1992) increasing evidence shows that they can sometimes reduce pest populations in agro-ecosystems (Mansour *et al.*, 1980; Riechert & Lawrence, 1997; Nyffeler & Benz, 1998; Young & Edwars,1990; Maloney *et al.*, 2003; Picchi *et al.*, 2016; Camerini, 2017). Therefore, in order to test such an hypothesis, we planned a research aimed at identifying the community of agrobiont spiders which can prey on *D. virgifera* in corn fields, assessing the extent of predation and the influence of landscape, if any, on natural enemies' response to the invader.

Material and methods

The experimental sites

The experimental sites were located in the Central Western Po floodplain. This area is crossed by the Po River, which works as a border demarcation between two agricultural areas whose agricultural patterns are significantly different: on one side a diversified landscape south of the Po River called Oltrepò Pavese (OP study area), on the other side a highly homogeneous area north of the Po River called Pavese (P study area) almost entirely devoted to rice cultivations.

According to the Italian Agricultural Census (2010), OP study area was mainly devoted to arable land (91.7%) where maize, wheat, barley and alfalfa were the most common crops and were rotated by the majority of farms (95.2%). Rice cultivation was missing.

In P study area 52.9% of farms have regularly followed a "crop plan" based on rice monoculture. Corn was not a widespread crop and a great part of agricultural land (88.6%) was devoted to rice production. (Italian Agricultural Census, year 2010).

In 2010 and 2011 two experimental sites were established in both study areas. The corn fields in OP study area were located in Bastida Pancarana (a.sl. 67 m) while in P study area they were placed in San Martino Siccomario (a.sl. 63m). The coordinates of the fields selected for sampling are shown in Table 1.

Table 1 - Experimental sites: coordinates and field size. Experimental sites in P area were the same in both years.

Experimental site	Year	Sampling site	Field size (ha)	Latitude	Longitude
OP	2010	a	6.8	45°05'15''.17 N	9°04'37''.76 E
OP	2010	b	0.4	45°05'24''.51 N	9°04'59''.83 E
P	2010	a	1.2	45°09'56''.70 N	9°07'43''.43 E
P	2010	b	1.1	45°09'44''.47 N	9°07'40''.90 E
OP	2011	c	0.3	45°05'17''.71 N	9°05'02''.83 E
OP	2011	d	1.5	45°05'24''.51 N	9°04'59''.83 E
P	2011	c	1.2	45°09'56''.53 N	9°07'43''.46 E
P	2011	d	1.1	45°09'44''.37 N	9°07'43''.17 E

Experimental plan and Diabrotica monitoring

In those experimental sites, sampling and observations aimed at:

- monitoring *D. virgifera* life cycle by means of chromotropic traps;
- assessing the density of potential preys (corn rootworm adults) on corn plants;
- analysing spider community composition in corn fields;
- identifying spider species which feed on *D. virgifera* and assessing the predation rate.

D.virgifera adults were monitored by means of chromotropic traps (Pherocon® AM) since corn rootworms adults are attracted by yellow coloured panels (Kuhar and Youngman,1995). The density of traps in each experimental site was 3/ha. They were placed along a diagonal transect crossing the field. From the end of June to the end of August each trap was weekly checked and renewed.

Density of *D.virgifera* was also estimated by counting adults on a sample of 100 corn plants along the rows of the crop (10 sub-samples - 10 plants /sub-sample). The sub-sample position within the field was set weekly by randomly generating 10 points by means of ArcView 9.3 GPS.

The following data were recorded:

- *D.virgifera* adults on corn plants;
- remains of *D.virgifera* adults caught in spiders webs;
- total number of spiders beside webs.

The percentage of *D.virgifera* adults preyed by spiders (P%) was calculated as follows:

$P\% = (N1/ N1+N2)*100$ where N1 is the number of victims (*D.virgifera* adults captured by spiders) and N2 is the number of unpreyed *D.virgifera* adults recorded on corn plants.

