The performance and preference of a specialist herbivore, *Catorhintha schaffneri* (Coreidae), on its polytypic host plant, *Pereskia aculeata* (Cactaceae)

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By

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DEDICATION

With humility to the Author of life, this thesis is dedicated to my beloved parents,
Mr E. P. Egbon and Mrs E. K. Egbon neé Otabor …

Their perseverance in life's long walk, trials and tribulations were unimaginably instrumental for the attainment of my academic feat. Although unworthy of sight-seeing, with an article of faith and without dining with the devil, they went through hell with calluses and scars to fend for me (and many others, including those with no blood ties).

In spite of the drama, they sacrificed consistently their comfort for our education. For these and much more, I owe them everything with love and thanks.

… and to everyone, who lifted me up.

ABSTRACT

Plant species moved beyond their natural ranges may be liberated into enemy-free spaces, where they increase resource allocation to fitness, rather than defence against natural enemies, and become invasive as suggested by the Evolution of Increased Competitive Ability (EICA) Hypothesis. Several cacti are notable invaders and are targeted for biological control. The leafy cactus, *Pereskia aculeata* Miller, introduced into South Africa from South America, has become a target for biological control after becoming invasive. The absence of natural enemies of *P. aculeata* in the introduced range may be the reason for its invasiveness. This thesis seeks to investigate the role of the evolution of increased competitive ability (enemy release) as the probable driver of *P. aculeata*'s success, and ascertain how the plant's intraspecific variation influences the impact, fitness of, and preference by its biological control agent, *Catorhintha schaffneri* Brailovsky and Garcia (Coreidae), in South Africa.

Enemy release and evolution of traits in *P. aculeata* were examined by quantifying plant growth parameters of fifteen genotypes of *P. aculeata* from both the native and invaded distribution of the plant. Ten genotypes of *P. aculeata* were used in testing the effect of agent herbivory (impact and damage) under similar conditions. These studies indicated that most invaded-range genotypes were more vigorous than the native genotypes. Rapid growth may account for the quick access of invasive genotypes of *P. aculeata* to tree canopies. *Catorhintha schaffneri* damage varied between genotypes but differences in the damage and impact from the agent could not be explained by whether the plant originated in the introduced or native distribution. In sum, while the growth of the invasive genotypes largely conforms to the EICA hypothesis, the impact of *C. schaffneri* did not support the hypothesis.

The influence of host variation in *P. aculeata* on the fitness of *C. schaffneri* within the context of local adaptation to plant genotypes from different localities was tested using agent survival, stage-specific and total developmental time, and the extent of damage to ten host genotypes. Maw's Host Suitability Index (HIS) and Dobie's Susceptibility Index (DSI) showed the preference by and performance of *C. schaffneri* on the different genotypes of the plant. *Catorhintha schaffneri* survived to the adult stage on 70% of genotypes tested. Evidence consistent with the assumption that *C. schaffneri* would be fitter on the native genotypes than the invasive genotypes due to local adaptation was not found. In addition, there was no evidence in

support of fitter agents on the invasive genotypes than on the native genotypes as proposed by EICA hypothesis. *Catorhintha schaffneri* developed equally well on the invasive genotypes of *P. aculeata* as on the native genotypes.

To establish whether host variation would affect diet selection by *C. schaffneri*, both nymphs and adults were examined in paired-choice and multiple-choice trials. The nymphs and adults chose their hosts regardless of host genotype differences. The agent may be good at selecting good succulent shoots from bad shoots, but is incapable of distinguishing a good host genotype from a poorer one.

This thesis shows, therefore, that *P. aculeata* and its array of genotypes in South Africa could be effectively controlled by *C. schaffneri*, as it has the potential to suitably utilise and impact the different genotypes of the weed in South Africa with neither any demonstrable preference nor local adaptation for the native genotypes. Consequently, the use of *C. schaffneri*, as a biological control agent in the weed biological control programme of *P. aculeata* remains promising, as the agent is insensitive to the intraspecific variation of the invasive host plants.

