Comparative study of the reproductive ecology of two co-occurring related plant species: the invasive *Senecio inaequidens* and the native *Jacobaea vulgaris*

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Background and aims – A previous study showed that the visitation rate by pollinators and the seed set of the exotic invasive *Senecio inaequidens* (Asteraceae) were higher compared to the native relative, *Jacobaea vulgaris*. The first aim of the present study was to assess if these results could be explained by differences in self-fertility, nectar rewards or floral display between the two species. Moreover, in a second step, we examined if the higher visitation rate on *S. inaequidens* has a negative effect on the reproductive success of *J. vulgaris*.

Methods – Self-fertility was estimated after self- and cross-hand pollinations. Nectar volume, total sugar concentration and sugar composition were analysed on plants cultivated under controlled conditions. In the field, in order to assess the effect of floral display and impact of the invasive on the pollination success of the native, insect behaviour was assessed by comparing visitation rates, number of visitors per 10 min observation and individual censuses. Floral display (density of capitula per unit area) was artificially modified by clipping or grouping inflorescences in both species.

Key results – In terms of self-fertility, seed sets were similarly low after self-pollination (11–12%) for both species. *S. inaequidens* produced lower amounts of nectar with lower sugar concentration compared to *J. vulgaris*. No influence of floral display was detected on insect visitation rates. The presence of *S. inaequidens* did not alter pollinator visits and seed set of *J. vulgaris*.

Conclusions – Other traits need to be investigated to explain the different visitation rates and reproductive success between the two species. The higher seed set of *S. inaequidens* could be due to a higher outcrossing rate, or more frequent pollinator movements between individuals.

Keywords – reproductive ecology, invasive success, floral display, nectar, self-fertility, pollinator, visitation rate, *Senecio jacobaea*, *Senecio inaequidens*, *Jacobaea vulgaris*, Asteraceae.

INTRODUCTION

The reproductive success of plants and their abundance are at least in part dependent on their breeding system (e.g. level of self-fertility) and the activity of pollinators. High fecundity has been frequently associated with high invasive success (Rejmánek & Richardson 1996, Grotkopp et al. 2002, Totland et al. 2006, van Kleunen et al. 2010). However, reproductive traits like breeding system and the attractiveness to pollinators, and the influence therefore on invasive success have still rarely been investigated (Richardson et al. 2000).

Self-compatibility, which may contribute to reproductive success, appears to be frequent in exotic invasive species (Richardson et al. 2000, Rambuda & Johnson 2004, Harmon-Threatt et al. 2009), although some examples of self-incompatible invasive species are known, including *Lythrum salicaria* (Brown et al. 2002) or *Prunus serotina* (Pairon & Jacquemart 2005, Pairon et al. 2010). In case of self-incompatibility, efficient pollen transfer among individuals is required to produce seeds and efficiency of the attraction of pollinators can conceivably influence reproductive success. Hence, floral traits that increase attractiveness, i.e. showy and numerous flowers, high nectar production, UV patterns, floral odours, could be linked to invasiveness. This hypothesis was recently supported for ornamental alien species (Lambdon et al. 2008).

As invasive plants are generally well integrated in local plant-pollinator webs (e.g. Lopezaraiza-Mikel et al. 2007, Aizen et al. 2008, Vilà et al. 2009), their high attractiveness

might induce changes in pollinator services to co-flowering native plants (reviewed in Bjerknes et al. 2007, Morales & Traveset 2009). Such changes in pollinator behaviour may imply consistent effects on the reproductive success. In some cases, invasives reduced pollinator visits to native plants (e.g. Brown et al. 2002, Moragues & Traveset 2005, Bartomeus et al. 2008). The reduction in pollinator visits can result in a decreased fruit or seed set in native species (Chittka & Schürkens 2001, Brown et al. 2002). For example, a recent study in Japan showed that the presence of the invasive *Taraxacum officinale* (Asteraceae) reduced the seed set of the native congener *T. japonicum* (Kandori et al. 2009). A recent quantitative review which compiled data from forty studies confirmed overall negative impacts of invasive species on visitation rates and reproductive success of native species (Morales & Traveset 2009).

Interestingly, some cases of positive impacts are also documented (Moragues & Traveset 2005, Larson et al. 2006, Bartomeus et al. 2008, Nielsen et al. 2008). They were interpreted as a facilitation effect due to an increase in the total amount of resources for pollinators which are not drawn away from the native plants by the invasive ones.

