

The impact of *Solanum elaeagnifolium*, an invasive plant in the Mediterranean, on the flower visitation and seed set of the native co-flowering species *Glaucium flavum*

Thomas Tscheulin · Theodora Petanidou ·
Simon G. Potts · Josef Settele

Received: 15 July 2008 / Accepted: 9 March 2009 / Published online: 31 March 2009
© Springer Science+Business Media B.V. 2009

Abstract We examined the effect of the invasive *Solanum elaeagnifolium* (Solanaceae) on flower visitation patterns and seed set of the co-flowering native *Glaucium flavum* (Papaveraceae). We observed flowering *G. flavum* plants in invaded and uninvaded sites and found that *G. flavum* flowers in uninvaded sites received significantly more total visits. In addition, we hand-pollinated flowers on plants of *G. flavum* with (i) pure conspecific pollen, (ii) pure *S. elaeagnifolium* pollen and (iii) three different mixtures of the two types of pollen (containing 25, 50 and 75% invasive pollen). As a control, flowers were left unmanipulated or were permanently bagged. Seed set did not differ significantly between flowers receiving pollen

mixtures and pure conspecific pollen. However, in the open pollination treatment, seed set was significantly lower than in the 100% conspecific pollen treatment, which suggests pollen limitation. Bagged flowers had very low seed set. *G. flavum* was generally resilient against the deposition of *S. elaeagnifolium* pollen.

Keywords Silverleaf nightshade · Yellow-horned poppy · Invasive · Alien · Seed set · Pollination · Heterospecific pollen deposition

Introduction

Invasive plants pose a threat to biodiversity and ecosystem functioning (Pimentel et al. 2001; Traveset and Richardson 2006), because they may displace native plants and crop species due to competition for space, nutrients, water and light (Mack and D'Antonio 1998; Wardle et al. 1994; Weihe and Neely 1997). In addition, the presence of a co-flowering invasive can also affect native plants through the disruption of pollination services (see Bjerknes et al. 2007 for a short review; but see Bowman et al. 2008).

Competition for pollination services can potentially result in the deposition of heterospecific pollen (i.e. a reduction in pollen quality) or/and in lower visitation (i.e. a reduction in pollen quantity). Both processes may eventually lead to lower seed set of the native plants. In particular, invasives can potentially

T. Tscheulin (✉) · T. Petanidou
Laboratory of Biogeography and Ecology, Department
of Geography, University of the Aegean, University Hill,
81100 Mytilene, Greece
e-mail: t.tscheulin@geo.aegean.gr

T. Petanidou
e-mail: t.petanidou@aegean.gr

S. G. Potts
Centre for Agri-Environmental Research (CAER), School
of Agriculture, Policy and Development, University of
Reading, Reading RG6 6AR, UK
e-mail: s.g.potts@reading.ac.uk

J. Settele
Department of Community Ecology, UFZ – Helmholtz
Centre for Environmental Research, Theodor-Lieser-Str.
4, 06120 Halle (Saale), Germany
e-mail: Josef.Settele@ufz.de

affect pollinator–native plant interactions in a negative way in terms of visitation patterns (Brown et al. 2002; Chittka and Schürkens 2001; Larson et al. 2006; Moragues and Traveset 2005; Totland et al. 2006) and seed set (Brown et al. 2002; Chittka and Schürkens 2001; Grabas and Lavery 1999). However, in contrast, there have also been cases, where invasives had a positive effect in terms of visitation (Grabas and Lavery 1999; Larson et al. 2006; Moragues and Traveset 2005) and seed set (Brown et al. 2002; Chittka and Schürkens 2001; Grabas and Lavery 1999). There have also been cases, where invasives have had no effect on visitation (Aigner 2004; Grabas and Lavery 1999; Larson et al. 2006; Moragues and Traveset 2005) and seed set (Aigner 2004; Grabas and Lavery 1999; Moragues and Traveset 2005; Totland et al. 2006).

One potential reason for the positive effect of invasives could be that they provide additional resources for pollinators, which could lead to an overall increase in pollinator abundance, diversity, geographical range or even duration of their foraging season. This could potentially facilitate the pollination of the native plants (Feldman et al. 2004; Memmott and Waser 2002; Tepedino et al. 2008).

Although, on the other hand, invasives could attract pollinators that were previously monopolized by the native species, which could lead to lower visitation rates to the natives, the deposition of heterospecific pollen (Lopezaraiza-Mikel et al. 2007) and subsequently to reduction in seed set (Jacobi et al. 2005; Larson et al. 2006).

Flowers receive heterospecific pollen due to the inconstant foraging behaviour of many pollinators, especially generalists, which make up the vast majority of visitors to invasive plants (Lopezaraiza-Mikel et al. 2007; Memmott and Waser 2002). This can lead to lower seed set even in the absence of pollinator limitation (Kasagi and Kudo 2005; Petanidou et al. 1995a; Ruane and Donohue 2007). The reduction in seed set caused by the deposition of heterospecific pollen can come about in several ways, namely stigma clogging (Holland and Chamberlain 2007), stigma closing, pollen allelopathy, stylar clogging and stylar inhibition (see Brown and Mitchell 2001 and references therein). Heterospecific pollen deposition occurring between closely related species may lead to hybridization, a further threat to native flora (Brown and Mitchell 2001; Chapman

et al. 2005; Kasagi and Kudo 2005). In addition, heterospecific pollen deposition constitutes a loss of male gametes and therefore potentially reduces male fitness (McGuire and Armbruster 1991).

