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Egbon, IN, Paterson, ID, Compton, S orcid.org/0000-0002-1247-8058 et al. (1 more author) (2020) Evolution of growth traits in invasive Pereskia aculeata (Cactaceae): testing the EICA hypothesis using its specialist herbivore, Catorhintha schaffneri (Coreidae). Pest Management Science, 76 (12). pp. 4046-4056. ISSN 1526-498X

https://doi.org/10.1002/ps.5959

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Evolution of growth traits in invasive *Pereskia aculeata* (Cactaceae): testing the EICA hypothesis using its specialist herbivore, *Catorhintha schaffneri* (Coreidae)

Running title: **Evolution of growth traits in invasive *Pereskia aculeata***

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ABSTRACT

BACKGROUND: Species introduced into new habitats are fitter than their native populations as hypothesised by the 'Evolution of Increased Competitive Ability' (EICA). Here *Pereskia aculeata* Miller was used as a model to test EICA and explore how 'enemy release' may have influenced invasion success of its 400-year-old introduced populations (genotypes) compared to native ones. Plant growth traits (height and shoot length) of fifteen genotypes [four from the introduced range (South Africa), eleven from the native range (Brazil and Argentina, Venezuela and The Dominican Republic)] were assessed. Damage and impact of a shoot-feeding, sap-sucking specialist *Catorhintha schaffneri* Brailovsky & Garcia on ten genotypes were also compared. RESULTS: All, but one, invasive genotypes were significantly taller than the native genotypes. Though the invasive genotypes were relatively more damaged by herbivory than some of the native genotypes, the observed differences were not completely explained by their origins. Nonetheless, the findings partially supported the predictions of the EICA hypothesis, because invasive genotypes were generally taller than native genotypes, but did not fully support the hypothesis because they were not always more damaged than the native genotypes by *C. schaffneri*. CONCLUSION: The invasive genotypes had an advantage in the introduced range as they can climb neighbouring vegetation more quickly than the native ones, but the damage incurred by the invasive genotypes relative to the native genotypes only suggests that *C. schaffneri* would be as damaging in South Africa, where it serves as a biocontrol agent, as it is in the native distribution in Brazil.

Keywords: Sap-sucking bug, leaf cactus, biological control agents, common garden experiment, enemy release and plant-herbivore interactions.

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INTRODUCTION

Understanding the invasion process and what make a few alien plant species successful invaders have long fascinated ecologists.[1-4] Though rapid growth, prolific reproduction, and short life cycles of plants are deemed responsible for invasion success, the basic mechanism to explain it still remains elusive.[5-7] Successful invasion occurs due to the removal of some plant species from their natural enemies to enemy-free introduced ranges, where the escape from natural enemies (herbivory and diseases) allows the alien plant to become overabundant, according to the Enemy Release Hypothesis (ERH).[2,8]

Aside the ERH, a different framework dubbed the evolution of increase competitive ability (EICA) hypothesis, which suggests that invasive alien plants grow faster and are less defended against their natural enemies than the native-range (conspecific) populations of the plants, was birthed.[9] Blossey and Notzold[9] first proposed and tested this hypothesis using two separate populations of purple loosestrife, *Lythrum salicaria* Linneaus (Myrtales: Lythraceae) in which the invasive population from Ithaca (in U.S.A.) was fitter (as seen in growth and reproductive output) than the native European population from Lucelle (in Switzerland). The observed improvements in fitness traits were inversely linked with anti-herbivore defenses; presumably suggesting that the improved fitness in the invasive alien plants was mutually dependent on a downward regulation in costly biosynthesis of anti-herbivore defenses amid plant growth's demands for limited resources.[9] In that study, when the invasive and native populations were exposed to insect herbivory, the foliar feeder *Galerucella pusilla* Linnaeus (Coleoptera: Chrysomelidae) performed equally on both populations of *L. salicaria*, unlike the root feeder *Hylobius transversovittatus* Goeze (Coleoptera: Curculionidae), which performed better on the invasive population than the native ones.[9] The adaptive changes in the introduced plant populations were thought to be genetic rather than being ordinary plastic responses, and that these genetic changes may have arisen from their long history of 'enemy release'.[2,9,13]

Several studies have tested the EICA predictions, and some findings hold for some species,[10-14] but not all,[8,14-19] and the reasons for the observed ambiguities are probably context-specific in relation to either the alien plants (e.g., their history of enemy release) or the natural enemies (feeding habits).[9,11,16,20] For instance, in Blossey and Notzold's work[9] *H. transversovittatus* remarkably had the greatest impact against *L. salicaria*, but the plant's exposure to another natural enemy (*G. pusilla*, and perhaps many others) remained similar regardless of host origins. Such occurrences may be hinged on the herbivores' specialisations and feeding habits, and/or the malleability of plants' traits (e.g., reproductive, above- and below-ground traits) to insect herbivory (for review see Rotter and Holeski[20]). Thus far, records[9,20] have shown that insect herbivores with biting and chewing habits (e.g., Coleopterans and Lepidopterans) had improved performance on alien plant populations compared to the native counterparts, while the insects with piercing and sucking habits (e.g., Hemipterans) relatively had no variable effects (i.e., damage) on both populations. Such differential effects attributed to feeding habits of insect herbivores could, *inter alia*, have important implications on how biological control agents are selected and how hypotheses are tested. Hence, disentangling the role of enemy release/EICA in the invasion success of different introduced plants requires specific assessments of any herbivore-plant systems.

The focus here is on *Pereskia aculeata* Miller (Cactaceae), a polytypic host plant commonly known as leaf cactus, which is native to South and Central America, but introduced to Australia and South Africa[19,21-23] The plant model provides an avenue to test the response of a specialist herbivore with a piercing/sucking feeding habit against some predictions of the EICA hypothesis. In South Africa, *P. aculeata* is invasive and a target for biological control programme. Two biological control agents, a leaf-chewing beetle *Phenrica guerini* Bechyne (Coleoptera: Chrysomelidae) and a shoot-wilting bug *Catorhintha schaffneri* Brailovsky & Garcia (Hemiptera: Coreidae) were introduced in 1991 and 2014, respectively. Before the agents' introductions, *P. aculeata* has been present in South Africa for about 400 years.[23] Meanwhile, the invasive *P. aculeata* is genetically distinct from the native populations (to be subsequently referred to as native genotypes, likewise the invasives ones) and has been separated from its coevolved natural enemies since the 1600s when it was grown at Kew Gardens for horticultural purposes.[21-23] *Pereskia aculeata* has a disjunct native distribution in Venezuela and the Caribbean, northern Argentina and southern Brazil.[21,22] The invasive genotypes in South Africa are closely related to those from Rio de Janeiro in southeast Brazil.[22] In the Brazilian states of Santa Catarina and Rio de Janeiro, the plants are genetically and morphologically heterogeneous[21,22]. Beyond the intraspecific host variation, however, the history of the invasive genotypes is further complicated by the time they spent firstly as horticultural plants in gardens, and secondly since their introduction into South Africa.[21,22]

The morphological and/or genetic heterogeneity of both native and invasive populations of *P. aculeata* is also reflected in differences in the species composition, and relative abundance, of natural enemies that are associated with them in the native range.[21-25] Far fewer natural enemies occurred in the northern native region of *P. aculeata* distribution (i.e., the Dominican Republic and Venezuela) than in the southern region (i.e., Brazil and Argentina).[25] Specifically, examples of the heterogeneous insect communities are *Phenrica guerini* (Coleoptera: Chrysomelidae), which is only present in Rio de Janeiro (Brazil); *Pereskiophaga brasiliensis* (Coleoptera: Curculionidae), which is only present in Santa Catarina (Brazil); and the cerambycid, *Acanthodoxus machacalis* Martins and Monné, which is rare in Santa Catarina, but abundant in Rio de Janeiro.[25] Another natural enemy, *Catorhintha schaffneri*, is only found in south-east coastal Brazil including Rio de Janeiro, which is the origin of the invasive *P. aculeata* that is problematic in South Africa.[22,25] Although the release of the invasive *P. aculeata* from these natural enemies did not alter the impact of the weed's first biological control agent, *P. guerini* (which is a leaf-chewing insect),[18] this may not be the case for the new agent, *C. schaffneri*, which has a different feeding habit (that is, the piercing and sucking habit).[20,24]

In keeping with the EICA hypothesis,[9] the invasive *P. aculeata* may have evolved traits for increased growth, which could also increase their palatability to herbivores given their long history of enemy release since the 1600s. Given that multiple forms (genotypes) of *P. aculeata* exist and that notable differences abound in the assemblages of their natural enemies across the native range, each genotype may or may not differ in its fitness response to herbivory.[18,24] Thus, understanding the invasive *P. aculeata* in South Africa is essential to develop an effective management strategy. In light of EICA hypothesis, whether the invasive *P. aculeata* had undergone adaptive changes that will facilitate the allocation of more resources to growth relative to their native conspecifics was investigated. The investigation will help in deciphering whether enemy release and varying evolutionary histories with, or local adaptations to, different herbivore assemblages could have resulted in evolutionary changes in the invasive *P. aculeata*.

MATERIALS AND METHODS

Study system

The study organisms used were a single population of *C. schaffneri* and fifteen genotypes of *P.*

aculeata. The latter were sourced from both the native range in South and Central America, and the invasive range, in South Africa. All *C. schaffneri* used were from the same generation sourced from a breeding culture that was maintained under similar conditions of light, temperature, food and water regimes, within the biological control mass-rearing facility at Rhodes University, Grahamstown, South Africa. The culture was established from progenies of a population of twenty-three adults that were sourced from Brazil in 2012.[24]

For *P. aculeata*, Paterson *et al.*[22] established the origin of the invasive genotypes with DNA sequencing and an Inter-Simple Sequence Repeat (ISSR). Using neighbour joining, maximum parsimony and Bayesian analyses, they found that the genotypes of the introduced range were most similar to garden varieties of *P. aculeata* and then to the genotypes from the southern native range, which consist of Brazil, Argentina & Paraguay (Figure 1).[21,22] *Pereskia aculeata* from the northern native range (Venezuela and the Dominican Republic) were closely related to each other but distinct from South African genotypes and those of the southern native distribution.[22] The genetic analysis also revealed a high average genetic distance between the introduced genotypes and native genotypes most likely due to artificial selection as a horticultural entity.[22] The most closely related plants from the native range to the introduced-range genotypes are B7 and B8, followed by B1 & B2 (Figure 1).[22] In this current study, genotypes from Argentina and Brazil, which were designated as A and B in Paterson *et al.*,[22] were referred to as AR and BR respectively to keep all codes as two-lettered codes e.g., A3 is the same as AR3; all others genotypes remain unchanged.

