Title: Plant-pollinator interactions: Comparison between an invasive and a native congeneric species

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Running title: Plant-pollinator interactions in plant invasions

Abstract

Plant-pollinator interactions determine reproductive success for animal pollinated species and, in the case of invasive plants, they are supposed to play an important role in invasive success. We compared the invasive *Senecio inaequidens* to its native congener *S. jacobaea* in terms of interactions with pollinators. Visitor guild, visitation rate, and seed set were compared over three years in three sites in Belgium. Floral display (capitula number and arrangement) and phenology were quantified, and visiting insects were individually censused, i.e. number of visited capitula and time per visited capitulum. As expected from capitula resemblance, visitor guilds of both species were very similar (proportional similarity= 0.94). *Senecio inaequidens* was visited by 33 species, versus 36 for *S. jacobaea*. For both species, main visitors were Diptera, especially Syrphidae, and Hymenoptera. Visitation rate averaged 0.13 visitor per capitulum per 10 min for *S. inaequidens* against 0.08 for *S. jacobaea*. However, insects visited more capitula per plant on *S. jacobaea*, due to high capitula density (886 m^{-2} versus 206 m^{-2} for *S. inaequidens*), which is likely to increase considerably self-pollen deposition. Seed set of *S. jacobaea* was lower than that of *S. inaequidens.* We suggest that floral display is the major factor explaining the differences in insect visitation and seed set between the two *Senecio* species.

Keywords: pollination, invasion, *Senecio inaequidens*, *Senecio jacobaea*, visitor guild, visitation rate, seed set, floral display

1. Introduction

Invasive plants are exotic species introduced in new areas, that reproduce and disperse efficiently, to such an extent that they spread rapidly. It is increasingly clear that interactions with resident biota often play a role in the invasive success (Richardson et al., 2000). Among these interactions, pollination has comparatively received less attention (Bjerknes et al., 2007). Though, for insect-pollinated exotic species, reproductive success critically depends on attractiveness to local pollinators.

Some authors proposed that some exotic plants became invasive due to a high attractiveness to pollinators (Brown et al., 2002), and that they have negative consequences on reproductive success of native species. For example, *Impatiens glandulifera* and *Lythrum salicaria* had negative impacts on both visitation rate and reproductive success of the considered native species (Chittka and Schürkens, 2001; Brown et al., 2002). Studies on other invasive species showed mixed effects, depending on the native species considered, the year and/or the site, and positive effect has even been found in some cases (Moragues and Traveset, 2005; Larson et al., 2006). A recent review stressed the lack of long term studies, as most of them were carried out only over one or two years, and rarely throughout the whole vegetation season (Bjerknes et al., 2007). Moreover, most of these studies were performed on ornamental species, among the showiest invasive. For instance, attractiveness of *Lythrum salicaria* (Lythraceae) was explained by its larger floral display in comparison to the native *L. alatum*, which has less flowers per plant (Brown et al., 2002). Another example is *Impatiens glandulifera* (Balsaminaceae) which presents larger flowers and a higher rate of sugar production in nectar than *Stachys palustris* (Lamiaceae; Lambinon et al., 2004; Chittka and Schürkens, 2001). Only one study has so far investigated pollination of non ornamental invasive species (Larson et al., 2006).

Floral display (i.e. flower number and arrangement) and phenology are important factors determining attractiveness and composition of pollinator guilds (Kunin, 1997; Feldman, 2006). Congeneric pairs of invasive and native species are good models to investigate competition for pollinators, as they often share floral and/or ecological traits. Such models have already been used in order to disentangle biological or life history traits linked to invasion success (Goodwin et al., 1999; Gerlach and Rice, 2003; Sans et al., 2004). In the context of pollination, however, the only congeneric pair of species studied was *Lythrum salicaria* and *L. alatum* (Brown and Mitchell, 2001; Brown et al., 2002).

Here, we chose the pair *Senecio inaequidens – S. jacobaea* (Asteraceae), for their morphological similarities in capitula and for their ecological niche and phenological overlap. *Senecio inaequidens* D.C., the narrow-leaved ragwort, is native of South Africa and was unintentionally introduced in Europe as a contaminant of wool. It has spread rapidly, becoming one of the most troublesome invasive species in Europe (Schmitz and Werner, 2000). This species is mostly a pioneer of open vegetation on disturbed soils but also occurs in dry grassland. Even fewer data are available about interactions with pollinators. Some observations suggest only that, like a majority of Asteraceae species, *S. inaequidens* presents a generalist pollination syndrome (Ernst, 1998; Lopez-Garcia and Maillet, 2005). The native *S. jacobaea* L., the tansy ragwort, has a relatively broad ecological niche, occurring in open communities, like sand dunes, but also in diverse types of grasslands (Harper and Wood, 1957). Visitor species of *S. jacobaea* have been listed, and included many species of several insect orders: syrphids, solitary bees, bumblebees and butterflies (Harper and Wood, 1957).

In this paper, we compared pollination ecology of *S. inaequidens* and *S. jacobaea*, over three years in three sites where they occur in sympatry. We characterized pollinator guilds, compared visitation rate and reproductive success, and traits related to pollinator attractiveness in both species. Specifically, we address three questions: i) Do *S. inaequidens* and *S. jacobaea* share the same pollinator guild? ii) Are they equally visited by pollinators and iii) do they have similar reproductive success?

