

Seasonal habits of predation and prey range in aphidophagous silver flies (Diptera Chamaemyiidae), an overlooked family of biological control agents

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Abstract

Aphids are among the most widespread and serious groups of pests in agro-ecosystems, and predaceous arthropods have been proposed as biological control agents against them, including parasitic Hymenoptera, lacewings, ladybugs, hoverflies and silver flies (Diptera Chamaemyiidae). Chamaemyiidae is a small family of predaceous flies, including aphidophagous and coccidophagous species. Little is known about their ecology, and partial failures of Chamaemyiidae-based biological control programs against aphids may be due to poor synchronization of predator-prey seasonal habits. In this study, we investigated seasonality of predation and prey range of aphidophagous Chamaemyiidae. A field survey was conducted on crops and indigenous flora in seven agricultural sites in southern Turkey. Seventeen host plant families were surveyed for Chamaemyiidae presence and 371 silver flies were studied. All Chamaemyiidae larvae were observed to prey on adult and young instar aphids in the field. Silver fly total larval abundance reached a maximum in autumn and early winter. Six species were identified: *Leucopis annulipes*, *L. formosana*, *L. glyphinivora*, *L. revisenda*, *L. rufithorax* and *L. spyrothecae*. *L. glyphinivora* was more abundant in July, *L. annulipes* and *L. revisenda* in September-October, and *L. formosana* in October-December. *L. annulipes* was found to prey mostly on *Aphis gossypii*, *A. craccivora* and *A. fabae*. *L. formosana* on *A. gossypii*, *A. fabae* and *Toxoptera aurantii*. *L. glyphinivora* on *Brachycaudus cardui* and *Dysaphis plantaginea*. *L. revisenda* on *Myzus persicae*. Our results add baseline knowledge on seasonal habits of predation and prey range of aphidophagous Chamaemyiidae and may be helpful for biological control purposes.

Key words: aphid predator, cotton aphid, green peach aphid, mass-rearing, prey range, Turkey.

Introduction

Aphids (Homoptera Aphididae) are widespread and serious pests in agro-ecosystems, and among the most destructive pests in many temperate climate areas (Dedryver *et al.*, 2010). They cause massive losses in field crops, horticultural crops and even forest trees, as a result of both direct and indirect damage (Ebert and Cartwright, 1997). Direct damage arises from feeding and the injection of bioactive substances through the saliva, which interferes with plant physiology. Indirect damage is due mainly to virus transmission, honeydew excretion, and alteration of the microflora communities on plant foliage (Minks and Harrewijn, 1989; Blackman and Eastop, 2000). Many chemical insecticides have been used for control of aphid pests. The development of systemic aphicides started with formulation of organophosphate compounds (Gates, 1959; Pirone *et al.*, 1988; Casida and Durkin, 2013). Subsequently, carbamate insecticides and synthetic pyrethroids extended the range of effective products (Dedryver *et al.*, 2010). However, there are serious concerns about the use of insecticides for aphid management in agriculture, including high costs, hazards to human health (Weisenburger, 1993) and the environment (Desneux *et al.*, 2007; Heimpel *et al.*, 2013), and widespread development of pesticide resistance in numerous aphid species (Bos *et al.*, 2010; Bass *et al.*, 2011).

For use in more environmentally benign Integrated Pest Management programs, a wide array of natural

enemies has proven effective against aphids, including predaceous arthropods such as lacewings (Neuroptera Chrysopidae), ladybirds (Coleoptera Coccinellidae) (Ebert and Cartwright, 1997), hoverflies (Diptera Syrphidae) (Seagraves and Lundgren, 2012), parasitic Hymenoptera, mainly Braconidae (Aphidiinae) and Aphelinidae (Starý, 1970; Kavallieratos *et al.*, 2010; Boivin *et al.*, 2012; Ortiz-Martínez *et al.*, 2013; Benelli *et al.*, 2014a), and several Diptera families, including Cecidomyiidae (Muratori *et al.*, 2009) and silver flies (Chamaemyiidae) (Mitchell and Wright, 1967; McAlpine, 1971; Gaimari, 1991; Gaimari and Turner, 1996a).