In this study we examined the impact of the silverleaf nightshade, *Solanum elaeagnifolium* (Cavanilles), an invasive to the Mediterranean from America, on the pollination of the native yellow-horned poppy, *Glaucium flavum* (Crantz). *G. flavum* is part of the perennial vegetation of stony beaches along sections of the Mediterranean coast, where it co-flowers and shares pollinators with the invasive during summer (June–August). In this region flowering peaks in spring (March–May) (Petanidou and Ellis 1996; Petanidou et al. 1995b), with few plants coming into flower in summer (June–August). During the Mediterranean summer, however, the flowering plant species are visited by a much higher number of pollinator species compared to other seasons (Petanidou 2004). This implies the existence of ‘open niches’ for plant species, which may be utilized by silverleaf nightshade. In this study we (i) test whether the invasive affects pollinator visitation to native plants in natural populations and (ii) quantify the effect on seed set of depositing different proportions of invasive pollen on the stigmas of native plants. The study was carried out in 2006–2007 on Lesbos Island, Greece, where the invasive and native plants are sympatric in at least two sites. Prior observations confirmed that both plants share bee pollinators.

Materials and methods

Study species

Glaucium flavum is a perennial herb that occurs along the Mediterranean shores and the coasts of W. Europe in shingle or stable pebbles just above the beach (Thanos et al. 1989). Pebble and shingle beaches and their perennial vegetation have been listed as an Annex I habitat interest feature in the EU Habitat Directive 92/43/EEC (EU 1992). The large yellow flowers of *G. flavum* have four silky petals, which drop on the same day of anthesis after which the stigma is no longer receptive and the long-curved seed capsules develop (Tscheulin, personal observation).

Solanum elaeagnifolium is native to South and Central America and south-western states of the US (Boyd et al. 1984). Interest in the plant increased in the 1970s as silverleaf nightshade increasingly spread outside its native range. Today it is found in Australia, Algeria, Chile, Croatia, Cyprus, Denmark, France, Egypt, Greece, India, Israel, Italy, Morocco, Serbia and Montenegro, New Zealand, Pakistan, Puerto Rico, South Africa, Spain, Switzerland, Syria, Taiwan, Tunisia and Zimbabwe (Bouhache and Tanji 1985; Boyd et al. 1984; Mekki 2007). In Greece *S. elaeagnifolium* is widespread especially on the mainland and locally very abundant (Boratynski et al. 1992; Economidou and Yannitsaros 1975; Tscheulin et al. 2008). *S. elaeagnifolium* is deep-rooted, which is the main reason why control with herbicides has shown only limited success (Baye et al. 2007; Eleftherohorinos et al. 1993). It lowers crop yield through competition (Boyd and Murray 1982), may lower the quality of hay taken from infested fields (Boyd et al. 1984), and is toxic to livestock, such as sheep, cattle and horses. The flowers have poricidal anthers and are pollinated by bees (Buchmann and Cane 1989). Individual berries produce 24 to 149 seeds (Boyd and Murray 1982), which can add up to 5 to 100 million seeds per acre (Cooley and Smith 1971). Seeds may be dispersed by animal faeces, water, machinery and agricultural produce, and dried plants may also blow like tumbleweeds, spreading seed along the way (Boyd et al. 1984). *S. elaeagnifolium* can also spread by root fragments (Boyd and Murray 1982).

Study sites

We conducted hand-pollination treatments in an uninvaded population (*S. elaeagnifolium* absent) of *G. flavum* on a pebble beach, in Skala Vasilikon in the south of Lesbos Island, (39°08'54.98" N, 26°14'59.27" E) in July 2007. The population is more than 6 km away from the closest population of *S. elaeagnifolium*, a distance which is considered beyond the maximum foraging range of bees visiting *S. elaeagnifolium* (Gathmann and Tscharrntke 2002; Greenleaf et al. 2007; Kremen et al. 2004).

Observations of the flower visits to the flowers of *G. flavum* were carried out on two paired sites: beaches near Plomari and Skala Eressou with each one containing one invaded (38°58'15.77" N, 26°22'55.93" E and 39°08'20.27" N, 25°55'18.53" E,

respectively) and one uninvaded site (38°59'08.86" N, 26°18'53.12" E and 39°06'26.82" N, 25°57'53.17" E, respectively). Visits to the flowers of *S. elaeagnifolium* were observed in the two invaded sites. The invaded and uninvaded sites within a pair were at least 5 km apart and the two pairs were 40 km apart. We consider 5 km as a sufficient distance here, as flower visitation rather than seed set was measured and pollen transfer is therefore not an issue.