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CHAPTER 1 INTRODUCTION

1.1 OVERVIEW

Species translocation arises through either deliberate or unintentional introduction, across biogeographic barriers such as oceans, into novel habitats which are referred to as either the introduced or adventive range (Hillman 1893a & 1893b; Faulkner *et al.* 2016). Upon arrival, some non-native species progress through an 'introduction-naturalisation-invasion continuum' which results in the species becoming invasive by being overly abundant in the introduced range to the detriment of native species (Williamson & Fitter 1996; Richardson & Pyšek 2012). The negative effects of these introduced invasive species on human health, livelihood, forestry, agriculture, economies, biodiversity, and ecosystem functioning are costly and usually underestimated (OTA 1993; McFadyen 1995; van Wilgen *et al.* 2001; Pimentel 2002; Williams *et al.* 2010). If left unmanaged, biological invasions can result in significant socio-economic and environmental implications.

The aftermath of invasions are characterised by alterations of species composition and structure of ecological communities, ecosystem functions, nutrient recycling, and ultimately ecosystem modification (Richardson *et al.* 2000; Eads & Biggins 2015). Invasive alien species (IAS) can reduce ecosystem stability through a cascade of events that endanger keystone species (Richardson *et al.* 2000; Lowe *et al.* 2014; Eads & Biggins 2015). Such IAS are known as transformer species (Richardson *et al.* 2000). For example, the bacterium *Yersinia pestis* van Loghem (Enterobacteriales: Yersiniaceae) when introduced into the western North America decimated populations of herbivorous prairie dogs (*Cynomys* spp.) (Eads & Biggins 2015). This event encouraged the range expansion of introduced plant species such as the Russian thistle *Kali tragus* Linnaeus (Caryophyllales: Amaranthaceae) and *Prosopis* spp. (Fabales: Fabaceae), thereby completely transforming the grassland ecosystem into a degraded ecosystem (Ecke & Johnson 1952; Eads & Biggins 2015; Davidson *et al.* 2010). In this example, the bacterium exhibited the characteristics of a transformer species (Richardson *et al.* 2000) and the influence of the bacterium was exacerbated by its interaction with other non-native species causing an invasion meltdown (Simberloff & Holle 1999; Eads & Biggins 2015).

Invasive alien plants are capable of altering ecological stability, such as the prairie dog ecosystem (Eads & Biggins 2015), either following anthropogenic disturbances or, in some

cases, no disturbance at all. Richardson *et al.* (2000) considered species that are capable of altering ecological systems, trophic interactions and balance, as transformer species (or simply 'Transformers'). In relation to MacDougall and Turkington (2005) and Bauer (2012)'s categorisation of species as (i) passengers (ii) back-seat drivers and (iii) drivers of biodiversity loss, the transformers are neither passengers of change nor back-seat drivers on an 'ecological ride', but are the actual drivers, or causes, of biodiversity loss. The passengers and back-seat drivers both require ecological perturbation to establish and spread, unlike the drivers (Hill & Coetzee 2017; Eads & Biggins 2015). Despite their similarities, both passengers and back-seat drivers differ in their post-establishment impact status such that upon removal of the disturbance the impact of the passengers would subside but the impact of the back-seat drivers would not (Hill & Coetzee 2017). According to Hill and Coetzee (2017), problematic aquatic alien plants in South Africa, which proliferate following disturbance and nutrient enrichment from run-off (eutrophication), provide examples of back-seat drivers. In terrestrial ecosystem, the thistle *K. tragus* and *Prosopis* spp., which became predominant after the decimation of the prairie dogs, rode on the effects of *Y. pestis* to dominate the grassland and are therefore examples of back-seat drivers (MacDougall & Turkington 2005; Bauer 2012; Eads & Biggins 2015).