2. Material and methods

2.1. Study species

The invasive *Senecio inaequidens* is a perennial shrub reaching a maximum of 1m height and 1.5 m in diameter. Each stem ends by one or few capitula that can flower from May to December (Böhmer et al., 2000). One plant produces between 26 and 500 capitula per year, each of them bearing in average 93 florets, and between 75 and 95 achenes (Ernst, 1998; Sans et al., 2004; Lopez-Garcia and Maillet, 2005). Female flower stage within a capitulum lasts on average three days, and time between fertilization and achene dispersal varies between 17 and 35 days (Ernst, 1998). *S. inaequidens* is partially self-compatible, as hand self-pollinations produced 8% fruit set. Nevertheless fruit set following hand cross-pollinations and insect pollination was higher (respectively 76 and 77%; Lopez-Garcia and Maillet, 2005). Our preliminary results under controlled conditions confirmed the poor selfing capacity (11 ± 6% after hand self- and 80% ± 3 after cross-pollination; Mahaux, 2008).

Senecio jacobaea, native to Europe, is a biennial to perennial hemicryptophyte. After a rosette stage during one year or more, plants develop one stem of maximum 1.5 m height, which divides into ramets, each of them ended by numerous capitula. Plants flower between June and October (Harper and Wood, 1957; Lambinon et al., 2004). One single plant produces between 68 and 2489 capitula, each of them carrying 50 to 80 florets, and bearing 70 achenes in average (Harper and Wood, 1957). Nectar is present and pollen presentation occurs from 8 a.m. to 5 p.m., with a peak from 10 a.m. to 12 noon (59% of the daily pollen production). On one capitulum, anthers continue to dehisce over a period of 4-9 days (Harper and Wood, 1957). Achenes are dispersed approximately 23 days later (Fenner et al., 2002). *Senecio jacobaea* was considered as self-incompatible species, as fruit set was in general nul following self-pollination (Andersson, 1996). Our preliminary results suggest however that *S.*

jacobaea reacted to hand self- and cross-pollination in the same way than *S. inaequidens* (12 ± 4% of seed set after self- and 72 ± 5% after cross-pollination; Mahaux, 2008).

The two species present yellow capitula, with a diameter of 2-3 cm, ligules included. Disc florets are protandrous hermaphrodite, whereas ray florets are pistillate. Capitulum development is centripetal. In Belgium, the flowering periods of the two species overlap in July, but *S. inaequidens* can continue to flower until November.

2.2. Study sites

The present study was performed simultaneously in semi-natural invaded sites and in an experimental plot, in order to compare the results obtained in natural conditions with those obtained in homogenous conditions (substrate, plant density). Observations were carried out over three flowering seasons: 2005, 2006 and 2007.

The site Jardin Massart was established in a garden at the University in Brussels (see Table 1). Three rows of thirteen 0.5×0.5 m sub-plots were established, separated by 20 cm. In autumn 2004, 4 plants were planted in each monospecific sub-plot, as a checkerboard so that *S. inaequidens* and *S. jacobaea* were placed alternately in monospecific sub-plots. In order to increase diversity in the plot, the 156 individuals were collected from seven Belgian populations of *S. inaequidens* and nine populations for *S. jacobaea,* from contrasted habitats and distant from each other by 6 to 125 km. In 2005 and 2006, the dead individuals were replaced with new ones from the same populations. The plot was surrounded by a wide range of entomophilous species (*Helianthus annuus, Phacelia tanacetifolia, Hypericum patulum,* etc).

In 2005, the semi-natural invaded site was situated at Nossegem, in the outskirts of Brussels (see Table 1). Vegetation consisted in a shrubby grassland of approximately 250 m², on a NNW-facing slope of approximately 35% along a roadside. Entomophilous species were present, mainly *Buddleja davidii, Daucus carota, Hypericum* sp., and *Epilobium hirsutum*. Flowering plant density averaged 1.25 and 0.38 individuals m^{-2} for *S. jacobaea* and *S. inaequidens* respectively (in summer 2005). After the destruction of this site during the following winter, a second site was chosen in Antwerp (see Table 1). That site consisted in a 25 ha dry grassland on sandy soil, situated in an industrial area. Entomophilous species were present like *Diplotaxis tenuifolia, Cirsium arvense, Carduus crispus, Reseda lutea* etc. A homogenous 2000 m^2 area (based on vegetation) was selected for the observations, and flowering plant density was measured for both *Senecio* species (Table 1). Plant densities varied greatly between 2005 and 2006, plant density of *S. inaequidens* increased 10-fold. In contrast, that of *S. jacobaea* decreased 10-fold, mainly due to caterpillar herbivory (*Tyria jacobaeae*, Arctiidae).

2.3. Visitor guild: qualitative aspect

The visitor species were listed for each *Senecio* by capturing visiting insects. Insects landing on capitula were randomly collected on both species. Collected individuals were killed by diethyl-ether and fastened on a pin to be identified. Captures were carried out during sunny days between 9 a.m. and 5 p.m. from June to October, in 2005 at the Jardin Massart and Nossegem, and in 2006 at the Jardin Massart and Antwerp (approximately a total of 12h for each species and site). Identified species were then classified in six categories: large-size Syrphidae, small-size Syrphidae, other Diptera, Apidae, other Hymenoptera and other insects (see Table 2).

