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ORIGINAL PAPER

Below- and above-ground mutualisms impact two alien brooms in South Africa differently

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Abstract Mutualisms are important determinants of the invasion success of alien plants. If alien plants are specialized in their mutualistic requirements, or need different types of mutualistic partners (e.g., pollinators and seed dispersers), invasion success may be hampered. Many studies only consider one type of mutualistic relationship when examining the interaction ecology of alien plants, but most plants interact with multiple types of mutualists. In this study, we explored the interactions between two alien legumes, the brooms *Genista monspessulana* and *Spartium junceum*, and their associated below-ground (nitrogen-fixing rhizobia) and above-ground (pollinators) mutualists in South Africa. We conducted pollinator observations, breeding system experiments and collected root nodules to identify rhizobia via 16S rDNA sequencing. Both plants species were entirely dependent on pollinators for seed production. *Genista monspessulana* was pollinated largely by *Apis mellifera capensis* and was not pollen limited, while the larger flowers of *S. junceum* were only pollinated by the larger *Xylocopa* bees and was pollen limited. *Genista monspessulana* was nodulated by four distinct *Bradyrhizobium* (Alpha-proteobacteria) strains, while *S. junceum* was nodulated by one *Paraburkholderia* (Beta-proteobacteria) and two *Bradyrhizobium* strains. Low pollination rates in *S. junceum* suggest that its reproduction and natural spread in South Africa may be slow. The wider distribution of *S. junceum*, compared to *G. monspessulana,* thus likely reflects differences in historic propagule pressure between the two species. The role of rhizobia in limiting plant performance is less clear and the connection between nodulation rate and plant fitness requires further study.

Keywords Alien plant invasion · *Bradyrhizobium* · *Genista monspessulana* · Nodulation · Pollination · *Spartium junceum*

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Introduction

Mutualisms such as seed dispersal, nitrogen fixation and pollination are thought to be important factors that govern the invasion dynamics of alien plants

(Traveset and Richardson 2014; Le Roux et al. 2020). These mutualisms can aid (e.g. Levin 1970; Stanton 1987; Geerts and Adedoja 2021), limit (Traveset and Richardson 2020), or prevent (e.g. Nadel et al. 1992; Nunez et al. 2009; Stout et al. 2002) plant invasion. For example, there is no guarantee that introduced alien plants will encounter suitable mutualistic partners in their novel environments (Missed Mutualisms Hypothesis; Traveset and Richardson 2014). Naturally, when mutualistic partners are co-introduced with their alien host plants (e.g. Warrington et al. 2019) invasion is more likely (Hui and Richardson 2017; Le Roux et al. 2017).

Mutualisms span a continuum of interaction specificity and generalist alien plants are expected to readily co-opt new mutualistic partners while specialists are less likely to do so (Bascompte 2009; Wansell et al. 2022). Surprisingly few studies have considered the barriers posed to invasion success of alien plants based on their dependence on multiple mutualistic partners (but see Wolfe et al. 2005). For example, alien woody species often need mycorrhizal fungi or nitrogen-fixing symbionts to establish (Dickie et al. 2010; Nunez et al. 2009). Woody species also more frequently make use of uniparental reproductive strategies than other angiosperms. This strategy, when coupled with autonomous self-pollination, can compensate for pollen limitation (Rambuda and Johnson 2004).

The symbiosis between legumes and nitrogen-fixing rhizobia represents a physiological adaptation that has been linked with the invasiveness of legumes (Rodríguez-Echeverría et al. 2009, 2011). Rhizobia are free-living soil bacteria that form specialized structures called nodules on the roots of most legumes. Within root nodules, rhizobia convert atmospheric di-nitrogen into organic forms usable by the host plant, while receiving carbon-rich photosynthates in return (Spaink 2000). Since rhizobia are generally not known to be transmitted within seeds, they must be obtained from the soil environment in the novel range (Perez-Ramirez et al. 1998). Whilst many legumes are generalists, others are very specific in their rhizobium requirements and fail to form effective mutualisms in the novel range (see for example Parker 2001; Parker et al. 2006; Rodriguez-Echeverria et al. 2009; La Pierre et al. 2017; Le Roux et al. 2017). Thus, rhizobial interactions can influence the invasion success of legumes either by limiting establishment due to a lack of compatible host-symbiont associations in the new range or by facilitating growth in nutrient-poor soils when suitable symbionts are present (Le Roux et al. 2017; Simonsen et al. 2017).

High reproductive output enhances invasion success (Mason et al. 2008; Simberloff 2009; Catford et al. 2011), emphasizing the need for effective reproductive mutualisms by alien species in their new ranges. Around 87.5% of flowering plants require pollinators (Ollerton et al. 2011) and 80% are facultative or obligate out-crossers (also referred to as non-autogamous) (Brown 1990; Goodwillie et al. 2005). Being pollinator dependent thus represents a significant barrier to the establishment of alien plants, as they need to co-opt pollinators in the new range (Moodley et al. 2016). Indeed, outcrossing alien species are more pollen limited than co-occurring natives (Knight et al. 2005), while invasive woody species have higher self-compatibility rates than expected by chance (Rambuda and Johnson 2004). Outcrossing alien plants with generalist flowers are more likely to find reliable pollinators compared with those with specialized flowers (Adedoja et al. 2021; Baker 1974; Goulson and Derwent 2004; Stout et al. 2006). On the other hand, species that are dependent on specialist pollinators are more likely to be pollen limited (Faegri and van der Pijl 1979; Hopkins 1914). The constraints associated with pollinator specialization can be overcome when alien plants form novel pollinator interactions, they are co-introduced with effective alien pollinators or they evolve an increased capacity for autonomous selfing (e.g., Geerts and Pauw 2009; Geerts 2011; Ollerton et al. 2012; Rodger et al. 2010; Stout et al. 2002).

