

SPIDERS (ARANEAE) AS PREDATORS OF THE EXOTIC *Metcalfa pruinosa* (SAY, 1830) (HOMOPTERA: FLATIDAE) IN THE CENTRAL WESTERN PO FLOODPLAIN (NORTHERN ITALY)

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ABSTRACT

The planthopper *Metcalfa pruinosa* (Say, 1830) (Homoptera: Flatidae) was unintentionally introduced from North America to Europe and started its invasion from Italy in 1979. During 2004, a survey was carried out in a study area located in the central western Po floodplain (Northern Italy) in order to identify spiders feeding on *M. pruinosa*. Fourteen species of spiders preying upon the planthopper were recorded. The community of spiders mainly included species belonging to Agelenidae (27.4%), Linyphiidae (26.7%) and Araneidae (15.6%). Almost all remains of *M. pruinosa* found in the webs were adults of the pest, while the predation of juvenile stages was negligible.

During summer 2005 and 2006, more specific observations were carried out, respectively, on *Agelena labyrinthica* and *Linyphia triangularis* that were identified in 2004 as the most common planthopper predators.

M. pruinosa was regularly found in the webs of *A. labyrinthica*; predation rate was maximum in July and around the half of August, while it tended to decline during September.

L. triangularis modulated its density in response to the increase of *M. pruinosa* density. *M. pruinosa* was recorded as the dominant prey in *L. triangularis* webs all summer long as far as the prey could be available.

Key words: *Linyphia triangularis*, *Agelena labyrinthica*, predators, *Metcalfa pruinosa*.

INTRODUCTION

The constant increase of trade across the World tends to make the probability of human-assisted spread of living organisms beyond their native range easier (Hulme, 2009). Alien species are actually considered one of the main factors supporting the ongoing global environmental change (Vitousek *et al.*, 1997; Pimentel *et al.*, 2005; Gurevitch and Padilla, 2004). Due to their peculiar biological characters (such as small dimensions and high fecundity) insects are often accidentally introduced into areas outside their native range, where they can escape the controlling influence of their natural enemies. Therefore, when they successfully establish in such new areas, it may be difficult to control them, especially when the invader tends to play a complex role within the trophic web because of its poliphagy (Borges *et al.*, 2006).

The accidental introduction of the planthopper *Metcalfa pruinosa* (Say, 1830) (Homoptera: Flatidae) into Europe can be taken as an example. This invasive species is originally from America (Metcalf & Bruner, 1948; Wilson & McPherson, 1981).

M. pruinosa started its invasion from Italy. It was first recorded next to Treviso (north eastern Italy) in 1979 (Zangheri & Donadini, 1980). Then the species invaded all Italy, including the insular regions (Girolami & Conte, 1999). After Italy's colonization, in a few decades *M. pruinosa*, has spread over most Europe, finally reaching the Black Sea coast in 2009 (Preda &

Skolka, 2011). It has been recorded in several European Countries: France, Slovenia, Croatia, Switzerland, Spain, Serbia Montenegro, Austria, Hungary, Greece, Turkey, Bulgaria, Bosnia Herzegovina, Slovakia, Albania, Russia, and Romania (Della Giustina, 1986; Maceljski *et al.*, 1995; Lauterer & Malenovsky, 2002; Seliak, 2002; Drosopoulos *et al.*, 2004; Orosz & Der, 2004; Trenchev *et al.*, 2007; Gnezdilov & Sugonyaev, 2009; Strauss, 2010; Grozea *et al.*, 2011; Barbuceanu *et al.*, 2015).

In the native range, the species is not considered of any economic importance, while in Europe, *M. pruinosa* can potentially be responsible for damage to a wide range of host plants. The trophic niche differs greatly from one country to another (Grozea *et al.*, 2011). In Italy, this polyphagous insect feeds on a wide variety of woody and herbaceous plants, with over 200 species of hosts. Potential damage to roadside trees, forest tree nurseries, vineyards, orchards and soybean (Ciampolini *et al.*, 1987) is directly related to sap extraction and indirectly due to the production of wax and honeydew excretion (Lucchi, 1997).

Metcalfa pruinosa has one generation per year. In late summer and early autumn, the females insert eggs into pre-existing openings in the bark of twigs or excavate openings in soft corky bark. After overwintering in the twigs, the eggs hatch in late spring. The development of the five nymphal instars takes place throughout the summer and the first adults can be found by early July. Adults tend to spend night time for dispersion, calling behaviour and copulation, while feeding occurs during daylight (Wilson & Lucchi, 2007).