Pollen extraction and mixture preparation

Solanum elaeagnifolium pollen was collected using the standard method for pollen extraction from poricidal flowers by using a tuning fork (Hz 512) held against the anthers of a freshly open flower (Buchmann and Cane 1989). The method also proved effective in extracting pollen from *G. flavum* flowers, whose flowers are not poricidal. In order to determine the average number of pollen grains that can be extracted with this method per flower of either plant species we extracted pollen from 15 previously bagged, fully dehiscent, virgin flowers, randomly collected from different plants and suspended the pollen in 10 ml water for counting. For both species this was repeated with three lots of 15 flowers each. We then diluted tablets containing a known quantity of *Lycopodium* spores ($n = 13,500/\text{tablet}$) in the suspensions using an electric stirrer (vortex). This technique allows an estimation of the number of target particles (in this case pollen grains of the study plant species) using the ratio of target particles to *Lycopodium* spores and the known total number of *Lycopodium* spores (Petanidou et al. 2001). We counted pollen grains and *Lycopodium* spores under a light microscope at 400× magnification in subsamples (fields of view) of these suspensions until the ratio of pollen count: *Lycopodium* spores per subsample did not change the accumulated average by more than 1%. The mean number of *S. elaeagnifolium* pollen grains extracted with this method per flower was 81675 ± 1990 (mean \pm SE, $n = 3$, 15 flowers in each replicate), the average number of *G. flavum* pollen extracted per flower was 143835 ± 3120 (mean \pm SE, $n = 3$, 15 flowers in each replicate). All flowers providing pollen were first day, virgin flowers that had been covered with tulle bags before anthesis to prevent contact with flower visitors. The flowers were taken from potted plants, grown from seeds collected in Skala Eressou (39°08'22.02" N, 25°55'20.31" E) in

2006 (*S. elaeagnifolium*) and from plants in a wild population in Mytilene (39°06'46.54" N, 26°33'39.89" E; *G. flavum*).

Based on the mean numbers of pollen grains contained in their flowers, the ratio of *S. elaeagnifolium*:*G. flavum* flowers used to make up the respective pollen mixtures were as follows: 3:5 for the mixture 25%:75%; 7:4 for 50%:50%; and 11:2 for 25%:75%. With these ratios the mean percentages differed less than $\pm 0.5\%$ from the respective target percentage. We used multiples of those ratios to ensure better resilience against outliers. The pollen was extracted with the tuning fork and mixed in clean and dry Petri dishes using a toothpick.

Pollination treatments

All flowers for treatment on 46 randomly chosen experimental plants were covered with fine nylon tulle bags made from bridal veil before anthesis. The bags were removed for the application of pollen on the day of anthesis and immediately replaced after treatment. For hand-pollinations we used standard cotton bud sticks (CIEN, Germany), which we dipped into the pollen and applied the grains directly on the stigmas until they were visibly covered with pollen assuming that the pollen was fully mixed and the transfer occurred with equal facility. The number of conspecific pollen applied in pollen mixtures and pure conspecific pollen to each flower was an amount sufficient for full seed set in all pollination treatments. Over a period of 2 weeks in July 2007 all open flowers on experimental plants received one of the seven treatments on treatment days. The order of the treatment application was randomized for each plant. We aimed to apply all treatments once to each plant. The tulle bags were removed after the flowers had senesced. In addition, we marked flowers that were left untreated and uncovered ($n = 18$) to test for open pollination, and flowers that were left untreated but covered with tulle bags, which were removed after the flowers had senesced to test for spontaneous selfing ($n = 35$). The seed capsules were harvested in August and the seeds counted.

Pollinator visitation

Observation plots (1 m \times 1 m) were established randomly on observation days along a 50 m transect

in each invaded and uninvaded site so that every plot contained at least one flowering plant of *G. flavum* or *S. elaeagnifolium*. In each of four sampling rounds a minimum of six plots were observed per species in each of four sites in the case of *G. flavum* and two sites in case of *S. elaeagnifolium*. Observations were carried out in mid June, mid July, early August and late August 2006. In total we performed observations in 41 plots in uninvaded sites containing 43 *G. flavum* plants and 275 flowers, and in 42 plots in invaded sites containing 43 *G. flavum* plants and 221 flowers. In addition, we carried out observations in 48 plots in invaded sites containing 116 *S. elaeagnifolium* plants and 1061 *S. elaeagnifolium* flowers. Insect visitation to flowers was recorded for 3 min periods per plot and observation took place between 8.30 and 13.00 on sunny and calm days. In each census we recorded the total number of flowers, the visited number of flowers and the insect visitor in each observation plot. The flower visitors were identified at least to family level in bees when species or genus was not known.

Statistical analysis

After checking the seed set data for normality we carried out a one-way ANOVA followed by Tukey HSD post-hoc tests for the multiple comparisons to compare between pairs of pollination treatments in SPSS (2006).

We used general linear mixed models to assess the response of flower visitation to the explanatory fixed effect of site status (2 levels: invaded/uninvaded). Within the mixed model site, round and flower abundance were specified as random effects. Round (essentially a time effect) was not specified as a repeated measure within the model as plots were set up new on each occasion, it was however, still necessary to account for this blocking effect in time. The inclusion of within site flower abundance within each site accounts for any intercorrelation between flower abundance and visitation rate by the pollinators, independent of the treatment effect. In all cases the response variable of flower visits per plot was $\log_e n + 1$ transformed to normalize the data. The analysis was carried out using the Proc Mixed function within SAS version 9.1. As degrees of freedom were calculated using the Satterthwaites approach, denominator degrees of freedom can include decimals.

Results

Pollination treatments

Pollination with pollen mixtures containing 25, 50 and 75% *S. elaeagnifolium* pollen did not significantly lower seed set of *G. flavum* flowers compared to pollination with pure conspecific pollen (Fig. 1; Table 1). Pollination with pure *S. elaeagnifolium* pollen resulted in very low seed set, as did flowers in the spontaneous selfing treatment. The seed set in both these treatments was significantly lower than the seed set in flowers that received 100% conspecific

pollen, or any mixture of conspecific and invasive pollen, or flowers that were left untreated to test for open pollination. Open pollination produced significantly fewer seeds than flowers that were hand-pollinated with 100, 75 and 50% conspecific pollen.

