Long lasting summer flowerings of *Lythrum salicaria* as honeybee-friendly flower spots in Mediterranean basin agricultural wetlands

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Short title: Bee-friendly flower spots in wetlands

Abstract

The purple loosestrife, *Lythrum salicaria*, is a species native to Eurasia. It is rustic, produces both pollen and nectar, and may be a helpful food source for bees in Mediterranean wetlands, helping them to overcome hot summer periods characterised by food paucity. In this research, we investigated the flowering of *L. salicaria* plants in Mediterranean agricultural wetlands, as well as pollinator diversity and abundance. *L. salicaria* has a long lasting flowering, with more than 640 open flowers/plant from mid-July to early September. 476 pollinators belonging to 3 orders, 7 families and 15 species of insects were studied. Scanning electron microscopy showed that all species carried *L. salicaria* pollen on their body parts. Hymenoptera pollinators were the most abundant (427 specimens), followed by Diptera (26) and Lepidoptera (23). Apoidea abundance reached a maximum in August and social bees largely dominate (94.38%). *Apis mellifera* was the most abundant species, followed by 5 bumblebees, including the endangered *Bombus sylvarum*. Honeybee visits on *L. salicaria* were higher in August over the rest of the summer and early autumn, while no differences were detected for bumblebees, Halictidae and Megachilidae. Overall, we support the purple loosestrife as a suitable species for bee-friendly flower spots in Mediterranean agricultural wetlands, as well as in close proximity of beehives. The latter use may facilitate the honeybees collecting pollen for the colony, thereby ensuring robustness to overcome the winter season.

Keywords: Apoidea; colony collapse disorder; food paucity; pollinators' diversity; purple loosestrife; wildflower strips

1. Introduction

The purple loosestrife, *Lythrum salicaria* L. (Lythraceae) is a self-incompatible, perennial herb native to Eurasia, usually found in freshwater wetland habitats (Darwin, 1864; Stuckey, 1980; Colautti et al., 2010), and widely known as a source of plant metabolites with antioxidant and anti-inflammatory properties (Tunalier et al., 2007). In Europe, this species is present from Mediterranean basin countries to North Europa, in a variety of wetland habitats including lake-and seashores, riversides and fens (Olsson and Ågren, 2002). The purple loosestrife shows a high environmental plasticity at a wide range of latitudes (Olsson and Ågren, 2002; Mal and Lovett-Doust, 2005). Flowering occurs from mid- to late summer. *L. salicaria* bears flowers in dense terminal verticillate spikes. Flowers are actinomorphic, slightly zygomorphic, hermaphroditic, hypogynous (Richard et al., 1989) and characterized by trimorphic heterostyly (Mulcahy and Caporello, 1970). According to the length of the styles, any individual can be assigned to one of the following forms, long, mid and short, because of its genetic evolution preventing self-pollination (Levin and Kerster, 1973; O'Neil and Schmitt, 1993). Seed-set of this species was found to be proportional to plant population size, since small populations of reward-producing plants are likely to be less attractive to pollinators than large ones (Ågren, 1996; Waites and Ågren, 2004). *L. salicaria* can also regenerate from stem or rootstock fragments (Welling and Becker, 1990; Klips and Peñalosa, 2003).

The purple loosestrife has been accidentally introduced in North America and Australia (Stuckey, 1980; Hultén and Fries, 1986) and is now considered a wetland menace in North America, due to its ability to displace native wildlife food sources as

cattails (e.g. *Typha* spp.) (Blossey et al., 2001; Farnsworth et al., 2001). In the Eurasian native environment, its potential invasiveness is negligible, since it lives in equilibrium with other species typical of wetland ecosystems. While several efforts have been carried out to evaluate the potential of some phytophagous insects as biocontrol agents against purple loosestrife in North America (Batra et al., 1986; Hunt-Joshi et al. 2004), there is a lack of information on the potential benefits of this plant as food source for pollinators in Mediterranean wetland agro-ecosystems. To the best of our knowledge, knowledge on *L. salicaria* pollinators has been provided only for wild ecosystems of northern Sweden (Waites and Ågren, 2004) and Canada (Mai et al., 1992), reporting that purple loosestrife flowers produce both pollen and nectar and are visited mainly by honeybees, bumblebees and Lepidoptera. An analysis at specific level about pollinator identity is not available, as well as a survey about the abundance of pollinator species over the whole summer period.

