The presence of the invasive plant Solanum elaeagnifolium deters honeybees and increases pollen limitation in the native co-flowering species Glaucium flavum

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The presence of the invasive plant *Solanum elaeagnifolium* deters honeybees and increases pollen limitation in the native co-flowering species *Glaucium flavum*

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Received: 30 September 2011 / Accepted: 23 July 2012 / Published online: 7 August 2012
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**Abstract** Invasive plants can impact biodiversity and ecosystem functioning by displacing native plants and crop species due to competition for space, nutrients, water and light. The presence of co-flowering invasives has also been shown to affect some native plants through the reduction in pollinator visitation or through the deposition of heterospecific pollen on the native’s stigmas leading to stigma clogging. We examined the impact of the invasive plant *Solanum elaeagnifolium* Cavanilles (silver-leaved nightshade), native to South and Central America and South-western parts of North America, on the seed set of the native *Glaucium flavum* Crantz (yellow-horned poppy) on Lesvos Island, Greece. To do this we measured seed set and visitation rates to *G. flavum* before and after the placement of potted individuals of the invasive near the native plants. In addition, we hand-crossed *G. flavum* flowers with super-optimal amounts of conspecific pollen, bagged flowers to measure the rate of spontaneous selfing, and applied self-pollen to measure self-compatibility of *G. flavum*. The hand-selfing treatment resulted in very low seed set, which indicates that *G. flavum* is to a large degree self-incompatible and highlights the plant’s need for insect-mediated outcrossing. We show that the presence of the invasive significantly enhanced pollen limitation, although the overall visitation rates were not reduced and that this increase is due to a reduction in honeybee visitation in the presence of the invasive resulting in reduced pollination.

**Keywords** Pollination · Pollen limitation · Alien · Pollination services · Lesvos · Mediterranean

**Introduction**

Invasive plants have the potential to negatively impact biodiversity and ecosystem functioning (Pimentel et al. 2001; Traveset and Richardson 2006), because they compete with native plants and crop species for space, nutrients, water and light (Mack and D’Antonio 1998; Weihe and Neely 1997). In few studies, the presence of a co-flowering invasive has also been shown to affect native plants through the disruption of pollination services (see Bjerknes et al. 2007 for a short review).

Plants competing for pollination services can suffer from an increase of heterospecific pollen deposition and/or a reduction in visitation rates, which can both lead to a decrease in seed set. In particular, invasives have been shown to affect pollinator–native plant interactions negatively by lowering visitation rates...
In contrast, there are also cases where invasives were shown to have a positive effect in terms of visitation (e.g. Grabas and Laverty 1999; Larson et al. 2006; Moragues and Traveset 2005) and seed set (e.g. Brown et al. 2002; Chittka and Schürkens 2001; Grabas and Laverty 1999), or no effect on visitation (e.g. Aigner 2004; Grabas and Laverty 1999; Larson et al. 2006; Moragues and Traveset 2005; Thijs et al. 2012) and seed set (e.g. Aigner 2004; Grabas and Laverty 1999; Moragues and Traveset 2005; Thijs et al. 2012). The cases where invasives had a positive effect on the pollination of native plants were explained by arguing that they provide additional resources for pollinators, which could potentially lead to an overall increase in pollinator abundance, diversity, geographic range or even duration of their foraging season (Feldman et al. 2004; Memmott and Waser 2002; Tepedino et al. 2008).

Conversely, some invasives have been shown to attract pollinators that were previously monopolized by the native species, which could lead to lower visitation rates to the natives, deposition of heterospecific pollen (Lopezaraiza-Mikel et al. 2007; Matsumoto et al. 2010) and subsequently to reduction in seed set (Jacobi et al. 2005; Larson et al. 2006). Due to the inconstant foraging behaviour of many pollinators, especially generalists, which make up the vast majority of visitors to invasive plants, flowers may receive heterospecific pollen (Lopezaraiza-Mikel et al. 2007; Memmott and Waser 2002). As a direct result this can lower seed set in the natives even in the absence of pollinator limitation (Kasagi and Kudo 2005; Petanidou et al. 1995; Ruane and Donohue 2007). The reduction in seed set caused by the deposition of heterospecific pollen can be the result of several processes 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).