2.4. Quantitative observations in monospecific patches

Observations were performed in monospecific patches of *S. inaequidens* and *S. jacobaea*, during 10 minutes periods. One or several adjacent plants in full flower, forming a monospecific patch, were selected for each observation period. Before each observation period, the size of the patch was measured and the number of open capitula counted. A capitulum was considered as open if at least the ray florets were at the pistillate stage. Then, during 10 minutes periods, the number of visitors per category was counted. An insect was considered as a visitor when it landed at least on one open capitulum, for at least one second. Observations took place during sunny days (the same as for qualitative measures), between 9 a.m. and 5 p.m., at Nossegem (in 2005) and Antwerp (2006 and 2007) from June to October, and at the Jardin Massart (from 2005 to 2007) in July.

Approximately 80 visitors were censused for each *Senecio*, site and year. As a quantitative measure of the behavior, the number of capitula visited in the patch was counted and the time spent on each of them was noted. Relative frequencies of the six main categories of visitors were measured in 10 minutes periods in monospecific patches. Visitation rate of each *Senecio* was defined as the total number of visitors counted in 10 min divided by the number of open capitula in the patch.

Floral display of *S. inaequidens* and *S. jacobaea* (inflorescence arrangement) was quantified in terms of capitula density in the observed patches, i.e. the number of open capitula divided by the area covered by the plant. Flowering phenology was examined in terms of variation of the capitula density throughout the season. Phenology of plants in the Jardin Massart was not taken into account, as it was disturbed by artificial planting (plants ceased flowering in early August).

2.5. Seed set

Reproductive success was estimated as seed set, expressed as the number of achenes per capitulum divided by the number of florets. In 2006 and 2007, at Antwerp, twenty ripe capitula were collected individually (one or two per plant). Capitula were harvested in August or early September. In the site of the Jardin Massart, one capitulum in each sub-plot was harvested in August 2007.

2.6. Statistical analyses

Proportional similarity (PS) of the pollinator categories was calculated to measure the pollinator overlap between *S. inaequidens* and *S. jacobaea* (Grabas and Laverty, 1999). PS calculation is based on the relative visitation frequency of the six visitor categories. For each category, the lowest frequency between *S. inaequidens* and *S. jacobaea* was selected, then the six frequencies were summed (Costello and Colin, 2002). PS ranges from 0 to 1, a high PS value indicating a high pollinator overlap. Differences in relative frequencies between years and sites were tested with chi-square tests (the category "other insects" was pooled with "other Diptera" in order to reduce the number of null values).

Visitation rate and capitula density were analyzed by two-way ANOVAs, to test for effects of species and site, and interaction, with data of the overlapping flowering period (July) of the 3 sites and the 3 years pooled. Effects of species, year, and interaction were tested on data of three years in the Jardin Massart. For these analyses, data from the overlapping flowering period were used, for *S. inaequidens* and *S. jacobaea*. For *S. inaequidens*, effects of period, year and interaction were tested on data of Nossegem (2005) and Antwerp (2006 and 2007 pooled).

Seed set was analyzed by two-way ANOVA to test for effects of species, sites and interaction, with data following the overlapping flowering period of 2007, in the Jardin Massart and in Antwerp. For data of Antwerp, effects of year (2006 and 2007), species and interaction were tested.

Insect behavior, i.e. number of visited capitula and the time per capitulum, were not normally distributed. Non parametric Kruskal-Wallis tests were applied on all pooled data, to test for differences between insect categories, plant species, sites and years. As no effect of period was detected on behavior for *S. inaequidens*, data of the overlapping flowering period and the late season were pooled for the analyses.

Visitation rates and capitula densities were log transformed to reach normality and proportions were arcsin transformed. Statistical analyses were performed with STATISTICA 7 (Statsoft 2006).

3. Results

3.1. Floral display and phenology

Senecio jacobaea and *S. inaequidens* differed in their flowering phenology. Flowering period of *S. inaequidens* was long, as it staggered over 5 months, with a more or less constant capitula number of 269 ± 30 capitula m^{-2} (mean ± SE, see also Fig. 1). The flowering period of *S. jacobaea* was shorter, lasting only 2 months, with a peak in July (886 ± 86 capitula m^{-2}). In 2007 at Antwerp, however, the massive flowering was prevented by a strong herbivory pressure due to the caterpillars of *Tyria jacobaeae* in the early stage of flowering. Some eaten individuals of *S. jacobaea* developed new stems that flowered in the late season, responsible for a secondary flowering in mid October 2006, and a longer flowering period in 2007 (Fig. 1). During the overlapping flowering period, *S. inaequidens* and *S. jacobaea* differed significantly in their floral display as mean capitula density of *S. jacobaea* was 4-fold higher than that of *S. inaequidens* (ANOVA: $F= 95.5$, $p<0.001$; Fig. 2). Capitula density differed significantly between sites ($F= 17.7$, $p<0.001$) and in the Jardin Massart, a significant effect of year was detected ($F=26.8$, $p<0.001$).