In Italy, the natural enemy complex of *M. pruinosa* includes several indigenous predators, mainly birds and insects (Greatti *et al.*, 1994) and *Neodryinus typhlocybae* (Ashmead, 1893) (Hymenoptera, Dryinidae), a control agent introduced by the USA as a result of a biological fight project which started in 1979 (Mazzon & Girolami, 2002). The project was then developed in many urban and agricultural areas of Italy, which resulted in the establishment of the parasitoid (Alma *et al.*, 2005).

This paper summarizes the results of a research on indigenous spiders which are active against the planthopper in a study area located in the Po flood plain (Northern Italy), the main Italian agricultural district.

A preliminary survey was carried out in 2004 on the community of spiders feeding on *M. pruinosa* in some agro-ecosystems of the Padana plain. In 2005 and 2006, a research was planned in order to analyze predation patterns, phenology and response to *M. pruinosa* density of the spiders species *Agelena labyrinthica* (Clerck, 1757) and *Linyphia triangularis* (Clerck, 1757) which showed to be particularly active against *M. pruinosa*.

MATERIAL AND METHODS

Study area: The study area is located in the plain of Oltrepo Pavese, in the province of Pavia (central western Po floodplain) south of the river Po. The landscape mainly includes agricultural land devoted to the cultivation of cereals (wheat, barley, corn and sorghum), alfalfa, soybean, horticultural crops and poplar plantations. The main destination of agricultural production is arable land (91.7%). With regard to agricultural patterns, the greatest part of farms (95.2%) rotate crops (ISTAT, 2010).

The research started in summer 2004, when samples of spiders feeding on the planthopper were collected in three study sites. In addition, during this preliminary survey, samples were also taken from a study area located at foot of the hills in the southern part of Oltrepo Pavese,

where the main agricultural destination is grapevine cultivation. Table 1 shows the coordinates of sampling sites and the general description of the habitat.

Table 1 sampling sites (year 2004).

Sampling site	Latitude	Longitude	A.S.L. (m)	Habitat
Pancarana	45°04'48".63 N	9°03'22".09 E	64	Shrubs next to willow woods and poplar plantations
Castelletto Branduzzo	45°04'02".55 N	9°05'33".32 E	64	Hedgerows next to soybean and alfalfa cultivations
Bressana Bottarone	45°05'41".57 N	9°08'20".24 E	61	Shrubs next to corn fields
Casteggio	44°59'30".25 N	9°08'07".31 E	157	Hedgerows next to vineyards

Spiders found on webs with remains of the planthopper (neanids, nymphs and adults of *Metcalfa pruinosa*) were captured and preserved in a 70% ethyl alcohol solution until their identification by means of a stereoscopic microscope. Spider samples are stored in the entomological collection of "Istituto A.Cairolì" in Pavia (Italy).

During summer 2005 and 2006, more specific observations were carried out, respectively, on *Linyphia triangularis* (Fig.1) and *Agelena labyrinthica* (Fig.2) which in 2004 had been identified as the most common planthopper predators.



Figure 1 *Linyphia triangularis* and *Metcalfa pruinosa*

Figure 2 *M. pruinosa* remains on *Agelena labyrinthica* web

The phenology of *M. pruinosa* predation by *A. labyrinthica* was studied during summer 2005 in the study area of Bressana Bottarone (Table 1) by initially marking 20 webs occupied by the spider. Webs were built on *Rubus* sp., *Eupatorium cannabinum* and *Equisetum arvense*. From July to September, webs were regularly visited every 2 days and preyed *M. pruinosa* remains were counted and removed from the webs. All the webs abandoned by spiders or damaged by storms and hail were not computed in the survey, but from time to time, they were replaced by marking new nearby webs occupied by *A. labyrinthica*. Thus, the number of monitored webs was not constantly equal to the initial one (N=20) but the replacement of damaged webs maintained the number of monitored web within the range N=15÷20.