All the known members of Chamaemyiidae have larvae that prey on soft-bodied homopterans, mainly Aphidoidea and Coccoidea. Chamaemyiidae are small, silvery grey flies (about 1-5 mm long) found in all zoogeographic areas, although the majority of known species are in the Palaearctic Region. Worldwide, silver flies comprise more than 300 species (Raspi, 2013). The family exhibits characteristic trophic specialization at the generic or sub-generic level (Raspi, 1983; 1988; 2003; 2006; Gaimari and Raspi, 2002), and there is evidence of their importance for biological control of a number of homopterous pests of economic importance, including adelgids and aphids (Smith and Coppel, 1957; Clark and Brown, 1962; Mitchell and Wright, 1967; Culliney *et al.*, 1988; Mills, 1990; Greathead, 1995), as well as mealy bugs and scales (Tiensuu, 1951; Gaimari, 1991). Information for mass rearing and colony maintenance have been provided for both aphidophagous and

coccidophagous Chamaemyiidae (Gaimari and Turner, 1996b; Canale *et al.*, 2002; Loni and Raspi, 2002).

Despite silver flies' potential as biological control agents, little is known about their behavioral ecology (e.g., courtship and mating: Gaimari and Turner, 1997; Benelli *et al.*, 2014b; prey-finding behavior, McLean, 1992; Gaimari and Turner, 1997; Fréchette *et al.*, 2008) or the predator-prey phenology for given ecological habitats (Mizuno *et al.*, 1997). However, such knowledge has major implications for homopterous pest control (Benelli, 2015), as it has been reported that some partial failures of Chamaemyiidae-based biocontrol programs may be linked to poor synchronization of predator and prey phenology and/or inadequate searching ability of the flies (Mitchell and Wright, 1967). Furthermore, information on tri-trophic associations is a pivotal background in ecological and biodiversity studies (Alikhani *et al.*, 2013, and references therein). In this study, we examine the seasonal habits of predation and the prey range of six species of aphidophagous Chamaemyiidae, during a field survey conducted in seven agricultural sites of southern Turkey.

Materials and methods

Field sites

Experiments were carried out from January to December 2011 in fruit orchards [*Citrus aurantium* L., *Citrus limon* L., *Cydonia oblonga* Mill., *Malus domestica* Borkh., *Prunus avium* L., *Prunus persica* (L.) Batsch, *Pyrus communis* L., *Vitis vinifera* L.]; horticultural crops (*Brassica oleracea* L., *Capsicum annuum* L., *Cucumis sativus* L., *Cucurbita maxima* L., *Lactuca sativa* L.); cereal crops (*Zea mays* L.), legumes (*Vicia faba* L.), cotton (*Gossypium hirsutum* L.) and other indigenous plants and weeds, located in seven sites of Southern Turkey: Adana (37°0'0"N 35°19'16.8"E), Antalya (36°54'0"N 30°41'0"E), Gaziantep (37°3'33"N 37°22'57"E), Hatay (36°11'56"N 36°9'38"E), Mersin (36°48'0"N 34°38'0"E), Niğde (37°58'0"N 34°40'45"E) and Osmaniye (37°4'30"N 36°15'0"E). No pesticides were used in any of the surveyed sites.

Collection, rearing and identification of Chamaemyiidae

Six samples/month (1 every five days) were collected at each site for different aphid species. Both crops and indigenous plants and weeds were haphazardly selected and surveyed for the presence of aphid populations. In each sampling for each surveyed aphid species on a given host plant, 400 aphids were examined searching for Chamaemyiidae larval instars preying on them. Aphid specimens were sampled and identified to species level using the key and descriptions of Blackman and Eastop (2000) and references therein. All Chamaemyiidae larval specimens were directly observed preying on aphid adults and or young. Each silver fly larva was collected using a fine brush (diameter 2 mm) and transferred in a glass vial (diameter 5 cm, length 10 cm), moistened with a wet cotton wick and immediately transferred to the University of Çukurova, Citrus Pest laboratory.