Flower visitation

A total of 47 visits to *G. flavum* flowers were observed in the invaded sites and 122 visits to *G. flavum* flowers were observed in the uninvaded sites (Table 2). Site status (invaded/uninvaded) had a significant effect on *G. flavum* flower visitation

Fig. 1 Seed set per pollen treatment: 100% *Glaucium flavum* (=100% conspecific pollen), open pollination, 100% *Solel* (=100% *S. elaeagnifolium* pollen), spontaneous selfing, and mixtures of *Glaucium flavum* and *Solanum elaeagnifolium* pollen as stated in graph. Treatments sharing the same letter above their bars are not significantly different

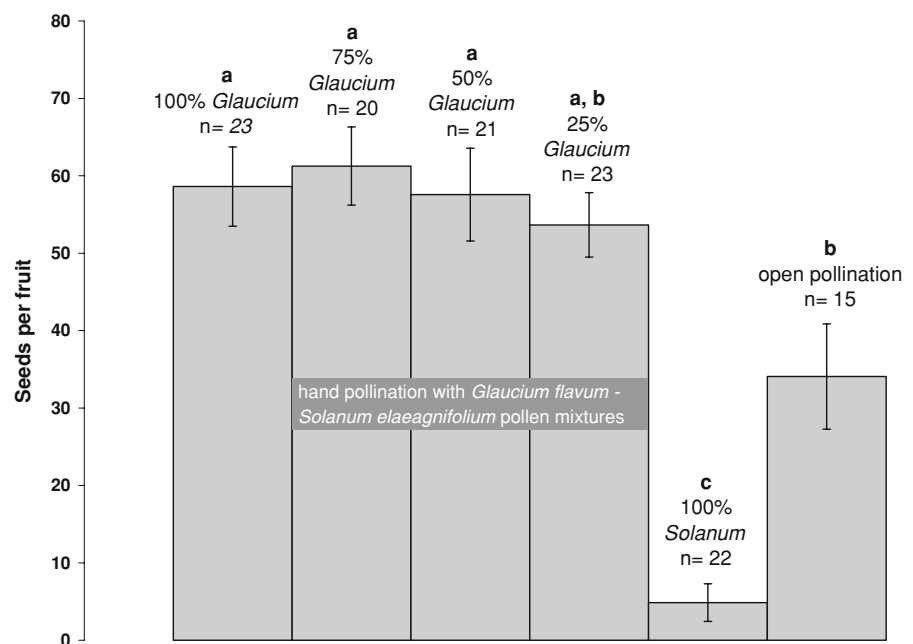


Table 1 P-values for multiple comparisons of all pollination treatments using Tukey HSD post-hoc test

Treatment	100% <i>G. f.</i>	100% <i>S. e.</i>	Open pollination	Spontaneous selfing	75% <i>G. f./</i> 25% <i>S. e.</i>	50% <i>G. f./</i> 50% <i>S. e.</i>
100% <i>S. e.</i>	<0.001					
Open pollination	0.013	0.001				
Spontaneous selfing	<0.001	1	0.002			
75% <i>G. f./</i> 25% <i>S. e.</i>	1	<0.001	0.005	<0.001		
50% <i>G. f./</i> 50% <i>S. e.</i>	1	<0.001	0.024	<0.001	0.998	
25% <i>G. f./</i> 75% <i>S. e.</i>	0.986	<0.001	0.092	<0.001	0.907	0.996

Dependent variable: seed set

Table 2 Main results from flower observations in invaded and uninvaded sites pooled over site and round

	Uninvaded	Invaded	
	<i>G. flavum</i>	<i>G. flavum</i>	<i>S. elaeagnifolium</i>
Plots	41	42	48
Plants	43	43	116
No. of flowers in plots	275	221	1061
Mean no. of flowers per plot	6.71	5.26	22.1
SE of no. of flowers per plot	0.44	0.35	2
Total no. of observed flower visits	122	47	156
Flower visitation rate ^{-h}	8.87	4.25	2.94

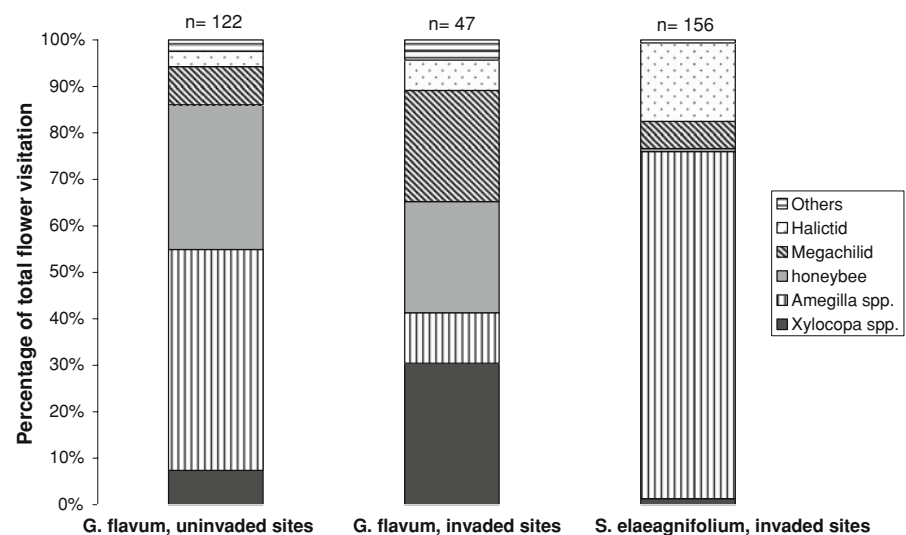
($F_{1,76.8} = 5.62$, $P = 0.02$). *Glaucium flavum* flowers received on average 4.25 visits/h in the invaded and 8.87 visits/h in the uninvaded sites during the hours of observation.