Notably, pollinator abundance and diversity are declining in many agricultural areas worldwide (Biesmeijer et al., 2006; Potts et al., 2010; Bommarco et al., 2012; Bartomeus et al., 2013). This seems mostly due to large-scale agricultural intensification (Decourtye et al., 2010), since the loss of semi-natural habitats and overuse of agrochemicals have strong detrimental effects on nesting and foraging activities of pollinators and other beneficial arthropod species (Rundlöf et al., 2008; Brittain et al., 2010; Williams et al., 2010; Wratten et al., 2012). In particular, the health of wild and managed honeybees is declining substantially over the last decade, both in Europe and USA (Becher et al., 2013). Huge losses of honeybee colonies have been observed worldwide, particularly in USA from 2006, when colony collapse disorder (CCD) was described (Oldroyd, 2007). CCD seems due to multiple stressors, such as

occurrence of epidemiological factors affecting insect health (e.g. diseases and parasites) (Cox-Foster et al., 2010; Le Conte et al., 2010), overuse of pesticides and other agrochemicals (Becher et al., 2013), and loss of flower-rich plant communities associated with traditional agricultural landscapes (Potts et al., 2010). Pollinator communities may be supported thanks to field margins, other buffer zones and set-aside fields. Such areas offer a suitable environment for soil-nesting bee pollinators, as well as for Lepidoptera species that require particular plant species on which to oviposit (Saarinen, 2002; Nicholls and Altieri, 2013; Rollin et al., 2016). Since abundance and diversity of wild and managed bees is strictly related to the availability of continuous floral resources (Wratten et al., 2012), the establishment of flower-rich plant communities within or around intensively farmed landscapes increases the availability of pollen and nectar resources supporting Apoidea populations (Carvell et al., 2007; Decourtye et al., 2010; Haaland et al., 2011; Fabian et al., 2013; Benelli et al., 2014).

In this scenario, we hypothesise that purple loosestrife can be a key source of nectar and pollen for Apoidea in Mediterranean wetlands, helping them to overcome hot summer periods characterised by nectar and pollen paucity. In this study, we described the long lasting flowering dynamics of *L. salicaria* plants in intensively managed agricultural wetlands of Italy. Pollinator diversity and abundance were also investigated, in order to determine if this rustic species could be proposed to enhance floral diversity supporting Apoidea populations in agricultural areas of Mediterranean basin countries.

2. Experimental

2.1. Experimental site, flowering dynamics and abundance of pollinators

Investigations were carried out during *L. salicaria* flowering (from early June to late September 2014) in a wetland agro-environment of a Tuscan area (Agnano 43.73 N, 10.48 E, Pisa, Italy). No beekeeping activities were detected in a radius of 5 km from the study site. The studied area, surrounded by herbaceous field crops (i.e. wheat, maize and sunflower cultivations), is characterised by clay soils (2 m a.s.l.). *L. salicaria* plants were located along field drainage canals. In this area, three homogeneous *L. salicaria* strips (length: 30 m, width: 3 m) growing along the filed drainage channels were selected. For each strip, ten *L. salicaria* plants have been randomly selected and marked, and used both for evaluation of flowering-dynamics and pollinators' abundance. *L. salicaria* flowering dynamics was surveyed following the methods reported in Benelli et al. (2014). From June to September 2014, samplings were carried out every ten days (three replicates for each sampling) and the number of open flowers per plant was noted.

Insects were captured, using an entomological net, during their foraging on *L. salicaria* flowers. From July 1st to September 30th, five samplings per month (one each five days) were carried out. For each sample date, two observation periods were chosen: morning (from 11:00 to 12:00) and early afternoon (from 14:00 to 15:00) (Benelli et al., 2014; Canale et al. 2014). Collected specimens were kept separately in plastic test tubes with cork shavings imbued with ethyl acetate until they could be prepared for identification. Insects were dry mounted, observed using a binocular microscope (Leica ES2, Germany) and identified at a species level. A sample ranging from two to four specimens for each species was observed with an environmental scanning electron microscope (ESEM, hereafter) (FEI QuantaTM 200, Hillsboro, USA) to ensure the

presence of *L. salicaria* pollen (**Supplementary Data Fig. S1**) on the insect body, thus allowing us to legitimate the species as pollen foragers on the plant (Ollerton et al., 2003; Benelli et al., 2014; Canale et al., 2014). Voucher specimens of all species were stored in entomological boxes and kept at the Department of Agriculture, Food and Environment of the University of Pisa. During summer 2014, appreciable flowering of honeybee-visited flora was recorded by direct observation (Canale et al., 2015). Observations were conducted in a radius of 500 m from the plants of *L. salicaria* flower strips surveyed for abundance of insect pollinators.