Studies of reproductive biology of invasive species seldom include comparisons between closely related taxa with similar pollinator guilds and floral characters. This kind of comparative approach has been strongly advocated as a tool to investigate the causes of invasiveness (Morales & Traverset 2009, van Kleunen et al. 2010) as well as of rarity (Rymer et al. 2005), or for studying the evolution of plant-pollinator interactions in general (Jacquemart & Thompson 1996, Barrett 2010).

In a previous study (Vanparys et al. 2008), we found that the invasive *Senecio inaequidens* DC. (Asteraceae), accidentally introduced in Europe, had (i) a higher visitation rate by pollinating insects and (ii) a higher seed set compared to the native relative, *Jacobaea vulgaris* (formerly *Senecio jacobaea* L.; Pelser et al. 2007).

The percentage of self-fertility is poorly documented for both species. They were reported to be poorly self-fertile (Andersson 1996, Lafuma & Maurice 2007) although some individuals of *S. inaequidens* can have a high level of self-fertility (López-García & Maillet 2005). A comparison of the levels of self-fertility between *S. inaequidens* and *J. vulgaris* under the same experimental conditions is thus needed to disentangle the causes of differences in seed set.

Nectar rewards are likely to influence pollinator visits, but this has only rarely been investigated (Nicolson et al. 2007). The pollination success of the invasive *Impatiens glandulifera*, for example, has been ascribed to high nectar production (Chittka & Schürkens 2001). In the congeneric species pair *Taraxacum officinale* (invasive in Japan) / *T. japonicum* (native), nectar appears to be a key factor favouring the invasive (Kandori et al. 2009). Nectar reward for our two species still needs to be investigated.

In NW Europe, *S. inaequidens* and *J. vulgaris* have overlapping flowering period and share the same generalist pollinator guild (Vanparys et al. 2008). Both species have similar capitula with yellow ray florets. However, we observed that the two species present different floral displays, as capitula

of *S. inaequidens* plants are loosely arranged while they are grouped into a relatively compact corymbose inflorescence in *J. vulgaris*. It can thus be hypothesized that this difference in floral display might result in a higher visitation rate and reproductive success. Vanparys et al. (2008) found that the foraging behaviour of insects differed between the two species, as they visited a higher number of capitula per foraging trip on *J. vulgaris* compared to *S. inaequidens*. This may result in a higher deposition of self-pollen and, therefore a decreased seed set for *J. vulgaris*, especially if the species is self-incompatible or suffers from inbreeding depression. Competition for pollinator services could result in a decrease of the reproductive success of the native species (Morales & Traveset 2009).

In the first part of this study, we performed field and controlled experiments to test three traits that could account for the higher reproductive success of *S. inaequidens* in comparison to *J. vulgaris*. Our questions were: (i) does *S. inaequidens* have a higher level of self-fertility? (ii) does it offer higher nectar rewards in quantity and/or quality? (iii) does its floral display play a role in the visitation rate?

In the second part of this study, we performed a field experiment to test if the invasive *S. inaequidens* can alter insect visits, foraging behaviour and seed set of *J. vulgaris*.

MATERIALS AND METHODS

Study species

Senecio inaequidens DC. (Asteraceae), the South African ragwort, was unintentionally introduced in Europe as a wool contaminant in the end of the 19th century. The species is now invasive in Europe and spreads mainly in anthropogenic habitats like roadverges and wastelands but is also often found in more natural communities like dry open grasslands. It is a perennial chamaephyte up to 1 m in height, often much ramified, with each stem ending in one or a few capitula, forming a loose floral display (270 capitula m^{-2}). A single plant produces 26 to 500 capitula each year, with approximately 90 florets/capitulum, 74% of them developing a viable achene (Ernst 1998, Sans et al. 2004, López-García & Maillet 2005, Vanparys et al. 2008). In Belgium, the flowering period staggers from June to late October (Vanparys et al. 2008).

Jacobaea vulgaris, formerly *Senecio jacobaea* L., the tansy ragwort, is native to Europe and is commonly found in a wide range of mesic grasslands, and more occasionally in ruderal habitats like roadsides or wastelands (Harper & Wood 1957). The species is a biennial to perennial hemicryptophyte up to 1.5 m in height, generally with a single stem ending in a corymbose, relatively compact inflorescence (up to 890 capitula m^{-2}). A single plant produces 68 to 2500 capitula per year, each comprising approximately 65 florets, 54% of them developing a viable achene (Harper & Wood 1957, Vanparys et al. 2008). In Belgium, most plants flower in July or August, but the flowering period can extend to October due to resprouting after herbivore damage (Vanparys et al. 2008).