Here we investigated the mutualist interactions associated with resource acquisition (rhizobia) and reproduction (pollination) of the two alien legume (Fabaceae) species, *Genista monspessulana* and *Spartium junceum*, in South Africa. It has been suggested that poor performance of *S. junceum* in South Africa compared to its the native range (based on seed set) is related to ineffective rhizobium and pollinator interactions, in line with the Missed Mutualisms Hypothesis (Geerts et al. 2013; Le Roux et al. 2020). Both *G. monspessulana* and *S. junceum* form mutualistic relationships with rhizobia and struggle in nutrient poor soils in the absence of compatible rhizobia (Gonzalez-Andres and Ortiz 1999). Even though *G.*

monspessulana and *S. junceum* nodulate with a broad range of rhizobial strains under greenhouse conditions, in their alien range in US they do not associate with resident native rhizobia (La Pierre et al. 2017). This aligns with growing evidence that shows invasive legumes often utilize rhizobium communities that differ from those of native legumes (Lafay and Burdon 2006; Rodriguez-Echeverria 2010; Weir et al. 2004). Furthermore, both *G. monspessulana* and *S. junceum* are considered widespread invaders in several countries, despite suboptimal seed production in some of these countries (Parker and Haubensak 2002; Paynter et al. 2010; Simpson et al. 2005). In South Africa, both species have been introduced over a century ago but remain restricted in their distribution to small populations, mostly confined to disturbed sites, with limited spread into undisturbed vegetation (Geerts et al. 2013). Whilst residence time and climatic suitability have been ruled out as potential reasons for their limited spread in South Africa (Geerts et al. 2013), the role of mutualisms remains unknown. To test whether the Missed Mutualisms Hypothesis holds true for *G. monspessulana* and *S. junceum* in South Africa, we ask whether (1) pollinator and (2) rhizobium mutualisms are potentially limiting the invasion success of these two legumes.

Methods

Study species

Genista monspessulana has a large native range, including southern Europe, northwest Africa and temperate western Asia (GRIN 2011). The species is a major invader in California (Cal-IPC 2006) and is listed as a weed of national significance in Australia (Paynter et al. 2003). It is also invasive and widespread in New Zealand and Chile (Parsons and Cuthbertson 2001; Pauchard et al. 2008). *Spartium junceum* is native to the southern Mediterranean region of Europe, North Africa, the Canary Islands and temperate west Asia (GRIN 2011). The species is a prohibited pest plant in parts of Australia (Thorp and Wilson 1998) and is invasive in the California and the Pacific Northwest of the USA, Chile, Hawaii, and Peru (Bossard 2000; Castro et al. 2005; DiTomaso and Healy 2007; Ochoa and Antrade 2003). Both species have been introduced into several other countries where they are not considered to be major invaders, such as Bolivia, Colombia, Ecuador and South Africa (GBIF 2011; Henderson 2006; ILDIS 2011).

Genista monspessulana and *S. junceum* are perennial shrubs that grow up to 3–5 m tall. They flower profusely during spring and summer with typical yellow zygomorphic legume flowers. Seed pods burst open to disperse seeds 3–4 m from parent plants, with secondary seed dispersal by ants and water (Adams and Simmons 1991; Bossard 2000; Oliveras et al. 2007; Parsons and Cuthbertson 2001). Mature *G. monspessulana* plants annually produce ~ 1200 seeds in California, > 35,000 in Australia and ~ 2500 in the native range (Herrera et al. 2011). *Spartium junceum* plants produce between 7000 and 10,000 seeds per plant annually (Bossard et al. 2000).

Incapable of autogamous selfing, and with a specialized pollination system known as "trip pollination", *G. monspessulana* and *S. junceum* potentially rely on specific pollinators for reproduction (Cordoba and Cocucci 2011). Trip pollination occurs when the fused keel petals of the zygomorphic flowers are pushed down by pollinators heavy enough to split the petals and release the reproductive parts of the flower (Westerkamp 1992). The larger flowers of *S. junceum* require much more force, and thus heavier pollinators, to trip the flowers compared with the smaller flowers of *G. monspessulana* (Parker and Haubensak 2002; Parker et al. 2002). *Spartium junceum*'s flowers are therefore considered to be more specialized for trip pollination than those of *G. monspessulana* (Galloni et al. 2008). Once tripped, a flower remains tripped and is easily distinguished from untripped flowers.

In its native range, *S. junceum* flowers are tripped and pollinated by large *Xylocopa* bees (Galloni et al. 2008) such as *X. violacea* (Galloni and Cristofolini 2003). *Genista monspessulana* is probably pollinated by honeybees and solitary bees in its native range, but this has not been formally documented. In its invasive range in Argentina, *S. junceum* is pollinated by *Megachile* spp., *Bombus bellicosus* and *X. augusti* (Cordoba and Cocucci, 2011) while *G. monspessulana* in its invasive range in California is pollinated by *Apis mellifera* and *B. vosnesenskii* (Parker et al. 2002).

There is little data to determine whether the *Bradyrhizobium* strains associated with invasive *S. junceum* and *G. monspessulana* are related to those nodulating other broom species in native Europe. La

Pierre et al. (2017) suggested that the *Bradyrhizobium* strains associated with *S. junceum* (Quatrini et al. 2009) and *G. monspessulana* in their native ranges are closely related to those that nodulate these species in their alien range in the US.