3.2. Visitor guilds

A total of 358 insects were captured and identified. Forty-three species were identified, belonging to 14 families and four orders (Table 2). The most important family in terms of number of species was the Syrphidae (17 species). *Eristalis tenax* (large-size Syrphidae) was the most abundant species collected each year in all sites. *Episyrphus balteatus, Sphaerophoria scripta* and *Syritta pipiens* (small-size Syrphidae) were also present in all sites. Other species were not collected in all sites, and 8 of them were only represented once. Besides these few abundant species, 6 species were collected only on *S. jacobaea*: *Autographa gamma, Pieris rapae* (Lepidoptera), *Sicus ferrugineus, Conops flavipes, Physocephala rufipes* (Conopidae) and *Scaeva pyrastri* (Syrphidae). Two species were collected only on *S. inaequidens*: *Metasyrphus latifasciatus* and *Cheilosia* sp. (Syrphidae). *Episyrphus balteatus* was captured more frequently on *S. jacobaea* than *S. inaequidens* (22 and 10 individuals respectively). In total, *S. inaequidens* and *S. jacobaea* were visited respectively by 33 and 36 species.

During the overlapping flowering period, Syrphidae were the most frequently observed visitors in term of categories (small- and large-size pooled: 57% in total; Fig. 3), followed by Apidae (18%), other Hymenoptera (19%), and other Diptera (12%). Other insects were very rarely observed (<1%). The global proportional similarity (all sites and years pooled) of visitor guilds during the overlapping flowering period was very high between *S. inaequidens* and *S. jacobaea* (PS=0.94). Proportional similarity ranged from 0.67 to 0.93, indicating a wide overlap of the visitor guilds in all sites and years (Fig. 3). During the late season, *S. inaequidens* was still visited by large-size Syrphidae (59%), small-size Syrphidae (19%), other Diptera (12%), Apidae (2%) and other Hymenoptera (7%; Fig. 3). Differences between years and sites were detected in the relative frequencies of the categories (χ^2= 319 and 342 respectively, $p<0.001$ in both cases).

3.3. Visitation rate and visitor behavior

Visitation rate (visitors per capitula) was significantly higher for *S. inaequidens* than for *S. jacobaea* (Fig. 4, Table 3). *Senecio inaequidens* attracted more visitors in proportion to the number of capitula as we counted in average 0.13 ± 0.01 visitor per capitula per 10 min in *S. inaequidens* patches, against 0.07 ± 0.01 in *S. jacobaea* patches. For both species, visitation rate varied between sites and years (Table 3). For *S. inaequidens*, no differences were detected between the overlapping flowering period and the late season (Table 3).

Insects visited more capitula in *S. jacobaea* patches than in *S. inaequidens* patches (Kruskal-Wallis: H= 11.0, $p<0.001$; see Table 4 for details). Visitor categories differed significantly in the number of visited capitula (H= 115, p<0.001). The highest numbers of visited capitula were measured for large-size Syrphidae and Apidae. Time spent on a capitulum did not differ between *S. inaequidens* and *S. jacobaea* (H= 0.31, p=0.58), but varied significantly among visitor categories (H= 37.5, p<0.001): the small-size Syrphidae spent the longest time per capitulum, whereas Apidae were the most rapid visitors (Table 4). For *S. inaequidens*, neither the number of visited capitula, nor the time spent per capitulum differed between the overlapping flowering period and the late season (Kruskal-Wallis: H=0.19, p=0.27 and H=1.00, p=0.32 respectively). Insect behavior varied between years, both in terms of number of visited capitula (H=79.3, p<0.001) and of time per capitulum (H=9.31, p=0.02). The numbers of visited capitula varied between sites (H=70.1, p<0.001), but the time per capitulum did not (H=0.79, p=0.67). In total, visitors remained rarely more than 5 minutes in an observed patch. All the insect visitors were observed collecting nectar, and some individuals of large-size Syrphidae, Apidae and other Hymenoptera collected pollen.

3.4. Seed set

The reproductive success, i.e. seed set, significantly differed between the two species (ANOVA: F=23.9, p<0.001). *Senecio inaequidens* had a mean seed set of 74%, against 54% for *S. jacobaea* (Fig. 5). No effect of site (F= 0.07, p=0.80) was detected but the interaction species*site was significant (F=7.4, p=0.008), because difference between the two *Senecio* was less strong at the Jardin Massart than at Antwerp. In Antwerp, there was no difference between seed set of 2006 and 2007 (F=0.5, p=0.49).

4. Discussion

4.1. Visitor guild

This study showed that *Senecio inaequidens* and *S. jacobaea* shared the same visitor guild, in terms of diversity, of species identity and of relative frequencies of insect categories. This result is not surprising, since both species have very similar capitula morphology. Though a wide range of insect taxa visited the two *Senecio* species, Syrphidae represented more than half of the visits, and *Eristalis tenax* (large-size Syrphidae) was by far the most abundant visitor. Moreover, large-size Syrphidae are potentially efficient pollinators, as they are quite mobile on capitula, moving rapidly on a capitulum (and visiting most of the open florets), and between capitula, as they visited many capitula per unit of time. It can thus be assumed that large-size Syrphidae, especially *E. tenax*, are of major importance in the pollination of *S. inaequidens* and *S. jacobaea*. These results are consistent with the list of floral visitors reviewed by (Harper and Wood, 1957) for *S. jacobaea*, where *E. tenax* was cited, but without quantitative information. Results are also consistent with the assumption that *Senecio* presents a generalist pollination syndrome (Schmitt, 1980; Proctor et al., 1996). The similarity in visitor guild between the two *Senecio* means that they are potential competitors for pollination services, as their ecological niches overlap widely.