2.2. Data analysis

Concerning *L. salicaria* flowering dynamics, differences in the mean number of open flowers, were analysed by JMP 7 (SAS 1999) by using a general linear model with one fixed factor (i.e. the number of open flowers). Averages were separated by the Tukey-Kramer HSD test. A probability level of $P < 0.05$ was used for the significance of differences between values.

Differences in the mean abundance of different orders of insect pollinators were analysed using the general linear model described above with two fixed factors, the period and the pollinator's order. Averages were separated by the Tukey-Kramer HSD test. A probability level of $P < 0.05$ was used for the significance of differences between values.

Since bees, with special reference to honeybees and bumblebees, were the main insect pollinators on *L. salicaria* flowers, differences in the mean abundance of different bee species over time were analysed the above-mentioned general linear model with two

fixed factors, the period and the bee species. Averages were separated by the Tukey-Kramer HSD test. A probability level of $P < 0.05$ was used for the significance of differences between values.

3. Results

In summer and early autumn 2014, we found a significant effect of the sampling period on *L. salicaria* flowering dynamics ($F = 87.953$; $d.f. = 11$; $P < 0.0001$). *L. salicaria* maximum flowering occurred during early August, with values ranging from 722 ± 68 to 798 ± 71 open flowers per plant (o.f.p.) (mean $\pm$ SD) (**Fig. 1**). The number of open flowers per plant was still high in late July (694 ± 92 o.f.p.), late August (658 ± 68 o.f.p.) and early September (642 ± 58 o.f.p.), while it was lower in mid September (373 ± 43 o.f.p.) and June (58 ± 15 o.f.p.) (**Fig. 1**).

Concerning insect pollinators, 476 specimens, belonging to 3 orders, 7 families and 15 species were identified (**Supplementary Data Table S1**). During focal observations, we observed honeybees, *A. mellifera*, foraging on *L. salicaria* flowers, with their pollen baskets (*corbiculae*) filled by the dark-coloured *L. salicaria* pollen (**Supplementary Data Fig. S1**). ESEM investigations highlighted that all identified insect species carried *L. salicaria* pollen grains (**Supplementary Data Fig. S2**) on their body parts, particularly third-leg femora [e.g. pollen grains mass-packed in the pollen baskets of honeybees, *A. mellifera* (**Supplementary Data Fig. S3**) and mouthparts]. These evidences allowed us to legitimate the recorded insect species as pollen foragers on purple loosestrife.

L. salicaria was served by insect pollinators belonging to Hymenoptera, Diptera and Lepidoptera. Hymenoptera pollinators were the most abundant (427 observed specimens), followed by Diptera and Lepidoptera (26 and 23 specimens, respectively). The mean abundance of insect pollinators from different orders is significantly affected by the sampling period ($F = 13.225$; $d.f. = 2$; $P < 0.0001$), the insect order ($F = 188.826$; $d.f. = 2$; $P < 0.0001$), and their interaction ($F = 9.557$; $d.f. = 4$; $P < 0.0001$). Mean abundance of Hymenoptera reached a maximum in August, while no significant differences were found in mean abundance of Diptera and Lepidoptera pollinators over time (**Fig. 2**).

Bees were the main insect pollinators on *L. salicaria* flowers. The mean abundance of bee species was significantly affected by the sampling period ($F = 18.488$; $d.f. = 2$; $P < 0.0001$), the species ($F = 72.136$; $d.f. = 7$; $P < 0.0001$), and their interaction ($F = 6.345$; $d.f. = 14$; $P < 0.0001$). Social species dominated, with 94.38% individuals compared to 5.62% solitary species. The most abundant species is the honeybee, *A. mellifera*, followed by five bumblebee species [*Bombus lapidarius* (Linnaeus), *B. pascuorum* (Scopoli), *B. rupestris* (Fabricius), *B. sylvarum* (Linnaeus) and *B. terrestris* (Linnaeus)], the halictid *Halictus maculatus* Smith and the megachilid *Rhodanthidium septemdentatum* (Latreille) (**Fig. 3**). Mean honeybee abundance was higher in August over the rest of the summer. Honeybee visits on *L. salicaria* flowers decreased from late summer to early autumn, when blooming was less abundant and more flowering species, including Asteraceae, Dipsacaceae and Solanaceae become available to bees (**Table 1**). No differences in abundance of pollinators visiting *L. salicaria* over time were detected for other bee species (**Fig. 3**).