Study sites

Spartium junceum is widely distributed in South Africa but in small populations, mostly found along roadsides (Fig. 1a), and is the most abundant in the Fynbos biome (Geerts et al. 2013). We conducted breeding system experiments for this species at two sites and pollinator observations at six sites (Appendix 1). Root nodules were collected from three sites as plants at other sites had no nodules (Appendix 1).

Genista monspessulana only occurs in the Fynbos biome and is mostly found in disturbed areas (Geerts et al. 2013). We conducted breeding system experiments at one site and pollinator observations at two sites for this species (Appendix Table 3). Root nodules for this species were collected at four sites (Appendix Table 3). All plants targeted for these collections had root nodules.

Pollinators and floral "tripping" rates

The identities of pollinator species, number of flowers visited, and pollinator behavior were recorded during the spring of 2012. Pollinator observations were conducted when pollinators were most active, i.e. late morning till early afternoon, and for most sites were done once (Appendix 1). During these observations

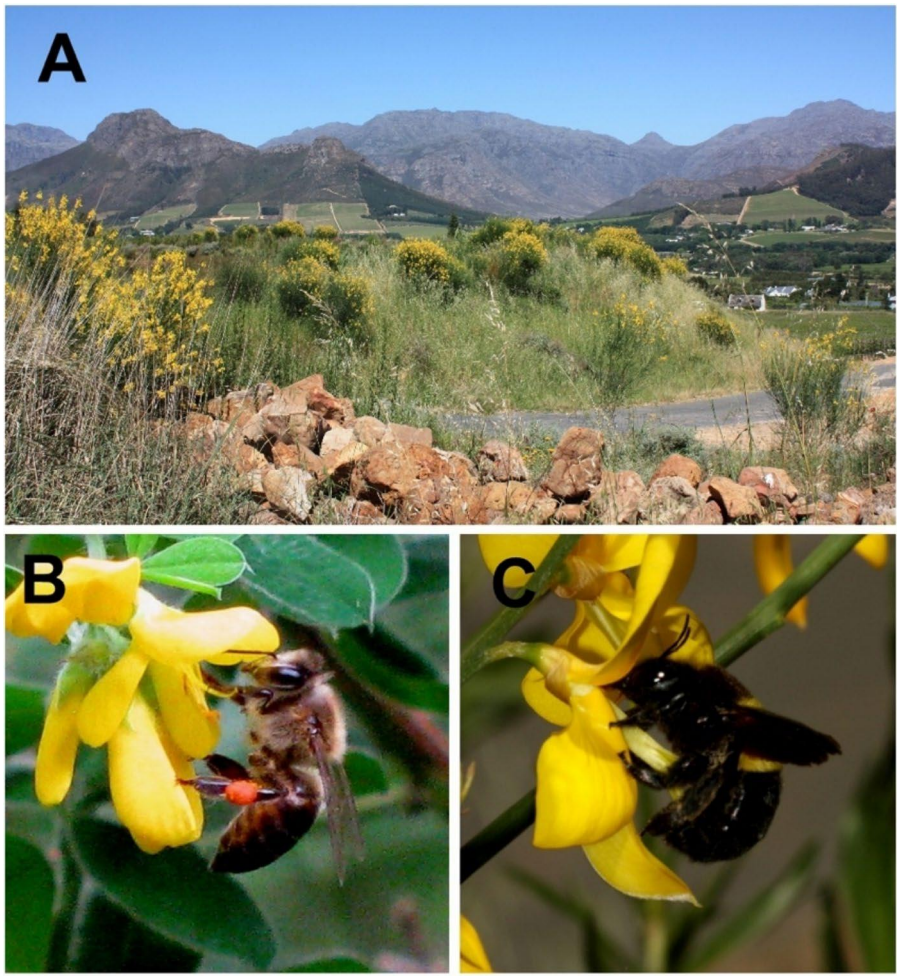

Fig. 1 **a** Typical *Spartium junceum* habitat in South Africa; **A** few plants scattered along a disturbed road side. **B** *Apis mellifera* bee tripping and pollinating a *Genista monspessulana* flower. **C** The larger and heavier *Xylocopa caffra* (carpenter bee) tripping and pollinating a *S. junceum* flower (Photographs by Sjirk Geerts and Ethan Newman)

several other plant species pollinated by honeybees were in flower in the vicinity of *G. monspessulana.* Similarly, native plants known to be pollinated by *Xylocopa* bees, such as *Psoralea* and *Virgilia* spp., occurred near *S. junceum* individuals. *Spartium junceum* was observed for a total of 23 h (six populations) and *G. monspessulana* for six hours (three populations; Appendix 1). Inclement weather was avoided. At each site, all flowers from multiple plants that were clearly visible from the chosen observation point were counted and visitation to these flowers recorded. Pollinator behavior was scored as legitimate (i.e., pollinators tripping flowers and touching reproductive parts of the flower) and illegitimate (i.e., pollinators unable to trip flowers, or visiting flowers that were already tripped). Visitation frequencies were calculated as the number of pollinator visits per flower per hour.

To corroborate observed pollinator visitation rates, floral tripping rates were scored as a snapshot in time of pollinator activity. Tripping rates for each species were determined early in the morning for at least 200 haphazardly selected flowers per site on a given day (range 200–1500 flowers). Sites were not all visited on the same day, but over the peak flowering period for both species in 2012.