4.2. Visitation rate, insect behavior and seed set

Senecio inaequidens had a higher visitation rate than *S. jacobaea*, and thus a higher probability for a capitulum to be visited (in 10 min) by an insect coming from another individual. This is due to differences in floral display, i.e. capitula number per plant. On a per plant basis, *Senecio inaequidens* was less attractive than *S. jacobaea* (9.0 ± 0.7 visitors per 10 min and 11.5 ± 1.0, respectively), but on a per capitula basis, visitation rate was higher for *S. inaequidens* (0.13 ± 0.12 and 0.07 ± 0.03 for *S. jacobaea*). However, visitation rate calculation only takes into account the number of insects that visited at least one capitulum. The probability for a capitulum to be visited in 10 min can be estimated by including the mean number of capitula visited by censused insects. The probability was very similar, with 54 ± 5% for *S. inaequidens* versus 53 ± 5% for *S. jacobaea*. Thus the lower visitation rate of *S. jacobaea* is compensated by the tendency of insects to visit more capitula per plant, which can also be explained by the higher capitula density. Capitula are densely displayed in *S. jacobaea*, to such an extent that capitula are in contact with each other. From the point of view of insects, it should be a substantial energetic advantage, as they can move between capitula without flying (Thomson, 1981; e.g. optimal foraging theory *sensu* Waddington in Real, 1983). We observed indeed that visitors of *S. jacobaea* walked from one capitulum to the nearest. From the point of view of the plant, high capitula density can be an advantage as it increases attractiveness (Schmitt, 1983) and a disadvantage as it increases within-plant pollination and selfing rate. The plant's dilemma between attractiveness and cross pollination level has already been discussed for other species (Klinkhamer and de Jong, 1993; Vrieling et al., 1999). In this context, *S. jacobaea* presents a floral display maximizing attractiveness, while that of *S. inaequidens* is less attractive, but limits self-pollination by ways of geitonogamy.

Other studies showed higher visitation rates on the showy invasive *Impatiens glandulifera* and *Lythrum salicaria*, in comparison with less showy co-flowering species, *Stachys palustris* and *Eupatorium perfoliatum*, respectively (Grabas and Laverty, 1999; Chittka and Schürkens, 2001). In these cases, results were attributed to higher attractiveness, i.e. larger floral display, and/or nectar. In contrast, higher visitation rate of the non ornamental *S. inaequidens* is explained by a smaller floral display, which decreases plant attractiveness, but increases capitula visitation rate.

This difference in floral display could explain the lower seed set of *S. jacobaea* (55%) in comparison with *S. inaequidens* (78%), due to pollinator behavior. In our study, the relationship between floral display and visitation rate is rather clear, but further studies are needed to establish the link between visitation rate and seed set. Other factors than insect pollination can indeed influence seed set, such as availability of nutrient resources (Ivey et al., 2003). Pollen addition experiments in the field (supplemental hand cross-pollinations), for instance, should determine whether pollen deposit by insects is a limiting factor of seed set. To our knowledge, our study is the first one that compared seed set of invasive and native congeners under the same conditions.

Other studies showed a pollinator-mediated impact of an invasive on a native species. The presence of *Impatiens glandulifera*, for instance, had a negative impact on seed set of the native *Stachys palustris* (Chittka and Schürkens, 2001). Here we did not test the impact of *S. inaequidens* on reproductive success of *S. jacobaea*, but our results suggest that the *S. inaequidens* is not more attractive to pollinators, and thus it is quite improbable that the lower seed set of *S. jacobaea* was due to a pollinator shift to the invasive species.

4.3. Spatiotemporal aspects

Visitor guild varied between sites and years. Differences between sites are probably due to the different co-flowering species, as they consisted of contrasted vegetation types (common garden, open or closed grassland). Spatiotemporal fluctuation in pollinator relative abundance is well documented, linked to environmental conditions (Horvitz and Schemske, 1990; Cane and Payne, 1993). Visitation rate and insect behavior also varied between sites and years, as it has been reported in other studies (Ivey et al., 2003; Moragues and Traveset, 2005; Larson et al., 2006). But whatever the year or the site, capitula density was always higher for *S. jacobaea* and visitation rate was always higher for *S. inaequidens*.

For *S. inaequidens*, no seasonal effect was detected between the mid-summer (July), and the late season (September – October), which means that the long flowering period of this species is useful, as capitula are still visited in the late season. Moreover, we observed seed production until November. Some authors had suggested that a long flowering period could constitute an advantage for invasive plants (Reichard and Hamilton, 1997).