Each Chamaemyiidae larva was reared under laboratory conditions (24 ± 4 °C; R.H. = 60 ± 10%; 16:8 h photoperiod). Each specimen was transferred to a 5 L plastic mesh cage (length 40 cm, diameter 30 cm), and allowed to develop on its original aphid preys and host plant (i.e. those observed in the field for each specimen). Original plant parts (i.e. buds or branches with length about 30 cm, depending on the plant species and abundance of aphid preys), with their proximal end submerged in a 250 cc beaker filled with tap water, were stored in each cage. For each cage, aphid-infested plant parts were renewed every two days. The procedure was carried out until pupariation of each Chamaemyiidae larva (maximum duration: 30 days).

After emergence, adult silver flies were transferred to clean glass vials (diameter 5 cm, length 10 cm), provided *ad libitum* with water through a cotton wick and fed on a dry diet composed of yeast extract and sucrose mixture, at the ratio of 1:10 (w/w) (Canale *et al.*, 2002; Benelli *et al.*, 2014b), resembling their natural diet usually consisting of Homoptera honeydew as well as sweet secretions from plant nectaries. After three days, they were cooled for 10 min at -20 °C and sent to University of Pisa (Italy) for identification following Gaimari and Raspi (2002) and Raspi (1983; 2003; 2008; 2013). Voucher specimens of all species are stored in the Department of Agriculture, Food and Environment, University of Pisa.

Data analysis

Seasonal differences in total larval abundance of Chamaemyiidae flies were analyzed by line by using a weighted generalized linear model with one fixed factor: $y = X\beta + \varepsilon$ where y is the vector of the observations (number of Chamaemyiidae for each sampling date), X is the incidence matrix, β is the vector of fixed effect (month) and ε is the vector of the random residual effects. Each sampling date represented a replicate ($\alpha = 0.05$). Seasonal differences in abundance of each Chamaemyiidae species were analyzed by using a generalized linear model with two fixed factors (Chamaemyiidae species and month). Abundance value of each Chamaemyiidae species per month represented a replicate ($\alpha = 0.05$).

Abundance of *Leucopis annulipes* Zetterstedt, *Leucopis formosana* Hennig, *Leucopis glyphinivora* Tanasijtshuk, *Leucopis revisenda* Tanasijtshuk, *Leucopis rufithorax* Tanasijtshuk and *Leucopis spyrothecae* Raspi preying on different aphids was analyzed by using a generalized linear model with one fixed factor (aphid prey). Abundance of each Chamaemyiidae species preying on a given aphid species per month represented a replicate ($\alpha = 0.05$). Data concerning *L. rufithorax* and *L. spyrothecae* were not analysed due to the low number of total collected individuals (i.e. 10 for *L. rufithorax* and 1 for *L. spyrothecae*).

Results

A total of 371 Chamaemyiidae larvae were observed preying on adult and young instar aphids in the field. They were collected and reared in laboratory conditions

until adult emergence. Seasonal abundance of Chamaemyiidae preying on aphids infesting agricultural crops and spontaneous flora was depicted in figure 1. A significant effect of season was found on silver fly total abundance ($\chi^2 = 360.283$; $d.f. = 11$; $P < 0.0001$). Maximum abundance was reached in mid-summer (July) and early autumn (September-October). However silver fly larvae were still preying on aphids in November and December (figure 1).

Six Chamaemyiidae species were identified: *L. annulipes*, *L. formosana*, *L. glyphinivora*, *L. revisenda*, *L. rufithorax* and *L. spyrothecae* (figure 2). A significant effect of the silver fly species ($\chi^2 = 151.997$; $d.f. = 5$; $P < 0.0001$), season ($\chi^2 = 152.901$; $d.f. = 11$; $P < 0.0001$) and their interaction ($\chi^2 = 553.259$; $d.f. = 55$; $P < 0.0001$) was found. *L. glyphinivora* was more abundant in July, *L. annulipes* and *L. revisenda* in September-October, and *L. formosana* in October-December (figure 2). A list of host plant species found in each site during the year was given in supplemental material table S1.