Plant breeding system experiments

To establish the importance of pollinators in the seed production of *G. monspessulana* and *S. junceum*, branches bearing flowers were enclosed in fine-mesh pollinator exclusion bags whilst in the bud phase. Branches were kept bagged throughout the flowering period. Bagged flowers were counted and subjected to one of three treatments: (1) tripping of flowers and the addition of outcross pollen by hand, (2) tripping of flowers and the addition of self-pollen (different flower on the same plant, i.e., geitonogamy) by hand, and (3) untripped flowers (i.e., autonomous seed production). Pollen donor plants used for outcrossing were at least 10 m away from recipient plants. Flowers on a nearby unbagged branch on each of the experimental plants were assigned to two further treatments: (1) flowers left open to pollinators to determine natural levels of pollination and pod and seed production and (2) the addition of outcross pollen to naturally pollinated flowers to determine whether flowers are pollen limited.

After flowering, pods and seeds were harvested and counted. It has been argued that the reallocation of resources between branches on a plant may result in pollen limitation on a specific branch, even when the plant is limited by other resources rather than by pollination (Stephenson 1981; Zimmerman and Pyke 1988). However, in a closely related species, the Scotch broom (*Cytisus scoparius*), no evidence for resource reallocation was found (Parker 1997) and therefore this was not considered to impact our results.

Rhizobium collection and phylogenetic analysis

Up to ten individuals of *G. monspessulana* or *S. junceum* were carefully excavated at each site to collect root nodules. The root nodules were placed and stored on silica gel. In the laboratory, root nodules were rehydrated using distilled water, surface sterilized, and rhizobia isolated and cultured following protocols described by Somasegaran and Hoben (2012). Rhizobial colonies were recovered on yeast extract-mannitol (YEM) agar plates supplemented with Congo red (Vincent 1970) at 28 °C. Gram-staining was carried out using the Gram's staining kit (Sigma Aldrich) according to the manufacturer's specifications.

Single pure colonies of isolated rhizobia were picked, lysed in boiling water and used as PCR templates (i.e. colony PCRs). The 16S rRNA gene was amplified and fragments were sequenced using the primers E9F (Farelly et al. 1995), 341F (Muyzer et al. 1993), 536R and 907F (Lane et al. 1985), 1100R (Lane 1991) and 1512R (Weisburg et al. 1991). Each 50 µL PCR reaction contained approximately 50 ng of genomic DNA, 200 µM of each dNTP (AB gene, supplied by Southern Cross Biotechnologies, Cape Town, South Africa), 25 pmol of each primer, 5 U Taq DNA polymerase (SuperTherm JMR-801, Southern Cross Biotechnologies, Cape Town, South Africa), 1×PCR reaction buffer, 1.5 mM $MgCl_2$. PCR followed a cycle of initial denaturation of 95 °C for 5 min; 35 cycles at denaturation at 94 °C for 30 s, annealing at 58 °C for 60 s, elongation at 72 °C for 90 s; and final extension at 72 °C for 10 min. PCR products were purified using the

QIAquick PCR Purification Kit (Qiagen, supplied by Whitehead Scientific, Cape Town, South Africa) and sequenced using the ABI PRISM Big Dye Terminator Cycle Sequencing Ready Reaction kit and an automated ABI PRISM 377XL DNA sequencer (PE Applied Biosystems, Foster City, California, USA). DNA sequencing data were deposited in the Genbank online repository (accession numbers PV266733-PV266770).

16S rDNA sequences for rhizobia associated with *G. monspessulana* and *S. junceum* were compared to 16S rRNA gene sequences available from GenBank using BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi). A multiple sequence alignment of selected reference rhizobium strains (obtained from above GenBank search and our isolates was created using CLUSTALX (Thompson et al. 1997) as implemented in BioEdit (Hall 1999). We reconstructed a phylogeny based on Bayesian search criteria as implemented in the MrBayes program (Ronquist and Huelsenbeck 2003) and the GTR+I model based on Akaike scores obtained from jModeltest version 2.1.4 (Darriba et al. 2012). Topological support for the tree was inferred as posterior probabilities.

Statistical analyses

Floral tripping rates between sites were analyzed using one-way ANOVAs. Two measures of plant fitness were determined: percentage of flowers producing pods and the number of seeds produced per pod. Percentage pod production was determined by dividing the number of pods produced by the total number of flowers (flowers not producing pods, leave a scar on the stem). To compare the proportion of flowers that produced pods between the five treatments (i.e. (1) outcross pollen, (2) geitonogamy, (3) autonomous, (4) control and, (5) pollen addition) a generalized linear model (GLM) with a binomial error structure and a logit link function was used. Chi-squared tests were used to compare generalized linear models with and without treatment as a factor. The proportion fruit set was the response variable, and the reproductive treatments the predictor variable. The seed set data were checked for normality with residual and Q-Q plots and analyzed using a generalized linear model (GLM) with Poisson error structure and a log link function. Over-dispersion of the GLMs was then checked using the function *dispersiontest* of the *AER* R package (Kleiber and Zeileis 2008). All statistical analyses were conducted in R (R Development Core Team 2019).

Results

Floral "tripping" rates and pollinators

Floral tripping rates for *G. monspessulana* ranged between 82 and 93% and were similar between sites (ANOVA, $F_{2,\ 37}=3.11$, $P>0.05$). Four pollinator taxa visited and tripped the flowers of this species: Cape honeybees, *Apis mellifera* subsp. *capensis*; Carpenter bee species, *Xylocopa capitata* and *X. caffra*; and Colletidae bees. Cape honeybees (Fig. 1b) and the Colletidae bees were the most frequent visitors (Table 1).