Spider monitoring and sampling

The spider community was studied by weekly collecting all spiders from webs. A random sample of 20 corn plants was weekly set by using ArcView 9.3 GPS in the same way above described. Spiders on plants were sampled by means of an aspirator and brought to the lab for identification. Adults of *D. Virgifera* coming from chromotrophic traps or preyed by spiders were also observed with a stereomicroscope for sex discrimination.

Supplemental collection

In addition to systematic surveys carried out in 2010 and 2011, supplemental observations were carried out inside a broader area in July 2011. A sample was taken from each one of 8 fields both in OP and P study areas, in order to achieve a confirmation of the results coming from systematic sampling, on a broader geographic scale. Fields were located across a transect of respectively 8.5 Km (OP study area – mean field size 4.1±2.4 ha) and 14.6 Km in length (P study area - mean field size 2.6±1.7 ha). *D.virgifera* predation and spiders' community composition were studied according to the methods already described.

Statistical analysis

Data coming from the research were statistically elaborated using "Biostat" software . Data sets were first analyzed to test their normal distribution according to five specific tests: Kolmogorov-Smirnov/Lilliefor, Shapiro-Wilibuitk, D'Agostino Skewness, D'Agostino kurtosis, D'Agostino Omnibus.

As data sets distribution did not agree with a normal pattern, only non parametric tests were applied. The Mann-Whitney U test was used to compare differences between two independent groups of data. Contingency tables were used to record and analyze the relation between two or more categorical variables.

Results and discussion

D.virgifera population trends

D.virgifera population dynamics in 2010 and 2011 as resulting from trapping are reported in Figures 1-2. *D. virgifera* adults trapping provided some basic information on population trends. In summer 2010 the number of records was low until the beginning of July, but a sharp increase was recorded around the half of the month. An earlier emergence of adults was recorded in 2011 in comparison with the previous year. The abundance of trapped corn rootworm adults in OP experi-

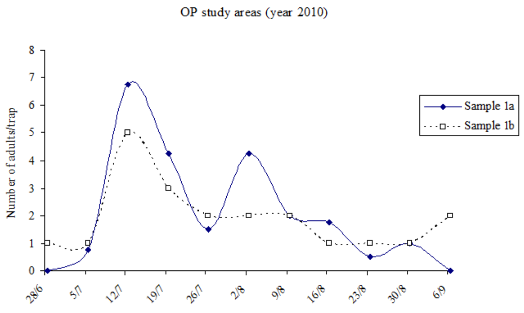

Fig. 1a - Number of D.virgifera adults weekly trapped in OP experimental sites (year 2010)

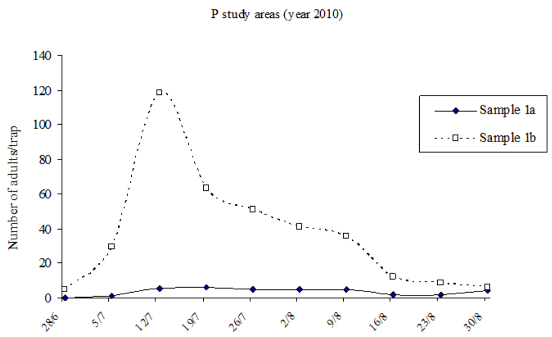

Fig. 1b - Number of D.virgifera adults weekly trapped in P experimental sites (year 2010)

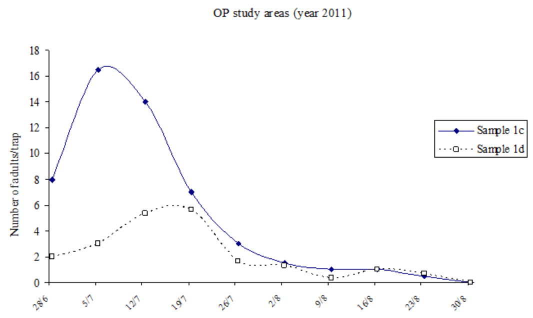

Fig. 2a - Number of D.virgifera adults weekly trapped in OP experimental sites (year 2011)

mental sites was not significantly different both in 2010 and 2011 (Mann Withney test - P>0.05). A significant difference occurred between P experimental sites in 2010 (Mann Withney test - P<0.001) but such a trend was not confirmed in 2011.