Aphid prey species was found to play a significant role on abundance of the surveyed Chamaemyiidae species (table 1). Concerning silver fly prey range, *L. annulipes* larvae were found to prey mostly on *Aphis gossypii* Glover, *Aphis craccivora* Koch and *Aphis fabae* Scopoli (figure 3). *L. formosana* prey predominantly on *A. gossypii*, *A. fabae* and *Toxoptera aurantii* Boyer de Fonscolombe (figure 4). *L. glyphinivora* larvae prey mostly on *Myzus persicae* Sulzer, *Brachycaudus cardui* (L.) and *Dysaphis plantaginea* (Passerini) (figure 5). Also *L. revisenda* was predominantly found preying on *M. persicae* (figure 6). In our survey, we found only a single larva of *L. spyrothecae*, and it was preying on

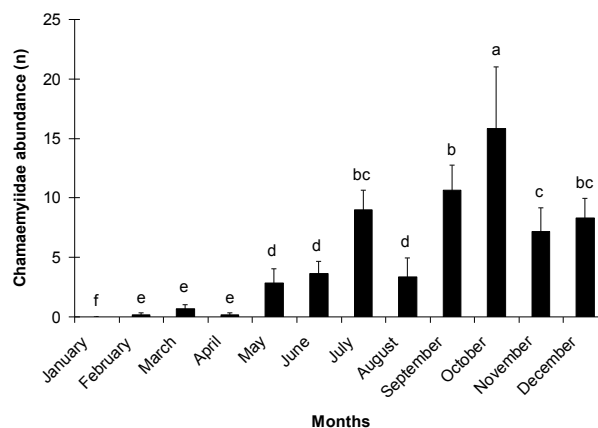


Figure 1. Seasonal abundance of silver flies (Diptera Chamaemyiidae) preying on aphids on crops and spontaneous flora in Turkey. Values are means of six samplings/month. Different letters above each column indicate significant differences. T-bars indicate standard errors (generalized linear model, $P < 0.05$).

Table 1. Effect of the aphid prey species on abundance of the main Chamaemyiidae species found in agricultural areas of Turkey; $d.f.$ = degrees of freedom. Asterisks indicate significant differences (generalized linear model, $P < 0.05$).

Chamaemyiidae	χ^2	$d.f.$	P -value
<i>Leucopis annulipes</i>	62.028	16	<.0001 *
<i>Leucopis formosana</i>	214.751	5	<.0001 *
<i>Leucopis glyphinivora</i>	79.189	16	<.0001 *
<i>Leucopis revisenda</i>	47.205	4	<.0001 *