In many arid ecosystems, invasive alien cacti are transformer species that are drivers of biodiversity loss. Although native to the Americas, many cacti have naturalised outside their native distribution where they often form dense infestation with negative implications for the natural ecosystem and agriculture (OTA 1993; Moran *et al*. 2013; GISD 2015). During the late 18th Century, in an attempt to farm cochineal for its red dye (carmine) in India, the incorrect species of cochineal insect, *Dactylopius ceylonicus* (Green) (Hemiptera: Dactylopiidae) from South America, was introduced to India instead of *D. coccus* Costa, which can be viably farmed for the dye. *Dactylopius ceylonicus* was therefore introduced into India inadvertently on a problematic invasive species, the drooping prickly pear, *Opuntia monacantha* Haw. (Caryophyllales: Cactaceae). Although the intention was to farm the cochineal rather than control the alien plant, this accidental introduction became the world's earliest weed biological control programme (Moran *et al.* 2013). In 1903 Australia attempted to bring cactus under control, drawing from the Indian experience with the first successes from biological control being recorded in 1914 (Moran *et al.* 2013). Meanwhile, South Africa had initiated biological control on *O. monacantha* in 1913 with successful outcomes as the agent spread around the

country (Moran *et al.* 2013). In Henderson's (2001) compilation of alien weeds and invasive plants in South Africa, eight (57.1% of) cacti species out of 14 were transformers. Although high, it could have been more than that if not for the success of biological control against some cacti such as *O. monocantha* and many other cactus species (Moran & Zimmermann 1991; Henderson 2001; Klein 2011, updated 2016).

The historic accounts of biological control successes against invasive alien cacti may lead people to believe that biological control is a rapid solution to invasive alien plant problems, but in most cases, it requires long-term studies, and control is achieved only after a number of years (Moran *et al.* 2013; Dlugosch *et al.* 2015). The outcome of weed biological control is usually not easy to predict and, because it may take several years before an agent results in control of an invasive alien plant, it has been suggested that the success or failure of a project should only be assessed some 20 years after release (McFadyen 2000).

Pereskia aculeata Miller (Cactaceae) (the topic of this thesis) is an invasive and a transformer species in South Africa (Klein 1999; Henderson 2001). Native and endemic species are threatened as *P. aculeata* outcompetes them for light and space, and kills large forest trees, which later collapse under its weight (Klein 1999; Henderson 2001; Paterson *et al.* 2011). Consequently, conservationists, forest managers and biological control practitioners are seeking sustainable methods to halt its spread and reduce the negative impacts of the plant in South Africa. Although chemical and mechanical control have been considered, biological control had taken precedence because it is ecologically friendly and self-sustainable after successful establishment (Moran & Zimmermann 1991; Klein 1999). This is unlike other control methods, which require continuous input, such as re-application of chemicals and their negative impacts on the environment and non-target species (Moran & Zimmermann 1991; Klein 1999; Carlson 1962).

The initial attempt at managing the infestations of *P. aculeata* using a biological control agent began with *Phenrica guerini* Bechyné (Coleoptera: Chrysomelidae) but this agent did not reduce *P. aculeata* populations to an acceptable level (Klein 1999). A new biological control agent, *Catorhintha schaffneri* Brailovsky and Garcia (Hemiptera: Coreidae), was then released in 2014 and initial post-release evaluations have indicated that it may be more successful than the

previous agent (Paterson *et al.* 2014a, b; Klein 2011 updated 2016). The *C. schaffneri* population that was released in South Africa was collected from two sites in southern Brazil, but the majority of the collected individuals came from a single site (ID Paterson, Rhodes University, pers. comm.). The agent is a specialist herbivore and is expected to have low levels of intraspecific variability because only 23 individuals were collected in the native distribution (Paterson *et al.* 2014a). The target plant, *P. aculeata* is also genetically depauperate in South Africa, but is highly polytypic and polymorphic in the indigenous range (Leuenberger 1986; Paterson *et al.* 2009). This thesis investigates the performance and preference of the *C. schaffneri* biological control agent population on the different forms of *P. aculeata* from both the native and introduced distribution.