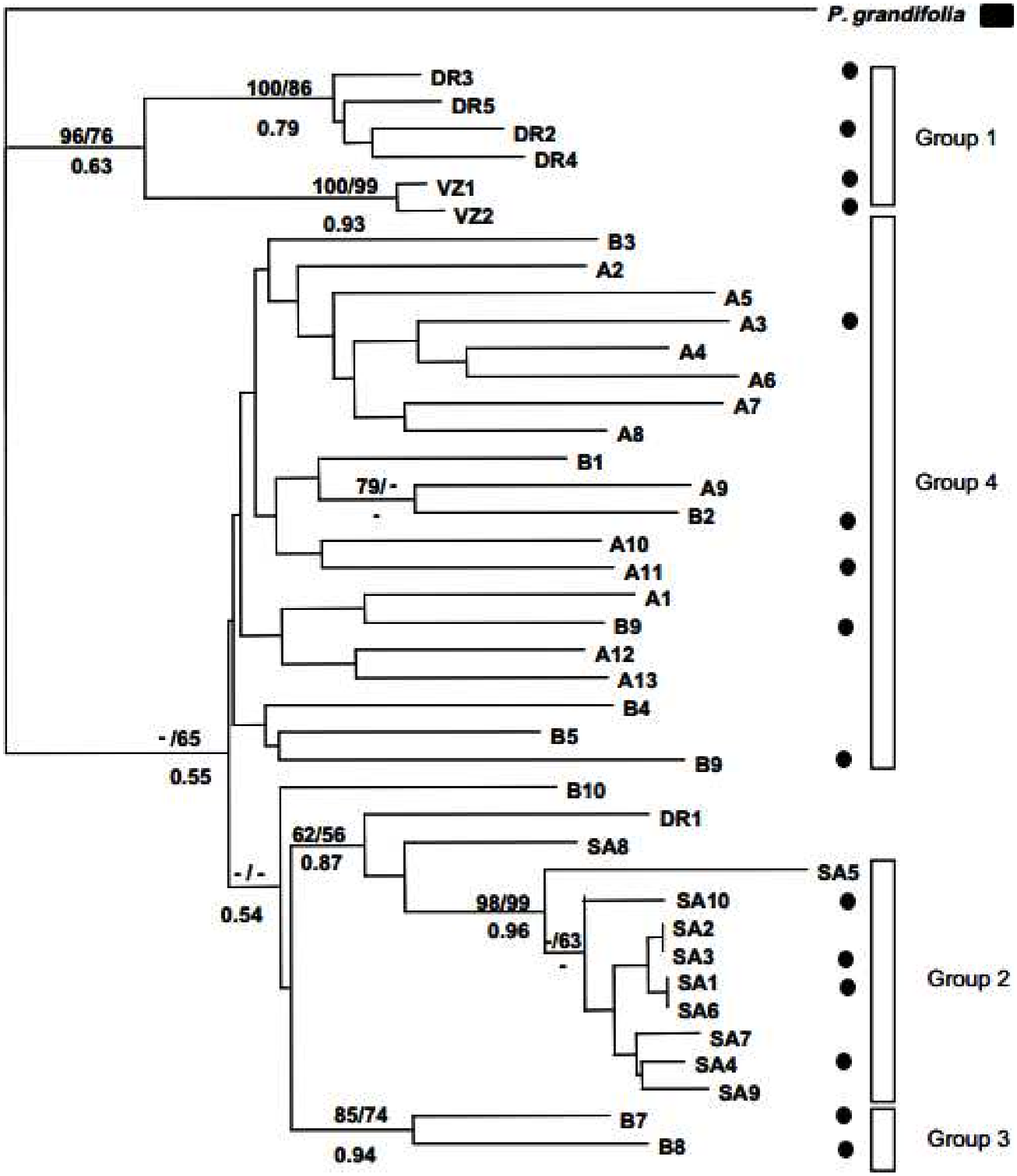

Figure 1 Genetic relationships of *Pereskia aculeata* using neighbour-joining tree constructed from ISSR data excluding bootstrap values and posterior probabilities lower than 0.5. The neighbour-joining bootstrap values/parsimony bootstrap values were provided above and the Bayesian posterior probabilities provided below each node as adapted from Paterson *et al.*[22]. Dots beside the vertical group bars represent the genotypes selected for this study.

Of the 40 genotypes[22], 15 were selected for this current study (Figure 1); of which eleven genotypes of *P. aculeata* were sourced from two distinct native regions (northern and southern ranges: as defined relative to the equator), and four from the invasive range.

A genotype was obtained from Punta Cana (DR2) and another from Pedernales (DR3), in the Dominican Republic. Two genotypes (VZ1 & VZ2) were sourced from Caracas, Venezuela and Misiones (AR3 & AR11), Argentina (Table 1). While five genotypes were obtained from Brazil namely: Paraná (BR2), Santa Catarina (BR6 & BR9), and Rio de Janeiro (BR7 & BR8) –the probable origin of the invasive genotypes.[22] The invasive genotypes were collected from Knysna (SA1) in Western Cape Province, Port Alfred (SA3) and Port St. Johns (SA4) both in Eastern Cape Province, and Kosi Bay (SA10) in KwaZulu-Natal Province, South Africa (Table 1).[22] While the native genotypes from the northern native range are from several thousands of kilometres away from the source of the *C. schaffneri* population in Santa Catarina, Brazil, those from Brazil and Argentina were less than a few hundred kilometres away (Table 1). Geographic coordinates[24] showed that the genotypes BR6 and BR9 are approximately 40 km apart and occur in the same area as *C. schaffneri*. All selected genotypes were genetically unique as illustrated by their genetic distance from BR9, which was geographically close to the source of the *C. schaffneri* population used here. Although a few individuals of *C. schaffneri* were sourced about two to four kilometres away from the origin of BR9 (cf.[24,] Table 1), BR9 cannot be assumed as the same genotype as those on which the few agents were collected.

Table 1 Sources of genotypes, relative geographic and genetic distances relative to BR9, which was collected Santa Catarina Province (Brazil). [†]Country: SA = South Africa, VZ = Venezuela, DR = Dominican Republic, BR = Brazil, AR = Argentina. [‡]The distance (km) away from Santa Catarina as measured using Google Earth^{TM}. [§]Sourced with permission; adapted from Iain Paterson's unpublished report, and published report[21] for geographic distribution.

Distances							
P. aculeata[†]	Location	Ranges (Regions)	Reference sites	Latitude	Longitude	Geographic[‡]	Genetic[§]
SA1	Knysna	Invasive (Invasive)	Undocumented	34.03333° S	23.06667° E	6,915	0.60714
SA3	Port Alfred	``	``	33.59661° S	26.88815° E	7,270	0.61404
SA4	Port St. Johns	``	``	31.61562° S	29.54164° E	7,570	0.60377
SA10	Kosi Bay	``	``	26.96366° S	32.81116° E	8,050	0.60714
VZ1	Caracas	Native (Northern native)	Venezuela Site 11	10.45000° N	66.80583° W	4,390	0.56000
VZ2	Caracas	``	Venezuela Site 12	10.45000° N	66.80583° W	4,390	0.57692
DR2	Punta Cana	``	Dom. Rep. Site 2	18.59777° N	68.46744° W	5,300	0.67273
DR3	Pedernales	``	Dom. Rep. Site 3	17.79383° N	71.46854° W	5,347	0.63158
BR2	Paraná	Native (Southern native)	Brazil Site 3	23.37200° S	51.06522° W	450	0.52000
BR6	Santa Catarina	``	Brazil Site 9	27.05392° S	48.58772° W	40	0.64151
BR7	Rio de Janeiro	``	Brazil Site 10	23.01594° S	43.42358° W	850	0.40000
BR8	Rio de Janeiro	``	Brazil Site 11	22.93318° S	42.61041° W	850	0.53488
BR9	Santa Catarina	``	Brazil Site 12	26.76676° S	48.64097° W	-	-
AR3	Misiones	``	Argentina Site 15	25.63683° S	54.55278° W	430	0.65385
AR11	Misiones	``	Argentina Site 8	26.32808° S	54.61508° W	430	0.61111

Plant propagation

Each genotype was grown to a large plant from cuttings taken from the field. Several cuttings were then taken from a single plant to replicate each genotype. Cuttings of 8-10 cm long were propagated individually, immediately after pruning from their parent plants. Each genotype was replicated twenty times in a growth medium of 3 parts loamy soil to 1 part wood chips, but cuttings that failed to sprout were excluded from data collection and analysis. Plant bags of dimensions 125 x 100 x 225 mm were filled with the growth medium to three centimetres below the brim and watered to saturation three days before the cuttings were propagated. Five grams of 3:1:5 slow-release N-P-K Wonder™ fertiliser and $MgSO_4$ were added per bag. Plants were thereafter watered weekly; and the ambient temperature and relative humidity were obtained using *hygrochron* iButton® at a resolution of 0.5 °C and 0.04% RH (Model DS 1923; Maxim Integrated Products, San José, CA, USA). All the (invasive and native) plants used here were exposed to similar watering and fertilising regimes in the experimental garden.

Growth of genotypes of *Pereskia aculeata*

Growth parameters were quantified from total shoot length (sum of all growing shoots per plant) and plant height (highest growth point from soil surface) 60 days after propagation. Although Blossey and Notzold[9] used plant height and biomass, the use of biomass was impractical in this study as the plants were needed for other trials. Shoot length was used here as it is a good parameter for assessing plant vigour and it correlates with biomass in preliminary assessment and relates directly to the negative impacts of herbivory on the plants. Shoot length has also been used in previous studies.[9,24] Ten plants were sampled destructively to establish the relationship between shoot lengths and biomass (dry weights). Shoot lengths were measured singly using a standard metric tape and the shoots were then removed from the plant and placed in properly labelled envelopes before drying in a PROLAB™ oven at 90°C for two days. The dried materials were then removed and weighed immediately on an AR2140 Adventurer™ OHAUS scale with a readability of 0.0001g. All shoot lengths were measured from the base of the stem (that is the areole on which the shoot sprouted on the initial cutting) to the last apical node on which the youngest leaves were borne (at the meristematic tips). Plant height was measured as vertical length using the single most-upright (tallest) shoot, which was measured (to the nearest cm) from the basal stem of the plant (at the soil surface) to the highest level of the shoot tip. For the 'growth' parameter, the number of leaves was not measured as our preliminary assessment (not shown here) showed that they strongly correlate with shoot length. Shoot height was measured the same day as the measurement taken for shoot lengths. At sixty days after planting, when these data were obtained, the plant shoots were still upright.