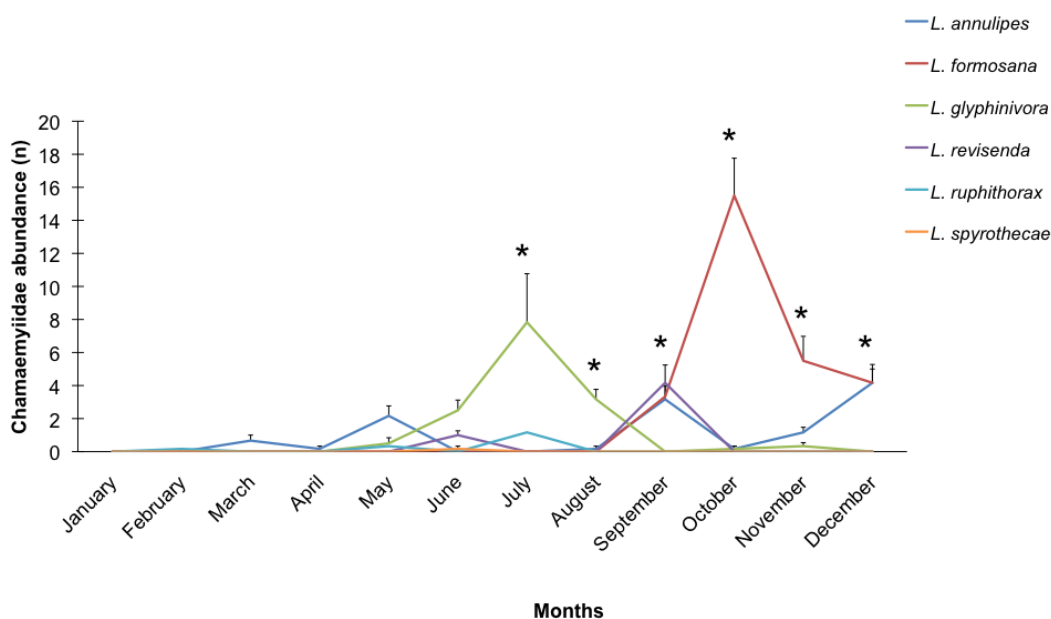


Figure 2. Seasonal abundance of six Chamaemyiidae species (*L. annulipes*, *L. formosana*, *L. glyphinivora*, *L. revisenda*, *L. rufithorax* and *L. spyrothecae*) preying on aphids on crops and spontaneous flora in Turkey. Values are means of six samplings/month. Within each month, asterisks indicate significant differences among species abundance. T-bars indicate standard errors (generalized linear model, $P < 0.05$). (in colour at www.bulletinofinsectology.org)

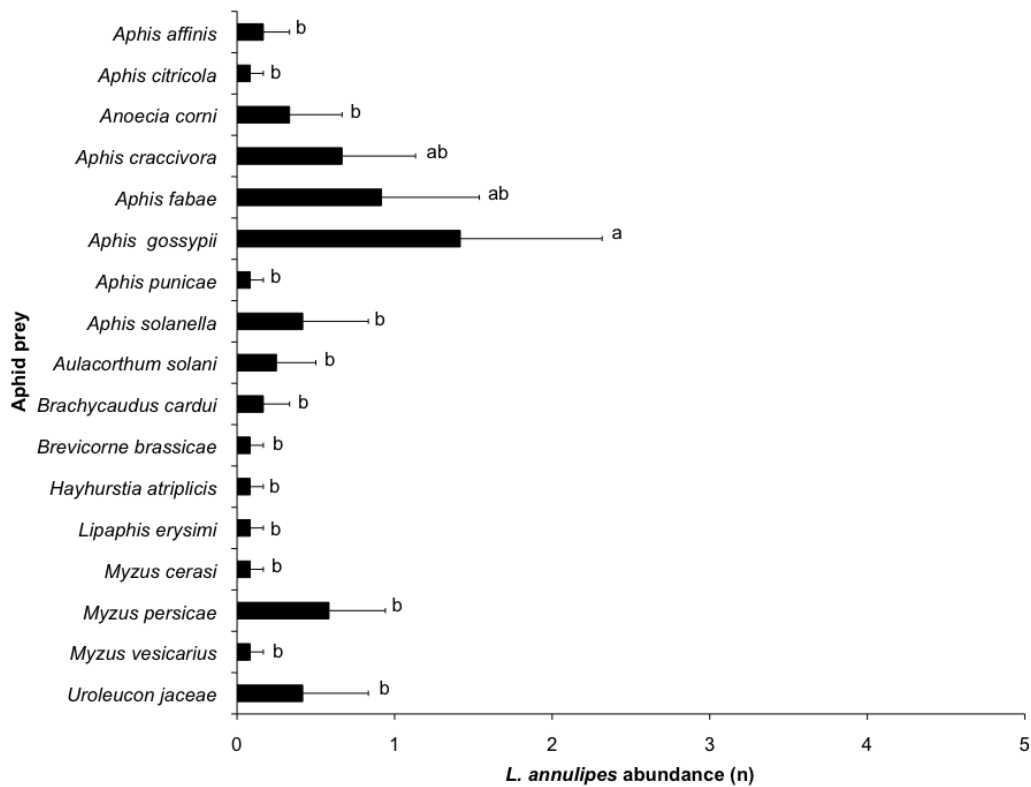


Figure 3. Prey range and abundance of *L. annulipes* on crops and spontaneous flora in Turkey. Different letters on each bar indicate significant differences. T-bars indicate standard errors (generalized linear model, $P < 0.05$).

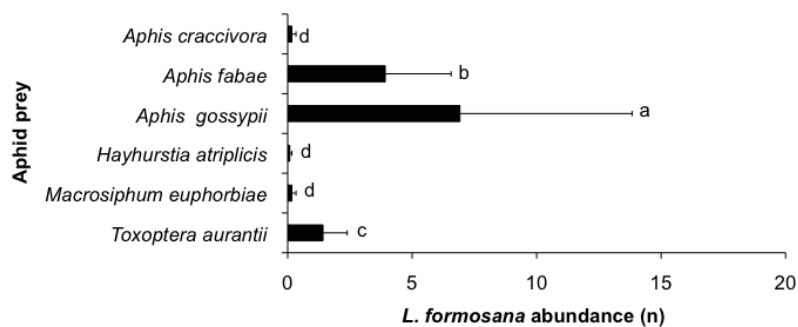


Figure 4. Prey range and abundance of *L. formosana* on crops and spontaneous flora in Turkey. Different letters on each bar indicate significant differences. T-bars indicate standard errors (generalized linear model, $P < 0.05$).