All recorded visits in the invaded sites were by bees and 97.5% of visits in the uninvaded sites were by bees (2.5% fly visits, mainly Syrphids). In the uninvaded sites, the number of visits to *G. flavum* flowers by *Amegilla* spp. accounted for almost 50% of all visits compared to just 10% in invaded sites (Fig. 2). In contrast to the uninvaded sites, *G. flavum* was mainly visited by *Xylocopa iris* and *X. violacea*, and Megachilid bees in the invaded sites, which accounted for over 50% of the total visits. A total of 156 *S. elaeagnifolium* flowers were visited in the invaded sites, which translates to 2.94 visits/h. *Amegilla* spp. accounted for almost 75% of all visits to *S. elaeagnifolium* flowers.

Discussion

Pollen treatments

Generally *G. flavum* was very resilient against the deposition of *S. elaeagnifolium* pollen in our experiment. We found that *S. elaeagnifolium* pollen did not significantly impact the seed set of *G. flavum* at pollen percentages of up to 75%/25% (invasive/conspecific pollen). The seed set lowering processes associated with the deposition of heterospecific pollen, such as stigma clogging (Holland and Chamberlain 2007), stigma closing, pollen allelopathy, stylar clogging and stylar inhibition (see Brown and Mitchell 2001 and references therein) do not seem to have any major effects at the tested percentages of invasive pollen deposition. It should be noted, however, that under natural conditions, when *G. flavum* is pollen limited,

Fig. 2 Composition of flower visitors to *Glaucium flavum* and *Solanum elaeagnifolium* in invaded and uninvaded sites

any deposition of invasive pollen on *G. flavum* flowers could still have a negative impact on its reproduction due to the expected decrease in conspecific pollen grain numbers deposited by pollinators visiting both plants.

Our findings are in contrast with other studies, which investigated the effects of pollen from invasive species on seed set in sympatric and co-flowering native species and found significant reductions in the natives' seed set after hand-pollination with a mixture of invasive and conspecific pollen compared to pure conspecific pollen (Brown and Mitchell 2001; Moragues and Traveset 2005). Jakobsson et al. (2008), however, hand-pollinated two native species with mixtures of conspecific and invasive pollen and found no significant difference in seed set to open-pollinated control flowers.

Seed set in the open pollination treatment was significantly lower than in the 100% conspecific pollen treatment, which suggests that *G. flavum* was pollen limited. The seed set in this treatment was not significantly different from seed set in flowers that had been hand-pollinated with 25% *G. flavum* and 75% *S. elaeagnifolium* pollen. This suggests that natural visitation was low and/or the pollinator efficiency was low, that is, pollinators generally deposited few pollen grains or pollen mixtures containing small ratios of conspecific to heterospecific pollen. It is also possible that the sample size was too small to produce a difference.

Pollination with only invasive pollen resulted in very low seed set. This finding is in contrast with Moragues and Traveset's (2005) study on the impact of *Carpobrotus* spp. on the native flora. There, pure *Carpobrotus* spp. pollen that was applied to stigmas of *Cistus salvifolius* and *C. monspeliensis* resulted in seed set that was not significantly different from the seed set in the open pollination (control) treatments. They did not, however, bag flowers before and after treatment so that successful pollination might have taken place before the application of the treatment or high amounts of conspecific pollen deposited after the application of the treatment might have compensated for the experimental deposition of heterospecific pollen.

Spontaneous selfing, achieved by excluding pollinators before and during anthesis, resulted in very low seed set. This result is in accord with Eisikowitch (1979), who showed that spontaneous selfing in several populations of *G. flavum* in Israel occurred only rarely, in 1.8% of flowers.

The seed set of bagged flowers (spontaneous selfing) and flowers that received only *S. elaeagnifolium* pollen was not significantly different. As hybrids between both plants are highly unlikely, this suggests that spontaneous selfing can still occur at its natural level even after the application of *S. elaeagnifolium* pollen.

Pollinator visitation

We observed significantly lower visitation rates for *G. flavum* in sites, where *S. elaeagnifolium* was present compared to sites, where *S. elaeagnifolium* was absent. Several other studies have found a negative impact of invasive plants on the visitation of native plants (Bartomeus et al. 2008; Brown et al. 2002; Chittka and Schürkens 2001). Conversely, Aigner (2004) observed no significant difference in visitation to the native whether or not two invasives were present, and Bartomeus et al. (2008) and Lopezaraiza-Mikel et al. (2007) showed that invaded plots had significantly higher flower visitation. In the latter case, however, the pollen transport networks were dominated by alien pollen grains in the invaded plots and consequently higher visitation may not have translated in facilitation for pollination.

As *G. flavum* is pollen limited, the observed lower visitation rate in invaded habitats can be expected to eventually lead to a reduction in seed set through fewer conspecific pollen grains arriving on the stigmas, which in turn may impact the abundance of the plant in invaded sites and lead to inbreeding depression.