Damage and Impact of *Catorhintha schaffneri* on *Pereskia aculeata*

Ten genotypes of *P. aculeata* were grown from cuttings under similar conditions and replicated ten times; of which (i) five plants serve as controls (i.e., herbivore-free) and (ii) the others for herbivore-inoculation, similar to a method used previously.[25] However, in that study, the assessment had been carried out on a whole plant stem, whereas an apical portion of a single shoot was used here by pruning off other shoots thus restricting feeding to a single shoot. The apical portion of the shoot was standardised by marking off the topmost ten centimetres using a xylene-free permanent black marker and the marks are hereafter referenced as standardised reference marks (srm). The 'srm' brought uniformity to the apical shoot lengths of 10 cm succulent portions that were exposed to *C. schaffneri* across all genotypes. All test/control plants were singly confined to 60 x 40 x 40 cm cages made from an aluminium wire (Ø = 2 mm), screened using an Organza™ fabric, and set up under a 10% shade house. On a set of five plants, five adults (2♂:3♀) of less than 7 days old *C. schaffneri* were introduced, to be later referred to as herbivore-inoculated plants, while the others (control plants) had no insects. Numbers of leaves above 'srm' on both sets of plants were counted before and after the trials. After ten days, the apical shoot lengths were altered either by growth (in control) or herbivory (in herbivore-inoculated plants). Shoot lengths were measured before and after the trial using a measuring tape. In the damage and impact assessments, five genotypes of the initial 15 genotypes (selected in growth assessment) were excluded 'arbitrarily', and these genotypes were BR2, AR11, VZ1, DR2 and SA3.

Catorhintha schaffneri has a lifespan that averages twenty-five days,[24] the chosen age limit (of ≤ 7 days) eliminated any probable effects of senescence on their feeding behaviour during the trial. Thus, at the end of the ten-day trial, surviving insects would have been seventeen days old or less. All trials were conducted under same ambient weather conditions, which were between 24.6 ± 0.3 °C and 25.2 ± 0.4 °C and between 67.4 ± 1.1 % and 75.8 ± 1.0% RH within the shade house. Damage inflicted by *C. schaffneri* on the plants was defined as the difference between the 'before' and 'after' herbivore-induced changes in shoot length on treated plants only, and impact was computed as the differences between damaged plants and their respective control plants.

Statistical analysis

Parameters for plant traits (height and total shoot lengths after 60 days) did not satisfy the assumptions of a parametric test, hence they were analysed using a generalised linear mixed model, GLMM (Gaussian family with log link function; for rationale, see review.[26] At each higher level of fixed effect, the corresponding lower level was treated as random effects and the different levels of fixed effects were range (invasive and native), region (invasive, northern native and southern native ranges), countries (Argentina, Brazil, Dominican Republic, Venezuela and South Africa) and lastly, the genotypes. The global significance of fitted models was tested using type III ANOVA. Observed significant differences were followed by *posthoc* tests based on general linear hypotheses with 'tukey contrast' and adjusted against type I (false-positive) error using Bonferroni correction, and were automatically separated by compact letter display.[27]

For impacts, the assumptions of the parametric test were satisfied so the data were analysed with ANOVA followed by a pairwise *posthoc* test based on Fishers' LSD method in 'multcomp' R package. For agent's damage (damaged shoots and number of wilted leaves) on different genotypes, Shapiro-Wilk (W) and Levene's tests demonstrated that the data did not satisfy parametric assumptions. Hence, the non-parametric tests: Kruskal- Wallis H, Mann-Whitney U, one-sample Wilcoxon Signed Rank tests were adopted where appropriate and significant differences were separated using *posthoc* Kruskal-Wallis multiple comparisons (*kruskalmc*) in R 3.3.3[27]. The genotypes in all figures were arranged in similar order as follows: the invasive South African genotypes, native Brazilian genotypes from Rio de Janeiro where the invasive genotypes originated from, and then to the genotypes from Santa Catarina where the insect was sourced. The other genotypes thereafter were those from Argentina, Venezuela and the Dominican Republic.

RESULTS

Growth of genotypes of *Pereskia aculeata*

Plant height

At the first level of fixed effects (range), the invasive genotypes grew taller than the native genotypes of *P. aculeata* as shoot height showed significant range effect (difference in geographic localities) with an average height of 23.47 ± 0.66 cm and 19.04 ± 0.37 cm for invasive and native genotypes, respectively (t statistic = -2.14, $p < 0.05$; Table 2). Collectively, the four genotypes from the invasive range on average were 23.20% taller than the average heights of the native-range genotypes (Table 2). At the second level of fixed effects (region), invasive genotypes from South Africa and the northern native genotypes had a mean height of 23.47 ± 0.66 cm and 21.39 ± 0.65 cm respectively, while the southern native genotypes were relatively shorter at 17.66 ± 0.39 cm. The invasive and northern native genotypes were not significantly different from each other but were 27% taller than the average heights of the Brazilian genotypes and this difference was statistically significant (F = 10.63, df = 2, p = 0.005). At third level of fixed effects (national scale, or countries), the invasive genotypes from South Africa were significantly taller than the average shoot heights of the genotypes from the Dominican Republic and Brazil (F = 145.87, df = 4, $p < 0.001$; Table 2), but not statistically different from the genotypes from Argentina and Venezuela. The differences at the individual level (genotypes) revealed that not all invasive genotypes grew significantly taller than other native genotypes. The genotype SA10 was statistically shorter than other invasive genotypes but had similar average height compared to the Brazilian genotypes (Figure 2).

Total shoot lengths

At the first level of fixed effects (range), the invasive genotypes grew generally longer than the native genotypes, but there was no statistical difference, unlike at the other (regional and country) levels (Table 2). The average shoot lengths of invasive and native genotypes of *P. aculeata* were 38.74 ± 1.33 cm and 31.82 ± 0.89 cm, respectively. Although the range effects on these measurements did not differ significantly (t statistic = -1.54, $p = 0.12$), regional effects were significant (Table 2). The genotypes from the southern native and invasive ranges had similar average lengths of 35.02 ± 1.20 cm and 38.74 ± 1.33 cm respectively, which were 25% and 32% longer than the average shoot lengths of the genotypes from the northern native range. In the northern native range but at the national scale, the Venezuelan genotypes of *P. aculeata* (VZ1 and VZ2) and those from the Dominican Republic (DR2 and DR3) were statistically similar. Both DR and VZ genotypes had significantly shorter average total shoot lengths than the genotypes from Argentina (AR3 and AR11) and all the South African genotypes that is, SA1 from Knysna, SA3 from Port St. Johns, SA4 Port Alfred and SA10 from Kosi Bay (Table 2; $F = 50.46$, df = 4, $p < 0.05$;). Generally, the analysis revealed that there was a significant genotypic differences (Figure 4; $F = 154.28$, df = 14, $p < 0.001$). Consequently, a *posthoc* test with Tukey contrast and Bonferroni adjustment to minimise false-positive errors revealed that three out of four invasive genotypes were among the fastest growers in terms of the average total shoot lengths, which also included two other genotypes from Misiones (Argentina), and one each from Santa Catarina and Rio de Janeiro (Brazil) (Figure 3).

Table 2 Summary and analysis of the traits of *Pereskia aculeata* using a generalised linear mixed model with random effects.

		Plant height (cm)	Total shoot length (cm)
Fixed Effects	Sample size	Mean ±SEM	Mean ±SEM
Range			
Invasive	72	23.47 ± 0.66^{a}	38.74 ±1.33^{a}
Native	194	19.05 ± 0.37^{b}	31.82 ± 0.89^{a}
t statistics		-2.14*	-1.54^{ns}
Region			
Invasive	72	23.47 ± 0.66^{a}	38.74 ±1.33^{a}
Northern native	72	21.39 ± 0.65^{ab}	26.40 ± 0.99^{b}
Southern native	122	17.66 ± 0.39^{b}	35.02 ± 1.20^{a}
F-statistic		10.63**	38.07***
Country			
Argentina	39	21.21 ± 0.64^{b}	39.66 ± 1.75^{a}
Brazil	83	16.00 ± 0.36^{c}	32.84 ± 1.51^{b}
Dominican Republic	37	18.32 ± 0.63^{c}	27.83 ± 1.43^{bc}
South Africa	72	23.47 ± 0.66^{ab}	38.74 ± 1.33^{a}
Venezuela	35	24.63 ± 0.89^{a}	24.89 ± 1.35^{c}

F- statistic	145.87***	50.46***

Groups with same letters within the same column are not significantly different ($p < 0.05$). Significance codes: *** Significant at $p < 0.001$; ** $p < 0.01$; *$p < 0.05$; ^{ns} $p > 0.05$.

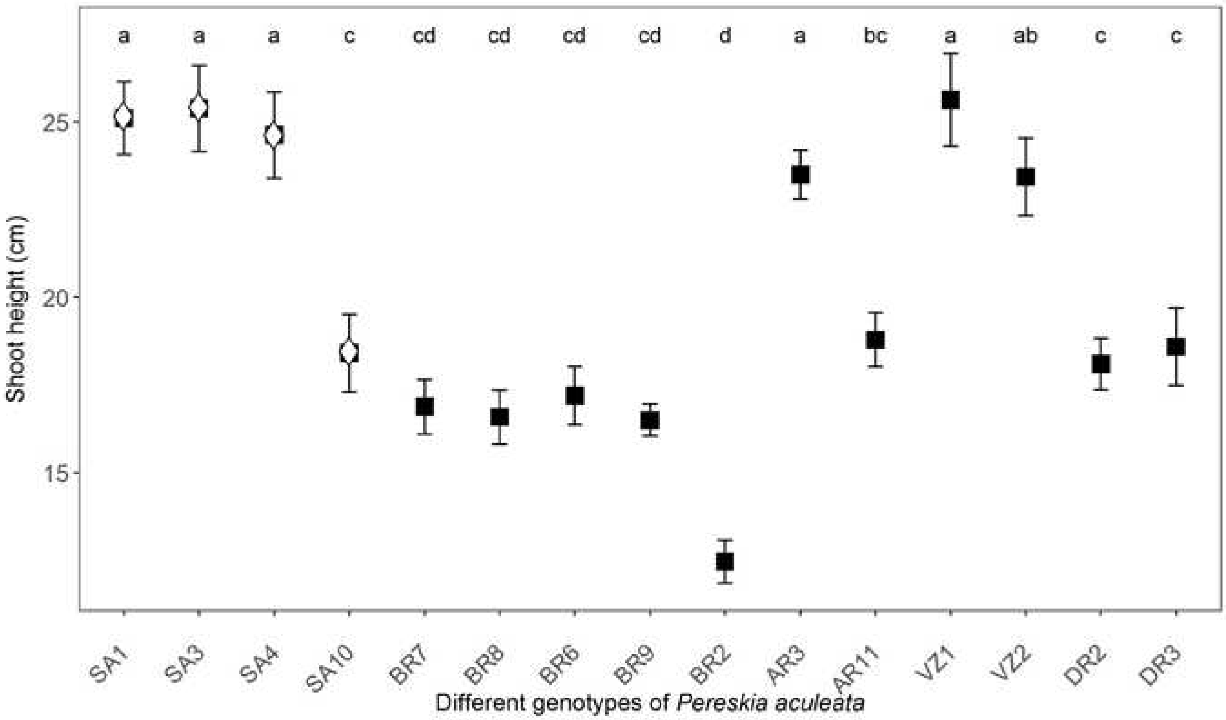

Figure 2 Means of plant heights of different genotypes of native and invasive *Pereskia aculeata*, sixty days after cultivation. Bars represent SEM. Significant differences among means were represented by the letters above each bar.