Pemphigus vesicarius Passerini. Furthermore, we collected 10 specimens of *L. rufithorax* preying on *Nasonovia ribisnigri* (Mosley), *Myzus cerasi* (F.), *Hyalopterus pruni* (Geoffroy) and *A. gossypii* (1, 4, 2 and 3 larvae, respectively). For all Chamaemyiidae species, a detailed list of predator-prey associations found in each site during the year was given in supplemental material table S1.

Discussion

Silver fly species preying on aphids

Our results shed light on seasonal habits of predation in aphidophagous Chamaemyiidae. In agreement with previous research, we found that these predaceous insects are widely present in a number of agricultural habitats, ranging from fruit orchards to horticultural

crops and intensively managed cereal and legume cultivations (Gaimari and Turner, 1996; 1997; Gaimari and Raspi, 2002; Raspi, 2003; 2013 and references therein). We identified six aphidophagous Chamaemyiidae species in agricultural areas of southern Turkey: *L. annulipes*, *L. formosana*, *L. glyphinivora*, *L. revisenda*, *L. rufithorax* and *L. spyrothecae*. Previous data concerning predation habits of these *Leucopis* silverflies are mostly qualitative (Tanasijshtshuk, 1986). *L. glyphinivora* is a widely distributed species throughout the Holarctic, probably the most widely polyphagous of all *Leucopis* species, feeding on about sixty aphid species on more than one hundred host plants. *L. annulipes* is widespread in the Palearctic region, recorded as predator on dozens of different aphid species in about twenty genera. Also *L. rufithorax* and *L. revisenda* are widely distributed in the Palearctic, preying on more than twelve and twenty