1.2 PERESKIA ACULEATA *MILLER (CACTACEAE)*

1.2.1 Taxonomy and Evolution

The genus *Pereskia* was named in honour of Nicholas F. Peiresk, a French patron of botany (Leuenberger 1986; Janick & Paul 2008). Specimens of this genus were first collected by Plumier between 1689 and 1695 in the West Indies, but their descriptions existed only in manuscripts (Hunt 1984). Although these were pre-Linnaean collections (Leuenberger 1986 [and references therein]; Sloane 1696; Commelin 1697), '*Pereskia*' was first adopted in Plumier's descriptive phrase as *P. aculeata*, that is '*Pereskia aculeata, flore albo, fructo flavenscente*' as described in an unpublished manuscript '*Botanica americanarum…*', and later became *P. guamacho* (Hunt 1984; Leuenberger 1986). In 1703, Plumier's collections of leafy cacti were listed by Linnaeus in '*Species plantarum*' as *Cactus pereskia* L. (Cactaceae), at a time when it was already cultivated in Europe, but no formal description of any species had been made (Leuenberger 1986).

Subsequently, the genus was revised to *Pereskia* as documented in the 'Gardener's Dictionary', 4th edition (Miller 1754), but was only described formally in the 8th edition (Miller 1768). This formal description of *Cactus pereskia* gave rise to *Pereskia aculeata* Miller 1768. Hence, Plumier's first descriptive term was resurrected. This led to the preservation of 'pereskia' from

the species epithet and may have accounted for the seeming confusion about the right authorship of *Pereskia aculeata* as to whether it was Plumier or Miller in some later literature (Leuenberger 1986), but the latter is the rightful author being the first to formally describe and publish the plant's description.

Other authors later described additional species in the genus *Pereskia*, but only fifteen species, excluding *P. aculeata*, were recognised in the most recent review of the genus (Leuenberger 1986). The other species of *Pereskia* are *P. humboldtii* Britton and Rose, *P. diaz-romeroana* Cárdenas, *P. weberiana* Schumann, *P. lynchnidiflora* De Candolle, *P. aureiflora* Ritter, *P. guamacho* Weber, *P. zinniiflora* De Candolle, *P. portulaciflora* (Linnaeus) De Candolle, *P. quisqueyana* Liogier, *P. bleo* (Kunth) De Candolle, *P. nemorosa* Rojas Acosta, *P. sacharosa* Grisebach, *P. grandifolia* Haworth, *P. bahiensis* Gürke, and *P. stenantha* Ritter (Leuenberger 1986 p.36,). However, a few years after Leuenberger's review of the genus, an additional species *P. marcanoi* Areces-Mallea was described (Areces-Mallea 1992), making *Pereskia* a genus with 17 species.

Biogeographic analysis of *Pereskia*'s evolution showed that they are closely related to the ancestors of Cactaceae (Schumann 1899). Based on the Wegener theory of continental drift and separation of the climatic belt, Backeberg (1942) demonstrated that the common ancestral link of Cacti was within the tropical 'West Indian-Middle American region' (Leuenberger 1986). The basal position of *Pereskia* and its relationship to the ancestors of Cactaceae are evident in the shared morphological and anatomical traits of their flowers and stems, for example, the stem-based photosynthesis (Leuenberger 1986; Edward *et al.* 2005).

Although Schumann (1899) sought the closest protocactaceous relatives of *Pereskia* among Portulacaceae and Aizoceae, Buxbaum (1949) relied on the assumption that *P. aculeata* was the most primitive member of Cactaceae to conclude that they evolved from Phytolacaceae (*Serguieria*). The evolutionary history of *Pereskia* remained unclear and speculative (Bailey 1962; Boke 1954, 1966; Leuenberger 1986), but recent assessments have favoured the Schumann's school of thought, which supports Portulacaceae as the closest relatives of Cactaceae (Carolin 1993; Hershkovitz & Zimmer 1997; Cuénoud *et al.* 2002; Nyffeler 2007; but see Nyffeler & Eggli 2010).