As regards Diptera pollinators, we observed four species of hoverflies (Diptera: Syrphidae), *Eristalis arbustorum* (L.) (total: 4 specimens), *Sphaerophoria scripta* (L.) (3 specimens), *Sphaerophoria rueppelli* (Wiedmann) (6 specimens), and *Volucella zonaria* (Poda) (13 specimens). Three species of Lepidoptera foraging on *L. salicaria* flowers were also identified, including *Polyommatus icarus* (Rottemburg) (14 specimens), *Pieris brassicae* (Linnaeus) (5 specimens), and *Zygaena transalpina* (Esper) (4 specimens) (**Supplementary Data Table S1**).

4. Discussion

Results showed that, in Mediterranean basin countries, *L. salicaria* is characterised by a long lasting flowering, from mid-July to early September, with more than 640 open flowers per plant. The availability of *L. salicaria* pollen and nectar in mid-summer and early autumn can be crucial for the survival of pollinators living in Mediterranean wetlands, since only few plant species provide food to them [see also Benelli et al. (2014) for a recent study on bee-friendly flora in sandy agricultural areas of Italy].

This research highlights the ecological and functional generalization of purple loosestrife flowers, since a number of different insect species pollinate them, suggesting that the plant has not evolved specific plant-pollinator associations (Ollerton et al., 2007; Canale et al., 2015). *L. salicaria* flowers are mainly visited by long-tongued Apoidea, and their abundance reached a maximum in August. In agreement with previous observations conducted in Northern Sweden islands by Waites and Ågren (2004), social bees largely dominates, with special reference to the honeybee and bumblebees.

In our experiments, Apoidea visits on *L. salicaria* bloomings decreased in early autumn, when more flowering plants (mainly Asteraceae, Dipsacaceae and Solanaceae) were available to bees (see also Canale et al., 2015). However, the pollinator abundance on these alternative food sources was not quantified. Further research on this issue is needed, since in North America has been highlighted that *L. salicaria* act as a strong competitor for pollinators against other plant species (e.g. *Mimulus ringens* L.), reducing their seed set (Flanagan et al., 2010).

Information on the specific identity of bumblebee pollinators serving *L. salicaria* have been never provided, with the only exception of preliminary records of *B. lapidarius* and *B. pascuorum* reported by Batra et al. (1986) for Northern Yugoslavia and Poland, respectively. In coastal Mediterranean wetlands, we identified five bumblebee species foraging on *L. salicaria* flower spots, including *B. sylvarum*, a species threatened by habitat loss and intensification of agriculture, now declining in numbers across Europe (Goulson et al., 2008) and included in the United Kingdom Biodiversity Action Plan. *L. salicaria* production of both pollen and nectar in periods characterised by food paucity for pollinators in Mediterranean agro-ecosystems, is of particular importance for bumblebees. Indeed, it is well known that a continuous and readily available supply of food is crucial for their successful establishment and growth (Fussel and Corbet 1992; Goulson et al., 2008), and flower availabilities during colony foundation (early spring) and colony reproduction (mid to late summer) have been suggested as key resource bottlenecks in European landscapes (Rundlöf et al., 2014). However, the most frequent insects on flowers of a given weed are not necessarily the most efficient pollinators. Indeed, pollen transport depends on the particular morphology of the insect body parts (e.g. pollen baskets or hairs, and on the speed of

insects while handling flowers). For instance, the rapid visits performed by long-tongued bees may result in reduced pollen transport (Fenster et al., 2004). On the other hand, the electrostatic forces occurring on pollen can guarantee adhesion to the insect, even if it is lacking in hairy structures (Armbruster, 2001).