D.virgifera predation by spiders in experimental sites (systematic samplings)

The predation of *D.virgifera* by spiders was regularly observed both in 2010 and in 2011 in OP experimental sites (Table 2).

Table 2 - Predation rates in OP experimental sites. "Preys": *D.virgifera* adults found in spider webs

Year	Experimental sites	Preys	D.virgifera (preyed+unpreyed)	Predation(%)
2010	Bastida P. (a).	25	62	40.3
2010	Bastida P. (b).	8	59	13.6
2011	Bastida P. (c).	9	23	39.1
2011	Bastida P. (d).	11	111	9.9

D.virgifera adults density was the highest in July (Fig.1,2) but predation rates were negligible in that period, while they gradually increased in August (Fig.3a,3b) when abundance of *D.virgifera* adults was going to decline.

Systematic sampling in OP study area also revealed that during 2010 *D. virgifera* females were more prone to be captured by spiders in comparison to males (contingency table, p<0.05), but such a trend was not confirmed in 2011.

In OP experimental sites *D. virgifera* was one of the most common preys of spiders both in 2010 and 2011. Out of 202 victims found in the same webs where also *D.virgifera* adults were trapped, only aphids (48%)

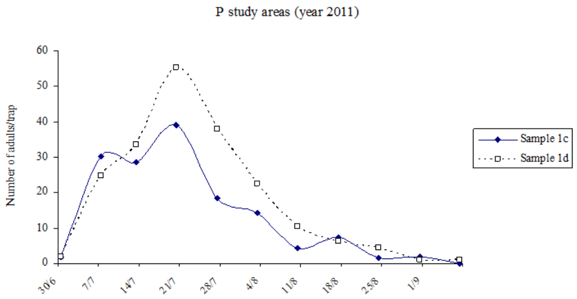

Fig. 2b - Number of D.virgifera adults weekly trapped in P experimental sites (year 2011)

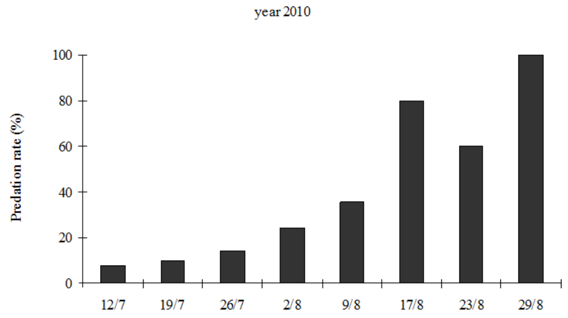

Fig. 3a - Percentage of D.virgifera adults preyed on by spiders in OP sampling sites (years 2010)

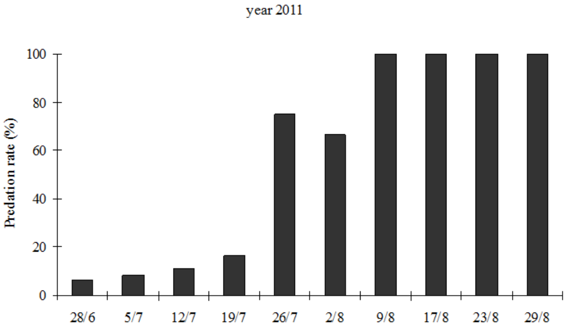

Fig. 3b - Percentage of D.virgifera adults preyed on by spiders in OP sampling sites (years 2010)

were more frequently recorded. Given the small size of aphids, *D.virgifera* (26.7%) represented an important food intake in terms of biomass.