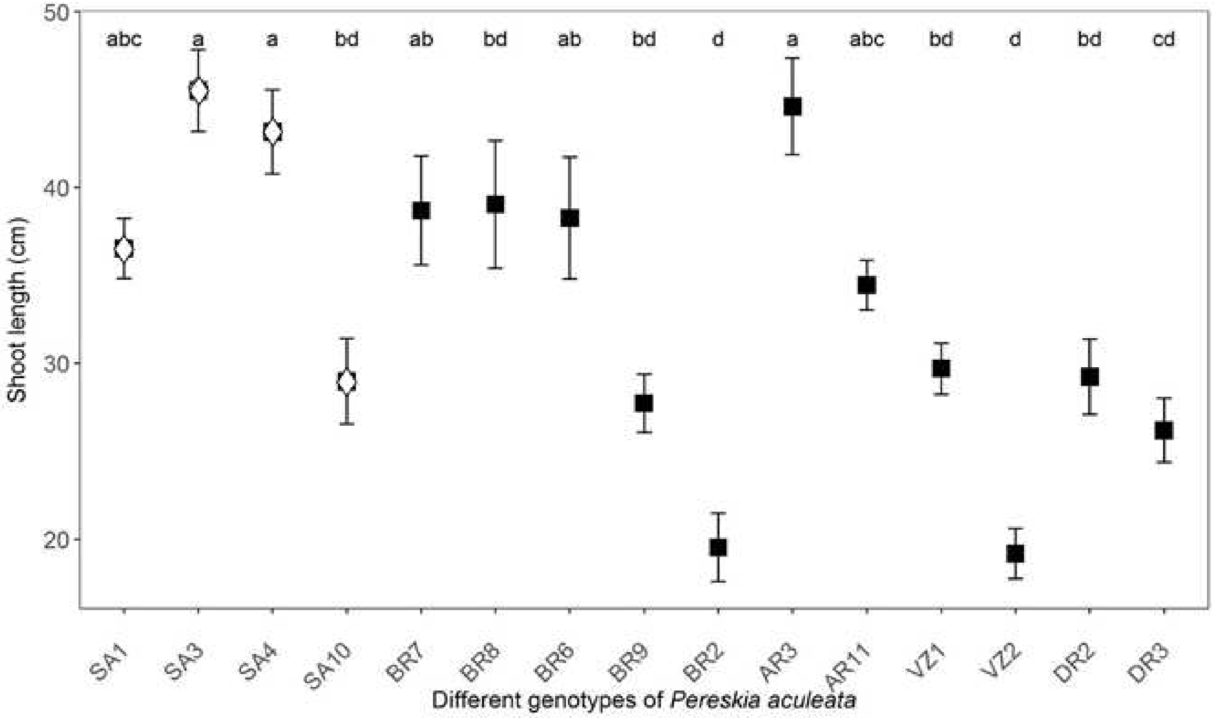

Figure 3 Means of total shoot lengths of different native and invasive genotypes of *Pereskia aculeata* after sixty days of growth. Bars represent SEM and significant differences among means were represented by different letters above each bar.

Damage of *Catorhintha schaffneri*

Generally, although all the ten genotypes exposed to *C. schaffneri* were damaged within ten days, only four were significantly ($p < 0.05$) damaged when compared with their initial shoot lengths unlike the other six genotypes as illustrated by a 'one-sample Wilcoxon Signed-Rank test' (Figure 4). The least damaged shoot was on AR3 from Misiones (Argentina), which had an average of 2% of the standardised apical portion (srm) damaged (that is, wilted) by *C. schaffneri*, while as high as 95% of the srm on BR6 from Santa Catarina (Brazil) was damaged (Figure 4). The other genotypes that incurred remarkably more damage than their respective initial shoot lengths were SA4 (median = 4.6, $p = 0.03$), SA1 (median =1.85, $p = 0.03$), BR6 (median = 0.95, $p = 0.03$) and BR8 (median = 2.3, $p = 0.03$), while the remaining six genotypes incurred an insignificant damage of 2% to 31% (Figure 4). The comparative effects of herbivory on all herbivore-inoculated plants as demonstrated using a one-way Kruskal-Wallis *H* test shows that the native genotype BR6 was significantly ($p < 0.05$) more damaged than the Argentina genotype (AR3); more so than a genotype from Santa Catarina (BR9). Nonetheless, BR6 did not incur significantly higher damage compared with any other genotypes, whether native or invasive ($H_9 = 27.43$, $p = 0.001$).

Impact of *Catorhintha schaffneri* on shoot length

For impact, the Mann-Whitney *U* test revealed a significant halt in growth for each herbivore-inoculated plants (for all genotype; $U = 25$; $p < 0.05$) compared to their respective control plants. Also, there was a significant impact on four genotypes namely SA1, BR8 (Rio de Janeiro), BR6 (Santa Catarina) and SA4 (ANOVA: $F_{(9, 40)} = 3.48$, $p = 0.003$; Figure 5), but the native genotype BR8 was not significantly more impacted than BR7 (Rio de Janeiro). Nonetheless, BR7 was significantly less impacted than SA1 (an invasive genotype) and BR6, (a native genotype) as shown from a Fishers' LSD *posthoc* test. The BR7 genotype was less impacted than SA1, but similar to other invasive genotypes like SA4 and SA10 (Figure 5). The least impacted genotypes were the invasive genotype (SA10), and the native genotypes outside the agent's natural range (DR3 and AR3) [i.e., non-local host plants], and interestingly another native genotype within the agent's natural range of Santa Catarina (BR9).

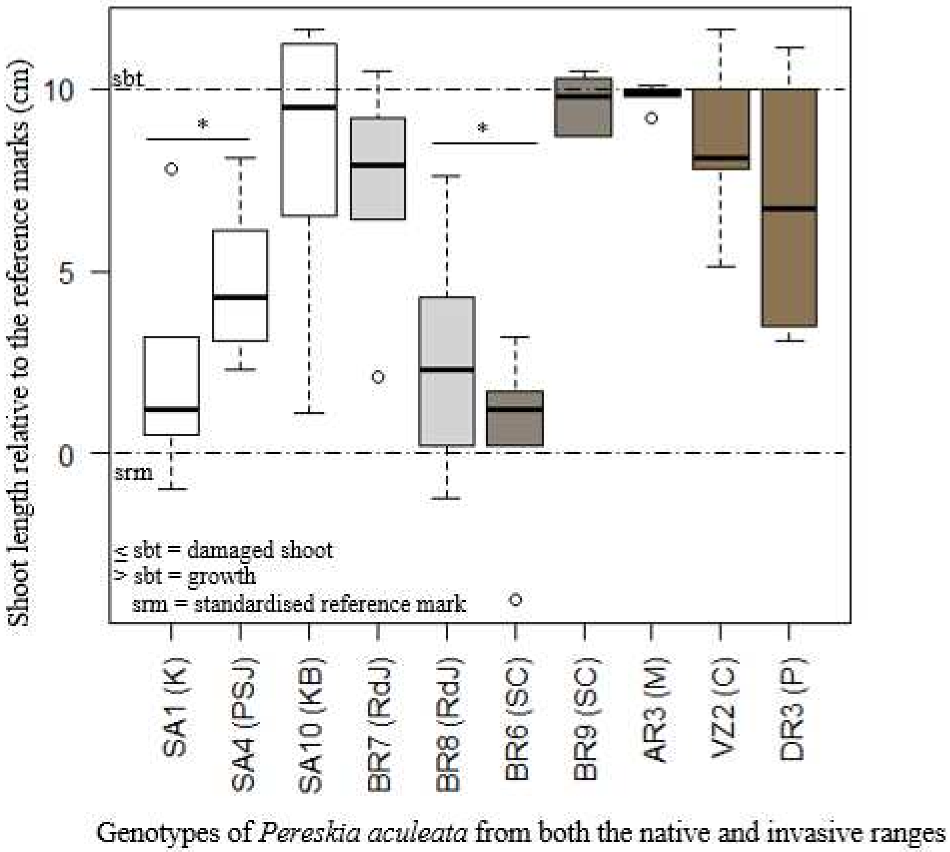

Figure 4 Damage of *Catorhintha schaffneri* on shoot lengths of *Pereskia aculeata* at a fixed level of herbivory (2♂:3♀). Colour representations: white –the invasive genotypes from South Africa; light gray –the Brazilian native genotypes from Rio de Janeiro while dark gray –those from Santa Catarina; brown –the native genotypes from Argentina, Venezuela and the Dominican Republic. Line 'sbt' is the height of the shoot tips at the beginning of the trial (ten centimetres above the standardised reference marks, line 'srm'). The box plots depict medians, 25th and 75th percentiles and minimum and maximum values. Horizontal bars above the boxes SA1 and SA4, BR8 and BR6 signify a significant damage relative to the sbt. Codes in parentheses are the sources of tested plants: C = Caracas, K = Knysna, KB = Kosi Bay, M = Misiones, P = Pedernales, PSJ = Port Saint Johns, SC = Santa Catarina, RdJ = Rio de Janeiro (cf. Table 1).

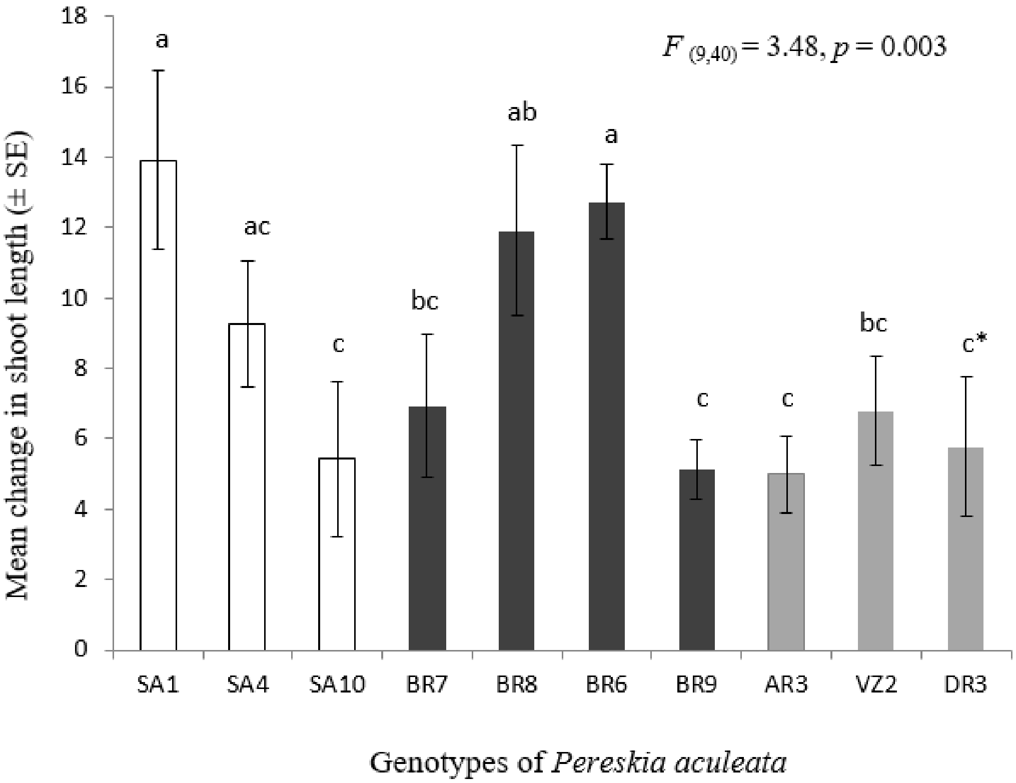

Figure 5 Impact of *Catorhintha schaffneri* on the apical shoot of genotypes of *Pereskia aculeata*. This was represented as the differences between controls and inoculated plants. Colour representations: white –the invasive genotypes from South Africa; black –the Brazilian native genotypes from Rio de Janeiro and Santa Catarina; grey –the native genotype from Argentina, Venezuela and the Dominican Republic. Notes: *The shoots of DR3 were drooping and pale, unlike others wherein top-down wilting of shoots were observed along with several 'split shoots.'

