Testing for Ecological and Genetic Allee Effects in the Invasive Shrub *Senna didymobotrya* (Fabaceae)

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For an introduced plant species to become invasive, it must be able to reproduce even in initially small populations. We tested for Allee effects (reduced reproductive performance of individuals in small populations) in the nonclonal, buzz-pollinated shrub *Senna didymobotrya* in its invasive range in South Africa. The species is self-compatible, but we found that in its invasive range in South Africa it requires pollinators to set seed. Nearly all stigmas (90%) received pollen, but natural fruit set was very low (3–20%). Pollen receipt and fruit set were not significantly correlated with population size. We thus found no evidence for an ecological Allee effect arising from pollen limitation in small populations. Offspring seedling performance, measured in terms of stem volume and leaf area, was also not significantly correlated with the number of plants in the source population, indicating that genetic Allee effects, such as inbreeding depression, are either absent or of such a small magnitude that they would be unlikely to limit further spread of *S. didymobotrya* in South Africa.

Key words: alien plant; Baker's rule; *Cassia didymobotrya*; nonindigenous plant; pollination; population size; *Xylocopa* spp.

There is still little understanding of why some introduced species become invasive and others do not (Enserink 1999). Interactions with other organisms in the invasive range are likely to be key factors in determining invasiveness. Most research, however, has focused on interactions between invasive plants and competing native plants, herbivores, or pathogens (Crawley, 1987; Blossey and Nötzold, 1995; Keane and Crawley, 2002), whereas mutualisms with other organisms such as pollinating insects have received much less attention (Richardson et al., 2000).

For an introduced plant species to become invasive, it must be able to reproduce. Self-compatible plants are more likely to be successful invaders because they can sire their own seeds if initial population sizes are small, limiting the availability of mates (Baker, 1955, 1967). This has been called Baker's law by Stebbins (1957). Despite self-compatibility, pollinators are required to move pollen from anthers to stigmas when introduced plants lack autonomous self-fertilization mechanisms (Parker, 1997). Even when potential pollinators are present, individual reproductive success of introduced plants, which at least initially grow as solitary individuals or in small populations, may be reduced by negative effects of small population size (i.e., Allee effects; Stephens et al., 1999).

Plant fitness in small populations may be reduced as a consequence of both ecological and genetic factors. Small populations often attract fewer pollinators, which results in reduced seed set as a consequence of pollen limitation (Jennersten, 1988; Ågren, 1996; Groom, 1998; Forsyth, 2003; Ward and Johnson, 2005). Reduced pollen deposition in small populations constitutes an ecological Allee effect. Moreover, the quality of deposited pollen often declines with population size, which can result in genetic Allee effects (Ellstrand and Elam,

1993). Plants in small populations often have fewer available mates, which results in higher rates of selfing and crosses with related individuals (i.e., biparental inbreeding) (Raijmann et al., 1994; Routley et al., 1999). This might result in lower germination rates and offspring performance as a consequence of inbreeding depression (Menges, 1991).

Allee effects could be responsible for the generally long lag period between introduction and spread of an alien species (Lewis and Kareiva, 1993; Veit and Lewis, 1996). Allee effects may also facilitate eradication of alien invasive species because management can be restricted to keeping populations small rather than removing each single individual (Liebhold and Bascompte, 2003). The consequences of population size on reproductive success through either ecological or genetic Allee effects have mainly been studied for native plant species suffering from habitat fragmentation (Fischer and Matthies, 1998; Fischer et al., 2000; Mavraganis and Eckert, 2001; Hooftman et al., 2003; Ward and Johnson, 2005) and only rarely for alien invasive plants (Cappuccino, 2004; Davis et al., 2004a, b).

We studied the effect of population size on reproductive success of the tropical shrub *Senna didymobotrya* in its invasive range in South Africa. *Senna didymobotrya* is nonclonal and as a consequence, relies solely on seed production for population growth and dispersal to new sites. The species is self-compatible and buzz-pollinated (Dulberger, 1981; Rambuda and Johnson, 2004). We first tested if the species is capable of seed production in the absence of pollinators. We then tested whether natural fruit set is pollen limited and if it depends on the presence and number of available mates in the population (i.e., whether there is an ecological Allee effect). Moreover, to test whether offspring quality is affected by population size (i.e., whether there is a genetic Allee effect), we germinated seeds and grew the offspring of solitary shrubs and shrubs from small and large populations in a common greenhouse environment.