Here we examine the impact of the invasive plant *Solanum elaeagnifolium* Cavanilles (silver-leaf nightshade) on the visitation to and seed set of the native *G. flavum* Crantz (yellow-horned poppy). These species serve as an excellent system for study given that they co-occur in coastal habitats in the Mediterranean, have a prolonged period of overlapping flowering times, and share pollinators (Tscheulin et al. 2008, 2009). We hypothesize that the native *G. flavum* would receive fewer visits but would not necessarily produce fewer seeds in the presence of *S. elaeagnifolium* based on our previous work in this system (Tscheulin et al. 2009; Vila et al. 2009), which indicates that the presence of the invasive lowers visitation rates to the native but even high proportions of manually applied invasive pollen does not significantly reduce seed set in the native. In contrast to our previous work in this system where we used invaded and uninvaded stands of the native, we used potted individuals of the invasive to better control for confounding factors and we measured the seed set of *G. flavum* under natural conditions before and after the addition of the invasive.

**Materials and methods**

**Study species**

Both study species, *G. flavum* and *S. elaeagnifolium*, co-occur in coastal habitats in the Mediterranean, have a prolonged period of overlapping flowering times in summer, and share pollinators (Tscheulin et al. 2008, 2009).

*Glaucium flavum* is a perennial herb that typically occurs along the Mediterranean shores and the coasts of W. Europe in shingle or stable pebbles just above the beach (Thanos et al. 1989). Such pebble and shingle beaches and their perennial vegetation have been designated as special areas of conservation in Annex I of 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. An average plant...
produces above 400 seed capsules during a season (Thomas Tscheulin, personal observation).

*Solanum elaeagnifolium* is native to Central America and South-western parts of North America (Boyd et al. 1984; Brunel 2011). It is a known invasive in all countries of the Mediterranean Basin, Australia, Chile, Australia, India, New Zealand, Pakistan, Puerto Rico, South Africa, Taiwan and Zimbabwe (Bouhache and Tanji 1985; Boyd et al. 2011). The sites are a minimum distance of 5 km away from each other. During all rounds and in rounds 2 and 3. In each round and site we conducted 4 days of flower observations and flower treatments.

Table 1 Schedule of experimental activities carried out in each site and round, in the morning (AM) and afternoon (PM)

<table>
<thead>
<tr>
<th>Activity</th>
<th>Day 1 Invasive present</th>
<th>Day 2 Invasive present</th>
<th>Day 3 Invasive present</th>
<th>Day 4 Invasive present</th>
</tr>
</thead>
<tbody>
<tr>
<td>Setting up pots (9.00)</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Removal of pots (16.00)</td>
<td></td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AM observation Glaucium</td>
<td>X</td>
<td></td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>PM observation Glaucium</td>
<td></td>
<td></td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>AM observation Solanum</td>
<td>X</td>
<td></td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>PM observation Solanum</td>
<td></td>
<td></td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Open pollination</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hand-crossing</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neotaxaneous selfing</td>
<td></td>
<td></td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>Hand-selling</td>
<td></td>
<td></td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Stigma collection</td>
<td>X</td>
<td></td>
<td></td>
<td>X</td>
</tr>
</tbody>
</table>
the spontaneous selfing treatment, in which the flowers remained covered throughout anthesis. For hand-pollinations we used the anthers of dehiscing flowers, which we daubed onto the receiving stigmas and thus applied the grains directly on the stigmas until they were visibly covered with pollen assuming that the transfer occurred with equal facility in each replicate and treatment. The number of pollen applied to each flower by far exceeded the expected number of ovules. We applied the spontaneous selfing and hand-selfing treatment once to one flower on each experimental plant in round 1 in the Mytilene population (totaling 50 flowers per treatment) and the hand-crossing treatment once to one flower on each experimental plant in all rounds and sites (totaling 329 flowers per treatment). In the spontaneous selfing treatment flowers were left untreated but covered with tulle bags, which were removed after the flowers had senesced. All pollen-donor flowers were first day, virgin flowers that had been covered with tulle bags before anthesis to prevent contact with flower visitors. The flowers for the hand-crossing treatment were taken from *G. flavum* plants at a distance of at least 10 m. The flowers for the hand-selfing treatment were taken from the same *G. flavum* plants as the pollen receiving flower. All tulle bags were removed after the flowers had senesced.