In NW Europe, *J. vulgaris* is the closest relative of *S. inaequidens* presenting similar ecological characteristics. Indeed, both species can be found coexisting in the same habitats (wastelands, grasslands), their flowering periods overlap

and they are visited by the same pollinator guild (Vanparys et al. 2008). Both species are generalist for pollination, being mainly visited by Hymenoptera (e.g. *Bombus* spp., *Apis mellifera* and solitary bees), Syrphidae species (large Syrphidae like *Eristalis* spp. and small Syrphidae like *Episyrphus balteatus*), other Diptera (Calliphoridae), and, to a lesser extent, Coleoptera and Lepidoptera (Harper & Wood 1957, Ernst 1998, Vanparys et al. 2008).

S. inaequidens as well as *J. vulgaris* have yellow-flowered capitula 2–3 cm in diameter with centripetal development, starting with the 12–15 ligulate ray florets (Harper & Wood 1957, Scherber 2002). Tubular disc florets are hermaphroditic and protandrous whereas ray florets are pistillate.

Experiments under controlled conditions

In summer 2007 and 2008, plants were cultivated in growth chambers at Louvain-la-Neuve (Université catholique de Louvain, Belgium, 50°39'57.9"N 4°37'8.82"E). Temperature was kept at 22/18 ± 1.5°C (day/night) and relative humidity at 69 ± 11%. Light was supplied by Philips HPIT 400W lamps (Philips lighting S.A. Brussels, Belgium) and the day/night cycle was of 16 h/8 h. Photon flux density at the top of the canopy was approximately of 190 µmol $m^{-2}s^{-1}$. In 2007, thirty plants per species, from four populations in central Belgium separated from each other by 5 to 70 km, were planted in five L-pots (one plant per pot) filled with a mixture of sand and compost (1:3, v:v). They were kept in open greenhouse before being transferred to the growth chambers.

Self-fertility – Hand self- and cross-pollinations were conducted in the growth chamber to assess the level of self-fertility of the two species. Recipient capitula were chosen at early stage of flowering, i.e. with two or three rays of open florets. Cross-pollinations were performed by brushing each recipient capitulum with two donor capitula harvested on two other individuals, randomly selected. Self-pollinations were performed in the same way but with donor capitula harvested on the same plant as the recipient capitulum. Pollinations were daily repeated until all florets of the capitulum were at the female stage (five days).

Ten individuals of each species received both treatments and were used to investigate the individual variability between self- and cross-fertility. Once the capitula were ripe, they were separately collected to assess the seed set.

Nectar – In summer 2008, twenty potted plants were placed in the growth chamber at least five days before starting nectar sampling. At the flowering peak, one capitulum on each individual was selected with all florets opened, anthers and stigmas were removed to avoid pollen contamination. Each capitulum was put in a 1.5 ml Eppendorf and centrifuged for nectar extraction (National Labnet Co. C1200, 6000 rpm for 90 sec, (Dafni et al. 2005). Nectar was then collected with glass capillary tubes of 0.5 µL or 5 µL depending on the nectar quantity (Hirschmann® Laborgerate, Eberstadt, Germany). The nectar volume was estimated by measuring the length of the nectar column in the capillary tube.

The same extraction method was applied to analyze sugar concentration and composition of nectar. Total sugar concentration was measured with a low volume hand-refractometer

(Eclipse Handheld refractometer, Bellingham & Stanley Ltd, Tunbridge Wells, UK) and was expressed as a percentage of sucrose in nectar mass (w/w). As nectar volume per capitulum of *S. inaequidens* was often under detection level, nectar extracted from two or three capitula from the same individual was pooled. Sugar ratio was estimated from thirteen additional nectar samples (approximately 2 µl) stored in capillary tubes at -80°C until analyses. Sugar composition was determined by gas chromatography, with a Perkin-Elmer Autosystem XL equipped with a split injector (1/20) and helium as the carrier gas (flow of 1 mL/min). The injector and detector temperatures were maintained at 250 and 350°C respectively. Sugar analyses were performed in the Centre Apicole de Recherche et d'Information (CARI asbl, Louvain-la-Neuve, Belgium).

Field experiments

Floral display – In July 2008, ten flowering individuals of *J. vulgaris* were collected from roadside grasslands at Louvain-la-Neuve. Flowering individuals of *S. inaequidens* were collected in two wasteland populations in Brussels and one roadside population at Louvain-la-Neuve.