We asked the following specific questions: (1) Does *S. didymobotrya* in its invasive range in South Africa depend on

the presence of suitable pollinators for seed production? (2) Is seed production of shrubs of *S. didymobotrya* pollen limited, and if so, does it depend on the presence and number of other flowering *S. didymobotrya* shrubs in the population? (3) Do seed germination rates and the size of offspring of *S. didymobotrya* depend on the presence and number of flowering *S. didymobotrya* shrubs in the population?

MATERIALS AND METHODS

Study species- -The peanut butter cassia *Senna didymobotrya* (Fresen.) Irwin & Barneby (Fabaceae), previously known as *Cassia didymobotrya*, is an evergreen shrub or small tree of up to 4 m high and native to tropical Africa. The species has been introduced into South Africa, where it is now invasive in grasslands, coastal scrub, woodland, roadsides, riverbanks, and wastelands (Henderson, 2001). *Senna didymobotrya* has also been introduced into Central and North America, the Middle East, southern Asia, and some islands in the Caribbean and in the Indian and Pacific oceans (http:/www.ildis.org/LegumeWeb/6.00/taxa/619.shtml; accessed 21 July 2004).

Although individual shrubs of *S. didymobotrya* do not flower continuously, flowering individuals can be found in South Africa throughout the year. Numerous inflorescences are produced from meristems in the axils of compound leaves and consist of yellow flowers arranged in a 10–20 cm long raceme. Inflorescences can produce >50 flowers but there are only 1–8 flowers open simultaneously. Flowers consist of five sepals (12–17 mm long), five petals (19–24 mm long), one sickle-shaped, enantiostylous (i.e., curves either to the left or the right) pistil (25–35 mm long), and 10 stamens of different sizes. The three uppermost stamens have small sterile anthers (1–3 mm long). The four central stamens and the lowermost single stamen produce fertile pollen and function as feeding anthers (4–6 mm and 5–7 mm long, respectively), while the two large, lateral, curved stamens have anthers (10–15 mm long) that deposit pollen on the back of the pollinators.

The flowers of *S. didymobotrya* are self-compatible and buzz-pollinated (Dulberger, 1981), meaning that pollen is only released by high frequency vibrations. In the study area, the main insect that buzz-pollinates the flowers is the giant carpenter bee *Xylocopa flavorufa* (Rambuda, 2001; M. van Kleunen, personal observation) that can carry large amounts of pollen (>10 000 grains) on its back (Rambuda, 2001). The shape of stigmas of *S. didymobotrya* is crateriform (i.e., funnel-shaped), as in most other species of *Senna* and the closely related genus *Cassia* (Owens, 1985; Dulberger et al., 1994). Pollen grains can only reach the stigmatic surface inside the cavity after passing through a small pore at the tip of the pistil. The pistil of each flower has the potential to develop into a flat bean up to 15 cm long. Each of these fruits has 9–17 compartments, which can each contain one single seed of ca. 7 mm long and 4 mm wide.

Breeding system experiment- -To test whether flowers of *S. didymobotrya* in its invasive range in South Africa are self-compatible and whether they can set seed in the absence of pollinators by apomixis (i.e., seed production without fertilization) or autonomous self-fertilization (i.e., self-fertilization without assistance of pollinators), we performed a breeding system experiment on 10 shrubs in a natural population in Pietermaritzburg, South Africa. On 20 October 2003, we enclosed four inflorescences on each of the 10 shrubs in bags made of fine nylon mesh with openings of 0.3 × 0.3 mm to exclude pollinators and marked a fifth open-pollinated inflorescence as a control. To test for self-compatibility, we hand-pollinated, over a 2-wk period, all open flowers on one of the bagged inflorescences on each shrub with pollen from the same shrub (selfing treatment) and on another with pollen from another shrub (outcrossing treatment).

Hand-pollinations were carried out by opening a large lateral anther by using two pairs of tweezers and dipping the tip of the pistil several times into the pollen mass of the opened anther. To test for seed set through apomixis, we emasculated all emerging flower buds on the third bagged inflorescence on each shrub over a 2-wk period. To test for autonomous seed set, we left the fourth bagged inflorescence on each shrub as a control. Two weeks after the start of the experiment, when 4–19 flowers had opened per inflorescence,

we removed all bags and scored the fruit set of these flowers 1 wk later. Because bags on four of the shrubs had been vandalized during the experiment, we could only collect data for six of the 10 shrubs.