4.4. Conclusion

This study provided the first quantitative observations of insect visitation of the invasive *S. inaequidens*. The two *Senecio* species presented contrasted flowering strategy, but shared the same visitor guild. Flowering of *S. inaequidens* was staggered in time and space, while that of *S. jacobaea* was shorter and densely displayed. In terms of visitation rate, the flowering strategy of *S. inaequidens* was more efficient than that of *S. jacobaea*. We suggest that the higher reproductive success of *S. inaequidens* is not explained by a higher attractiveness, but by a better flowering strategy, that limited self-pollination by within-plants movements of pollinators. Since attractiveness of *S. inaequidens* was not higher than that of *S. jacobaea*, the lower seed set of *S. jacobaea* was probably not due to the presence of *S. inaequidens*, we do not expect a pollinator-mediated negative impact of *S. inaequidens* on *S. jacobaea*. Further studies will focus on this assumption.

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Table 1 : Characteristics of the 3 study sites. Adult plant density expressed in number of flowering plants m⁻² measured in summer.

				Adult plant density	
site	Location	Vegetation type	Year	*S. inaequidens*	*S. jacobaea*
Massart (M)	50° 48’ 49.89’’ N 4° 26’ 18.47’’ E	Experimental garden	2005, 2006, 2007	3.5	3.5
Nossegem (N)	50° 52’ 18.30’’ N 4° 30’ 39.44’’ E	Shrubby grassland	2005	0.38	1.25
Antwerp (A)	51° 14’ 36.40’’ N, 4° 23’ 15.03’’ E	Dry grassland	2006,	0.56	0.87
			2007	5.5	0.1

Table 2 : Insect species collected visiting *S. inaequidens* and *S. jacobaea*, in 2005 (at Nossegem and Jardin Massart) and 2006 (at Antwerp and Jardin Massart), classified in the 6 main insect categories. 1: only one insect collected ; x: at least 2 insects collected ; xx more than 15 insects collected. *Bombus terrestris* or *B. lucorum* (non distinguishable).

		Insect species	Category	*S. inaequidens*	*S. jacobaea*
Diptera	Calliphoridae	Non identified	Other Diptera	x	x
	Conopidae	*Conops flavipes*	Other Diptera		x
		Physocephala vittata	Other Diptera		x
		Sicus ferrugineus	Other Diptera		x
	Sarcophagidae	Non identified	Other Diptera	1	x
	Syrphidae	*Cheilosia* sp.	Small-size Syrphidae	x	
		Episyrphus balteatus	Small-size Syrphidae	x	xx
		Eristalinus sepulchralis	Large-size Syrphidae	x	x
		Eristalis arbustorum	Large-size Syrphidae	x	x
		Eristalis intricarius	Large-size Syrphidae		1
		Eristalis nemorum	Large-size Syrphidae	x	x
		Eristalis sp.	Large-size Syrphidae	xx	xx
		Eristalis tenax	Large-size Syrphidae	x	1
		Helophilus pendulus	Large-size Syrphidae	x	x
		Metasyrphus corollae	Large-size Syrphidae	x	
		Metasyrphus latifasciatus	Large-size Syrphidae	1	
		Scaeva pyrastri	Large-size Syrphidae		x
		Sphaeroforia scripta	Small-size Syrphidae	x	x
		Sphaeroforia sp.	Small-size Syrphidae	x	x
		Syritta pipiens	Small-size Syrphidae	x	x
		Syrphus sp.	Small-size Syrphidae	1	x
		Syrphus vitripennis	Small-size Syrphidae		1
	Tachinidae	*Eriothrix rufomaculata*	Other Diptera	1	1
		Gymnosoma rotundatum	Other Diptera	1	
		Tachina fera	Other Diptera	x	1
		Non identified	Other Diptera	x	x
Hymenoptera	Andrenidae	*Andrena flavipes*	Other Hymenoptera	x	x
	Apidae	*Apis mellifera*	Apidae	x	x
		Bombus hypnorum	Apidae	1	
		Bombus pratorum	Apidae		1
		Bombus lapidarius	Apidae	x	x
		*Bombus terrestris**	Apidae	1	x
		Nomada sp.	Apidae	x	1
	Halictidae	*Halictus* sp.	Other Hymenoptera	x	x
		Heriades truncorum	Other Hymenoptera	x	x
		Lasioglossum sp.	Other Hymenoptera	x	x
		Megachile lapponica	Other Hymenoptera	1	
	Sphecidae	*Cerceris rybiensis*	Other Hymenoptera		1
Lepidoptera	Hesperiidae	*Thymelicus lineolus*	Other insect	x	1
	Lycaenidae	*Polyommatus icarus*	Other insect		x
	Noctuidae	*Autographa gamma*	Other insect		x
	Pieridae	*Pieris rapae*	Other insect	1	1
Mecoptera	Panorpidae	*Panorpa* sp.	Other insect	x	1
		Total number of species: 43		33	36

Table 3 : ANOVA for visitation rate on log transformed data.