Floral tripping rates for *S. junceum* varied between 63 and 85%, with only one site (Delheim) having significantly lower tripping rates (14%) compared to other sites (ANOVA, $F_{5,\ 32}=41.29$, $P<0.001$). This site also had the lowest pollinator visitation rate (Table 1). Three *Xylocopa* species (*X. capitata, X. caffra and X. rufitarsus*) were the only pollinators observed, all of which were heavy enough to trip flowers (Table 1). *Xylocopa caffra* (Fig. 1c) was observed at all sites and was the most abundant pollinator. *Xylocopa* bees were observed to do searching flights and inspections of flowers and to visit fewer tripped than untripped flowers (Table 1). A few other potential pollinators (a Halactid bee, *Pieris brassicae* and *Pachnoda sinuate*) were observed attempting to gain access to nectar occasionally (<0.0003 visits/flower/hour in all cases), but these species were unable to trip the flowers. Cape honeybees were present at all sites but were never observed to visit *S. junceum* flowers.

Breeding system

Seed pod production by *G. monspessulana* differed significantly between treatments ($P<0.001$ from a Chi-squared test comparing generalized linear models with and without treatment as a factor; Fig. 2a, Table S1). Specifically, pollinator exclusion led to no pod production while open hand-pollinated flowers had similar seed pod production to naturally pollinated flowers, indicating no pollen limitation for the species (Fig. 2a, Table S1). There was no difference

Table 1 Pollinator species and visitation rate to *Genista monspessulana* and *Spartium junceum* across their distribution range in the Cape Floristic Region of South Africa

Site	Visitor species	Visits/flower/hour all flowers	Visits/flower/hour untripped flowers
Genista monspessulana			
Op-die-Berg	Family Colletidae	0.3400	
	Xylocopa capitata	0.0400	
	Total	**0.3800**	
Newlands forest	*Apis mellifera*	0.0500	
	Total	**0.0500**	
Constantia	*A. mellifera*	0.0100	
	X. caffra	0.0026	
	Total	**0.0126**	
Spartium junceum			
Delheim road Stellenbosch	*X. caffra*	0.0110	0.0110
Paarl Charleston Hill rd	*X. caffra*	0.0213	0.0311
	X. capitata	0.0067	0.0036
	Total	**0.0280**	**0.0347**
Somerset West Avontuur	*X. caffra*	0.0389	0.0925
	X. capitata	0.0009	0.0019
	Total	**0.0412**	**0.0991**
Franschoek Le Pineta	*X. rufitarsus*	0.0023	0.0127
	X.caffra	0.0094	0.0444
	X. capitata	0.0009	0.0032
	Total	**0.0126**	**0.0720**
Stellenbosch	*X. rufitarsus*	0.0090	0.0582
	X. caffra	0.0450	0.2908
	X. capitata	0.0160	0.0162
	Total	**0.0700**	**0.3651**
Stellenbosch Uniepark	*X. caffra*	0.0333	0.0333

For *S. junceum* untripped flowers are included, for *G. monspessulana* this was not possible since honeybees sometimes trip flowers still in bud, which is not possible to observe without touching the bud/flower

in seed production among all treatments except for natural pollinated flowers that produced significantly more seeds per pod than all other treatments ($P < 0.001$ from a Chi-squared test comparing generalized linear models with and without treatment as a factor; Table 2; Fig. S1).

Seed pod production by *S. junceu*m differed significantly between treatments ($P < 0.001$ from a Chi-squared test comparing generalized linear models with and without treatment as a factor; Fig. 2b, Table S2). Specifically, pollinator excluded flowers did not produce seed pods and open hand-pollinated flowers produced significantly more pods than naturally pollinated flowers (Table 2; Fig. 2b), indicating pollinator limitation. Seed production did not differ significantly between treatments for this species ($P = 0.3$ from a Chi-squared test comparing generalized linear models with and without treatment as a factor; Table 2; Fig. S2). When combining pod and seed production, hand-pollinated flowers of *S. junceum* produced an average of 5.5 seeds per pod (505 seeds from 92 flowers) compared to the 3.5 seeds per pod (3011 seeds from 849 flowers) for naturally pollinated flowers.

Rhizobium collection and phylogenetic analysis

We observed marked difference in the nodulation of *G. monspessulana* and *S. junceum* in the field. *Genista monspessulana* plants were heavily nodulated at all sites and we counted 50 or more nodules from five plants/site. *Spartium junceum* had low nodulation with no plants producing nodules at four sites. At two sites we counted 70 nodules (from 10 plants) for this species. We isolated pure rhizobium colonies for 30