Natural fruit set and pollen limitation in small and large populations- -To test whether fruit set on shrubs of *S. didymobotrya* is pollen limited and whether this depends on the presence and number of other flowering *S. didymobotrya* shrubs in the populations, we first performed a pollen-supplementation experiment. In the period of 22–30 September 2003, we visited 58 populations of *S. didymobotrya* in a 100 × 125 km area in the southwestern part of the Province of KwaZulu-Natal, which also encloses a part of the Province of the Eastern Cape. Populations ranged in size from 1 to 200 flowering shrubs and were separated from each other by at least 300 m.

In each population, we chose at random one shrub for supplemental hand-pollinations. On these experimental shrubs, we counted the number of inflorescences and estimated its height in 0.5-m classes. To test for the effects of supplemental hand-pollination and the quality of supplemented pollen, we randomly chose, when possible, nine inflorescences on each experimental shrub for three treatments. On three of the inflorescences, we hand-pollinated all open flowers with pollen from the same shrub (self-pollen supplementation). On three others, we hand-pollinated all open flowers with pollen of a shrub from another population (outcross-pollen supplementation). The three remaining inflorescences were used as controls. Inflorescences were marked with pieces of colored wool, and treated flowers in each inflorescence were marked with a black spot at the base of their pedicels with a waterproof marker.

About 3 wk later, on 14 and 17 October 2003, we revisited each population and determined fruit set on the marked inflorescences. When a fruit had developed, we counted the number of seed chambers (i.e., the potential number of seeds) and the number of them filled with a seed. To increase the sample size for natural fruit set on the inflorescences in the control treatment, we also counted on the three control inflorescences the number of fruits and number of scars of pedicels of flowers that had not developed into fruits below the lowest marked flower up to a total of 20.

Because the results of the pollen supplementation experiment and subsequent pollen counts revealed that our hand-pollination treatment did not bring sufficient amounts of pollen to the stigmatic surface (see Results section), we could not use these data to test for pollen limitation. Therefore, we revisited the populations from 25–28 November 2003 to test for pollen limitation by assessing the amount of pollen deposited in the stigmatic cavity of pistils on open-pollinated flowers. Because some of our initial populations had been cleared, we also visited new ones to increase the total number of populations to 60.

On one flowering shrub in each population, we collected the pistils of 10 flowers, when possible on different inflorescences that had just finished flowering and put them in Eppendorf tubes. We counted the number of inflorescences on and estimated the height of these shrubs in 0.5-m classes. In addition, to test whether natural fruit set changes during the year, we also counted the number of fruits and pedicel scars on five inflorescences of each shrub at this second census. Because the stigmas are crateriform, the stigmatic surface is inside the pistil and not visible on fresh pistils. Therefore, we first fixed the pistils for 2 h in a solution of three parts 95% ethanol and one part glacial acetic acid (Carnoy's solution), then softened and cleared the pistil tissue by putting it for 24 h in a 4 mol/L NaOH solution, and finally stained the pollen for at least 4 h in a solution of aniline blue in 0.1 mol/L K_2HPO_4 (Martin, 1959). Thereafter, we counted the number of pollen grains in the stigmatic cavity with a fluorescence microscope.

Seed characteristics and size of offspring for small and large populations- -To test whether the quality of seeds and offspring of shrubs of *S. didymobotrya* depends on the presence and number of flowering *S. didymobotrya* shrubs in the population, we collected seeds from each of the shrubs used in the pollen supplementation experiment. We weighed one batch of 30 seeds per shrub and calculated the average mass per seed. On 6 November 2003, we sowed 30 seeds from each of the 58 shrubs in each of three pots (10 seeds each) with a diameter of 10 cm filled with potting compost. To

speed up germination, we scarified each seed slightly with sand paper before sowing. After 3 wk, when there were no more new seedlings emerging, we counted the total number of seedlings per pot.

Thereafter, we planted one randomly chosen seedling of each pot into a new pot and thinned the remaining seedlings to one per pot. This way we had six offspring per mother shrub. Ten weeks after sowing the seeds, we measured the height of the single stem and the diameter of its first internode on these plants and counted their total number of leaves. On the largest leaf of each plant, we counted the number of leaflets and measured the length and width of one of the two leaflets at the tip of the rachis. From these data, we calculated, as measures of plant performance, stem volume, by multiplying the stem height with the area of the stem cross section [π ($0.5 \times$ diameter)^2], and the total leaf area, by multiplying the number of leaves per plant with the number of leaflets per plant and the area per leaflet (which was estimated as the area of an oval: $0.8 \times$ length $\times$ width).