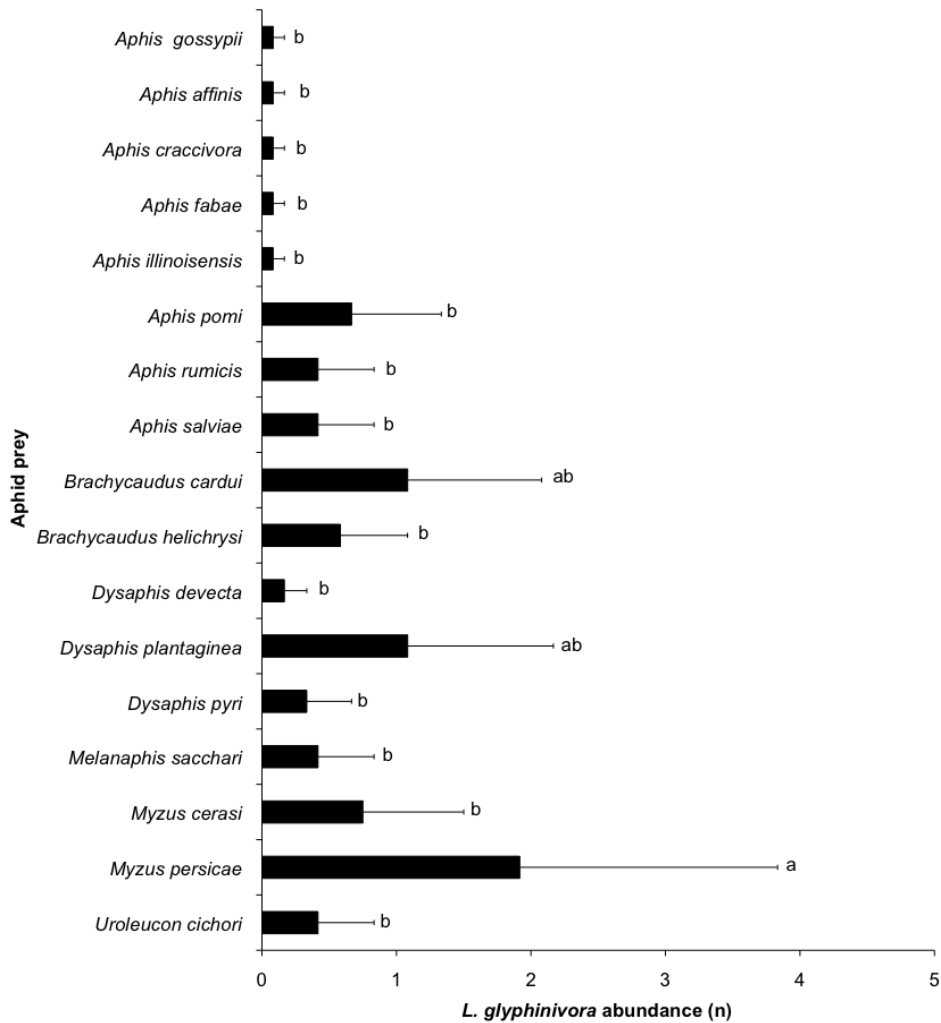


Figure 5. Prey range and abundance of *L. glyphinivora* on crops and spontaneous flora in Turkey. Different letters on each bar indicate significant differences. T-bars indicate standard errors (generalized linear model, $P < 0.05$).

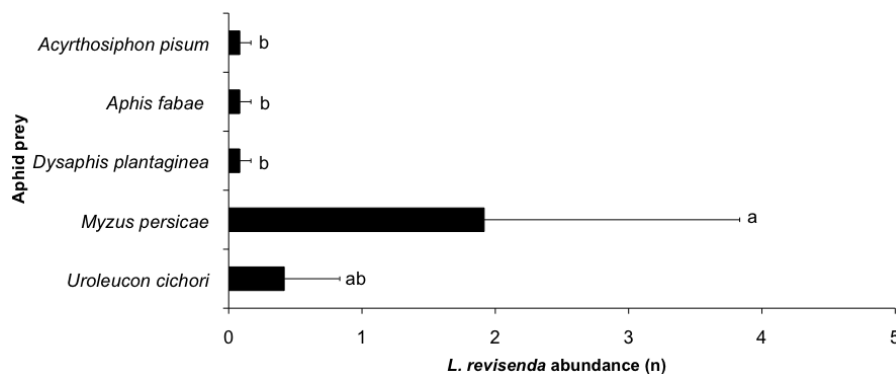


Figure 6. Prey range and abundance of *L. revisenda* on crops and spontaneous flora in Turkey. Different letters on each bar indicate significant differences. T-bars indicate standard errors (generalized linear model, $P < 0.05$).

aphid species, respectively. *L. formosana* is widespread through much of the Old World, feeding on many species of aphids (Raspi, 1985; 1996; 2008; Tanasijtshuk, 1986). Conversely, *L. spyrothecae* seems to be the only species limited in distribution, with a narrow host range, since it preys in galls of *Pemphigus* on *Populus* plants (Raspi, 2003).

Seasonal abundance of silver flies

The presence of Chamaemyiidae on these farms was affected by the season, with silver fly total abundance reaching a maximum in autumn and early winter. Several differences in seasonal activity were found among the Chamaemyiidae species. For instance, the majority of *L. glyphinivora* larvae were found during mid-

summer, while other species, such as *L. formosana*, *L. annulipes* and *L. revisenda* were mostly active in the autumn (see also Azab *et al.*, 1965; Tanasijtshuk, 1986). It has been widely reported that many aphid natural enemies showed different seasonal habits. For instance, in Chilean alfalfa fields, Coccinellidae are responsible for high levels of predation throughout the year, although the species responsible varied from spring to summer and autumn, while Syrphidae have been mainly found in spring and summer, Nabidae mainly in summer, and spiders only in autumn (Ximenez-Embun *et al.*, 2014, see also Grez *et al.*, 2007). Similar evidence for seasonal variations of predaceous arthropod populations feeding on aphids infesting herbaceous crops and fruit orchards have been also found in Iran (Rakhshani *et al.*, 2009), USA (Wheeler, 1977; Dutcher *et al.*, 2012; Gontijo *et al.*, 2012), Canada (Firlej *et al.*, 2012) and Japan (Nakashima and Akashi, 2005), and this seems also related to response to prey abundance (Rakhshani *et al.*, 2009; Ximenez-Embun *et al.*, 2014).