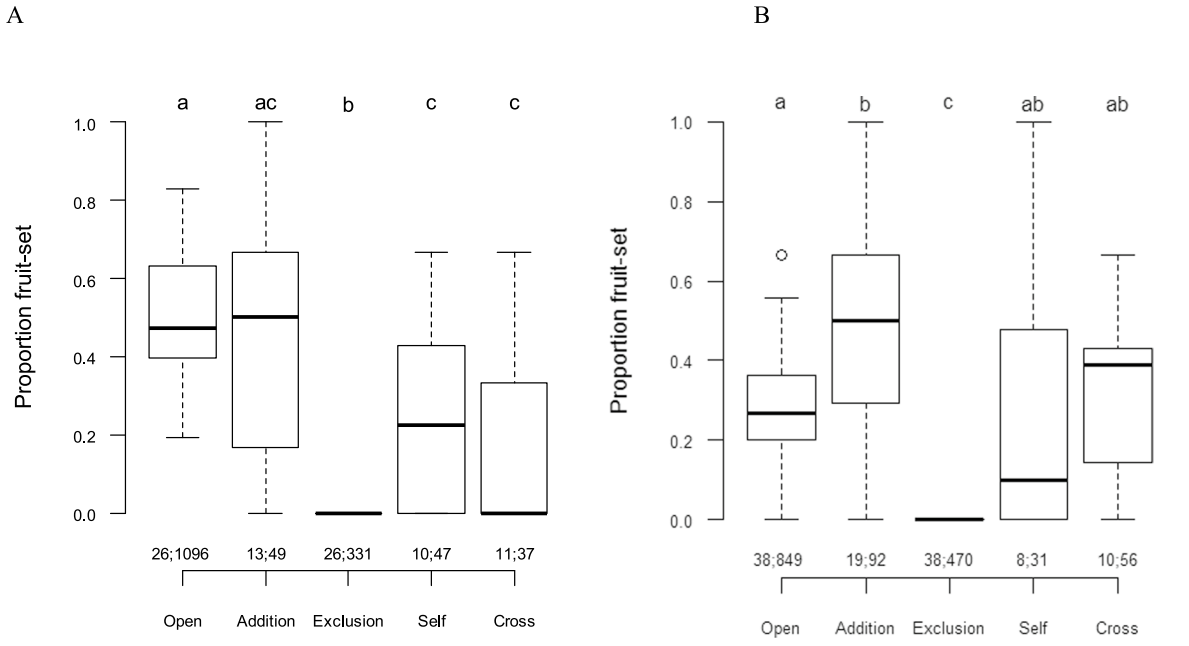

Fig. 2 Pod set (*i.e.* proportion of flowers producing fruit) comparison for **A** *Genista monspessulana* and **B** *Spartium junceum* for the different pollination treatments (letters above bars indicate significant differences between treatments). Numbers below bars indicate the number of plants and the number of flowers per treatment. Bold lines indicate the median and boxes show the quartile ranges. The dotted lines show either 1.5 times the interquartile range or the point furthest from the median, whichever is less

and eight root nodules from *G. monspessulana* and *S. junceum*, respectively.

Our DNA sequence alignment consisted of 1363 characters, including 18 gaps (indels) of between 1 and 25 bp. All rhizobia isolated from *G. monspessulana*, and most isolated from *S. junceum*, were identified as *Bradyrhizobium* species. Seven *G. monspessulana* isolates (i.e. isolates FBRM1-7) shared 100% DNA sequence similarity with *B. huanghuaihaiense*, while two (isolates FBRM12, 18) were closely related to *B. yuanmingense* (98.3% DNA sequence similarity; Fig. 3). The remaining *G. monspessulana* strain (i.e. isolates FBRM8-11; FBRM13-17 and FBRM19-30) did not show high relatedness to any *Bradyrhizobium* reference strains included in our analysis (Fig. 3). Two isolates of this strain were also isolated from *S. junceum* (i.e. isolates SBRM1, 2). For *S. junceum*, one strain (i.e. isolates SBRM4, 6, 7) were closely related to *B. canariense* and another (isolates SBRM5, 8) were identified a beta-rhizobium species from the genus *Paraburkholderia* (Fig. 3).

Discussion

In support of the Missed Mutualisms Hypothesis, we found that the invasion success of a historical widely used alien garden plant in South Africa, *Spartium junceum,* is potentially hampered by pollen limitation and suboptimal nodulation. Coupled with regular wildfires in the country's Fynbos biome, our findings may explain why this species has not spread extensively and formed dense thickets as has been observed elsewhere in the world (Geerts 2021). Even though the species is found over a large geographic area in South Africa, its populations are typically small and restricted to disturbed roadsides and it is not invading natural vegetation. In contrast, we found that *G. monspessulana* in South Africa is not pollen limited and able to nodulate profusely with four distinct *Bradyrhizobium* lineages. The localized distribution of this species in South Africa likely relates to it not having been traded or utilized as a horticultural plant (S. Geerts, pers. obs.) and ongoing control efforts to prevent its further spread (Geerts et al. 2013; Nsikani and Geerts 2024).