Analyses- In the breeding-system experiment, we tested for differences in the presence or absence of fruit set between the five treatments by using a chi-square test. In the other experiments, we tested for effects of the presence and number of other flowering *S. didymobotrya* shrubs on pollen receipt, fruit set, number of seeds per fruit, average mass per seed, seed germination rate, and size of offspring with hierarchical analyses of variance (Type I sum of squares) using the statistical package SPSS (SPSS, Chicago, Illinois, USA). The presence of other flowering shrubs (i.e., the effect of solitary vs. non-solitary shrubs) was considered a fixed factor and was fitted before the number of flowering *S. didymobotrya* shrubs, which was log_{10}-transformed.

To account for geographic effects, we grouped the populations into six geographic regions and used this as a blocking factor in the analyses. Moreover, to account for differences in size and floral display between shrubs, we fitted in the ANOVA height of shrubs and number of inflorescences per shrub as covariates before the presence and number of flowering *S. didymobotrya* shrubs in the populations. Further, to account for effects of the mass per seed on germination rates and offspring size, we included the average mass per seed of each shrub as a covariate in the analyses of germination rate and offspring size. To account for the effect of repotting half of the seedlings, we included it as a fixed factor in the analyses of offspring size. To determine the direction of the correlations between significant covariates and traits of interest, we calculated Pearson's correlation coefficients when the covariate had been fitted first in the ANOVA model and partial correlation coefficients when the covariate had been fitted after other covariates in the ANOVA model, thereby controlling for these other covariates.

In the pollen supplementation experiment, the supplementation treatment was considered a fixed factor. Because of the low number of marked flowers per inflorescence in the pollen supplementation experiment, we combined the three inflorescences per treatment for each shrub. For the analyses of other traits, for which we had replicated measurements on each shrub (i.e., inflorescence was the level of replication), we included shrub as a random factor and nested it within geographical region and the presence of other flowering shrubs. The proportion of fruit set and seed germination were arcsine square-root transformed prior to analyses to achieve normality and homoscedasticity.

RESULTS

Breeding system—None of the inflorescences in the four bagging treatments set fruit, while the proportion of flowers setting fruit in the control was 0.305 ± 0.103 (mean ± 1 SE). This difference in presence or absence of fruit set between the control and bagging treatments was significant ($\chi^2 = 24$, df $= 4$, $P < 0.001$). This indicates that pollinators are required for seed production in *S. didymobotrya*. The absence of fruit set in both the emasculated and control bagged inflorescences indicates that reproductive assurance through either apomixis or autonomous selfing is not possible in *S. didymobotrya*.

The absence of fruit set in both hand-pollination treatments (self- and cross-pollinated) suggests that our hand-pollination

treatment was not effective in bringing pollen into the stigmatic cavity onto the stigmatic surface. This was confirmed by pollen counts using a microscope, which showed that hand-pollination ($N = 15$) resulted on average (± 1 SE) in only 2.53 ± 0.93 pollen grains in the stigmatic opening, whereas flowers visited by carpenter bees ($N = 4$) had 58.50 ± 21.73 (in one-tailed t test, $t = 2.573$, adjusted df $= 3$, $P = 0.041$).

Pollen limitation in small and large populations—In the pollen supplementation experiment, there was no significant difference in the proportion of flowers setting fruit between the control (mean $\pm$ upper 1 SE/lower 1 SE $= 0.025 \pm 0.010/$ 0.008) and supplemental hand-pollination with self- ($0.034 \pm 0.011/0.010$) and cross-pollen ($0.028 \pm 0.010/0.009$; $F_{2,163} = 0.22$, $P = 0.800$). Neither was the effect of supplemental hand-pollination affected by the presence and number of flowering shrubs in the population ($F_{2,163} = 0.77$, $P = 0.463$, and $F_{2,163} = 0.35$, $P = 0.708$, respectively). This was most likely a consequence of the low effectiveness of our hand-pollination treatment in bringing pollen into the stigmatic cavity (see section Breeding system). Therefore, we additionally tested for pollen limitation by counting the number of pollen grains deposited in the stigmatic cavity of open-pollinated flowers.