In order to assess the influence of the floral display on insect visits and behaviour, an experiment was conducted outdoors in the experimental gardens at the University campus of Louvain-la-Neuve. Insect pollinators were abundant at the time of the experiment due to the proximity of a mesic grassland with many insect-pollinated species. The experimental design consisted of four 1 × 1 m plots, each of which with four "pseudo-plants" of *S. inaequidens* or *J. vulgaris* were placed. "Pseudo-plants" were created by manipulating floral display as follows:

(i) *S. inaequidens* with low capitula density (LD): unmodified plants, with 115 ± 17 (SD) capitula m^{-2} (14 to 52 capitula/plant)

(ii) *J. vulgaris* with low capitula density (LD): plants modified by clipping to 253 ± 29 capitula m^{-2} (18 to 50 cap/plant)

(iii) *S. inaequidens* with high capitula density (HD): plants modified by forming tight bunches of flowering stems with 821 ± 76 capitula m^{-2} (18 to 27 cap/plant)

(iv) *J. vulgaris* with high capitula density (HD): unmodified plants with 902 ± 89 capitula m^{-2} (14 to 60 cap/plant)

Insect visitations and foraging behaviour were assessed during 10 min-periods of observation. Every day the number of open capitula (i.e. with at least all ray florets at the pistillate stage) on the selected plants was counted. The number of visitors per 10 min was then censused, for a total of 136 10 min-periods. Insect visits were observed on each plot between 13h00 and 17h00 (no rain, temperature between 20 and 25°C). An insect was considered as a visitor when it landed on at least one open capitulum. The visitation rate to a plant was calculated as the total number of visitors per 10 min divided by the number of open capitula on the plant. Each visitor was assigned to one of the four categories: Hymenoptera, large-sized Syrphidae, small-sized Syrphidae and other Diptera. The foraging behaviour was observed for a total of 423 visitors individually censused. The number of visited capitula

on the selected plant(s) and the time spent on each capitulum were measured.

Pollinator-mediated impact – The pollinator-mediated impact of *S. inaequidens* on *J. vulgaris* was estimated at two different study sites. Both sites consisted of a mown grassland of approximately 120 × 55 m. They were homogenous and poor in terms of flowering plants. The vegetation was dominated by grasses, with a height of 10 to 25 cm. *Trifolium repens* was the only insect-pollinated species flowering during the experiment, covering a maximum of 10% of the site area. One site (A) was located between a golf course and meadows, in the North of Louvain-la-Neuve (50°41'8.0''N 4°36'14.4''E) and another site (B) was located between cereal fields, in the East of Louvain-la-Neuve (50°40'14.25''N 4°38'7.51''E).

At each site, three plots with seven *J. vulgaris* plants were established next to a plot with 0, 5 or 25 flowering plants of *S. inaequidens*. Plant density in a plot was of 5 to 7 per m².

A total of 180 10 min-periods of insect observation were performed between August 11th and 28th, 2008, according to the same protocol as in the previous experiment. In addition, 452 insects were individually censused on both plant species to study their foraging behaviour. The visitors were classified into four categories: large-size Syrphidae, small-size Syrphidae, other Diptera (other than Syrphidae) and Hymenoptera. In order to estimate if *S. inaequidens* influences the visitor guild of *J. vulgaris*, the relative proportions of the four categories were compared between the plots of *J. vulgaris*.

The reproductive success (seed set) of *J. vulgaris* was measured on fifteen plants with 8–17 ripe capitula per plot collected in early September. Capitula were individually collected and the number of viable achenes was counted. Ripe seeds can be recognized by their dark colour and their thickness, while undeveloped or aborted seeds are generally white and thin (Vanparys et al. 2008). Seed set was then calculated as number of seeds/ovules ratio.

Statistical analyses

The difference in seed set resulting from hand self- and cross-pollinations was tested for each species with two-way ANOVA on arcsin transformed data.

The difference between *S. inaequidens* and *J. vulgaris* in nectar quantity and composition were tested with a t-test. Volume data were square root transformed and sugar percentages were arcsin transformed before analyses.

The effect of floral display, species and their interaction were tested with two-way ANOVAs. Analyses were applied to two descriptors of plant attractiveness, i.e. the visitation rate and the number of visitors per 10 min, and the two descriptors of insect behaviour, i.e. the time per capitulum and the number of capitula visited per trip. All variables were log-transformed before analyses.