Furthermore, we observed some Diptera and Lepidoptera species foraging on purple loosestrife flowers. The four Diptera flower visiting species all belong to Syrphidae family; the role of hoverflies as pollinators has been probably underestimated in the past, pointing out that they feed most on nectar using their long proboscis (Katzourakis et al., 2001; Waites and Ågren, 2004). However, it has been demonstrated that hoverflies can use labellar food furrows to feed on pollen (Schuhmacher and Hoffmann, 1982; Canale and Loni, 2010), thus improving their diet with a protein-rich food. The role of Lepidoptera as pollinators seems less efficient over bees and flies, since they use a long proboscis to feed on nectar, and only few pollen grains of purple loosestrife have been found on their body parts during scanning electron micrograph observations.

Overall, we support the purple loosestrife as a suitable species for bee-friendly flower spots in Mediterranean agricultural wetlands, as well as in close proximity of beehives. The latter use may facilitate the honeybees collecting pollen for the colony, thereby ensuring robustness to overcome the winter season (Benelli et al., 2014). In agreement with the green payment guidelines proposed by EU Common Agricultural Policy 2014-2020 (e.g. ecological focus areas embedded in intensively managed arable lands), purple loosestrife flower spots may help to reduce the degradation and fragmentation of Mediterranean agricultural wetlands (Canale et al., 2015), which is one of the main causes responsible for the decline of biodiversity and abundance of insect pollinators.

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References

- Ågren, J., 1996. Population size, pollinator limitation, and seed set in the self-incompatible herb *Lythrum salicaria*. *Ecology*, 77, 1779-1790.
- Armbruster, W.S., 2001. Evolution of floral form: electrostatic forces, pollination, and adaptative compromise. *New Phytologist*, 152, 181-183.
- Bartomeus, I., Ascher, J.S., Gibbs, J., Danforth, B.N., Wagner, D.L., Hedtke, S.M., Winfree, R., 2013. Historical changes in northeastern US bee pollinators related to shared ecological traits. *Proceedings of the National Academy of Science USA*, 110, 4656-4660

303 Batra, S.W.T., Schroeder, D., Boldt, P.E., Mendl, W., 1986. Insects associated with
304 purple loosestrife (*Lythrum salicaria* L.) in Europe. Proceedings of the
305 Entomological Society of Washington, 88, 748-759.

306 Becher, M.A., Osborne, J. L., Thorbek, P., Kennedy, P. J., Grimm, V., 2013. Review:
307 Towards a systems approach for understanding honeybee decline: a stocktaking
308 and synthesis of existing models. Journal of Applied Ecology, 50, 868-880.

309 Benelli, G., Benvenuti, S., Desneux, N., Canale, A., 2014. *Cephalaria transsylvanica*-
310 based flower strips as potential food source for bees during dry periods in
311 European Mediterranean basin countries. PLOS ONE, 9, e93153.

312 Biesmeijer, J.C., Roberts, S.P.M., Reemer, M., Ohlemüller, R., Edwards, M., Peeters,
313 T., Schaffers, A.P., Potts, S.G., Kleukers, R., Thomas, C.D., Settele, J., Kunin,
314 W.E., 2006. Parallel declines in pollinators and insect-pollinated plants in
315 Britain and the Netherlands. Science, 313, 351-354.

316 Blossey B., Skinner L. C., Taylor, J., 2001. Impact and management of purple
317 loosestrife (*Lythrum salicaria*) in North America. Biodiversity and
318 Conservation, 10, 1787-1807.

319 Bommarco, R., Lundin, O., Smith, H.G., Rundlöf, M., 2012. Drastic historic shifts in
320 bumble-bee community composition in Sweden. Proceedings of the Royal
321 Society Series B Biological Sciences, 279, 309-315.

322 Brittain, C.A., Vighi, M., Bommarco, R., Settele, J., Potts, S.G., 2010. Impacts of a
323 pesticide on pollinator species richness at different spatial scales. Basic and
324 Applied Ecology, 11, 106-115.

325 Canale, A., Loni, A., 2010. Insects visiting olive flowers (*Olea europaea* L.) in a
326 Tuscan olive grove. Redia XCII, 95-98.

327 Canale, A., Benelli, G., Benvenuti, S., 2014. First record of insect pollinators visiting
 328 *Muscari comosum* (L.) Miller (Liliaceae-Hyacinthaceae), an ancient
 329 Mediterranean food plant. *Plant Biosystems*, 148, 889-894.

330 Canale, A., Benelli, G., Benvenuti, S., Raspi, A., 2015. Insect pollinators of the late
 331 winter flowering *Rhamnus alaternus* L., a candidate for honeybee-friendly
 332 scrubland spots in intensively managed agricultural areas. *Plant Biosystems*, doi:
 333 10.1080/11263504.2014.993742.