Of the analyzed flowers ($N = 546$) in the pollen receipt study, 89.7% contained pollen grains (mean $= 69.3$, range: 1–220) inside their stigmatic cavities by far exceeding the average fruit set of 20.3%. Nevertheless, the proportion of flowers that set fruit was positively correlated with the number of pollen grains in the stigmatic cavities of flowers on the same shrub (Pearson's $r = 0.346$, $N = 60$, $P = 0.007$) indicating that fruit set is partly pollen-limited. Pollen receipt varied among shrubs and was correlated positively with the height of the shrubs (Table 1; partial r controlling for the number of inflorescences $= 0.461$, df $= 57$, $P < 0.001$) but was not affected by the presence and number of flowering *S. didymobotrya* shrubs in each population (Table 1, Fig. 1a).

For the 39 shrubs that were measured at both the first census in October 2003 and the second census 5 wk later, the proportion of flowers that set fruit increased from on average ($\pm$ upper 1 SE/lower 1 SE) $0.031 \pm 0.009/0.008$ to $0.203 \pm 0.028/0.027$ (paired t test, $t_{38} = 8.48$, $P < 0.001$) between the two censuses. There was significant variation in fruit set among individual shrubs at both censuses (significant shrub effects in Table 1), but this variation was not related to the presence or number of flowering *S. didymobotrya* shrubs in each population (Fig. 1b, Table 1). At the second census, however, fruit set was significantly positively correlated with shrub height (Table 1; partial r controlling for the number of inflorescences $= 0.344$, df $= 57$, $P = 0.008$). Both the number of seed compartments (i.e., potential number of seeds) and the actual number of developed seeds per fruit were not affected by the presence ($F_{1,33} = 1.964$, $P = 0.170$, and $F_{1,33} = 0.289$, $P = 0.594$, respectively) or number ($F_{1,33} = 0.296$, $P = 0.590$, and $F_{1,33} = 0.283$, $P = 0.598$, respectively) of other flowering *S. didymobotrya* shrubs in the population.

Seed characteristics and size of offspring for small and large populations—The average mass per seed of shrubs was not affected by the presence ($F_{1,43} = 0.041$, $P = 0.841$) or number ($F_{1,43} = 0.424$, $P = 0.518$) of other flowering *S. didymobotrya* shrubs in the population. The proportion of seeds that germinated was positively correlated with average seed mass (Table 2; Pearson's $r = 0.636$, $N = 54$, $P < 0.001$) and

TABLE 1. Summary of analyses of variance of effects of the presence and number of other flowering *Senna didymobotrya* shrubs in each population on pollen receipt and fruit set of *S. didymobotrya* in its invasive range in South Africa. Fruit set was scored in October 2003 (first census) and five weeks later in November 2003 (second census). We corrected for the height of and number of inflorescences on each measured shrub by including them as covariates and for effects of the geographical region by including it as a factor in the analyses. Proportion of fruit set was arcsine square-root transformed for analysis.

Effect	Number of pollen deposited in stigmatic cavity			Proportion of fruit set at first census			Proportion of fruit set at second census		
	df	MS	F	df	MS	F	df	MS	F
Number of inflorescences	1	21112	3.72	1	0.1300	2.27	1	0.0290	0.18
Height of shrub	1	81573	14.37***	1	0.0360	0.64	1	1.2210	7.38**
Geographical region (G)	5	6765	1.16	5	0.1792	3.14*	5	0.2652	1.60
Presence of flowering shrubs (P)	1	1734	0.30	1	0.0503	0.88	1	0.1281	0.77
Number of flowering shrubs	1	45	0.01	1	0.0203	0.36	1	0.0129	0.08
Shrub (G, P)†	50	5826	3.25***	48	0.0570	1.91**	50	0.1654	3.84***
Residual	486	1795		117	0.0299		226	0.0431	

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

† Shrub was nested in "geographical region" and "presence of flowering shrubs."

was negatively correlated with shrub height (Table 2; partial *r* controlling for the mass per seed and the number of inflorescences $= -0.324$, df $= 49$, $P = 0.020$) but was not significantly affected by the presence and number of other flowering *S. didymobotrya* shrubs in each population (Table 2).

Stem volume of offspring varied significantly among mother shrubs and was on average 20.9% greater when other flowering *S. didymobotrya* shrubs were present in the population than when there was only a single flowering shrub (Fig. 2a). This effect of other flowering *S. didymobotrya* shrubs, however, was not significant (Table 2). Total leaf area, on the other hand, was not affected by the presence of other flowering shrubs per se. However, when other flowering shrubs were present, total leaf area tended to increase with the number of flowering *S. didymobotrya* shrubs, but this correlation was not significant (Fig. 2b; Table 2).