The trend of *M. pruinosa* predation by *L. triangularis* was analyzed in summer 2006 in the study area of Castelletto di Branduzzo (Table 1) where 10 webs were marked and monitored. The occurrence of preys (*M. pruinosa*) was recorded every two days; remains of preys were removed from webs and identified in the lab. Webs were built on *Equisetum palustris*. Those webs were often built so close one to the other that there was no clear separation between silks. The number of spiders on them tended to change from time to time, therefore both the number of victims and spiders were recorded on each survey. In addition to systematic records, more specific controls were carried out in the same study area. Since a higher density of *L. triangularis* webs was observed on *Equisetum* plants where *M. pruinosa* density was at epidemic levels, it was hypothesized that such a high density of spiders could be supported by a similar high density of *M. pruinosa* adults. In order to test this hypothesis, 7 square plot areas (0.5 x 0.5 m) including *Equisetum palustris* plants were traced and monitored both in the area where *M. pruinosa* density was high and in a control area where the level of planthopper abundance appeared to be much lower. The following data were recorded in three different moments (29-07; 08-08; 18-08) of *M. pruinosa* life cycle:

- number of spiders (*L. triangularis*) on webs
- number of *M. pruinosa* caught by spiders (victims were removed from webs)
- number of unharmed *M. pruinosa* resting on *Equisetum palustris*.

Statistical analysis was performed by means of a BioStat Pro 5.9.8 software. The normality of data distribution was assessed via five specific statistical tests: Kolmogorov-Smirnov/Lilliefors, Shapiro-Wilk, D'Agostino Skewness, D'Agostino kurtosis and D'Agostino omnibus. T test was applied to compare means from two samples when data distribution resulted to be normal.

Mann-Whitney test was applied in order to compare medians between two groups of data when normality of data distribution was not confirmed by normality tests,

Chi-square test was used to test for equality of proportions between samples. Correlation between data was assessed by calculating the Spearman coefficient, a measure of the strength of a linear association between two variables denoted by r_s .

RESULTS

As a result of the preliminary survey of spiders feeding on *M. pruinosa* carried out in 2004, fourteen species of spiders preying upon *M. pruinosa* were recorded. Except for *Synaema globosum*, the list includes only spiders able to weave a web. As the research was focused on identifying spiders making webs which can prey upon the invader, these data do not imply that spiders actively preying victims without weaving webs are uncommon predators of *M. pruinosa*.

The community of spiders (N=135) feeding on *M. pruinosa* (Figure 3) mainly includes spiders belonging to Agelenidae (27.4%), Linyphiidae (26.7%), and Araneidae (15.6%). *Agelena labyrinthica* and *Linyphia triangularis* were the most common species and were surveyed in each sampling site. Spiders were collected from ecotones (hedgerows, shrubs bordering woods and fields) where aggregations of *M. pruinosa* were commonly recorded.

Figure 4 displays the trend of *M. pruinosa* predation by *Agelena labyrinthica* (summer 2005). The great part of preys were adults of the planthopper (91.8% - N = 257). Except for the first and the last samples, *M. pruinosa* was regularly found in webs; predation rate was maximum in July and around the half of August, while it tended to decline in September.

The trend of *M. pruinosa* predation by *L. triangularis* is displayed by Figure 5. Some

traits of this graph resemble the ones of Fig. 4: predation rate is maximum in July, while a decrease is recorded during the second half of August.

Figure 6 shows the correlation between *L. triangularis* density on webs and the amount of *M. pruinosa* predation (number of adult remains found on the webs). A positive relation occurs (Spearman correlation - $r_s = +0.83$ - $P < 0.01$). All spiders on webs were *Linyphia triangularis*, except for a specimen of *Argiope bruennichi* which permanently joined the complex of *L. triangularis* webs. *M. pruinosa* trapped by *L. triangularis* webs were mainly adults (97.7%; $N = 173$).

In Figure 7, the frequency distribution of preys trapped in *L. triangularis* webs is shown: *M. pruinosa* is significantly (Chi square test - $P < 0.01$) the most common victim. Even if the biomass of preys was not measured and some preys could not be computed in the final data since they did not let trace on webs, the dominance of *M. pruinosa* among the spider preys states that during summer *M. pruinosa* worked as an important food intake for the predator.

Table 2 summarizes data coming from square plot areas (0,5 x 0,5 m) including *Equisetum* plants differently colonized by *M. pruinosa*. Plots (A area) including plants infested with the planthopper ($N=7$) were compared to the same number ($N=7$) of plots where *M. pruinosa* density was significantly lower (B area).

Plants density and height are not significantly different (t test, $P > 0.05$); therefore those factors cannot justify the significant difference in spider abundance inside the compared areas (Mann Whitney test, $P < 0.01$). The difference in terms of victims density and predation rate is significant, too (Chi square test, $P < 0.01$).