Effect	SS	df	MS	F	p

Effect of site on data of the overlapping flowering period in the 3 sites (all years pooled):					
Site	**0.09**	**2**	**0.04**	**55.93**	**<0.001**
Species	**0.02**	**1**	**0.02**	**19.80**	**<0.001**
Site x species	4x10^{-3}	2	2x10^{-3}	2.91	0.057
Error	0.13	168	7x10^{-4}		
Effect of year on data of the overlapping flowering period in site of Jardin Massart:					
year	**0.02**	**2**	**0.01**	**9.01**	**<0.001**
species	**0.01**	**1**	**0.01**	**8.54**	**0.005**
Year x species	2x10^{-4}	2	9x10^{-5}	0.07	0.934
Error	0.09	72	0.001		
Effect of period on data of *S. inaequidens* at the sites of Nossegem and Antwerp (2 years pooled):					
year	**0.03**	**2**	**0.01**	**22.02**	**<0.001**
period	4x10^{-4}	1	4x10^{-4}	0.63	0.429
Year x period	7x10^{-4}	2	4x10^{-4}	0.62	0.542
Error	0.05	82	6x10^{-4}		

Table 4 : Number (Nb) of visits per 10 min, in patches of *S. inaequidens* (I) and *S. jacobaea* (J), for each category, number of visited capitula and time spent per visited capitulum (mean ±SE). Kruskal-Wallis tests: H values. ns p>0.05. *p<0.05. **p<0.01. ***p<0.001.

Means±std err.	Visitor categories									
	Large-size Syrphidae		Small-size Syrphidae		Other Diptera		Apidae		Other Hymenoptera	
	I	J	I	J	I	J	I	J	I	J
Nb visits per 10 min	3.4±0.2	3.0±0.3	0.9±0.1	1.1±0.2	0.9±0.1	1.2±0.1	1.0±0.1	1.8±0.2	1.2±0.2	1.5±0.2
Nb visited capitula	5.4±0.4	7.1±0.6	2.5±0.5	3.3±0.9	2.9±0.3	4.9±0.7	5.9±0.7	9.3±1.0	3.2±0.2	4.0±0.5
Species effect	5.2*		0.005 ns		6.8**		3.8 ns		0.07 ns	
Time per capitulum	8.4±0.5	8.2±0.5	16.3±2.4	15.5±2.8	9.9±1.4	10.5±0.8	7.0±0.5	6.2±0.5	9.7±1.0	7.6±0.5
Species effect	0.17 ns		0.52 ns		4.3*		4.5*		1.4 ns	

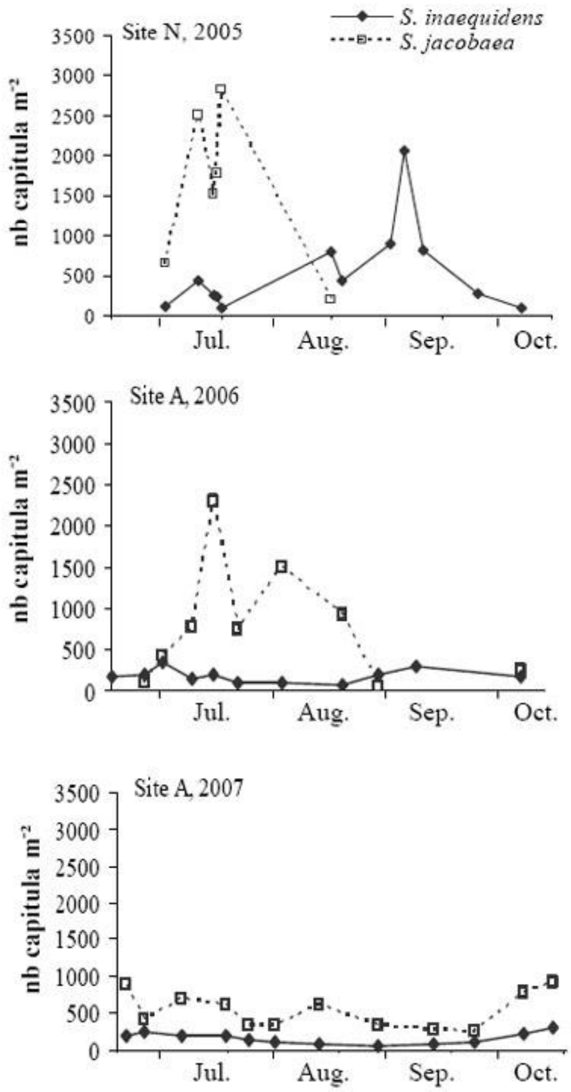

Fig. 1 : Phenology of *S. inaequidens* and *S. jacobaea* in the semi-natural study sites, Nossegem (N) in 2005 and Antwerp (A) in 2005 and 2006, quantified by the number of open capitula per square meter in the patches.

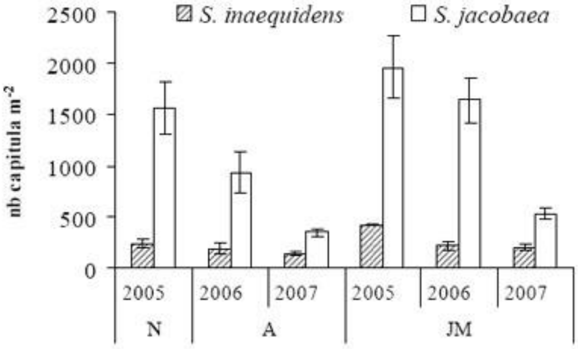

Fig. 2 Floral display, expressed as mean (±SE) capitula density of *S. inaequidens* and *S. jacobaea* for each site and year, in the patches observed during the overlapping flowering period, in the sites of Nossegem (N), Antwerp (A) and Jardin Massart (JM).