DISCUSSION

Breeding system and pollination of S. didymobotrya—The ability of isolated plants of *S. didymobotrya* to set seed as successfully as those in large populations is likely to arise from a favorable combination of self-compatibility and attractiveness to pollinators. Although we did not succeed in depositing pollen on the stigmatic surface of pistils by hand, previous studies in both Israel and South Africa have shown that *S. didymobotrya* is fully self-compatible (Dulberger, 1981; Rambuda and Johnson, 2004). The absence of fruit set in emasculated flowers and control flowers on bagged inflorescences in the present study indicates that *S. didymobotrya* cannot set seed by either apomixis or autonomous selfing. This means that *S. didymobotrya* relies on the service of pollinators for reproduction even though it is self-compatible.

Flowers that had been "buzz-pollinated" by the large carpenter bee *Xylocopa flavorufa* had 58.50 ± 21.73 ($N = 4$) pollen grains in their stigmatic cavities. This suggests that a specific buzzing frequency of the pollinator is required to both release pollen from the anthers and to deposit it into the stigmatic cavity (Dulberger, 1981). The fact that we were not able to deposit pollen on the stigmatic surface by simply dipping the tip of the pistil in the pollen mass is consistent with this unusual pollination syndrome. Even when we vibrated the pistil during hand pollination with a tuning fork the number of pollen grains in the stigmatic cavity only slightly increased from 2.53 ± 0.93 to 6.93 ± 2.25 (means ± 1 SE; $t_{28} = 1.80$, $P = 0.09$).

Although we do not have pollinator observations on *S. didymobotrya* in its native range in tropical Africa, *X. flavorufa* is known to occur in most of the native range of *S. didymobotrya* (C. Eardly, Agricultural Research Council, South Africa, personal communication), suggesting that *X. flavorufa* is

TABLE 2. Summary of analyses of variance of effects of the presence and number of other flowering *Senna didymobotrya* shrubs in each population on germination rate, stem volume, and total leaf area of offspring of *S. didymobotrya* in its invasive range in South Africa. We corrected for the average mass per seed and the height of and number of inflorescences on each measured shrub by including them as covariates and for effects of repotting of seedlings and the geographical region by including them as factors in the analyses. Proportion of germinated seeds was arcsine square-root transformed for analysis.

Effect	Proportion of germinated seeds			Stem volume (mm³)			Total leaf area (mm²)		
	df	MS	F	df	MS	F	df	MS	F
Average mass per seed	1	5.873	27.83***	1	341971	0.47	1	156496849	0.61
Re-potting	—	—	—	1	1243814	3.05	1	18599743	0.07
Number of inflorescences	1	0.153	0.73	1	39126	0.05	1	743368638	2.70
Height of shrub	1	1.225	5.80*	1	3789449	5.19*	1	424380601	1.54
Geographical region (G)	5	0.033	0.16	5	509139	0.70	5	99164687	0.39
Presence of flowering shrubs (P)	1	0.001	0.00	1	2521022	3.46	1	31099519	0.12
No. of flowering shrubs	1	0.277	1.31	1	580826	0.80	1	767496121	3.01
Shrub (G, P)†	42	0.211	4.91***	40	729664	1.79**	40	275216995	1.08
Residual	98	0.043		221	408189		218	255160110	

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

† Shrub was nested in "geographical region" and "presence of flowering shrubs."
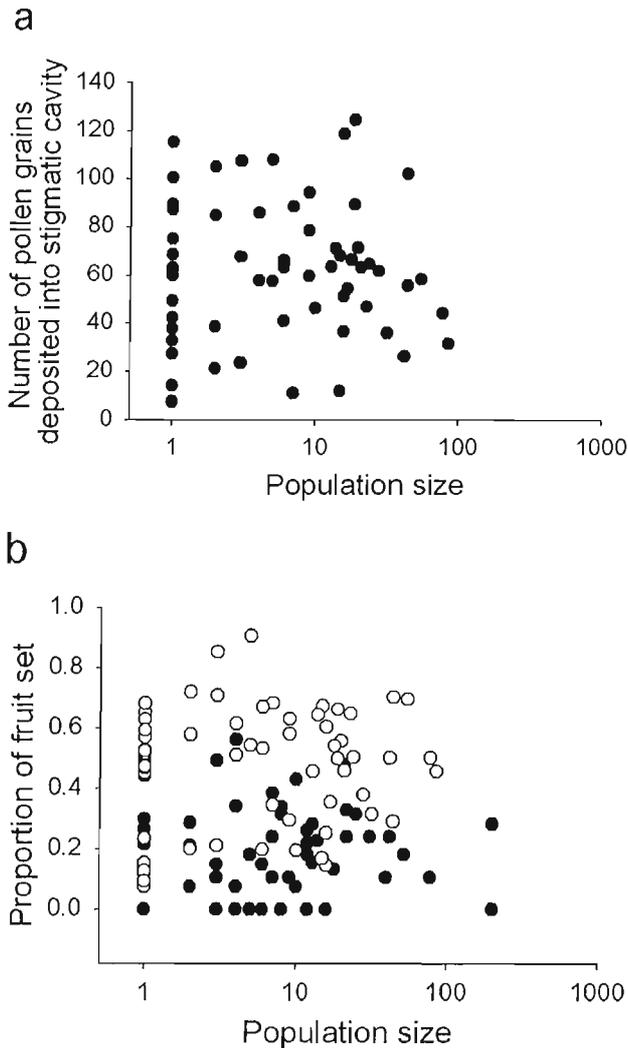
a