Predation of *D.virgifera* involved a restricted number of spider species, mainly belonging to Theridiidae. In more detail, predation seemed to be species specific, since the majority of *D. virgifera* adults (N = 39) were captured by *Phylloneta impressa* (66.7%) and *Theridion pictum* (15.4%) females. Other spider species involved in *D.virgifera* adults trapping were *Mangora acalypha, Theridion simile, Synaema globosum, Stemonyphantes lineatus, Hypsosinga pygmaea* and *Cyclosa oculata*. The leading role of *Phylloneta impressa* was somehow an expected data, since in summer 2009 a preliminary survey on spiders feeding on the corn rootworm in OP area had revealed that 90.5% of records (N = 21) were *Phylloneta impressa.*

No predation of *D.virgifera* adults was recorded in P study areas neither in 2010 nor in 2011. Given the role of Theridiidae as natural enemies of corn rootworm adults, it was hypothesized that the lack of predation in P experimental sites could be related to a different structure of spider communities in comparison with OP experimental sites. Such a hypothesis was confirmed by comparing the composition of spider communities resulting from sampling on corn plants (Table 3).

In 2011 a significant decrease of spiders abundance (N=14) was recorded in P experimental sites. Such a decrease can reasonably be related to the fact that in spring 2011 shrubs and weeds along borders of both sites had been removed by shredding.

Spider community composition in experimental sites (systematic samplings)

With regard to spider community composition, it has to be noticed that both in 2010 and 2011 the comparison between OP and P experimental sites resulted in a significant difference (P<0.01- contingency table - table 3). In 2010 (N=46) and 2011 (N=56) Theridiidae (40.1% on average) were the dominant group, together with Araneidae (36.2%) in OP experimental sites. In 2010 (N=38) and 2011 (N=14) spider community recorded in P experimental sites was dominated by Tetragnathidae (38.6% on average) and Araneidae (40.5%), while Theridiidae distribution frequency was of minor importance (7.6%).

Table 3 - Spiders' community composition (OP and P experimental sites). The abundance of spider species is expressed as a percentage value (mean value - years 2010 and 2011)

	OP experimental sites %	P experimental sites %
Fam.Theridiidae	*40.1*	*7.6*
Theridion sp.	4.9	1.9
Phylloneta impressa	30.3	1.9
Simitidion simile	1	1.9
Theridion pictum	3.9	1.9
Fam.Araneidae	*36.2*	*40.5*
Mangora acalypha	25.4	5.8
Hypsosinga sp.	-	15.5
Hypsosinga pygmaea	4.9	9.7
Singa hamata	2	-
Araneus sp.	-	3.8
Argiope bruennichi	2.9	1.9
Larinioides sp.	-	1.9
Araneus diadematus	-	1.9
Cercidia prominens	1	-
Tetragnathidae	*7.9*	*38.6*
Tetragnatha sp.	3.9	30.8
Tetragnatha montana	2	7.8
Tetragnatha extensa	2	
Linyphidae	*8*	*5.7*
Linyphia sp.	1	3.8
Microlinyphia pusilla	2	-
Linyphia triangularis	2	1.9
Frontinellina frutetorum	2	-
Stemonyphantes sp.	1	-
Clubionidae	*4.8*	-
Clubiona sp.	3.8	-
Clubiona pallidula	1	-
Sparassidae	*2*	-
Micrommata virescens	2	-
Thomisidae	*1*	-
Xysticus kochi	1	-
Pisauridae	-	*1.9*
Pisaura mirabilis	-	1.9
Saliticidae	-	*3.8*
Heliophanus sp.	-	1.9
Ballus chalybeius	-	1.9
Agelenidae	-	*1.9*
Agelena labyrinthica	-	1.9

D.virgifera predation by spiders (supplemental samples)

Results coming from the supplemental samples carried out in July 2011 confirmed the ones resulting from systematic samplings. *Phylloneta impressa* (37.5%) was the dominant species in OP study area (N=24), while in samples coming from P study area *P.impressa* frequency was lower (9.5% - N=21).