The pollinator-mediated impact of *S. inaequidens* on *J. vulgaris* was tested with two-way ANOVA. Data for visitation rates and numbers of visitors per 10 min were log transformed to achieve normality, data for seed set were arcsin transformed and effects of the number of *S. inaequidens* plants, the site and the interaction number × site were tested.
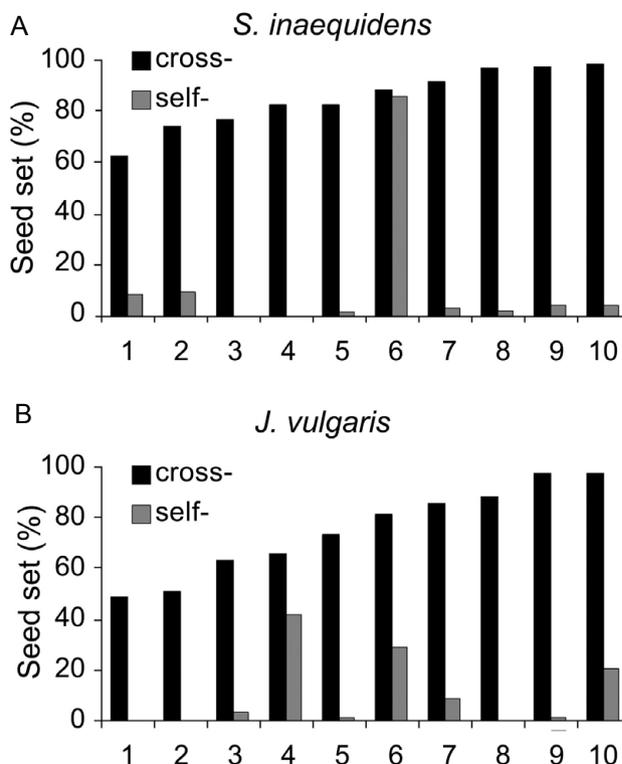

Figure 1 – Individual seed set (%) after hand self- and cross-pollinations on ten individuals of *S. inaequidens* (A) and *J. vulgaris* (B).

For the insects individually censused on *J. vulgaris*, differences between the two sites and among plots with different numbers of *S. inaequidens* plants (0, 5, 25) were compared with Kruskal-Wallis tests. The relative proportions of the insect categories visiting the three plots of *J. vulgaris* were compared by Chi-square tests.

All analyses were performed with SAS Enterprise Guide version 4.1. Means are given with their standard errors.

RESULTS

Self-fertility

For both species, hand self-pollination resulted in significantly lower seed sets compared to hand cross-pollination ($F_{1, 78} = 177.4$, $p < 0.0001$). Seed sets were similarly low for both species, with $12.2 \pm 4.0\%$ for *J. vulgaris* and $11.1 \pm 5.9\%$ for *S. inaequidens* (mean ± SD). On the other hand, cross-pollination resulted in similarly high seed sets for the two species, with $72.2 \pm 4.7\%$ and $80.3 \pm 3.1\%$ respectively. Neither species nor species × pollination interaction was significant ($F_{1, 78} = 1.36$, $p = 0.247$ and $F_{1, 78} = 1.27$, $p = 0.263$ respectively). Only one of the ten individuals of *S. inaequidens* appeared to be highly self-fertile (individual 6 in fig. 1A), as seed set obtained both by cross- and self-pollinations reached 88%. In contrast, none of the individuals of *J. vulgaris* exhibited such a high level of self-fertility, but after self-pollination two of them reached 41 and 29% of seed set (individuals 4 and 6 respectively, in fig. 1B).

Table 1 – Nectar quantities (means ± SE) per capitulum and sugar concentrations for *S. inaequidens* and *J. vulgaris*.
Statistical differences after t-test (t) are indicated as: *** p < 0.001, ** p < 0.01, * p < 0.05, ns: non significant (n = number of samples).

	n	*S. inaequidens*	n	*J. vulgaris*	*t*
nectar volume per capitulum (μl)	15	0.61 ± 0.10	20	3.41 ± 0.35	-7.61***
sugar concentration (%, w/v)	15	47.4 ± 1.18	20	59.7 ± 0.90	-8.42***
fructose (%)	13	46.55 ± 0.47	13	46.42 ± 0.51	0.18[ns]
glucose (%)	13	52.62 ± 0.52	13	50.36 ± 0.46	3.24**
sucrose (%)	13	0.83 ± 0.22	13	3.20 ± 0.49	-5.36***
sucrose/hexose ratio	13	0.008 ± 0.002	13	0.033 ± 0.005	-5.32***

Nectar

Nectar production per capitulum was five times higher for *J. vulgaris* than for *S. inaequidens* (table 1). In terms of per-floret production, the difference between species was even larger (per-floret volume: 0.0520 ± 0.0050 μl for *J. vulgaris* and 0.0065 ± 0.0011 μl for *S. inaequidens*; 67 and 94 florets per capitulum respectively; Vanparys et al. 2008). *J. vulgaris* produced a nectar 13% more concentrated than *S. inaequidens* (table 1). Sugar composition in nectar was very similar for the two species though sucrose concentration was higher for *J. vulgaris*. Nectar had a very low sucrose/hexoses ratio for both species (table 1). Both nectars are to the category "hexoses dominant" (Baker & Baker 1983).