b

Fig. 1. Relationship between the number of flowering *Senna didymobo-trya* shrubs and (a) pollen receipt within stigmatic cavities and (b) natural fruit set at census 1 (closed symbols) and census 2 (open symbols) for shrubs of *S. didymobotrya* in its invasive range in South Africa. The proportion of fruit set was arcsine square-root transformed.

a

b

Fig. 2. Relationship between the number of flowering *Senna didymobo-trya* shrubs in the population of origin and (a) stem volume and (b) total leaf area of offspring of shrubs of *S. didymobotrya* in its invasive range in South Africa.

also an important pollinator in the native range of *S. didymobotrya*. Dulberger (1981) found that in introduced populations of *S. didymobotrya* in Israel, *X. pubescens* is the main pollinator, indicating that although *S. didymobotrya* has a specialized pollination system, it does not depend on a single pollinator species for pollination.

Pollen receipt and fruit set—The high proportion (89.7%) of open-pollinated flowers containing pollen grains in their stigmatic cavities indicates that invasive populations of *S. didymobotrya* in the study area are frequently visited by pollinators. However, the number of pollen grains in the stigmatic cavities correlated positively with fruit set on the same shrub, indicating that fruit set on some shrubs may be pollen limited. On the other hand, overall fruit set was much lower than the number of flowers with pollen grains in their stigmatic cavi-
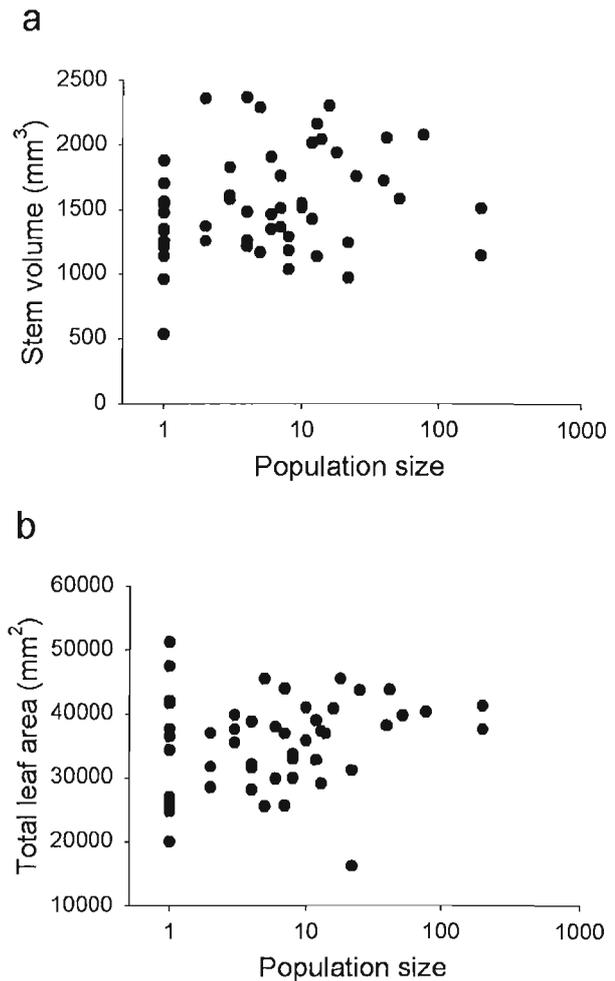
ties. This indicates that pollen did not always succeed in fertilizing the ovules or that a large proportion of pistils with fertilized ovules were aborted by the plant. A reason for the latter might be resource limitation (Stephenson, 1981). Two closely related species of *S. didymobotrya*, *Cassia grandis*, and *C. fasciculata*, also have relatively low proportions of natural fruit set, and in both species it increased with resource availability (Janzen, 1971; Lee and Bazzaz, 1982). The occurrence of most large populations of *S. didymobotrya* in river bank areas (M. van Kleunen, personal observation) suggests that a potential limiting resource in our study area might be the availability of water. Seasonal differences in precipitation might therefore also explain the significant increase in fruit set between both census dates in our study (the earlier one being in the drier winter months).