334 Carvell, C., Meek, W.R., Pywell, R.F. et al., 2007. Comparing the efficacy of agri-
 335 environment schemes to enhance bumble bee abundance and diversity on arable
 336 field margins. *J Appl Ecol* 44:29–40.

337 Colautti, R. I., White N.A., Barrett S.C.H., 2010. Variation of self-incompatibility
 338 within invasive populations of purple loosestrife (*Lythrum salicaria* L.) from
 339 Eastern North America. *International Journal of Plant Sciences*, 171, 158-166.

340 Cox-Foster, D.L., Conlan, S., Holmes, E.C., Palacios, G., Evans, J.D., Moran, N.A.,
 341 Quan, P.L., Briese, T., Hornig, M., Geiser, D.M., Martinson, V., van
 342 Engelsdorp, D., Kalkstein, A.L., Drysdale, A., Hui, J., Zhai, J., Cui, L.,
 343 Hutchison, S.K., Simons, J.F., Egholm, M., Pettis, J.S., Lipkin, W.I., 2007. A
 344 metagenomic survey of microbes in honey bee colony collapse disorder.
 345 *Science*, 318, 283-287.

346 Darwin, C., 1864. On the sexual relations of the three forms of *Lythrum salicaria*.
 347 *Journal of the Proceedings of the Linnean Society of London Botany* 8, 169-196.

348 Decourtye, A., Mader, E., Desneux, N., 2010. Landscape enhancement of floral
 349 resources for honey bees in agro-ecosystems. *Apidologie*, 41: 264-277.

350 Fabian, Y., Sandau, N., Bruggisser, O.T., Aebi, A., Kehrli, P., et al. (2013) The
 351 importance of landscape and spatial structure for hymenopteran-based food webs
 352 in an agro-ecosystem. *Journal of Animal Ecology*, 82, 1203–1214.
 353 Farnsworth, E.J., Ellis, D.R., 2001. Is purple loosestrife (*Lythrum salicaria*) an invasive
 354 threat to freshwater wetlands? Conflicting evidence from several ecological
 355 metrics. *Wetlands*, 21, 199-209.
 356 Fenster, C.B., Armbruster, W.S., Wilson, P., Dudash, M.R., Thomson, J.D., 2004.
 357 Pollination syndromes and floral specialization. *Annual Review of Ecology and*
 358 *Systematics*, 35, 375-403.
 359 Flanagan, R.J., Mitchell R.J, Karron, J.D., 2010. Increased relative abundance of an
 360 invasive competitor for pollination, *Lythrum salicaria*, reduces seed number in
 361 *Mimulus ringens*. *Oecologia*, 164, 445-454.
 362 Fussell, M., Corbet, S.A., 1992. Flower usage by bumble-bees: a basis for forage plan
 363 management. *Journal of Applied Ecology*, 29, 451-465.
 364 Goulson, D., Lye, G.C., Darvill, B., 2008. Decline and conservation of bumble bees.
 365 *Annual Review of Entomology*, 53, 191-208.
 366 Haaland, C., Naisbit, R.E., Bersier, L.F., 2011. Sown wildflower strips for insect
 367 conservation: a review. *Insect Conservation and Diversity*, 4, 60-80.
 368 Hultén, E., Fries, M., 1986. Atlas of North-European vascular plants north of the Tropic
 369 of Cancer. III. Koeltz, Königstein.
 370 Hunt-Joshi, T.R., Blossey, B., Root, R.B., 2004. Root and leaf herbivory on *Lythrum*
 371 *salicaria*: implications for plant performance and communities. *Ecological*
 372 *Applications*, 14, 1574-1589.

373 Katzourakis, A., Purvis, A., Azmeh, S., Rotheray, G., Gilbert, F., 2001. Macroevolution
 374 of hoverflies (Diptera: Syrphidae): the effect of using higher-level taxa in studies
 375 of biodiversity, and correlates of species richness. *Journal of Evolutionary*
 376 *Biology*, 14, 219-227.

377 Klips, R.A., Peñalosa, J., 2003. The timing of seed fall, innate dormancy, and ambient
 378 temperature in *Lythrum salicaria*. *Aquatic Botany*, 75, 1-7.

379 Le Conte, Y., Ellis, M., Ritter, W., 2010. *Varroa* mites and honey bee health: can
 380 *Varroa* explain part of the colony losses? *Apidologie*, 41, 353-363.