Impact on apical leaves

All herbivore-inoculated plants incurred some loss of apical leaves while control plants added leaves over the trial period. The least increase in apical leaves among the control plants was 24% on SA10, an invasive genotype from Kosi Bay, as opposed to the highest of 42% on a native genotype VZ2 from Caracas, Venezuela. The lowest loss of apical foliage on herbivore-inoculated plants was 22% on an Argentina genotype (AR3) as opposed to 93% on a native genotype BR6 from Santa Catarina, Brazil. The impact of herbivory on foliage losses (wilted leaves) as analysed using analysis of variance followed by Fisher's Least Significant Difference (LSD) showed that although the apical foliage losses were largely similar between the other genotypes, DR3, SA1, BR6, and BR8 were statistically more impacted than BR9 (ANOVA: $F_{(9,40)} = 2.27$, $p = 0.037$; Figure 6).

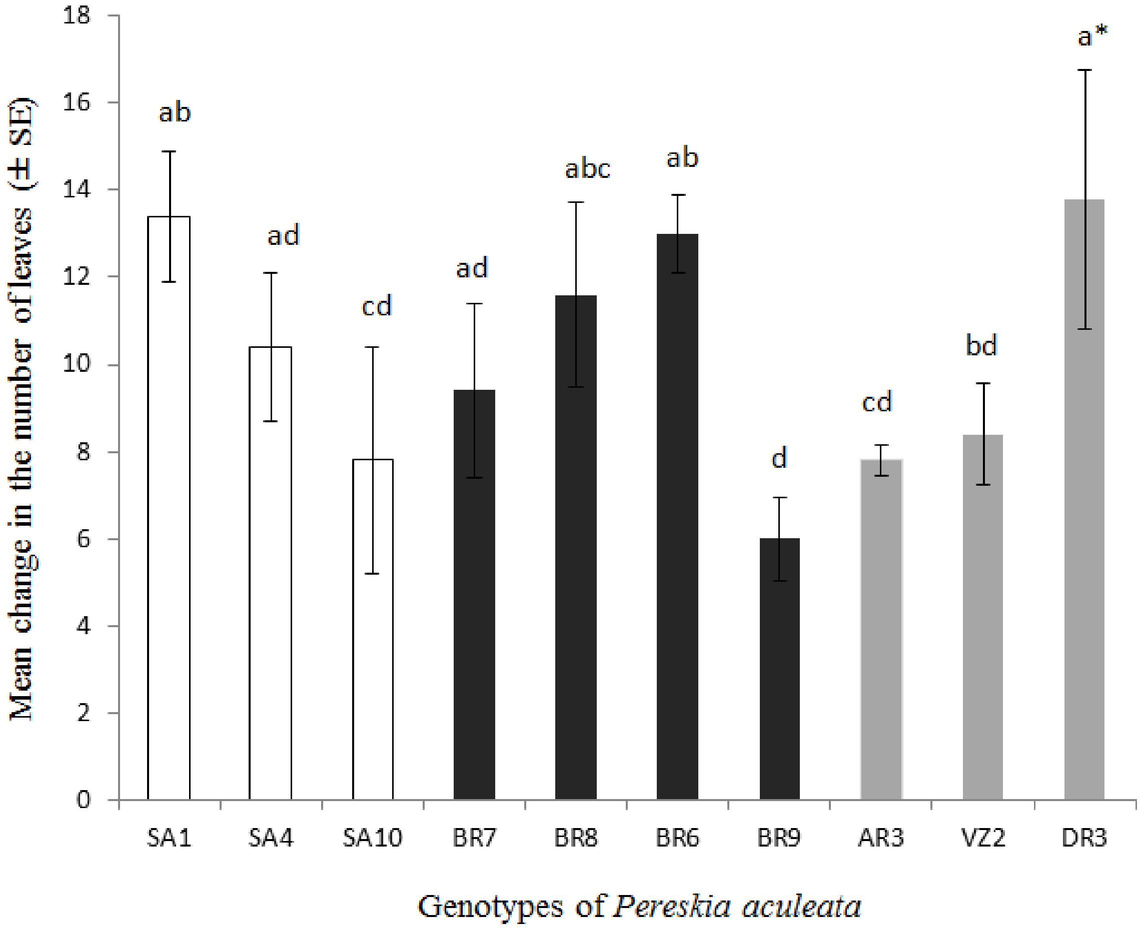

Figure 6 Impact of *Catorhintha schaffneri* on apical leaves among genotypes of *Pereskia aculeata* as differences between control and inoculated plants after ten days herbivory. Colour representations: white –the invasive genotypes from South Africa; black –the Brazilian native genotypes from Rio de Janeiro and Santa Catarina; grey –the native genotype from Argentina, Venezuela and the Dominican Republic. Note: *The value for DR3 must be interpreted with caution as most leaves were not wilted, but droopy and pale green and they remained attached to the shoot, unlike the others on which leaves were completely wilted or

had fallen off.

DISCUSSION

This study examined two predictions of the Evolution of Increased Competitive Ability (EICA) hypothesis: i) that invasive alien plant genotypes grow more vigorously, and ii) that they are more susceptible to a specialist natural enemy than their native-range genotypes.[9] The expectations were that the four invasive genotypes of *P. aculeata* would (i) grow taller and faster, and (ii) incur more damage from, and be more impacted by, *C. schaffneri* than the other eleven native-range conspecific genotypes.[9,22]

In keeping with the EICA hypothesis, the heights of the invasive-range genotypes relative to the native genotypes largely conformed to the first prediction; however, total shoot lengths were not always greater among the invasive genotypes than the native genotypes. The taller plants among the invasive genotypes support one of the EICA predictions as they differ from the Brazilian native plants. The Brazilian genotypes, especially those from Rio de Janeiro, are of particular interest for comparison, because genetic evidence suggests that they are the closest relatives of the invasive genotypes.[22] The invasive genotypes of *P. aculeata* had slightly greater shoot lengths than the natives but had a weak range and regional effects, which reflect a considerable variation within genotypes and between genotypes from the same range and region, respectively. The implication is that the range and regional factors could not explain the differences in plant vigour (shoot lengths) between genotypes, but did explain the increased heights among the invasive genotypes relative to their native conspecifics. Because the invasive-range genotypes grew taller than the native ones, they are more likely to climb onto neighbouring trees (and damage indigenous vegetation) than their native counterparts. Plants that can grow quickly are likely to be more competitive than those that do not, as they will get above other vegetation early in the growing season (spring) and outcompete other vegetation for light and space. Unlike the South American genotypes, which have never been noted for aggressive traits over their native flora (to the authors' knowledge), the invasive genotypes do have an advantage (early gains in shoot height) over the native-range genotypes and this occurrence may be explained by the EICA hypothesis. Other possible reason for the successful invasion of *P. aculeata* in South Africa, is enemy release.[2]

No evidence of non-native plant genotypes being more susceptible to the herbivore, *C. schaffneri,* was found in this study, which did not conform to the EICA hypothesis. For example, if (i) BR9 and BR7 were compared with SA1 and SA4, and (ii) BR8 and BR6 were compared with SA1 and SA4, the former pairs would have supported the EICA's prediction as opposed to the latter. Consequently, these findings suggest that the ecological outcomes of *P. aculeata* and *C. schaffneri* interactions are genotype dependent and that the EICA hypothesis cannot broadly predict the impact of *C. schaffneri* on its polytypic host. Several attempts at unravelling the mechanisms responsible for invasion success have generated ambiguous findings, possibly because different taxa and habitats respond differently or that each plant-herbivore system is unique.[9,20,28] For *P. aculeata* in this current study, there was insufficient evidence to suggest that *C. schaffneri* impacted the invasives more than the native genotypes; however, the outcome of a significant long-term difference cannot be ruled out. It is possible that the South African genotypes would be more susceptible either after a longer exposure time to the agent with multiple defoliation (shoot-wilting) events over many seasons or after high levels of agent released on them over a short period as compared to the conditions of our experiment.

Catorhintha schaffneri is not present across its entire host's native range and where it does occur the relative abundance varies; with higher densities found in the coastal sites of Porto Belo than in Penha, Brazil.[24,25] To investigate whether escape from herbivory by *C. schaffneri* has resulted in a change to *P. aculeata* in the invaded range, comparisons should only be made with plants from Santa Catarina and Rio de Janeiro, where *C. schaffneri* is present. *Catorhintha schaffneri* was quite damaging to BR8 from Rio de Janeiro and BR6 from Santa Catarina (both from Brazil) and the agent occurs in both localities (cf[25]), yet BR7 and BR9 were less damaged compared to the former despite being sourced from an area close to BR8 and BR6, and being genetically similar. Also, an invasive genotype, SA10, from Kosi Bay in South Africa suffered lower impact than the other invasive genotypes, which negates the EICA predictions. The hypothesised susceptibility patterns can therefore not be fully explained by either the origin of each genotype or the status of its enemy-free space. Consequently, the findings here only conform to prediction on vigour,[9] which explains alien plant invasion success, and did not to an enhanced host susceptibility to the agent. In sum, it suffices to say that the EICA hypothesis was partially supported.