In addition, *G. flavum* flowers in invaded sites received more visits from *Xylocopa* spp. and Megachilid bees, and fewer from *Amegilla* spp. The fact that *G. flavum* flowers received significantly fewer visits from *Amegilla* spp. in invaded sites can be attributed to *Amegilla* spp.'s apparent preference for *S. elaeagnifolium* flowers, which may be due to their ability to efficiently retrieve the extremely nitrogen and protein-rich pollen typical of *Solanum* flowers (Buchmann 1986) from the poricidal anthers of *S. elaeagnifolium*. Poricidal flowers can only be efficiently harvested by bees which sonicate (buzz) the anthers (Buchmann and Cane 1989) and *Amegilla* spp. are known to be buzz-pollinators (Hogendoorn et al. 2007). Almost 75% of all visits to *S. elaeagnifolium* in invaded sites were from *Amegilla* spp.,

which are the main visitors of *S. elaeagnifolium* on Lesvos (Petanidou, unpublished data). *S. elaeagnifolium*, therefore may act as a magnet species for *Amegilla* spp. The only other bee species recorded in invaded sites, which are able to sonicate were *Xylocopa violacea* and *X. iris*. Both showed relatively little interest in *S. elaeagnifolium* flowers, which might be due to their size and weight, and the resulting difficulty to land on *S. elaeagnifolium* flowers.

More work is needed to look at the behaviour of shared pollinators and the seed set of *G. flavum* in invaded sites of high *S. elaeagnifolium* and low *G. flavum* abundance to examine if the stronger preference of some of the shared pollinators for *S. elaeagnifolium* flowers can impact seed set in spite of the robustness of *G. flavum*'s reproductive system. Special attention needs to be paid to the constancy of the pollinators' foraging behaviour and the natural deposition of heterospecific pollen.

Acknowledgements This research project is financed by the E.U.-European Social Fund (80%), and the Greek Ministry of Development-GSRT (20%). Financial support was also received from the Helmholtz Centre for Environmental Research (UFZ) and the FP 6 Integrated Project "ALARM" (Assessing Large scale environmental Risks for biodiversity with tested Methods; GOCE-CT-2003-506675; www.alarmproject.net; Settele et al. 2005). We thank Christina Zografou for help with collecting field data, Ioannis Bazos for help with locating the *Solanum elaeagnifolium* populations on Lesvos, Andrew Grace for bee identifications and Ben Woodcock for statistical advice.

References

- Aigner PA (2004) Ecological and genetic effects on demographic processes: pollination, clonality and seed production in *Dithyrea maritima*. Biol Conserv 116:27–34. doi:10.1016/S0006-3207(03)00170-8
- Bartomeus I, Vilà M, Santamaría L (2008) Contrasting effects of invasive plants in plant-pollinator networks. Oecologia 155:761–770. doi:10.1007/s00442-007-0946-1
- Baye Y, Ameer A, Bouhache M, Taleb A (2007) Strategy for the chemical control of *Solanum elaeagnifolium* Cav. in Morocco. EPPO Bull 37:145–152. doi:10.1111/j.1365-2338.2007.01092.x
- Bjerknes AL, Totland Ø, Hegland SJ, Nielsen A (2007) Do alien plant invasions really affect pollination success in native plant species? Biol Conserv 138:1–12. doi:10.1016/j.biocon.2007.04.015
- Boratynski A, Browicz K, Zielinski J (1992) Chorology of trees and shrubs in Greece. Polish Academy of Sciences, Sorus, Poznan/Kornik
- Bouhache M, Tanji A (1985) Assessment of the stock of *Solanum elaeagnifolium* Cav. Yellow Nightshade seeds in the soil of the Tadla (Morocco). Weed Res 25:11–14. doi:10.1111/j.1365-3180.1985.tb00611.x
- Bowman G, Tarayre M, Atlan A (2008) How is the invasive gorse *Ulex europaeus* pollinated during winter? A lesson from its native range. Plant Ecol 197:197–206. doi:10.1007/s11258-007-9370-1
- Boyd JW, Murray DS (1982) Growth and development of Silverleaf Nightshade (*Solanum elaeagnifolium*). Weed Sci 30:238–243
- Boyd JW, Murray DS, Tyrl RJ (1984) Silverleaf Nightshade, *Solanum elaeagnifolium*, origin, distribution, and relation to man. Econ Bot 38:210–217
- Brown BJ, Mitchell RJ (2001) Competition for pollination: effects of pollen of an invasive plant on seed set of a native congener. Oecologia 129:43–49. doi:10.1007/s004420100700
- Brown BJ, Mitchell RJ, Graham SA (2002) Competition for pollination between an invasive species (Purple Loosestrife) and a native congener. Ecology 83:2328–2336
- Buchmann SL (1986) Vibratile pollination in *Solanum* and *Lycopersicon*: a look at pollen chemistry. In: D'Arcy WG (ed) Solanaceae systematic and biology. Columbia University Press, New York, pp 237–252
- Buchmann SL, Cane JH (1989) Bees assess pollen returns while sonicating *Solanum* flowers. Oecologia 81:289–294. doi:10.1007/BF00377073
- Chapman MA, Forbes DG, Abbott RJ (2005) Pollen competition among two species of *Senecio* (Asteraceae) that form a hybrid zone on Mt. Etna, Sicily. Am J Bot 92:730–735. doi:10.3732/ajb.92.4.730
- Chittka L, Schürkens S (2001) Metrology: successful invasion of a floral market. Nature 411:653. doi:10.1038/35079676
- Coolley AW, Smith DT (1971) Silverleaf Nightshade (White-weed) establishment from seed and clipped seedlings. Progress report of Texas A&M University, Texas Agricultural Experiment Station PR-3198
- Economidou E, Yannitsaros A (1975) Recherches sur la flore adventice de Grèce. V. Distribution et écologie de *Solanum elaeagnifolium* Cav. Rev Biol Ecologie Mediterranee 2:29–44
- Eisikowitch D (1979) Horned poppy, *Glaucium flavum*, in Israel: notes on its pollination and distribution. Isr J Bot 28:87–94
- Eleftherohorinos IG, Bell CE, Kotoula-Syka E (1993) Silverleaf Nightshade (*Solanum elaeagnifolium*) control with foliar herbicides. Weed Technol 7:808–811
- EU (1992) Council Directive on the conservation of natural habitats and of wild fauna and flora 92/43/EEC, OJ L 206. <http://eur-lex.europa.eu/LexUriServ/site/en/consleg/1992/L/01992L0043-20070101-en.pdf>. Accessed 4 Dec 2007
- Feldman TS, Morris WF, Wilson WG (2004) When can two plant species facilitate each other's pollination? Oikos 105:197–207. doi:10.1111/j.0030-1299.2004.12845.x
- Gathmann A, Tschamntke T (2002) Foraging ranges of solitary bees. J Anim Ecol 71:757–764. doi:10.1046/j.1365-2656.2002.00641.x
- Grabas GP, Lavery TM (1999) The effect of purple loosestrife (*Lythrum salicaria* L.; Lythraceae) on the pollination and reproductive success of sympatric co-flowering wetland plants. Ecoscience 6:230–242