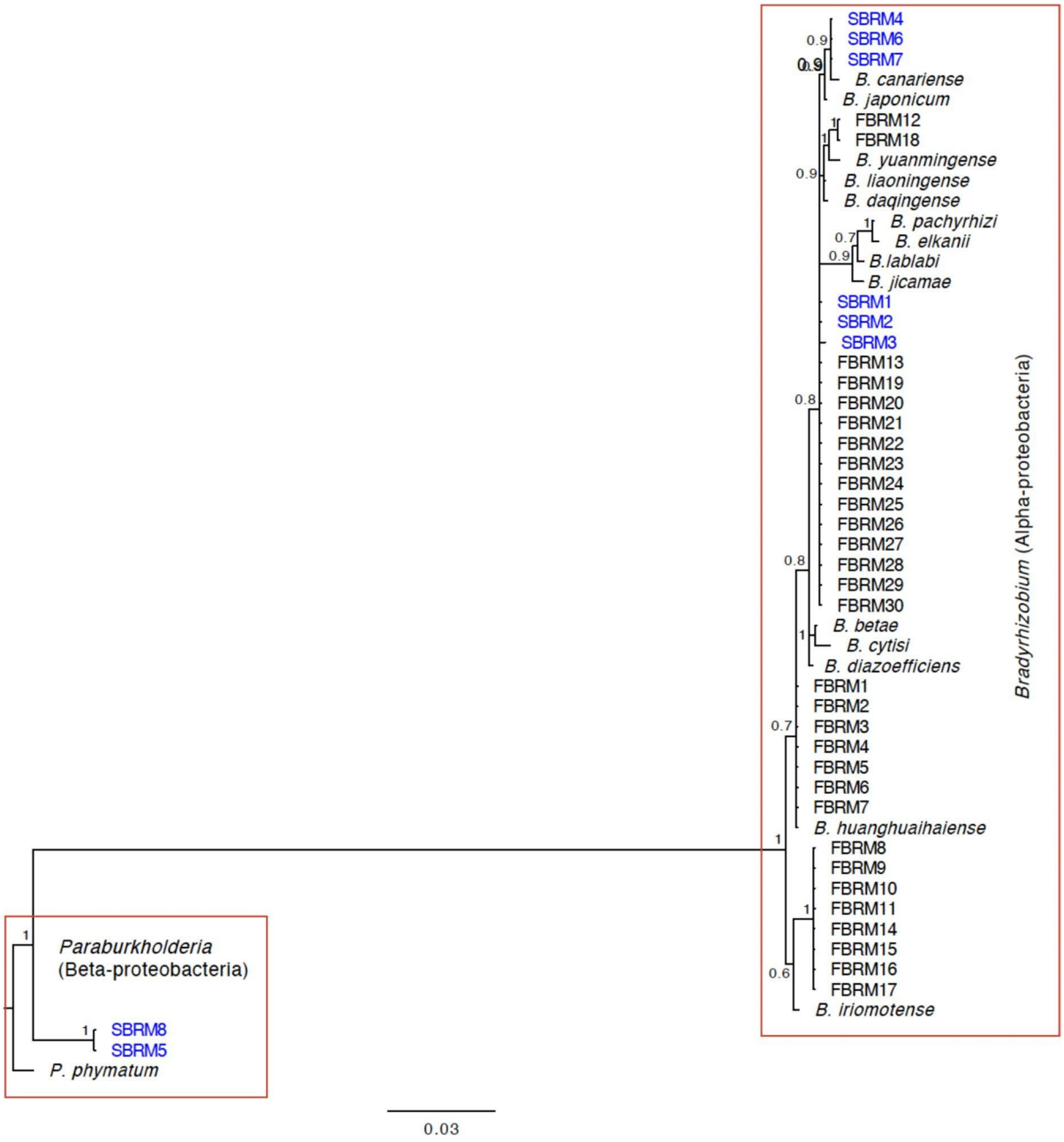

Fig. 3 Maximum likelihood phylogeny of representative members of the genus *Bradyrhizobium* and root nodule rhizobium isolates from *Genista monspessulana* and *Spartium junceum*. Tip labels starting with "SBRM" (in blue font) correspond to rhizobia isolated from *S. junceum* while those starting with "FBRM" (in black font) correspond to rhizobia isolated from *G. monspessulana*

The role of pollinators

Spartium junceum is dependent on a specific functional group of pollinators, i.e. large bees, and attracts *Xylocopa* bees as reliable pollinators in South Africa. Honeybees were abundant at all study sites but were never observed pollinating this species. Native legumes with large yellow flowers,

Table 2 Pod production (*i.e.* proportion of flowers producing fruit) and average number of seeds produced per pod across five treatments (i.e. (1) natural levels of pollination (2) pollinator exclusion, i.e. autonomous, (3) pollen addition via hand pollination to flowers open to pollinators, (4) hand cross pollination between different individuals, and, (5) hand self-pollination within an individual plant, i.e. geitonogamy) for *Genista monspessulana* and *Spartium junceum*

Treatment	Proportion pods produced per flower. Mean (range) (n)		Number of seeds per pod. Mean (range) (n)	
	G. monspessulana	*S. junceum*	*G. monspessulana*	*S. junceum*
Natural	0.50 (0.19–0.83)[a] (26;1096)	0.30 (0–0.67)[a] (38;849)	5.87 (3–8)[a] (26;260)	11.91 (3–19)[a] (37;221)
Autonomous	0 (0–0)[b] (26;331)	0 (0–0)[c] (38;470)	N/A	N/A
Pollen addition	0.44 (0–1)[ac] (13;49)	0.48 (0–1)[b] (19;92)	4.53 (3–7)[b] (10;19)	12.63 (7–19)[a] (18;40)
Cross	0.17 (0–0.67)[c] (11;37)	0.33 (0–0.67)[ab] (10;56)	4.14 (3–5)[b] (5;7)	10.90 (7–14)[a] (8;21)
Self	0.27 (0–0.67)[c] (10;47)	0.27 (0–1)[ab] (8;31)	3.91 (3–5)[b] (7;11)	12.56 (8–16)[a] (4;9)

Sample sizes (n) represent the number of plants and the number of flowers for each treatment. Superscript letters indicate significant differences between treatments within a species. See Tables S1 and S2 for detailed statistical analyses

such as *Cyclopia* spp., are also exclusively pollinated by *Xylocopa* bees (Shaw-Bonner et al. 2023). The large flowers of *S. junceum* are hard to trip, requiring heavy pollinators (Parker and Haubensak 2002; Parker et al. 2002). This, together with our observations that *Xylocopa* bees are less abundant than honeybees, probably explains why *S. junceum* is pollen limited in South Africa. These findings agree with previous studies on the species in other invaded regions also suggesting that pollen limitation, and thus seed set, might slow down invasion by this species (Parker and Haubensak 2002; Alexander and D'Antonio 2003). This contrasts with invasive legumes that usually have generalist flowers and that are rarely pollinator limited (e.g., Gibson et al. 2011; Geerts et al. 2016).