While a down-regulation of anti-herbivore defence as EICA had proposed may be a continuous process in *P. aculeata* that is perhaps yet to reach the point where the invasive genotypes are more susceptible than the native counterparts, it is noteworthy to state that the residence time of invasive *P. aculeata* is over 150 years[29] and it has been separated from its natural enemies as a horticultural plant for over 400 years;[21,30] both periods compare well to many other, but not all, invasive plant species that had become competitively enhanced. Although a complete support for the EICA hypothesis is lacking, it cannot be ruled out that the invasive genotypes do outgrow the native genotypes. Support for all the predictions in the hypothesis remains largely agent-host specific, and thus the generality of the EICA concept still remains elusive.[14,31,32] Even on *L. salicaria*, the predictions only favoured a root-feeder, *Hylobius transversovittatus* (belowground herbivory) and not a defoliator *Galerucella pusilla* (aboveground herbivory),[9] but a new record[33] has recently shown that a non-native older population of *Mimulus guttatus* Fisch. Ex DC. (Phrymaceae) responded to enemy release to a greater extent than its younger eastern North America population, in favour of the EICA hypothesis. The rationale for the different findings in support or against the predictions of the EICA hypothesis could be due to the differences in the plant-herbivore systems being studied, the temporal history of their release from the natural enemies and/or the feeding habits of specialist agents, which may influence their responses to native/invasive host differences (for rationale and review see Rotter and Holeski,[20] and Gruntman *et al.*[31]). Since most invasions are rather recent, it can be assumed that the genetic adaptations underlying the EICA hypothesis would not have played an important role in the case of *P. aculeata*, and evidences of age-dependent response to evolution of traits, in terms of enemy-release history abound in literature for different alien species.[31,33]

Additionally, in biological control programmes, it should not be assumed that the absence of negative effects due to intraspecific variation in a host plant against one agent, e.g., *P. guerini*,[18] could translate into similar results for another agent, e.g., *C. schaffneri* [see[9,32] for more examples]. Given that *P. guerini* was released on *P. aculeata* few years earlier than *C. schaffneri*, some of the invasive genotypes on which the former had established could have regained their anti-herbivore defences (e.g., Gruntman *et al.*[31]). Of the twelve sites of *P. aculeata* on which *P. guerini* was released in KwaZulu Natal and Eastern Cape Provinces in South Africa,[23] only one genotype from Port Alfred (SA3 –in impact trials) was among those studied here. Whether an earlier exposure to *P. guerini* had restored resource allocation to defences enough to undermine an expected higher performance of *C. schaffneri* on the invasive genotypes than the native ones seems unlikely because the invasive genotypes (SA1, SA3, and SA4) were equally utilised even though one had been previously exposed to *P. guerini*. Albeit *P. guerini* was absent in Knysna and Port St. Johns, there was no sufficient evidence to suggest that these genotypes were more impacted, or that the agent performed better on them, than either the invasive genotype (SA3) or the native genotypes from Rio de Janeiro and Santa Catarina. Consequently, any previous exposure to *P. guerini* is immaterial to the impact of *C. schaffneri* on the different genotypes of *P. aculeata* in South Africa.

CONCLUSION

The invasive genotypes of *P. aculeata* have acquired traits that enhance their invasive potential, but this has not resulted in an increased damage and impact incurred from the specialist agent, *C. schaffneri*. Evidence for variable impacts from the agent was found, but this variability cannot be explained by either genetic relationship among the plant genotypes or based on their geographic origins. This suggests that the biological control agent, *C. schaffneri* that was released on the invasive genotypes of *P. aculeata* would largely not be negatively influenced by the genotypic variation within its introduced range or by the geographic origin of the invasive genotypes, whose impacts were similar to those incurred by the native genotypes from Rio de Janeiro and Santa Catarina provinces. As was the case for other recent studies on different plant-insect systems,[8,14,32] the eco-evolutionary mechanisms of invasion success for *P. aculeata* could not be fully explained by the EICA hypothesis. The general implication of these findings for the biological control of *P. aculeata* in South Africa, and for weed biological control anywhere in the world, is that the extent of variability within an invasive alien plant needs careful considerations in managing invasive alien species using specialist natural enemies.

ACKNOWLEDGEMENTS

The anonymous researchers who at different times visited the natural homes of *Pereskia aculeata* and collected the plant materials from the native and invasive ranges prior to the commencement of this study are herewith recognised with thanks. The lead author is particularly grateful to DAAD, Bonn, Germany that provided the enabling funds (with the code number: A/14/93797-91560159) for his doctoral training at Rhodes University, South Africa. Thanks are also due to Maretha Boshoff, Ntyimkala Vuyani (*alias*

Majeke), Andre van Rooyen, Pendrick Kotelo and Phillippa Muskett for the technical supports, and to Helen Holleman for comments on one of the earliest versions. The South African Working for Water (WfW) Programme of the Department of Environmental Affairs: National Resource Management, and the South African Research Chairs Initiative of the Department of Science and Technology and the National Research Foundation of South Africa are acknowledged for providing additional funding. Any opinion, finding, conclusion or recommendation expressed in this material is that of the authors and the NRF (or any of the funders) does not accept any liability in this regard.

Authors' contributions

Testing the EICA hypothesis was suggested by MH; INE, IP, SC designed the experiments; INE performed the experiments, analyzed the data and wrote the paper under the guidance of, and contributions from, IP, SC and MH.

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Table 1 Sources of genotypes, relative geographic and genetic distances relative to BR9, which was collected Santa Catarina Province (Brazil). †Country: SA = South Africa, VZ = Venezuela, DR = Dominican Republic, BR = Brazil, AR = Argentina. ‡ The distance (km) away from Santa Catarina as measured using Google Earth™. §Sourced with permission; adapted from Iain Paterson's unpublished report, and published report[21] for geographic distribution.

Distances							
P. aculeata†	Location	Ranges (Regions)	Reference sites	Latitude	Longitude	Geographic‡	Genetic§
SA1	Knysna	Invasive (Invasive)	Undocumented	34.03333° S	23.06667° E	6,915	0.60714
SA3	Port Alfred	``	``	33.59661° S	26.88815° E	7,270	0.61404
SA4	Port St. Johns	``	``	31.61562° S	29.54164° E	7,570	0.60377
SA10	Kosi Bay	``	``	26.96366° S	32.81116° E	8,050	0.60714
VZ1	Caracas	Native (Northern native)	Venezuela Site 11	10.45000° N	66.80583° W	4,390	0.56000
VZ2	Caracas	``	Venezuela Site 12	10.45000° N	66.80583° W	4,390	0.57692
DR2	Punta Cana	``	Dom. Rep. Site 2	18.59777° N	68.46744° W	5,300	0.67273
DR3	Pedernales	``	Dom. Rep. Site 3	17.79383° N	71.46854° W	5,347	0.63158
BR2	Paraná	Native (Southern native)	Brazil Site 3	23.37200° S	51.06522° W	450	0.52000
BR6	Santa Catarina	``	Brazil Site 9	27.05392° S	48.58772° W	40	0.64151
BR7	Rio de Janeiro	``	Brazil Site 10	23.01594° S	43.42358° W	850	0.40000
BR8	Rio de Janeiro	``	Brazil Site 11	22.93318° S	42.61041° W	850	0.53488
BR9	Santa Catarina	``	Brazil Site 12	26.76676° S	48.64097° W	-	-
AR3	Misiones	``	Argentina Site 15	25.63683° S	54.55278° W	430	0.65385
AR11	Misiones	``	Argentina Site 8	26.32808° S	54.61508° W	430	0.61111

Table 2 Summary and analysis of the traits of *Pereskia aculeata* using a generalised linear mixed model with random effects.

Fixed Effects	Sample size	Plant height (cm)	Total shoot length (cm)
		Mean ±SEM	Mean ±SEM
Range			
Invasive	72	23.47 ± 0.66^{a}	38.74 ±1.33^{a}
Native	194	19.05 ± 0.37^{b}	31.82 ± 0.89^{a}
t statistics		-2.14*	-1.54^{ns}
Region			
Invasive	72	23.47 ± 0.66^{a}	38.74 ±1.33^{a}
Northern native	72	21.39 ± 0.65^{ab}	26.40 ± 0.99^{b}
Southern native	122	17.66 ± 0.39^{b}	35.02 ± 1.20^{a}
F-statistic		10.63**	38.07***
Country			
Argentina	39	21.21 ± 0.64^{b}	39.66 ± 1.75^{a}
Brazil	83	16.00 ± 0.36^{c}	32.84 ± 1.51^{b}
Dominican Republic	37	18.32 ± 0.63^{c}	27.83 ± 1.43^{bc}
South Africa	72	23.47 ± 0.66^{ab}	38.74 ± 1.33^{a}
Venezuela	35	24.63 ± 0.89^{a}	24.89 ± 1.35^{c}
F- statistic		145.87***	50.46***

Groups with similar letters within the same column are not significantly different ($p < 0.05$). Significance codes: *** Significant at $p < 0.001$; ** $p < 0.01$; *$p < 0.05$; ^{ns} $p > 0.05$.

Figure legends

Figure 1 Genetic relationships of *Pereskia aculeata* using neighbour-joining tree constructed from ISSR data excluding bootstrap values and posterior probabilities lower than 0.5. The neighbour-joining bootstrap values/parsimony bootstrap values were provided above and the Bayesian posterior probabilities provided below each node as adapted from Paterson *et al.*[18]. Dots beside the vertical group bars represent the genotypes selected for this study.

Figure 2 Means of plant heights of different genotypes of native and invasive *Pereskia aculeata*, sixty days after cultivation. Bars represent SEM. Significant differences among means were represented by the letters above each bar.

Figure 3 Means of total shoot lengths of different native and invasive genotypes of *Pereskia aculeata* after sixty days of growth. Bars represent SEM and significant differences among means were represented by different letters above each bar.

Figure 4 Damage of *Catorhintha schaffneri* on shoot lengths of *Pereskia aculeata* at a fixed level of herbivory (2♂:3♀). Colour representations: white –the invasive genotypes from South Africa; light gray –the Brazilian native genotypes from Rio de Janeiro while dark gray –those from Santa Catarina; brown –the native genotypes from Argentina, Venezuela and the Dominican Republic. Line ‘sbt’ is the height of the shoot tips at the beginning of the trial (ten centimetres above the standardised reference marks, line ‘srm’). The box plots depict medians, 25th and 75th percentiles and minimum and maximum values. Horizontal bars above the boxes SA1 and SA4, BR8 and BR6 signify a significant damage relative to the sbt. Codes in parentheses are the sources of tested plants: C = Caracas, K = Knysna, KB = Kosi Bay, M = Misiones, P = Pedernales, PSJ = Port Saint Johns, SC = Santa Catarina, RdJ = Rio de Janeiro (cf. Table 1).

Figure 5 Impact of *Catorhintha schaffneri* on the apical shoot of genotypes of *Pereskia aculeata*. This was represented as the differences between controls and inoculated plants. Colour representations: white –the invasive genotypes from South Africa; black –the Brazilian native genotypes from Rio de Janeiro and Santa Catarina; grey –the native genotype from Argentina, Venezuela and the Dominican Republic. Notes: *The shoots of DR3 were drooping and pale, unlike others wherein top-down wilting of shoots were observed along with several ‘split shoots.’