Floral display

The high-density (HD) pseudo-plants received significantly less visitors per 10 min (6.4 ± 0.6 for *S. inaequidens* and 8.2 ± 0.8 for *J. vulgaris*) compared to the low density (LD) pseudo-plants (9.3 ± 1.0 for *S. inaequidens* and 9.3 ± 0.7 for *J. vulgaris*; table 2). However, floral display did not influence the visitation rate (table 2). The visitation rate to HD pseudo-plants was of 0.13 ± 0.01 for *S. inaequidens* and 0.13 ± 0.03 for *J. vulgaris*. The visitation rate to LD pseudo-plants was of 0.16 ± 0.02 for *S. inaequidens* and 0.16 ± 0.03 for *J. vulgaris*. In terms of insect behaviour, no effect was detected on the number of visited capitula but insects spent more time on LD pseudo-plants (11.1 ± 0.8 sec for *S. inaequidens* and 11.6 ± 1.2 for *J. vulgaris*) compared to HD pseudo-plants (7.8 ± 0.6 sec for *S. inaequidens* and 8.5 ± 0.5 for *J. vulgaris*). In the two-way ANOVA, the species effect and the species × floral display interaction were not significant.

Pollinator-mediated impacts

There were differences in the taxonomic assemblage of insects between sites ($\chi^2 = 54.4$, p < 0.001) with other Diptera being proportionally more abundant in site B while small-sized Syrphidae being less frequent. In site A, the number of *S. inaequidens* plants altered the relative proportions of the four main insect categories ($\chi^2 = 12.6$, p = 0.01). The presence of *S. inaequidens* close to *J. vulgaris* decreased the relative frequency of Hymenoptera and other Diptera and increased the frequency of Syrphidae observed on *J. vulgaris*. In contrast, in site B, *S. inaequidens* had no effect on the visitor guild of *J. vulgaris* ($\chi^2 = 3.7$, p = 0.44).

The number of *S. inaequidens* plants had no impact on the number of visits to *J. vulgaris* (fig. 2, table 3). Site and site × number of *S. inaequidens* interaction were not significant. *S. inaequidens* and *J. vulgaris* did not differ in the number of visitors per 10 min (c. 8–9 visitors for either species, t-test: t = -0.67, p = 0.50) but the visitation rate was significantly higher for *S. inaequidens* (t = 5.42, p < 0.001, fig. 2A).

Insect foraging behaviour on *J. vulgaris* was not influenced by the number of *S. inaequidens* in terms of time per capitulum (table 4, Kruskal-Wallis: $\chi^2 = 0.04$, p = 0.98) but well in terms of number of capitula visited, as insects visited less capitula on *J. vulgaris* next to plots of 25 *S. inaequidens* ($\chi^2 = 0.03$, p = 0.98). On average, insects visited significantly more capitula on *S. inaequidens* compared to *J. vulgaris* (ta-

Table 2 – Results from ANOVA (F values) for the effects of the floral display and species on insect visits (in terms of numbers of visitors per 10 min and visitation rate) and on the foraging behaviour (in terms of number of visited capitula and time per capitulum).
Significance of F values: * p < 0.05, ** p < 0.01, *** p < 0.001. df = degrees of freedom.

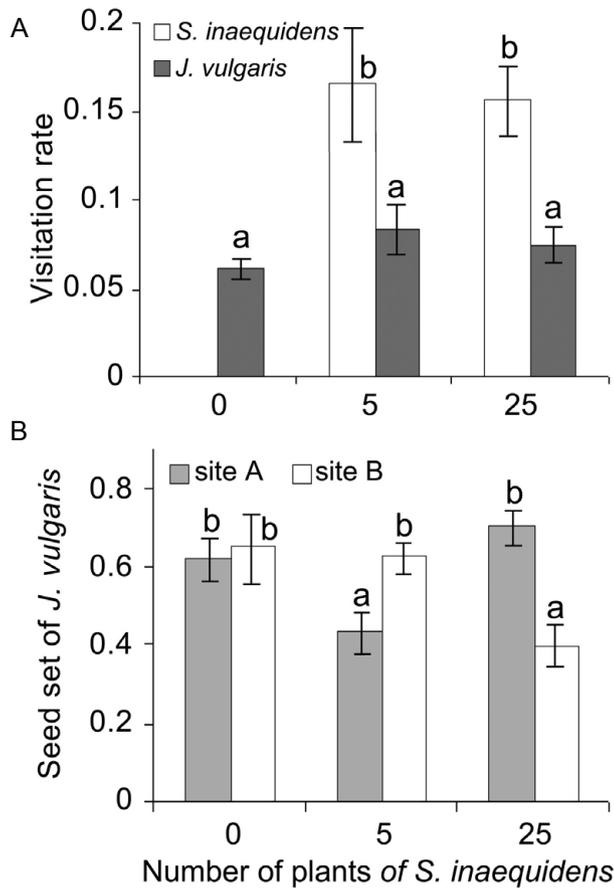|	df	insect visits		foraging behaviour	
		number of visitors per 10 min	visitation rate	number of visited capitula	time per capitulum
species	1	2.50	0.10	0.50	0.24
floral display	1	8.03**	2.49	0.28	18.32***
species × floral display	1	0.55	0.003	0.18	1.30
df residual	-	133	131	407	407