Pereskia aculeata is a 'complex of varieties, forms and clones', with some forms that are thought to have been developed as horticultural varieties (Leuenberger 1986; Paterson *et al.* 2009). Although Pfeiffer (1837) tried to describe all the forms, no reliable diagnostic traits were discovered, thus the species is better treated as a 'polymorphic species' (Leuenberger 1986), or loosely as a collection of 'morphotypes' (Moran & Zimmermann 1991), or even genotypes (Paterson *et al.* 2009). More recent work by Paterson *et al.* (2009) investigated the genetic relationships between these forms using molecular markers. The genetic data resulted in the conclusion that South African populations of *P. aculeata* originated from southern Brazil and that the population in southern Brazil and northern Argentina were genetically distinct from those in northern Venezuela and the Caribbean (Paterson *et al.* 2009).

1.2.2 Morphology and Habit

Pereskia exhibits diverse growth patterns typical of woody dicotyledonous plants (Buxbaum 1957). Some of its species, for example, *P. lynchnidiflora, P. zinniiflora* and *P. guamacho* possess both monopodial (terminal bud with subordinate lateral shoots) and sympodial (lateral buds with an inactive terminal bud) branching patterns (Leuenberger 1986). *Pereskia aculeata* (Fig. 1.1) is a clambering shrub and has similar branching patterns with *P. lynchnidiflora, P. zinniiflora* and *P. guamacho* (Leuenberger 1986; Henderson 2001). It grows as a vine and with support provided by neighbouring trees grows up into the canopy.

Pereskia species have different root systems, such as the deep-reaching taproots of *P. sacharosa* or shallow soil-surface roots in *P. lynchnidiflora*, and tuberous root systems among *P. quisqueyana* and *P. weberiana* (Leuenberger 1986). *Pereskia aculeata* has a shallow root system without tubers or a taproot (Leuenberger 1986). Deep root systems are crucial for water uptake during long periods of drought, while shallow roots allow for quick water uptake after brief rains (Leuenberger 1986; Edwards & Donoghue 2006). Since water is typically a limiting resource in arid places where cacti are abundant, such diverse (deep and shallow) root systems may confer a competitive advantage on *Pereskia* species over other plants through their ability to harness water efficiently after both light and heavy rains. Hence, these may facilitate the absorption of

water and other soil resources faster than other native floras, which could become disadvantaged in terms of water and nutrient uptake.