Figure 6 Impact of *Catorhintha schaffneri* on apical leaves among genotypes of *Pereskia aculeata* as differences between control and inoculated plants after ten days herbivory. Colour representations: white –the invasive genotypes from South Africa; black –the Brazilian native genotypes from Rio de Janeiro and Santa Catarina; grey –the native genotype from Argentina, Venezuela and the Dominican Republic. Note: *The value for DR3 must be interpreted with caution as most leaves were not wilted, but droopy and pale green and they remained attached to the shoot, unlike the others on which leaves were completely wilted or had fallen off.

Evolution of growth traits in invasive *Pereskia aculeata* (Cactaceae): testing the EICA hypothesis using its specialist herbivore, *Catorhintha schaffneri* (Coreidae)

Running title: **Evolution of growth traits in invasive *Pereskia aculeata***

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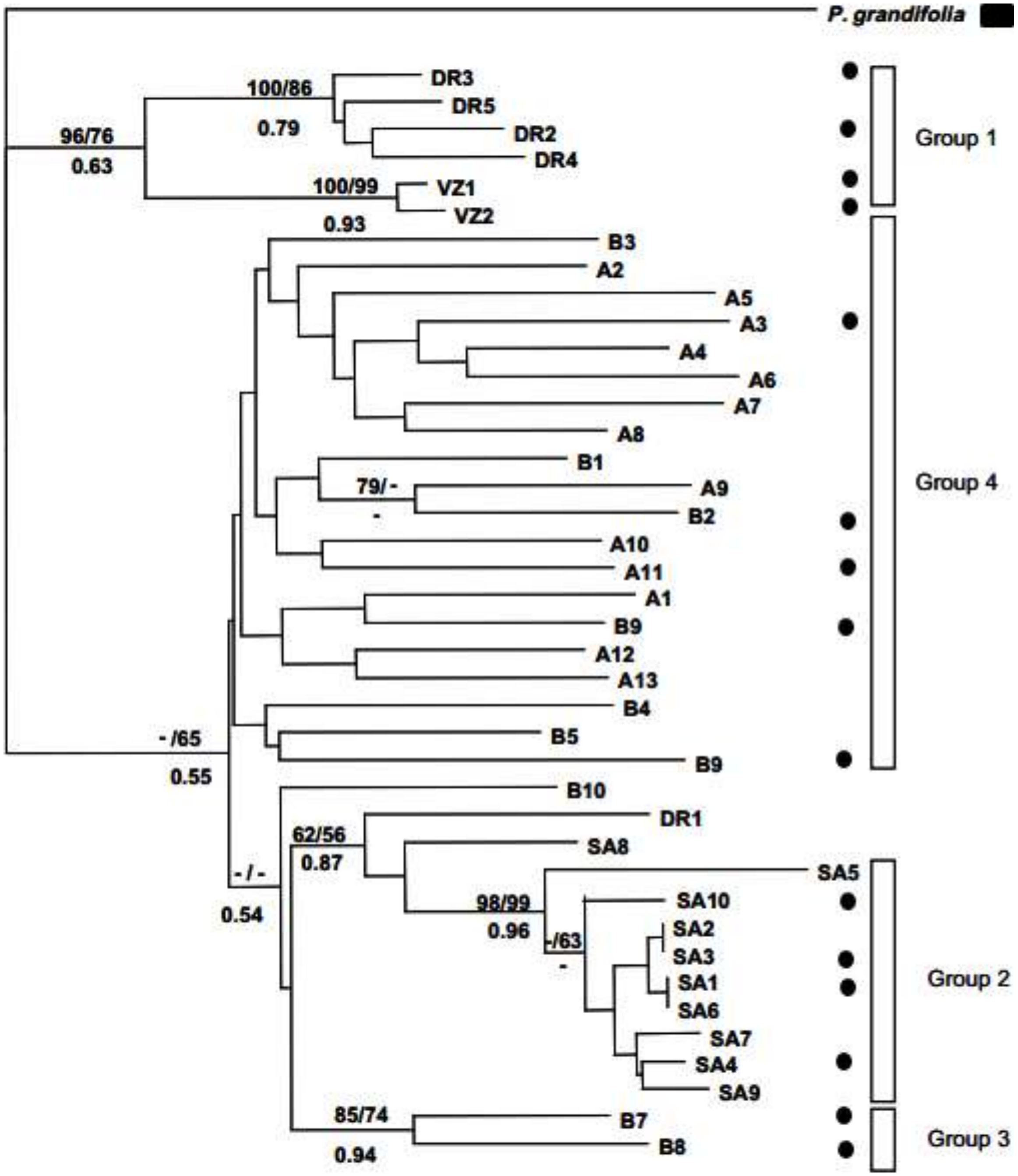

Figure 1 Genetic relationships of *Pereskia aculeata* using neighbour-joining tree constructed from ISSR data excluding bootstrap values and posterior probabilities lower than 0.5. The neighbour-joining bootstrap values/parsimony bootstrap values were provided above and the Bayesian posterior probabilities provided below each node as adapted from Paterson *et al.*[18]. Dots beside the vertical group bars represent the genotypes selected for this study.

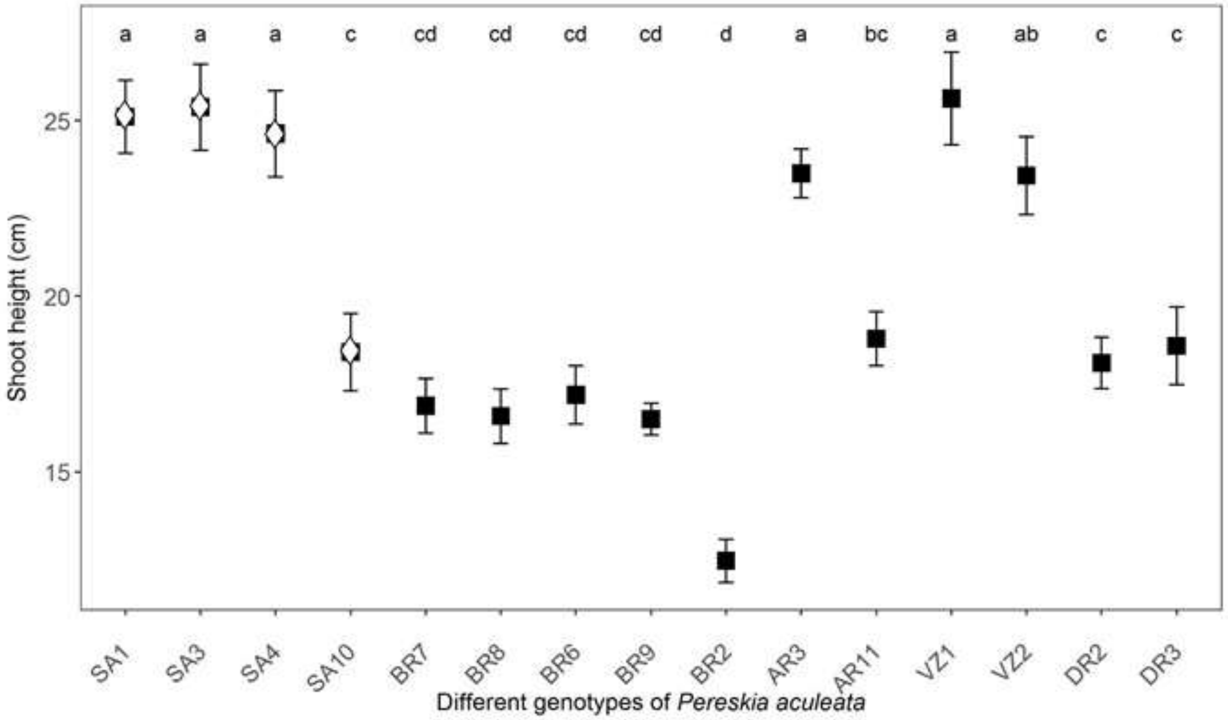

Figure 2 Means of plant heights of different genotypes of native and invasive *Pereskia aculeata*, sixty days after cultivation. Bars represent SEM. Significant differences among means were represented by the letters above each bar.

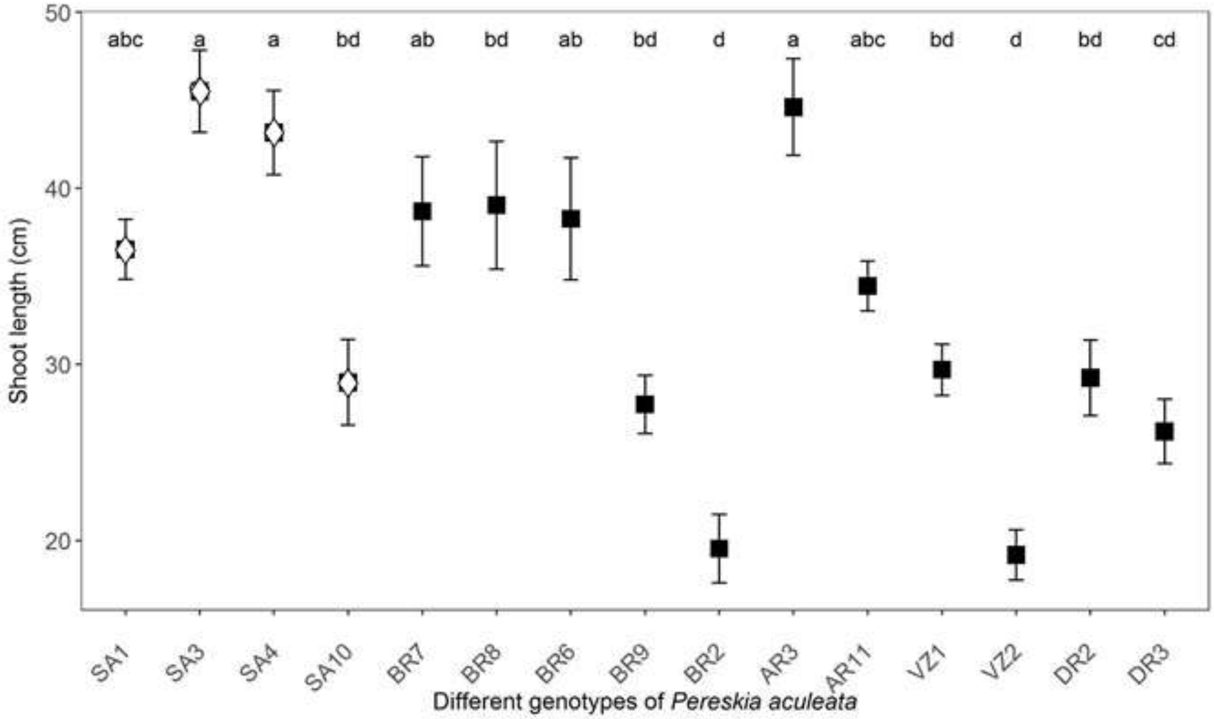

Figure 3 Means of total shoot lengths of different native and invasive genotypes of *Pereskia aculeata* after sixty days of growth. Bars represent SEM and significant differences among means were represented by different letters above each bar.