Prey range of aphidophagous silver flies

We found Chamaemyiidae larvae preying on aphids infesting herbaceous crops, fruit orchards and also neighborhood plants belonging to spontaneous flora. Concerning the latter, good examples are the genera *Capsella*, *Chenopodium*, *Cichorium*, *Cirsium*, *Mentha*, *Salvia*, *Sinapis*, *Solanum*, *Sorghum* and also *Populus*. Spontaneous flora is often a valuable resource to enhance agro-ecosystem stability and biodiversity, and particular attention should be devoted to agronomic practices supporting banker plants (i.e. non-crop plants infested by non-pestiferous aphids), since they can help Chamaemyiidae populations, providing food for larvae (i.e. aphid prey) and adults (i.e. honeydew, pollen, nectar and/or extra-floral nectaries) (Frank, 2010; Huang *et al.*, 2011; Jandricic *et al.*, 2014). More generally, spontaneous flora help a number of aphid natural enemies (e.g. other predaceous arthropods, as well as parasitic Hymenoptera), providing hosts and prey during food paucity periods, thereby creating natural enemy population reservoirs that can contribute to the success of biological control programs (see Benelli *et al.*, 2014c for a recent review).

Few quantitative studies are available on Chamaemyiidae trophic habits. However, it has been reported that aphidophagous silver flies may be polyphagous (e.g. *L. glyphinivora*) or oligophagous (e.g. *Leucopis palumbii* Rondani), with the sole exception of *Leucopis argentata* Hegger preying only on *H. pruni* on common reeds, *Phragmites australis* (Cav.) Trin. ex Steud (Raspi, 2008). In agreement with previous researches, our data showed a significant variation in prey range of aphidophagous species belonging to the genus *Leucopis sensu stricto* (Tanasijtshuk, 1986; Raspi, 2008; 2013). Both *L. annulipes* and *L. formosana* fed mostly on *A. fabae* and *A. gossypii*, two extremely polyphagous pests. In particular, *A. gossypii* is a pest of huge agricultural importance, since it attacks more than 900 plant species, including many field and greenhouse crops, and transmits over 50 plant viruses (Blackman and Eastop, 2000). In addition, *L. annulipes* commonly developed on *A. crac-*

civora, while another prey of *L. formosana* was the black citrus aphid, *T. aurantii*. In Turkish agricultural habitats, the most common prey of *L. glyphinivora* and *L. revisenda* was another important homopterous pest, the green peach aphid, *M. persicae*, for which chemical control tools show poor effectiveness, since several neonicotinoid-resistant strains are currently widespread in a number of geographic areas (Bos *et al.*, 2010; Bass *et al.*, 2011 and references therein).

Conclusions

Overall, our results report new prey aphid records and add basic knowledge to Chamaemyiidae ecological traits, with special reference to seasonality of predation and prey range. Our findings may have applied implications. First, basic information concerning prey range of aphidophagous silver flies may help to optimize their mass-rearing procedures (Gaimari and Turner, 1996; Canale *et al.*, 2002). Second, partial failures of silver fly-based biological control programs may be linked with poor synchronization of predator-prey seasonal habits and/or lack of searching ability of some Chamaemyiidae species against a targeted prey (Smith and Coppel, 1957; Mitchell and Wright, 1967; Gaimari, 1991). In this scenario, we believe that our results may be helpful to understand how these organisms could be used for biological control purposes, with special reference to horticultural crops in protected environments. This is particularly true for polyphagous silver flies that could be employed in their original habitats (Canale *et al.*, 2002).

Acknowledgements

The authors are grateful to Scientific and Technological Research Council of Turkey (TÜBİTAK/TOVAG-1050581) for financial support. Giovanni Benelli is supported by a MIS. 124 MODOLIVI Grant. Funds were also provided by the Italian Ministry of Education, University and Research (MIUR). Fund providers had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

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Received February 20, 2015. Accepted June 22, 2015.