381 Levin, D. A., Kerster, H. W., 1973. Assortative pollination for stature in *Lythrum*
 382 *salicaria*. *Evolution*, 27, 144-152.

383 Mai, T. R., Lovett-Doust, J., Lovett-Doust, L., Mulligan, G.A., 1992. The biology of
 384 Canadian weeds. 100. *Lythrum salicaria*. *Canadian Journal of Plant Science*, 72,
 385 1305-1330.

386 Mal, T. K., Lovett-Doust, J., 2005. Phenotypic plasticity in vegetative and reproductive
 387 traits in an invasive weed, *Lythrum salicaria* (Lythraceae), in response to soil
 388 moisture. *American Journal of Botany*, 92, 819-825.

389 Mulcahy, D.L., Caporello D., 1970. Pollen flow within a tristylous species: *Lythrum*
 390 *salicaria*. *American Journal of Botany*, 57, 1027-1030.

391 Mullin, B.H., 1998. The biology and management of purple loosestrife (*Lythrum*
 392 *salicaria*). *Weed Technology*, 12, 397-401.

393 Nicholls, M.S., 1987. Pollen flow, self-pollination and gender specialization: Factors
 394 affecting seed-set in the tristylous species *Lythrum salicaria* (Lythraceae). *Plant*
 395 *Systematics and Evolution*, 156, 151-157.

396 Nicholls, C.I., Altieri, M.A., 2013. Plant biodiversity enhances bees and other insect

397 pollinators in agroecosystems. A review. *Agronomy for Sustainable Development*, 33,
 398 257-274.

399 Oldroyd, B.P., 2007. What's killing American honey bees? *PLOS Biology*. 5, 1195-
 400 1199.

401 Ollerton, J., Johnson, S.D., Cranmer, L., Kellie, S., 2003. The pollination ecology of an
 402 assemblage of grassland asclepiads in South Africa. *Annals of Botany* 92, 807-
 403 834.

404 Ollerton, J., Killick, A., Lamborn, E., Watts, S., Whiston, M., 2007. Multiple meanings
 405 and modes: on the many ways to be a generalist flower. *Taxon*, 56: 717-728.

406 Olsson, K., Ågren, J., 2002. Latitudinal population differentiation in phenology, life
 407 history and flower morphology in the perennial herb *Lythrum salicaria*. *Journal*
 408 *of Evolutionary Biology*, 15, 983-996.

409 O'Neil, P., Schmitt J., 1993. Genetic constraints on the independent evolution of male
 410 and female reproductive characters in the tristylous plant *Lythrum salicaria*.
 411 *Evolution*, 47, 1457-1471.

412 Potts, S.G., Biesmeijer, J.C., Kremen, C., Neumann, P., Schweiger, O., Kunin, W.E.,
 413 2010. Global pollinator declines: trends, impacts and drivers. *Trends in Ecology*
 414 *and Evolution*, 25, 345-353.

415 Richards, J. H., Diggle, P.K. and Barrett, S.C.H., 1989. A comparison of floral
 416 development among tristylous taxa of *Lythrum*, *Oxalis*, and the *Pontederiaceae*.
 417 *American Journal of Botany*, 76, 53-57.

418 Rollin, O., Benelli, G., Benvenuti, S., Decourtye, A., Wratten, S.D., Canale, A.,
 419 Desneux, N., 2016. Weed-insect pollinator networks as bioindicators of

ecological sustainability in agriculture. A review. *Agronomy for Sustainable Development*, 36(1):1-22, doi: 10.1007/s13593-015-0342-x

Rundlöf, M., Nilsson, H., Smith, H.G., 2008. Interacting effects of farming practice and landscape context on bumblebees. *Biological Conservation*, 141, 417-426.

Rundlöf, M., Persson, A., Smith, H.G., Bommarco, R. 2014. Late-season mass-flowering red clover increases bumble bee queen and male densities. *Biological Conservation* 172: 138-145.

Saarinen, K., 2002. A comparison of butterfly communities along field margins under traditional and intensive management in SE Finland. *Agriculture, Ecosystems and Environment*, 90, 59-65.

Schuhmacher, H., Hoffmann, H., 1982. Zur Funktion der Mundwerkzeuge von Schwebfliegen bei der Nahrungsaufnahme (Diptera Syrphidae). *Entomologia Generalis*, 7, 327-342.