Table 2 Equisetum and spider density in study plots (standard error of the mean is reported)

Area	A	B
<i>Equisetum</i> density (plants/ m ²)	20.8 ± 0.9	18.3 ± 0.7
Plants medium height (cm)	57.8 ± 1.1	57.2 ± 1.4
Spiders/plant	1.47 ± 0.1	0.39 ± 0.07
Preyed <i>M. pruinosa</i> /plant	0.39 ± 0.08	0.07 ± 0.001
Unharmed <i>M. pruinosa</i> /plant	0.26 ± 0.03	0.04 ± 0.001

DISCUSSION

As generalist predators, spiders are not an insignificant component of terrestrial ecosystems and they can play a role in the biological control of insect pests in agro-ecosystems (Riechert & Bishop, 1990; Wyss *et al.*, 1995; Riechert & Lawrence, 1997; Maloney *et al.*, 2003; Thorbek *et al.*, 2004; Sanders *et al.*, 2008; Picchi *et al.*, 2016).

According to the definition proposed by Samu & Szinetà (2002), agrobiont spiders are species able to reach high dominance in agro-ecosystems. Except for *Theridion impressum*, which is one of the dominant species in corn cultivations around study areas (Camerini, personal observations), the complex of spiders preying upon *M. pruinosa* (Figure 3) does not include any species which can be classified as agrobiont. Anyway, those species are common in ecotones lying on the borders of fields and woods (Groppali, 2000; Groppali *et al.*, 2000) such as hedgerows, patches of uncultivated soil or road verges. The need for conservation of those habitats is universally recognized, therefore agricultural landscape management practices able to improve the availability of those "compensation areas" are fundamental for the conservation

of organisms such as spiders, that benefit from undisturbed ecotones (Le Viol *et al.*, 2008).

During the '90s, some damage to gardens and crops caused by the planthopper was recorded in the studied area and, more generally, in Italy. Farmers were therefore alarmed by the possible risk connected to the introduction of this new invader. Anyway, during the survey (2004-2006) *M. pruinosa* was rarely observed in fields of corn, soybean or vineyards, and the colonization of cultivations, if any, was usually limited to the shady margins of fields, so resulting in a negligible level of damage. After the successful establishment of this exotic species, a complex of limiting factors (like climate conditions, disturbance factors related to agro-ecosystems and natural enemies) is reasonably contributing to keep *M. pruinosa* population level under the threshold of economic damage in the Padana plain. Natural enemies include both generalist indigenous predators and the introduced *Neodryinus typhlocibae*, which successfully colonized the Padana plain and other Italian areas thanks to multiple release projects (Gervasini, 2000).

The results of this study suggest that several spider species can feed on *Metcalfa pruinosa* all summer long. Those species enrich the list of generalist predators (mainly insects and birds) working as natural enemies of the planthopper (Greatti *et al.*, 1994). Two of those spiders (*A. labyrinthica* and *L. triangularis*) were more frequently recorded.

On one hand, *A. labyrinthica* can be found quite commonly in the vegetation of uncut grassland, uncultivated field edges, low bushes and blackberry briar patches. There, it can feed on *M. pruinosa* adults starting from July, so preventing the reproduction of the planthopper.

On the other hand, Linyphiidae are known to be predators of phytophagous insects (Nentwig, 1983). This study seems to suggest that *L. triangularis* can modulate its density in response to an increase of *M. pruinosa*'s density. In such a condition, *M. pruinosa* becomes the dominant prey in *L. triangularis*'s webs all summer long, as far as the prey is available. This observation is only partially coherent with the usual feeding strategy of this spider, which over the season tends to feed on a constant complex of prey species, relying on a limited number of species for most of its food (Turnbull, 1962).

Further research is needed to assess the impact of spiders on population dynamics of *M. pruinosa*. The results of this survey reveals that the complex of indigenous spiders can potentially give a contribution to limit the outbreaks of the planthopper and at least two species tend to modulate their trophic niche in response to the increasing abundance of *M. pruinosa*.

ACKNOWLEDGEMENTS

Thanks to Carlo Pesarini for spiders identification and Monica Masanta for text revision.