- Greenleaf SS, Williams NM, Winfree R, Kremen C (2007) Bee foraging ranges and their relationship to body size. *Oecologia* 153:589–596. doi:[10.1007/s00442-007-0752-9](https://doi.org/10.1007/s00442-007-0752-9)
- Hogendoorn K, Coventry S, Keller MA (2007) Foraging behaviour of a blue banded bee, *Amegilla chlorocyanea* in greenhouses: implications for use as tomato pollinators. *Apidologie (Celle)* 38:86–92. doi:[10.1051/apido:2006060](https://doi.org/10.1051/apido:2006060)
- Holland JN, Chamberlain SA (2007) Ecological and evolutionary mechanisms for low seed:ovule ratios: need for a pluralistic approach? *Ecology* 88:706–715. doi:[10.1890/06-1283](https://doi.org/10.1890/06-1283)
- Jacobi CM, Ramalho M, Silva M (2005) Pollination biology of the exotic rattleweed *Crotalaria retusa* L. (Fabaceae) in NE Brazil. *Biotropica* 37:357–363. doi:[10.1111/j.1744-7429.2005.00047.x](https://doi.org/10.1111/j.1744-7429.2005.00047.x)
- Jakobsson A, Padron B, Traveset A (2008) Pollen transfer from invasive *Carpobrotus* spp. to natives—a study of pollinator behaviour and reproduction success. *Biol Conserv* 141:136–145. doi:[10.1016/j.biocon.2007.09.005](https://doi.org/10.1016/j.biocon.2007.09.005)
- Kasagi T, Kudo G (2005) Interspecific pollinator movements and heterospecific incompatibility: comparisons between *Phyllodoce caerulea* and *Phyllodoce aleutica* along snowmelt gradients. *Evol Ecol Res* 7:73–87
- Kremen C, Williams NM, Bugg RL, Fay JP, Thorp RW (2004) The area requirements of an ecosystem service: crop pollination by native bee communities in California. *Ecol Lett* 7:1109–1119. doi:[10.1111/j.1461-0248.2004.00662.x](https://doi.org/10.1111/j.1461-0248.2004.00662.x)
- Larson DL, Royer RA, Royer MR (2006) Insect visitation and pollen deposition in an invaded prairie plant community. *Biol Conserv* 130:148–159. doi:[10.1016/j.biocon.2005.12.009](https://doi.org/10.1016/j.biocon.2005.12.009)
- Lopezaraiza-Mikel ME, Hayes RB, Whalley MR, Memmott J (2007) The impact of an alien plant on a native plant-pollinator network: an experimental approach. *Ecol Lett* 10:539–550. doi:[10.1111/j.1461-0248.2007.01055.x](https://doi.org/10.1111/j.1461-0248.2007.01055.x)
- Mack RN, D'Antonio CM (1998) Impacts of biological invasions on disturbance regimes. *Trends Ecol Evol* 13:195–198. doi:[10.1016/S0169-5347\(97\)01286-X](https://doi.org/10.1016/S0169-5347(97)01286-X)
- McGuire A, Armbruster S (1991) An experimental test for reproductive interactions between two sequentially blooming *Saxifraga* species (Saxifragaceae). *Am J Bot* 78:214–219. doi:[10.2307/2445244](https://doi.org/10.2307/2445244)
- Mekki M (2007) Biology, distribution and impacts of Silverleaf Nightshade (*Solanum elaeagnifolium* Cav.). *EPPO Bull* 37:114–118. doi:[10.1111/j.1365-2338.2007.01094.x](https://doi.org/10.1111/j.1365-2338.2007.01094.x)
- Memmott J, Waser NM (2002) Integration of alien plants into a native flower-pollinator visitation web. *Proc R Soc Lond B Biol Sci* 269:2395–2399. doi:[10.1098/rspb.2002.2174](https://doi.org/10.1098/rspb.2002.2174)
- Moragues E, Traveset A (2005) Effect of *Carpobrotus* spp. on the pollination success of native plant species of the Balearic Islands. *Biol Conserv* 122:611–619. doi:[10.1016/j.biocon.2004.09.015](https://doi.org/10.1016/j.biocon.2004.09.015)
- Petanidou T (2004) Temporal patterns of resource selection in plant–pollinator communities in the Mediterranean: what can they really tell us? In: Arianoutsou MPV (ed) *Ecology, conservation and management of mediterranean climate ecosystems—Proceedings 10th MEDECOS conference Rhodos, CD*. Millpress, Rotterdam