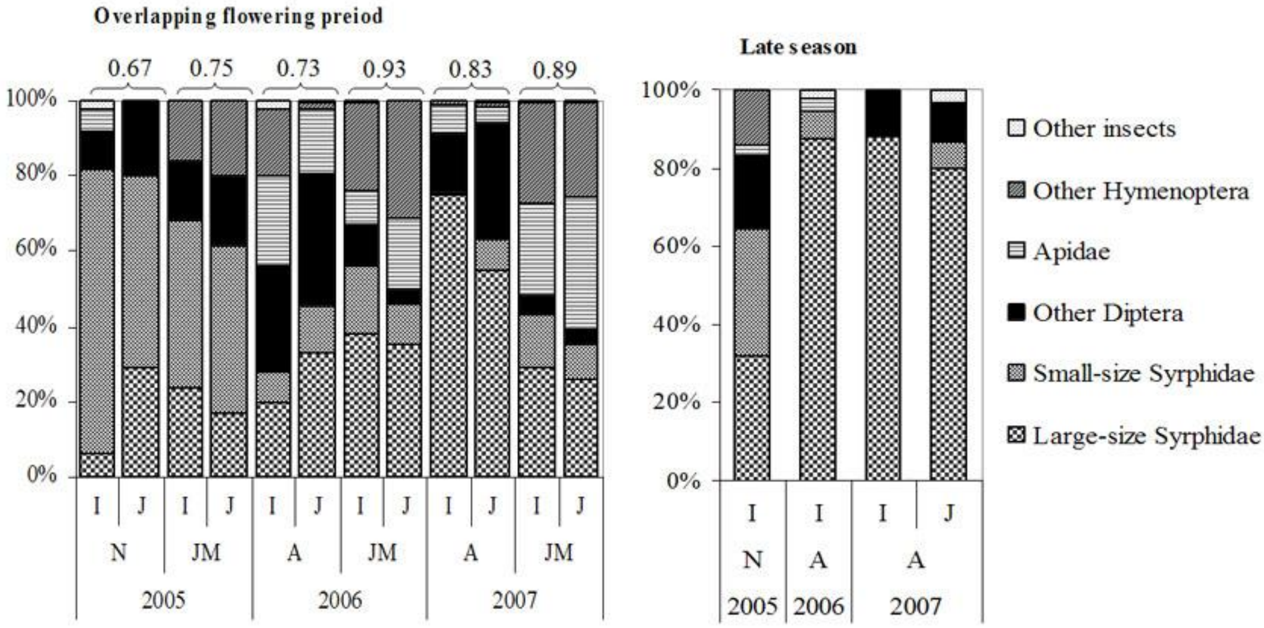

Fig. 3 : Relative frequencies in percent of the 6 main insect categories visiting *S. inaequidens* (I) and *S. jacobaea* (J) during the overlapping flowering period (July) in the three study sites: Nossegem (N), Antwerp (A) and Jardin Massart (JM); and during the late season (late August to October) in the sites of Nossegem and Antwerp. Numbers above are the proportional similarity (PS) for each pair.

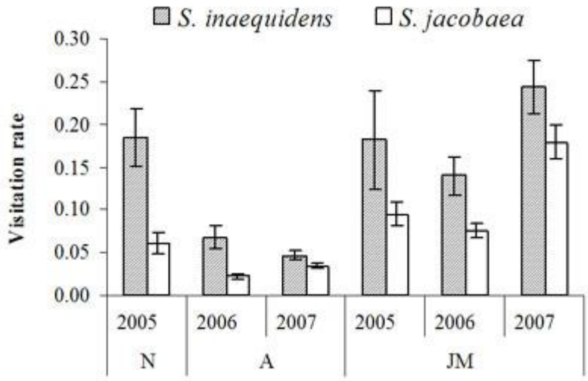

Fig. 4 : Visitation rates, expressed as the number of visitors per 10 min divided by the number of open capitula in the observed patches. Means (±SE) of *S. inaequidens* and *S. jacobaea* in the 3 sites (Nossegem (N), Antwerp (A) and Jardin Massart (JM)) over the 3 years, during the overlapping flowering period.

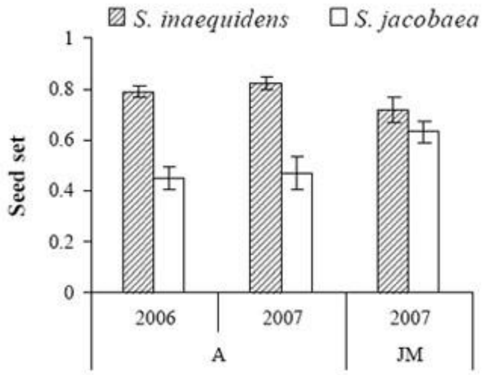

Fig. 5: Seed set (mean ± SE) of *S. inaequidens* and *S. jacobaea*, expressed as the number of achenes per capitulum divided by the total number of florets. Data of 2006 and 2007 at Antwerp (A) and of 2007 at the Jardin Massart (JM).