In addition, we marked one flower per experimental plant that was left untreated and uncovered to test for open pollination (free seed set) before (day 1) and after (day 3 & 4) the addition of the invasive plant (totaling 329 marked flowers in each group).

When mature all seed capsules were harvested and the seeds counted.

Pollen loads on stigmas

We examined stigmas of native flowers on mixed stands to assess the presence of *S. elaeagnifolium* pollen on them. To do this we collected 30 stigmas from *G. flavum* flowers each round before (on day 1) and after (on day 3) the addition of the potted invasive plants. The stigmas were collected, when the main pollinator activity was over (after 16.00), and inserted into small vials with alcohol (70 %) to fix pollen grains settled on stigmas. In the laboratory, stigmas were extracted and placed in distilled water, and the proportions of *G. flavum* and *S. elaeagnifolium* pollen grains were counted under a dissecting microscope.

Flower visitation

In each census we recorded the total number of flowers and the number of flower visits per insect visitor on each plant. Each flower visitor was classified as either honeybee, buzz-pollinator or other pollinator. This grouping was chosen as buzzing (sonicating) bees are more likely to be able to extract pollen from the poricidal *S. elaeagnifolium* flowers and therefore have a higher potential to impact on *G. flavum* seed set through the deposition of invasive pollen. The ‘buzz-pollinators’ belonged to *Bombus terrestris*, *Xylocopa violacea*, *Xylocopa iris*, *Pseudapis* spp. and *Amegilla* spp.; the ‘other pollinators’ belonged to non-buzzing Halictidae; other non-identified, non-buzzing bees; Syrphidae; and bee beetles (Trichiinae). Observations were carried out in the morning and afternoon for 3 min on each plant and time, and took place between 9.00 and 12.00 (morning observations) and 12.00 and 15.00 (afternoon observations) on sunny and calm days.

Each round we recorded flower visitation to *G. flavum* before (on day 1, in the morning and afternoon) and a minimum of 24 h after (on day 3 morning and day 4 afternoon) the introduction of *S. elaeagnifolium* (Table 1). We also recorded flower visitation to *S. elaeagnifolium* (on day 3 morning and day 4 afternoon).

Statistical analysis

We carried out separate Mann–Whitney U tests to compare seed set per fruit in the treatments hand-selfing, spontaneous selfing and the open-pollinated control in the Mytilene population, round 1, using SPSS, version 15.0 (SPSS 2006). Bonferroni corrections were applied.

Visits to flowers and flower number per plant were averaged over morning and afternoon observations, and pooled over round. Likewise seed set data per site were pooled over round. We calculated the pollen limitation experienced in the three populations of *G. flavum* for every round and treatment as follows:

\[
\text{Pollen limitation} = 1 - \frac{\text{Free seed set}}{\text{Hand cross seed set}}
\]

and Δ pollen limitation (Δpl) as a measurement of change of pollen limitation in presence of *S. elaeagnifolium* for every round as follows:
The predictor variables were treatment (presence or absence of the invasive), round and the differences between the *G. flavum* visitation rates per hour and flower ((visitation rates in the absence of the invasive) − (visitation rates in the presence of the invasive)) by the three main pollinator groups: Δ honeybee visitation rate per hour and flower, Δ buzz-pollinator visitation rate per hour and flower, and Δ other pollinator visitation rate per hour and flower. Two models were constructed using the response variables pollen limitation and Δ pollen limitation, which fulfilled the requirements of normality and homoscedasticity. Only models based on predictor variables that were not significantly correlated were constructed. We applied linear mixed effects models fitted by maximum likelihood using the “nlme function” in R, version 2.8 (R Development Core Team 2008). Within the mixed models the factor site was specified as random effect and round (essentially a time effect) as a fixed effect to account for the repeated measures. A nested design was applied with round being nested in site. For every response variable we constructed maximal models containing all predictor variables and their interactions. We then simplified the models using a stepwise backward elimination of the terms.