Genista monspessulana is pollen limited in its alien range in California despite attracting a range of pollinators such as *Bombus* spp., *Xylocopa* spp. and honeybees (Parker et al. 2002; Parker and Haubensak 2002). We found this to not be the case in South Africa, likely because of the abundance of native honeybees that are widely used in agriculture in the country. Both *G. monspessulana* and *S. junceum* in South Africa are highly dependent on pollinators for reproduction, with no pods produced when pollinators were excluded. Our geitonogamy hand pollination treatment led to the production of seeds, which is indicative of the ability of a single plant to reproduce in the presence of pollinators. Future research should consider the importance of beekeeping in enhancing the invasion success of *G. monspessulana*, since brooms flower in spring when honeybees are abundant in agricultural areas.

The role of nitrogen-fixing rhizobia

Both *Genista monspessulana* and *S. junceum* are nodulated by *Bradyrhizobium* strains in their native (Gonzales-Andres et al. 1999; Cardinale et al. 2008) and invasive (La Pierre et al. 2017) ranges. *Bradyrhizobium* belongs to the Alphaproteobacteria. The genus has a cosmopolitan distribution and nodulates a diversity of legumes (Lafay et al. 2006). While *Bradyrhizobium* is not commonly associated with native Fynbos legumes (Lemaire et al. 2015), some native (e.g. Avontuur et al. 2022) and invasive legumes (e.g. Warrington et al. 2019; Ndlovu et al. 2013) nodulate with members of this genus in South Africa. Most Fynbos legumes studied to date, however, nodulate with *Mesorhizobium* (Alpha-proteobacteria) or *Paraburkholderia* (formerly *Burkholderia*; Beta-proteobacteria) (Beukes et al. 2021; Mavima et al. 2022). Our findings suggest that *S. junceum* is possibly utilizing native Fynbos *Parabulkholderia* which is, to our knowledge, the first record of this species in association with beta-rhizobia.

In agreement with our findings, La Pierre et al. (2017) found *G. monspessulana* to nodulate with more diverse rhizobia in the US compared to *S. junceum*. Taken together, this suggests that *G. monspessulana* is more promiscuous in its nitrogen-fixing symbiont requirements than *S. junceum*. Quatrini et al (2002) found that native *S. junceum* is nodulated by rhizobia related to the *Bradyrhizobium japonicum* and *Bradyrhizobium liaoningense*. A lack of compatible *Bradyrhizobium* has also been linked to limited invasion success of broom species such as *Cytisus scoparius* in the US(Parker et al. 2006). Coupled with our observations of low nodulation by *S. junceum* in the field, it is conceivable that this species is tapping into any available rhizobium species, even when these are not the most effective symbionts for the species, as has been observed for other invasive legumes (e.g. see Shelby et al. 2016; La Pierre et al. 2017). Future work should test whether low levels of nodulation correspond to low plant growth (e.g., Le Roux et al. 2018) by comparing biological nitrogen fixation and its associated fitness consequences (e.g. vigor and seed set) for both legumes when associating with diverse South African rhizobia under controlled conditions (i.e. inoculation trials) or in the field. The latter was not possible in our field studies given that our breeding system experiments were only conducted at two sites and rhizobia were only collected at one of these.

In conclusion, low pollination rates in *S. junceum* in South Africa may impede its invasiveness. The role of rhizobia in limiting the performance of this species in the novel range, however, requires further investigation. The effects of these limiting factors could potentially be overcome through the evolution of growth and reproductive strategies that are less reliant on mutualists, the adoption of new mutualistic partners or a change in resource allocation (Moles et al. 2022). In contrast, *G. monspessulana* is not pollinator limited and nodulates profusely with a diverse set of rhizobia in South Africa. Thus, this species' restricted distribution in South Africa is likely due to its low introduction effort (i.e. low propagule pressure) and dispersal ability. Further research to establish whether this species has the potential to become a widespread and major invader in South Africa is warranted (Geerts et al. 2013).

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Declarations

Competing interests The authors have no relevant financial or non-financial interests to disclose.

Appendix 1

Table 3 Pollinator observation, breeding system experiments and rhizobia collection localities for *Genista monspessulana* and *Spartium junceum*

Site	Locality	Pollinator observations	Pollinator observation hours	Tripping rate (%)	Breeding system experiments	Rhizobia collection
Genista monspessulana						
Op-die-Berg	S33.02398 E19.31113	06–11-2012	1	86	No	Yes
Newlands forest	S33.97116 E18.44734	13–11-2012	3	82	No	No
Constantia (cellar)	S34.03826 E18.41222	04–11-2012	2	93	Yes	Yes
Tokai Manor	S34.05969 E18.41563	N/A		N/A	No	Yes
Tokai	S34.05968 E18.41872	N/A		N/A	No	Yes
Spartium junceum						
Stellenbosch (Delheim road)	S33.87581 E18.85943	26–11-2012	4.5	14	No	No
Paarl (Charleston Hill)	S33.72662 E18.98191	02–11-2012	5	82	No	No
Somerset West (Avontuur)	S34.02819 E18.82085	05–11-2012	5	63	No	No
Franschhoek	S33.91697 E19.13611	09–11-2012	4.5	82	No	No
Stellenbosch (Agricultural Research Council)	S33.92770 E18.87243	20–10-2011,07–11-2011	2	82	Yes	No
Stellenbosch (Uniepark)	S33.92711 E18.89050	20–10-2011	2	85	Yes	Yes
Uitkamp wetland Durbanville	S33.82215 E18.63659	N/A		N/A	No	Yes
Tygervalley road	S33.84318 E18.62534	N/A		N/A	No	Yes

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