The low abundance of *Theridiidae* spiders could justify the missing observation of *D.virgifera* predation in supplemental samples collected in P study area. On the contrary, in every OP supplemental sampling sites, evidence of predation was found, mainly due to *P.impressa* (85.7% - N=21). Factors which justify the ability of this spider in catching adults of *D.virgifera* are a usual occurrence in European corn fields, where it is common (Toth *et al.*, 2002, 2005; Moeser & Vidal S. 2005; Meissle & Romeis, 2009) and its predation habits. *P.impressa* females construct an inverted-cup-shaped retreat, which is covered with plant debris and spin a typical tangle web below it. Such an architecture works as an effective trap both for males and females of *D.virgifera* adults, which move on corn plants in search both for food (silk, pollen) and sexual partners. On the contrary, orb webs did not work as traps for corn rootworm adults; that's the reason why orb weaver spiders have been regularly recorded in corn fields, but did not have any role as natural enemies.

Trends of predation in relation to *D.virgifera* density are shown by Fig.4. The graph includes all predation data recorded from 2010 to 2011 (OP systematic and supplemental samplings). The effectiveness of predation tends to decrease with prey density ($P<0.01$).

Conclusions

In European Countries the complex of native pathogens, parasites and predators active against *D.virgifera* eggs and larvae includes a restricted number of species (Toepfer & Khulmann, 2004). Adults seem to be a bit more exposed to natural enemies attacks than larvae and spiders are the only potential predators of some importance which so far have been recorded in Europe (Tóth *et al.*, 2002; Grozea, 2010; Boriani *et al.*, 2005).

The assemblage of spiders recorded in the corn fields where *D.virgifera* predation was observed mainly included species belonging to Theridiidae, among which *Phylloneta impressa* played a key role.

Predation tended to be inversely dependant on victims density (Fig.4). This pattern can suggest a potential role for spiders in *D. virgifera* limitation within an integrated pest management strategy primarily based on crop rotation. Actually, the efficacy of crop rotation in limiting corn rootworm did not prove to be absolute (Gray *et al.*, 2009). Anyway, in European Countries colonized by *D.virgifera*, crop rotation is actually a recognized strategy recommended by the European Community (Commission Recommendation 2014/63/UE). In rural districts (i.e. OP study area) where rotation protocols imply the turnover of a variety of crops, *D.virgifera* density can be significantly limited; thus, when *D.virgifera* density is low or moderate, the action of spiders can work as a further pest-limiting potential factor.

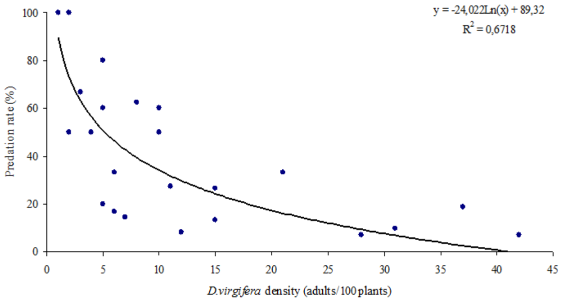

Fig. 4 - Predation of *D.virgifera* adults in relation to density. All data are from OP study areas (systematic and supplemental samples).

On the other hand, in agricultural areas where corn is not cultivated according to a rotation pattern, *D. virgifera* density tends to be so high that spiders fail to be effective predators.

The results of this study seem to give one more example of how the richness and abundance of potential natural enemies can be significantly affected by structure and composition of andscape surrounding crop fields (Clough *et al.*, 2005; Gardiner *et al.*, 2009).

In agricultural districts - such as P area - where landscape diversity is limited by monoculture, the scarce availability of uncultivated areas and the poor diversity of vegetation work as negative factors against spider communities.

In addition, rice fields proved to be an unsuitable habitat for *P.impressa* survival and dispersion. On a smaller scale, one more limiting factor of paddy landscape tends to negatively affect spiders and other natural enemies: herbicide spraying or shredding as a common practice to reduce weeds growing along strips of herbs bordering rice fields.

Further research is needed to assess the potential of spiders in affecting *D. virgifera* population dynamics in agricultural areas where corn is sown according to rotation patterns. Anyway, this study complies with the hypothesis that landscape heterogeneity is an ecological key factor for agrobiont spiders.

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