Stuckey, R.L., 1980. Distribution history of *Lythrum salicaria* (purple loose-strife) in North America. *Bartonia*, 47: 3-20.

Tunalier, Z., Koşar, M., Küpeli, E., Çaliş, İ., Başer, K., 2007. Antioxidant, anti-inflammatory, anti-nociceptive activities and composition of *Lythrum salicaria* L. extracts. *Journal of Ethnopharmacology*, 110, 539-547.

Waites, A.R., Ågren, J.O.N., 2004. Pollinator visitation, stigmatic pollen loads and among-population variation in seed set in *Lythrum salicaria*. *Journal of Ecology* 92, 512-526.

Welling C.H., Becker, R.L., 1990. Seed bank dynamics of *Lythrum salicaria* L.: implications for control of this species in North America. *Aquatic Botany*, 38, 303-309.

444 Williams, N.M., Crone, E.E., Roulston, T.H., Minckley, R.L., Packer, L., Potts, S.G.,
445 2010. Ecological and life-history traits predict bee species responses to
446 environmental disturbances. *Biological Conservation*, 143, 2280-2291.

447 Wratten, S.D., Gillespie, M., Decourtye, A., Mader, E., Desneux, N., 2012. Pollinator
448 habitat enhancement: benefits to other ecosystem services. *Agriculture,*
449 *Ecosystems and Environment*, 159, 112-122.

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Figure captions

Figure 1. Flowering dynamics of *Lythrum salicaria* plants growing in Mediterranean wetlands during summer 2014. Bars represent standard deviations. Different letters indicate significant differences among the number of open flowers per plant over time (general linear model, Tukey-Kramer test, $P < 0.05$).

Figure 2. Insect pollinators visiting *Lythrum salicaria* flowers in Mediterranean wetlands: mean abundance per sampling (5 samplings per month) of Hymenoptera, Diptera and Lepidoptera pollinators. Bars represent standard errors. Different letters indicate significant differences (general linear model, Tukey-Kramer test, $P < 0.05$).

Figure 3. Mean abundance per sampling (5 samplings per month) of Hymenoptera pollinators visiting *Lythrum salicaria* flowers in Mediterranean wetlands. *Apis mellifera* (AM), *Bombus lapidarius* (BL), *Bombus pascuorum* (BP), *Bombus rupestris* (BR), *Bombus sylvarum* (BS), *Bombus terrestris* (BT), *Halictus maculatus* (HM) and *Rhodanthidium septemdentatum* (RS). Bars represent standard errors. Different letters indicate significant differences (general linear model, Tukey-Kramer test, $P < 0.05$).

Figure 1

Figure 2

Figure 3

Table 1. Appreciable flowering of bee-visited flora in Mediterranean wetlands (Tuscany, Italy) during summer 2014. Observations were carried out in a radius of 500 meters from *Lytrum salicaria* plants monitored for pollinators’ abundance.

Plant species	Family	Month								
		July			August			September		
		Decads								
		1	2	3	1	2	3	1	2	3
<i>Abutilon theophrasti</i> Medicus	Malvaceae			•	•	•	•			
<i>Cichorium intybus</i> L.	Asteraceae		•	•	•	•	•	•	•	
<i>Cirsium arvense</i> (L.) Scop.	Asteraceae		•	•	•					
<i>Daucus carota</i> L.	Apiaceae			•	•	•	•	•	•	•
<i>Foeniculum vulgare</i> Mill.	Apiaceae	•	•	•	•	•	•			
<i>Glebionis segetum</i> (L.) Fourr.	Asteraceae	•	•							
<i>Iris pseudacorus</i> L.	Iridaceae	•	•							
<i>Lavatera trimestris</i> L.	Malvaceae	•	•	•	•	•				
<i>Malva sylvestris</i> L.	Malvaceae	•	•	•	•	•	•	•	•	
<i>Picris echioides</i> L.	Asteraceae			•	•	•	•	•		
<i>Pulicaria odora</i> (L.) Rchb.	Asteraceae							•	•	•
<i>Salpichroa rhomboidea</i> (Gill & Hook.) Miers	Solanaceae		•	•	•	•	•	•	•	•
<i>Scabiosa columbaria</i> L.	Dipsacaceae		•	•	•	•	•	•	•	•
<i>Solanum nigrum</i> L.	Solanaceae						•	•	•	•