Throughout the manuscript all error bars are SE.

**Results**

**Pollination treatments**

Due to the ongoing growth of the plants some seed capsules could not be found and did not enter the analysis. In total, we collected 262 and 235 seed capsules in the free seed set (open pollination) treatment in absence and presence of the invasive, respectively, and 240, 36 and 44 seed capsules in the hand-crossing, hand-selfing and spontaneous selfing treatment, respectively. The seeds in all seed capsules were counted. Flowers produced significantly fewer seeds in the selfing treatments compared to the hand-crossing treatment, which indicates that *G. flavum* is to a large degree self-incompatible (Fig. 1).

Pollen limitation, which exists even in the absence of the invasive, was significantly affected by the treatment (Fig. 2; Table 2). This shows that the presence of the invasive further enhances pollen limitation and therefore has a strong negative effect on seed production. The effects of round were not significant.

**Pollen loads on stigmas**

The *G. flavum* stigmas collected in the presence of *S. elaeagnifolium* had very low (below 1 % in every sample) invasive pollen on them (data not presented). In the light of the results of Tscheulin et al. (2009) that showed that the manual deposition of even high proportions of the invasive’s pollen did not affect seed set, the invasive pollen counts were deemed to be too low to significantly impact seed set and are therefore not considered further here.

**Flower visitation and pollen limitation**

*Glaucium flavum* and *S. elaeagnifolium* plants were observed for a total of 60 (including observations before and after the addition of the invasive) and 30 h, respectively. A total of 9,072 *G. flavum* flowers (4,501...
before and 4,571 after the addition of the invasive) and 4,679 *S. elaeagnifolium* flowers were observed of which the former received 1,662 (774 before and 888 after the addition of the invasive) and the latter 154 pollinator visits (Fig. 3; Table 3). Thus, *G. flavum* overall flower visitation rate increased slightly in the presence of the invasive due to the increase in buzz-pollinator visits. At the same time the flower visitation rates of honeybees and other pollinators decreased in the presence of the invasive. Although not recorded separately, very rarely insects (mainly *B. terrestris*) were seen visiting both *G. flavum* and *S. elaeagnifolium* flowers in the same bout (Tscheulin, personal observation).

**Table 2** Results of mixed effects models using a nested design with round (fixed factor) nested in site (random factor)

<table>
<thead>
<tr>
<th>Pollen limitation</th>
<th>Δ Pollen limitation</th>
</tr>
</thead>
<tbody>
<tr>
<td>df</td>
<td>F, P</td>
</tr>
<tr>
<td>Treatment</td>
<td>1, 6</td>
</tr>
<tr>
<td>Round n.s.</td>
<td></td>
</tr>
<tr>
<td>Δ Honeybee visitation</td>
<td>1, 3</td>
</tr>
<tr>
<td>Δ Buzzer visitation</td>
<td></td>
</tr>
<tr>
<td>Δ Small pollinator visitation</td>
<td></td>
</tr>
</tbody>
</table>

For every response variable we constructed maximal models containing all predictor variables and their interactions. We then simplified the models using a stepwise backward elimination of the terms. The variables in the top row are response variables, the variables in the first column are predictor variables

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 low seed set in the hand-selfing treatment indicates that *G. flavum* is to a large degree self-incompatible, which highlights the plant’s need for insect-mediated outcrossing and enhances the potentially negative pollination-related effects from invasive species (Tscheulin et al. 2009).