Figure 2 – Visitation rate (A) to *S. inaequidens* and *J. vulgaris* and seed set of *J. vulgaris* (B) in the plots with 0, 5 and 25 plants of *S. inaequidens*. Different letters indicate significant difference. Bars represent standard errors.

ble 4, χ^2 = 8.4, p = 0.004) but the time per capitulum did not differ between species (χ^2 = 0.43, p = 0.51). For both species, insect foraging behaviour was similar between the two sites both in terms of number of visited capitula and time per capitulum (χ^2 = 0.006, p = 0.98; χ^2 = 0.004, p = 0.95 respectively).

Seed set of *J. vulgaris* was not significantly influenced by the number of *S. inaequidens* (fig. 2B, table 4). No difference between sites was detected but the interaction site × number of *S. inaequidens* was significant for the seed set. Indeed, in

site A, the seed set of *J. vulgaris* decreased with the number of *S. inaequidens*, but not in site B.

DISCUSSION

Self-fertility

The hypothesis that the lower seed set of *J. vulgaris* compared to *S. inaequidens* is due to a lower percentage of self-fertility was not confirmed in the present study, as both species exhibited similarly low levels of self-fertility. Our results are in agreement with those of Lafuma & Maurice (2007) for *S. inaequidens* and of Andersson (1996) for *J. vulgaris*, who found very low seed sets after hand self-pollinations for both species (generally < 10%). However, we found a high level of self-fertility in one of our 10 individuals of *S. inaequidens*, in line with the findings of López-García & Maillet (2005). The higher seed set of *S. inaequidens* compared to *J. vulgaris*, as found by Vanparys et al. (2008), is thus not due to a higher self-fertility.

The seed set obtained for *J. vulgaris* after hand cross-pollinations under controlled conditions was 20% higher than that measured in the field (72 and 54% respectively; Vanparys et al. 2008). The difference was lower for *S. inaequidens* (80% and 74% with hand cross-pollination and in the field respectively). These results suggest that insect pollination may be less efficient than hand pollinations in *J. vulgaris*, implying that the plants may suffer from compatible pollen limitation. This idea is supported by the observation that insects visit more capitula per plant on *J. vulgaris* than on *S. inaequidens* and tend to move to the nearest capitulum (Vanparys et al. 2008) thus promoting geitonogamy. The hypothesis of pollen limitation needs to be tested by field experiments with pollen addition.

Nectar quality and quantity

Few studies so far have investigated the nectar production of invasive plants. However, in the case of *Impatiens glandulifera* and *Taraxacum officinale*, the high attractiveness to insects was ascribed to the their high nectar production (Chittka & Schürkens 2001, Lopezaraiza-Mikel et al. 2007, Kandori et al. 2009). In the present study, the higher visitation rate to *S. inaequidens* compared to *J. vulgaris* could not be explained by more attractive nectar. On the contrary, nectar amount was five times higher in the native *J. vulgaris* compared to *S. inaequidens*. Moreover, sugar concentration

Table 3 – **Results from ANOVA (F values) for the effects of the number of *S. inaequidens* plants (0, 5, 25) on the number of visitors per 10 min, on the visitation rate (log transformed) and the seed set of *J. vulgaris*.**
Significance of F values: * p < 0.05, ** p < 0.01, *** p < 0.001. df = degrees of freedom.

		insect visits to *J. vulgaris*		seed set of *J. vulgaris*
	df	number of visitors per 10 min	visitation rate	
number of *S. inaequidens*	2	0.80	1.11	2.39
site	1	0.18	0.13	0.65
number of *S. inaequidens* × site	1	0.97	1.04	9.33***
df residual	-	103	103	69

Table 4 – Behaviour of visiting insects according to the number of invasive plants: time per capitulum and number of visited capitula (both sites pooled).
Values indicate means ± SE.