Allee effects—Both pollen receipt and natural fruit set were not affected by the presence and number of other flowering *S. didymobotrya* shrubs in the population, indicating that pollen

limitation is not affected by population size. This implies that solitary shrubs and small populations of *S. didymobotrya* do not suffer from an ecological Allee effect through impaired pollinator visitation. A possible explanation for the absence of such an Allee effect might be that the pollinators are attracted by other flowering species in the *S. didymobotrya* populations and that pollinator abundance is therefore not determined by population size of *S. didymobotrya*. This, however, is an unlikely explanation because we did not notice carpenter bees visiting other plant species in our study populations, nor did we detect foreign pollen in the stigmatic cavities of *S. didymobotrya*. A more likely explanation is that as a consequence of their numerous large yellow inflorescences, even individual shrubs of *S. didymobotrya* are conspicuous and highly rewarding elements in the landscape. This might explain why even highly isolated shrubs were frequently visited by carpenter bees (M. van Kleunen, personal observation), resulting in high pollen receipt of flowers.

The fact that solitary shrubs and shrubs in small populations did not have decreased pollen receipt rates or fruit set, although they must have fewer available mates, suggests that they may have high rates of geitonogamous and pollinator-mediated autogamous selfing. This may result in a genetic Allee effect when there is inbreeding depression. We found only a tendency for a lower stem volume for offspring of isolated shrubs and a lower leaf area for the ones from shrubs in small populations, but these effects were not significant even though we corrected for maternal environmental carry-over effects by including seed mass as a covariable in the analyses. Therefore, we conclude that genetic Allee effects are absent or very small in magnitude for *S. didymobotrya* in its invasive range in South Africa.

Although Allee effects may have important consequences both for further expansion (Lewis and Kareiva, 1993; Veit and Lewis, 1996) and management aimed at eradication (Liebhold and Bascompte, 2003) of invasive species, other empirical studies on Allee effects in alien invasive plants are rare. Recently, Cappuccino (2004) studied Allee effects in experimental plots of the vine *Vincetoxicum rossicum* in its invasive range in Ontario (Canada). In this study, plants in plots with a larger number of *V. rossicum* individuals produced more biomass and follicles, but pollinator visitation rates were not affected by the number of individuals per plot. The author suggests that this ecological Allee effect was caused by reduced interspecific competition in plots with more *V. rossicum* plants. In another recent study, Davis et al. (2004a) found that the wind-pollinated grass *Spartina alterniflora* in its invasive range along the coast of Washington (USA) has lower seed set and germination rates when clones are relatively isolated than when they are part of nearby *S. alterniflora* meadows. In a subsequent study, Davis et al. (2004b) showed that the reduced seed set in relatively isolated clones of *S. alterniflora* is a consequence of pollen limitation. Although these recent studies show that alien invasive plants may suffer from Allee effects, the nature of the Allee effects differ and there are too few data to make generalizations.

Conclusions—Our study shows that even isolated plants of *S. didymobotra* have high levels of pollen receipt, despite their highly specialized pollination syndrome in which buzzing large insects are required to release pollen from the anthers as well as to deposit it into the stigmatic cavities. Pollen receipt, fruit set, number of seeds per fruit, and mass per seed are unrelated to the presence and number of other flowering shrubs in populations, indicating that there are no direct ecological Allee effects mediated through differences in pollinator visitation rates. Moreover, although offspring of solitary shrubs and shrubs in small populations tended to be slightly smaller when grown in a common greenhouse environment, this effect was not significant. Therefore, even though theory predicts lower invasion success for species such as *S. didymobotrya* that have a specialized pollination system and lack autonomous self-fertilization mechanisms (e.g., Baker, 1955, 1967), it is unlikely that the spatial extent or rate of invasion of *S. didymobotrya* in South Africa will be reduced by Allee effects.

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