- Petanidou T, Ellis W (1996) Interdependence of native bee faunas and floras in changing Mediterranean communities. In: Matheson A, Buchmann S, O'Toole C, Westrich P, Williams I (eds) *The conservation biology of bees*. Linnean Society Symposium series No 18. Academic Press, London, pp 201–226
- Petanidou T, Den Nijs JCM, Oostermeijer JGB (1995a) Pollination ecology and constraints on seeds set of the rare perennial *Gentiana cruciata* L. in the Netherlands. *Acta Bot Neerl* 44:55–74
- Petanidou T, Ellis W, Margaris N, Vokou D (1995b) Constraints on flowering phenology in a phryganean (East Mediterranean) ecosystem. *Am J Bot* 82:607–620. doi:[10.2307/2445419](https://doi.org/10.2307/2445419)
- Petanidou T, Ellis-Adam A, Den Nijs HCM, Oostermeijer JGB (2001) Differential pollination success in the course of individual flower development and flowering time in *Gentiana pneumonanthe* L. (Gentianaceae). *Bot J Linn Soc* 135:25–33. doi:[10.1111/j.1095-8339.2001.tb02365.x](https://doi.org/10.1111/j.1095-8339.2001.tb02365.x)
- Pimentel D, McNair S, Janecka J, Wightman J, Simmonds C, O'Connell C, Wong E, Russel L, Zern J, Aquino T, Tsomondo T (2001) Economic and environmental threats of alien plant, animal, and microbe invasions. *Agric Ecosyst Environ* 84:1–20. doi:[10.1016/S0167-8809\(00\)00178-X](https://doi.org/10.1016/S0167-8809(00)00178-X)
- Ruane LG, Donohue K (2007) Environmental effects on pollen-pistil compatibility between *Phlox cuspidata* and *P. drummondii* (Polemoniaceae): implications for hybridization dynamics. *Am J Bot* 94:219–227. doi:[10.3732/ajb.94.2.219](https://doi.org/10.3732/ajb.94.2.219)
- Settele J, Hammen V, Hulme PE, Karlson U, Klotz S, Kotarac M, Kunin WE, Marion G, O'Connor M, Petanidou T, Peterson K, Potts SG, Pritchard H, Pysek P, Rounsevell M, Spangenberg J, Steffan-Dewenter I, Sykes M, Vighi M, Zobel M, Kühn I (2005) ALARM—Assessing LARge-scale environmental Risks for biodiversity with tested Methods. GAIA—Ecological Perspectives in Science. *Humanit Econ* 14:69–72
- SPSS (2006) Version 15.0 for Windows. LEAD Technologies, SPSS Inc. All rights reserved, Chicago, USA
- Tepedino VJ, Bradley BA, Griswold TL (2008) Might flowers of invasive plants increase native bee carrying capacity? Intimations from Capitol Reef National Park, Utah. *Nat Areas J* 28:44–50. doi:[10.3375/0885-8608\(2008\)28\[44:MFOIPI\]2.0.CO;2](https://doi.org/10.3375/0885-8608(2008)28[44:MFOIPI]2.0.CO;2)
- Thanos CA, Georgiou K, Skarou F (1989) *Glaucium flavum* seed germination—an ecophysiological approach. *Ann Bot (Lond)* 63:121–130
- Totland Ø, Nielsen A, Bjerknes AL, Ohlson M (2006) Effects of an exotic plant and habitat disturbance on pollinator visitation and reproduction in a boreal forest herb. *Am J Bot* 93:868–873. doi:[10.3732/ajb.93.6.868](https://doi.org/10.3732/ajb.93.6.868)
- Traveset A, Richardson DM (2006) Biological invasions as disruptors of plant reproductive mutualisms. *Trends Ecol Evol* 21:208–216. doi:[10.1016/j.tree.2006.01.006](https://doi.org/10.1016/j.tree.2006.01.006)
- Tscheulin T, Petanidou T, Zografou C, Pantelis K (2008) Invading the Mediterranean: study of *Solanum elaeagnifolium*, a successful invasive plant from C. America. Earth Conference, University of the Aegean, Mitilini
- Wardle DA, Nicholson KS, Ahmed M, Rahman A (1994) Interference effects of the invasive plant *Carduus nutans* L. against the nitrogen fixation ability of *Trifolium repens* L. *Plant Soil* 163:287–297. doi:[10.1007/BF00007978](https://doi.org/10.1007/BF00007978)
- Weihe PE, Neely RK (1997) The effects of shading on competition between Purple Loosestrife and Broadleaved Cattail. *Aquat Bot* 59:127–138. doi:[10.1016/S0304-3770\(97\)00023-5](https://doi.org/10.1016/S0304-3770(97)00023-5)