	number of *S. inaequidens*		
	0	5	25
time per capitulum (sec)			
J. vulgaris	15.5 ± 1.7	15.8 ± 1.6	14.5 ± 1.1
S. inaequidens		20.6 ± 2.2	15.8 ± 1.5
number of visited capitula			
J. vulgaris	6.1 ± 1.0	6.6 ± 0.8	5.8 ± 0.6
S. inaequidens		4.7 ± 0.5	3.5 ± 0.3

in nectar was also higher in *J. vulgaris*. In comparison with other Asteraceae species (with similar floret size), nectar concentration was quite high, while nectar volume appears low even though nectar volumes vary greatly among species in Asteraceae (Schmitt 1983, Schultz & Dlugosch 1999, Torres & Galetto 2002, Chalcoff et al. 2006). For example, *S. integerrimus* produces 0.022 µL of nectar per floret, with 39% of total sugar concentration (Schmitt 1983). Most insect families prefer concentrated, low volume nectars (Petanidou et al. 2006). The high visitation rates observed in our studied species are thus not surprising. The nectar of most Asteraceae species has a larger proportion of hexoses than sucrose as found in our study (Torres & Galetto 2002). For example, the sucrose/hexose ratio of *Senecio pampeanus* is 0.01. It is known that Syrphids, which are major pollinators in our studied species, prefer such hexose dominant nectars (Petanidou et al. 2006, Nicolson et al. 2007).

Floral display

Our hypothesis that floral display would influence pollinator attractiveness was not supported, as capitula density had no effect on visitation rate. These results are not consistent with those of Andersson (1996) who showed that partial removal of the capitula on *J. vulgaris* plants had a slight negative effect on seed set. In his study, the effect was stronger when ligulate florets were removed and when plant density was high, suggesting that plant attractiveness results from the combination of several factors. As insects spent more time on capitula of LD (low density) pseudo-plants in our experiment, it could be expected that they visited fewer capitula, but this was not the case (table 2). This could mean that, when the distance between capitula is larger, insects stay longer on one capitulum, in line with the optimal foraging theory (Waddington 1983). This behaviour might increase geitonogamy, which in turn could decrease the seed set due to self-sterility (Jacquemart & Thompson 1996).

Neither the floral display nor the nectar production seemed to contribute to the higher pollination success (measured as the seed set) of *S. inaequidens*. Other traits can be involved. Thus preliminary results of UV pattern of the capitula appear to indicate slight differences between the two species (unpublished data). These patterns created by UV-reflectance of tubular disc florets and UV-absorbance of ligulate ray florets

were similar for both species. Nevertheless, as *S. inaequidens* has more florets per capitulum than *J. vulgaris* (Vanparys et al. 2008), the area of the UV-reflecting capitulum is larger for the invasive. This trait known to influence insect attractiveness could contribute to the higher visitation rate of *S. inaequidens*. Floral scents, which also contribute to plant attractiveness (Brodmann et al. 2008, Shuttleworth & Johnson 2009), might also differ between the two species. Other preliminary tests revealed a higher diversity and quantity of benzene and derivatives in scent of the invasive. Moreover, some terpenes (sabinene, β-pinene, β-myrcene) and terpenoids (linalool, β-terpineol) were identified in the floral scent of *J. vulgaris* and not in *S. inaequidens*.

Pollinator-mediated impacts of *S. inaequidens* on reproductive success of *J. vulgaris*

The lack of impacts of *S. inaequidens* on the visitation rate to *J. vulgaris* indicates that *S. inaequidens* does not turn the pollinators away from *J. vulgaris*, even when the invasive was abundant just beside the native plants. The lack of impacts on the seed set of *J. vulgaris* suggests that there is no pollen competition between the two plant species, although between-species pollinator movements were actually observed. These results do not agree with those of Morales & Traveset (2009). Their meta-analytical approach suggests that invasive species which are similar and closely related to natives are expected to be strong competitors for pollination.

Our results suggest that an invasive species can be more efficiently visited by pollinators (higher visitation rate and seed set; Vanparys et al. 2008) compared to a native neighbour, without interfering with the pollination and reproductive success of the latter.

CONCLUSION

This study shows that the higher visitation rate of *S. inaequidens* does not result from a higher nectar volume or sugar concentration compared to *J. vulgaris*, and is not due to its floral display. These results suggest that none of the studied traits play a role in the pollination success of *S. inaequidens*. Our study also shows that the higher seed set of *S. inaequidens* (Vanparys et al. 2008) could not be ascribed to a higher level of self-fertility. On the contrary, this higher seed set could be explained by a higher outcrossing rate, due to more frequent pollinator movements between individuals.

Finally, our results showed that there is no pollinator-mediated impact of *S. inaequidens* on *J. vulgaris*, at least in our experimental conditions.

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