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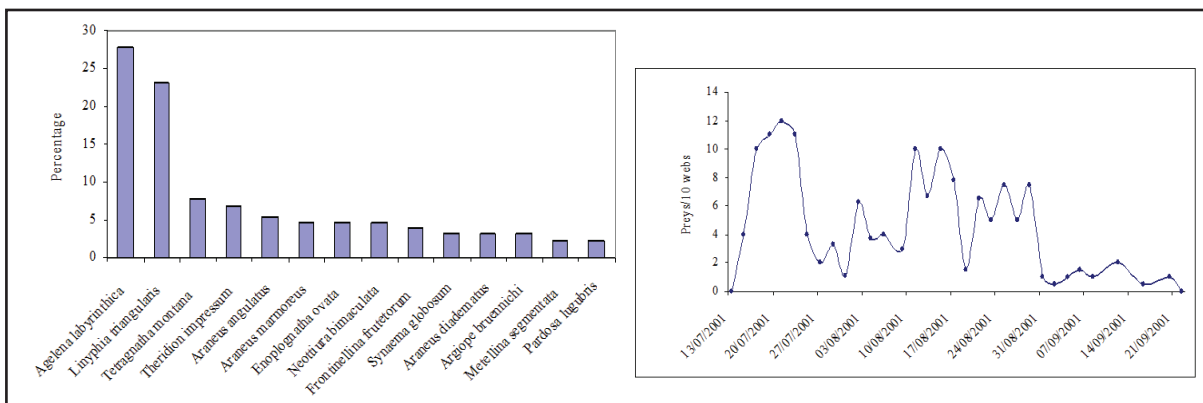


Figure 3 Frequency distribution of spiders feeding on *M. pruinosa* (year 2004; N = 135).

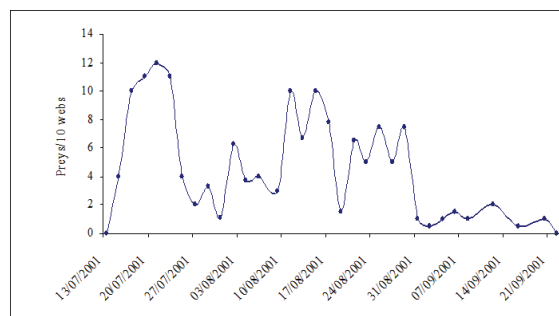


Figure 4 Trend of *M. pruinosa* predation by *Agelena labyrinthica* (predation rate is expressed as number of victims /10 spider web).

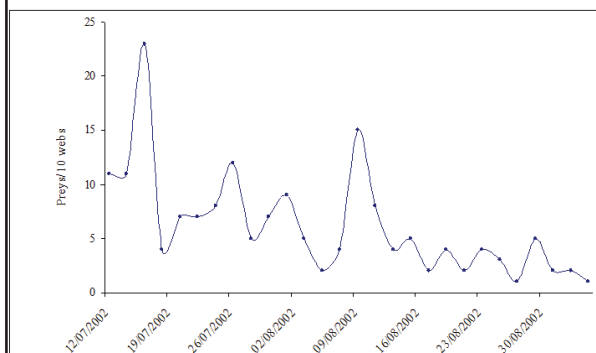


Figure 5 Trend of *M. pruinosa* predation by *Linyphia triangularis* (predation rate is expressed as number of victims /10 spider webs).

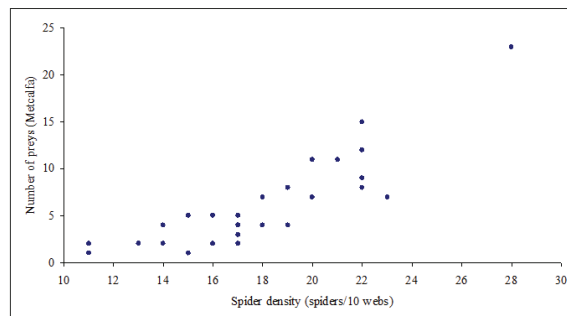


Figure 6 Correlation between spider density (*L. triangularis*) and prey density (number of adult *M. pruinosa* remains found on the webs).

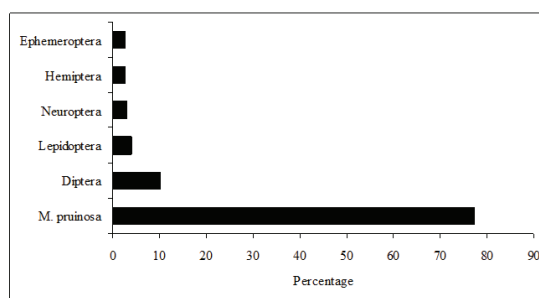


Figure 7 Frequency distribution of preys on *L. triangularis* webs (N = 224).