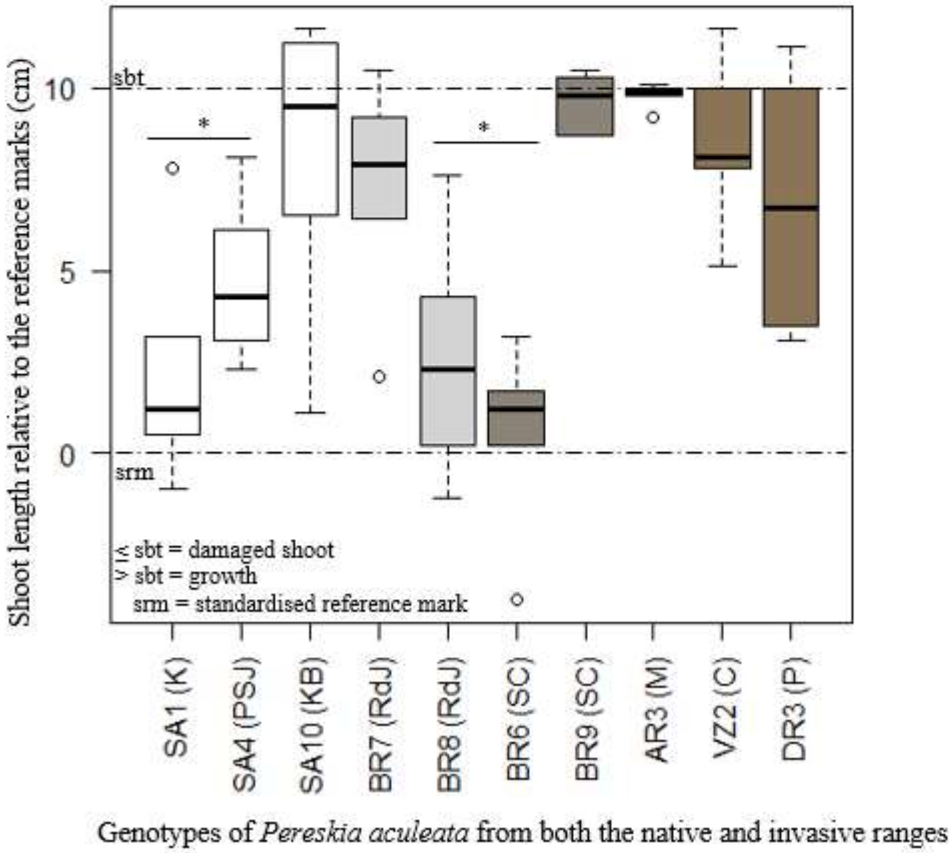

Figure 4 Damage of *Catorhintha schaffneri* on shoot lengths of *Pereskia aculeata* at a fixed level of herbivory (2♂:3♀). Colour representations: white –the invasive genotypes from South Africa; light gray –the Brazilian native genotypes from Rio de Janeiro while dark gray –those from Santa Catarina; brown –the native genotypes from Argentina, Venezuela and the Dominican Republic. Line ‘sbt’ is the height of the shoot tips at the beginning of the trial (ten centimetres above the standardised reference marks, line ‘srm’). The box plots depict medians, 25th and 75th percentiles and minimum and maximum values. Horizontal bars above the boxes SA1 and SA4, BR8 and BR6 signify a significant damage relative to the sbt. Codes in parentheses are the sources of tested plants: C = Caracas, K = Knysna, KB = Kosi Bay, M = Misiones, P = Pedernales, PSJ = Port Saint Johns, SC = Santa Catarina, RdJ = Rio de Janeiro (cf. Table 1).

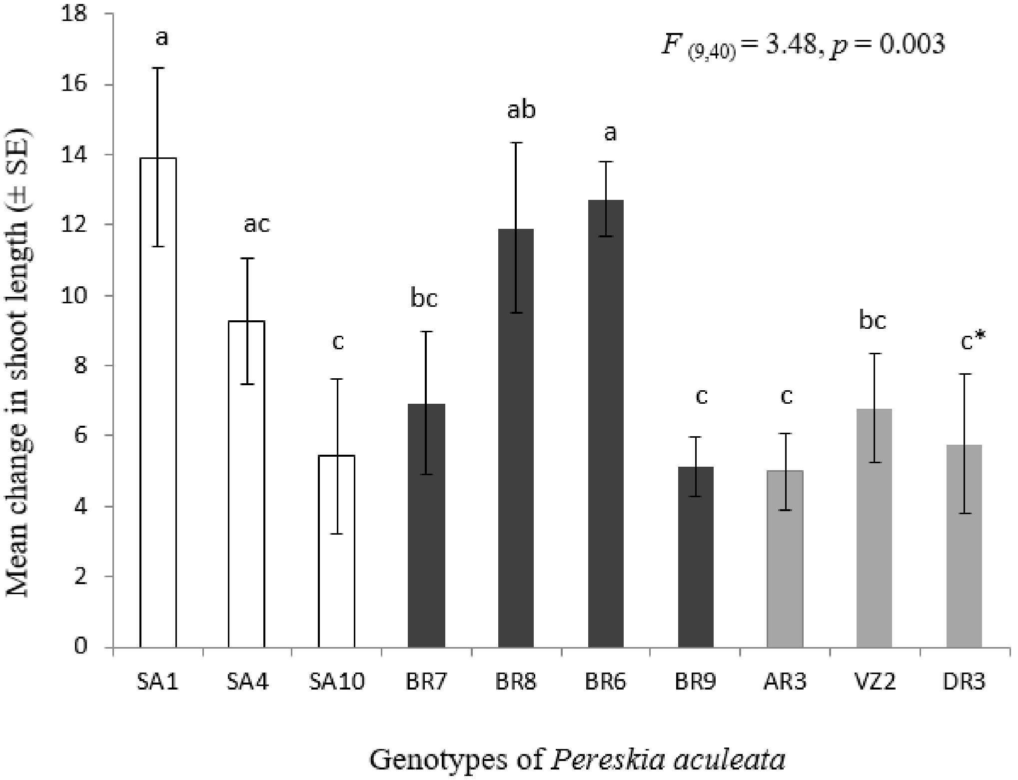

Figure 5 Impact of *Catorhintha schaffneri* on the apical shoot of genotypes of *Pereskia aculeata*. This was represented as the differences between controls and inoculated plants. Colour representations: white –the invasive genotypes from South Africa; black –the Brazilian native genotypes from Rio de Janeiro and Santa Catarina; grey –the native genotype from Argentina, Venezuela and the Dominican Republic. Notes: *The shoots of DR3 were drooping and pale, unlike others wherein top-down wilting of shoots were observed along with several 'split shoots.'

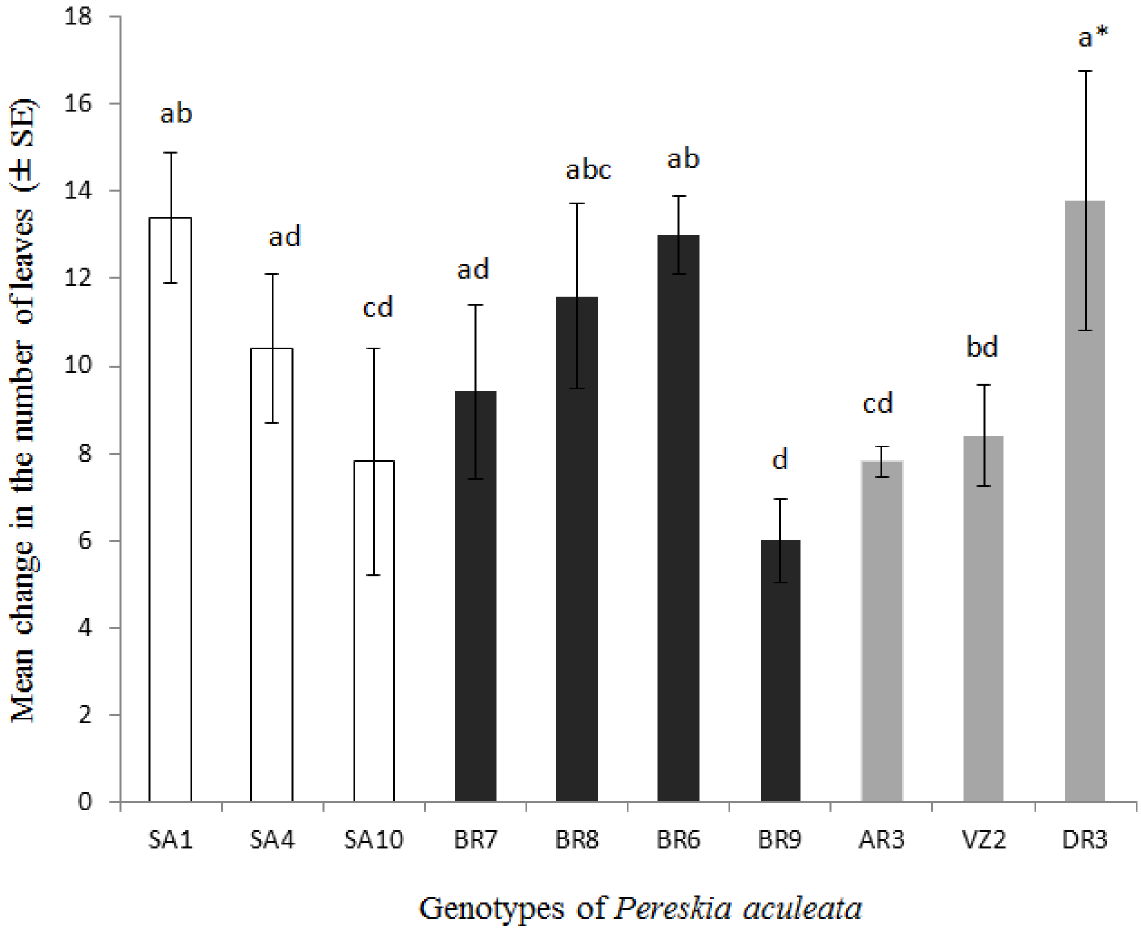

Figure 6 Impact of *Catorhintha schaffneri* on apical leaves among genotypes of *Pereskia aculeata* as differences between control and inoculated plants after ten days herbivory. Colour representations: white –the invasive genotypes from South Africa; black –the Brazilian native genotypes from Rio de Janeiro and Santa Catarina; grey –the native genotype from Argentina, Venezuela and the Dominican Republic. Note: *The value for DR3 must be interpreted with caution as most leaves were not wilted, but droopy and pale green and they remained attached to the shoot, unlike the others on which leaves were completely wilted or had fallen off.

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Graphic image