Flower visitation and pollen limitation

The presence of the invasive significantly enhanced pollen limitation in *G. flavum*, which resulted in a pronounced decrease in seed set in open-pollinated flowers in relation to hand-crossed flowers. This increase in pollen limitation was driven by a reduction in honeybee visitation in the presence of *S.*
S. elaeagnifolium. None of the other pollinator groups significantly influenced pollen limitation. We have previously shown that the presence of S. elaeagnifolium can cause an overall decrease in flower visitation to G. flavum by pollinators including honeybees where both plant species co-occur (Tscheulin et al. 2009). In contrast to the present study, however, Tscheulin et al. (2009) found a decrease in buzz-pollinator visitation in invaded habitats. As the two plant species in this study were not in competition for any resource other than pollinators we are confident that there were no non-pollination related effects on seed set. 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.

The increase in pollen limitation when the invasive was present could also stem from 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). As shown, however, in our previous work, these processes do not seem to have any major effects on G. flavum at even high levels of invasive pollen deposition (Tscheulin et al. 2009). This in combination with the results from the stigma pollen counts therefore rules out any major impact of heterospecific pollen deposition in the present study and suggests that solely the decrease of honeybee visits lead to the increase in pollen limitation when the invasive was present. Likewise Jakobsson et al. (2008) have found that invasive Carpobrotus spp. pollen had very little effect on the seed set of the native community because of the low frequency of pollen transfer.

Given that experimental flowers were hand-crossed every round prior to the placement of the potted individuals it could also be argued that resource limitation could have caused the decrease in seed production of G. flavum. However, in the light of the large number of flowers and seed caspules G. flavum produces naturally during a flowering season we consider any possible effect negligible.

Honeybees are known to show a high degree of flower constancy (Chittka et al. 1999 and references therein), which is one reason why they are often employed for the pollination of crops. As honeybees were never recorded on S. elaeagnifolium, which is probably due to the flowers’ lack of nectar glands and the presence of poricidal anthers it cannot be argued, however, that the invasive draws honeybees away from G. flavum. Chittka et al. (2009) have pointed out that there is a speed-accuracy trade-off in foraging pollinators that have to distinguish between profitable and less profitable flowers. The presence of the invasive may therefore increase the amount of time spent on decision-making in mixed stands, which might in turn render the visitation of G. flavum flowers less profitable leading to a decrease in visitation rates and ultimately a decrease in G. flavum seed set. This is also supported by Spaethe et al. (2006), who show that bees incur a cost when distinguishing between profitable and less profitable flowers. When this cost passes a threshold it could in turn potentially lead to bees avoiding a mixed patch altogether (Chittka, personal communication).

This study emphasizes the importance of honeybees for the pollination of G. flavum given that seed set

<table>
<thead>
<tr>
<th>Total number of visits</th>
<th>Flower visitation rate$^{-b}$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Glaucium</td>
</tr>
<tr>
<td></td>
<td>Invasive absent</td>
</tr>
<tr>
<td>Honeybees</td>
<td>251</td>
</tr>
<tr>
<td>Buzz-pollinators</td>
<td>404</td>
</tr>
<tr>
<td>Other Pollinators</td>
<td>119</td>
</tr>
<tr>
<td>Total</td>
<td>774</td>
</tr>
</tbody>
</table>

Table 3 Total number of visits and visitation rates (visits per hour and flower) by the three pollinator groups to G. flavum in the absence and presence of the invasive S. elaeagnifolium and to the invasive itself.
decreased significantly with the decrease of honeybee visits despite the overall increase in pollinator visits seen after the addition of *S. elaeagnifolium*. The presence of *S. elaeagnifolium* in natural populations of *G. flavum* therefore has the potential to indirectly impact on the reproduction of this native plant, which can be expected to ultimately have negative effects on the demography of the plant.

**Acknowledgments**  This research project was 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).

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The invasive plant *S. elaeagnifolium* enhances pollen limitation in the native *G. flavum*