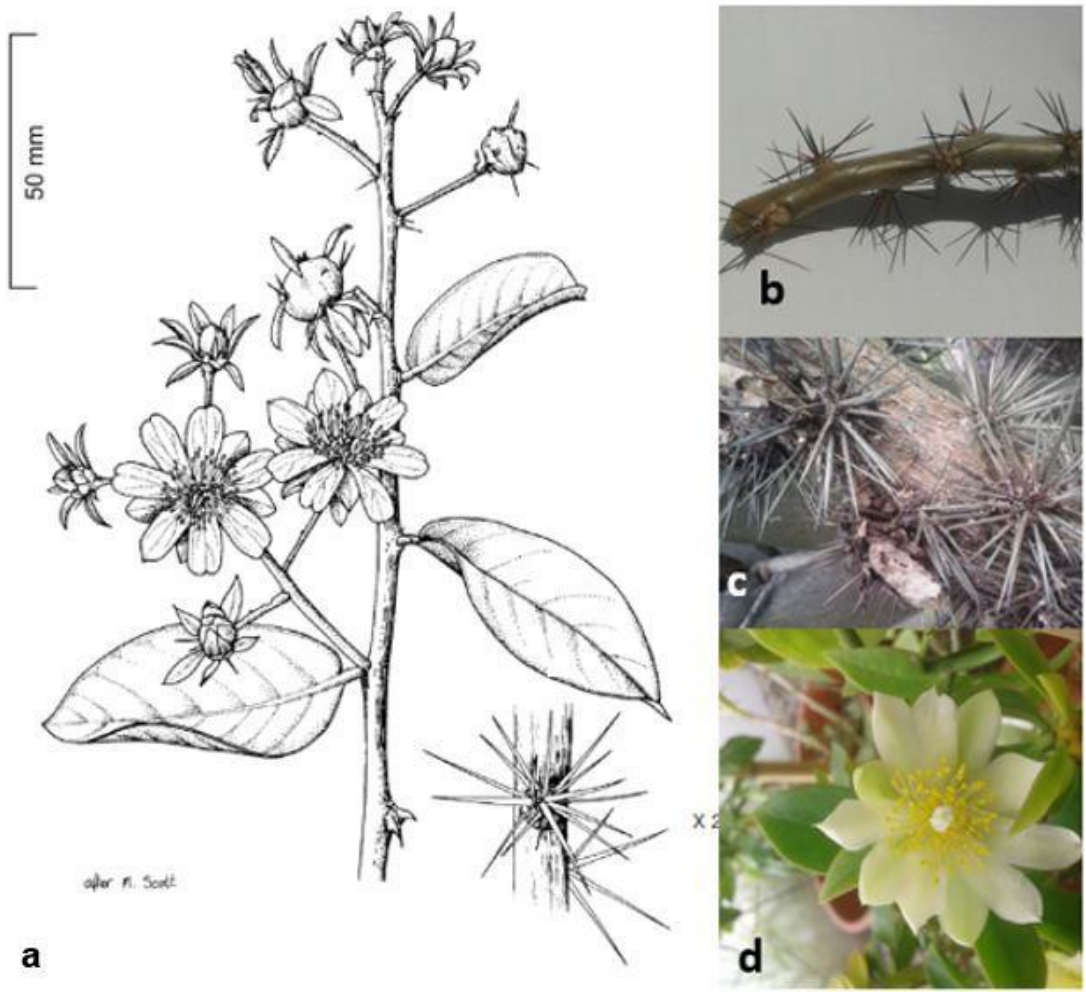

Fig. 1.1 The leafy cactus *Pereskia aculeata* (a-d). a) The apical section with flowers and fruits. b) A young stem with short slender spines. c) An older stem with long and numerous spines sourced from an infested sites in East London, South Africa. d) A full-grown flower from potted plants (Sources: a: drawn by G. Condy; as published in Paterson *et al.* 2011a; Image credit for b- d: I.N. Egbon).

The young stems (shoots) of *Pereskia* are fleshy, succulent and relatively spineless, but become woody and covered in dense spines with age. As it is characteristic of cacti, *P. aculeata* has areoles. These are auxiliary outgrowths (Leuenberger 1986; Nyffeler & Eggli 2010) with

contracted short shoots (brachyblasts) and are a site for trichome and spine production (Applequist & Wallace, 2003; Nyffeler & Eggli 2010). Long after leaves are shed, the areoles continue to produce spines (Leuenberger 1986). Pereskias, including *P. aculeata*, are often spineless at the distal end of their shoot apex, but extremely spiny at the basal end of the stem, because of the indefinitely spiniferously active areoles (Leuenberger 1986).

1.2.3 Biology and Distribution

Pereskia aculeata is native to South America, parts of North America and the Caribbean (Fig. 1.2; Leuenberger 1986), across broad ecological zones with diverse geological formations and vegetation zones. Records of the plant in Florida, U.S.A., and southern Mexico are considered naturalised populations from garden escapes rather than native populations (Leuenberger 1986). The northern native range consists of northern Venezuela, Guyana and the Caribbean Isla while the southern native range consists of populations in Paraguay, northern Argentina and south-eastern Brazil (Leuenberger 1986). The northern and southern ranges are separated by a distance of over 4000 km as well as biogeographic barriers such as the Amazon rainforest (Leuenberger 1986).

Pereskia aculeata is an invasive alien plant in South Africa, Australia and U.S.A (Hawaii) (Henderson 2001; Anon 2003; Imada 2012; SHDLNR, 2013). It has naturalised on Moloka'i Island, Hawaii, where it is a target for chemical and physical control (Imada 2012; SHDLNR, 2013). *Pereskia aculeata* has also naturalised in Australia, where it is confined to the coastal subtropical districts of Queensland and New South Wales (Anon 2003; 2011). Its occurrence in Australia was initially not considered problematic, however, in recent times, it has been reported that *P. aculeata* is gradually becoming invasive and the plant is now considered a potential target for biological control in the country (I.D. Paterson, pers. comm. 2017). In South Africa, *P. aculeata* has invaded seven out of nine provinces, but it is most abundant and damaging in the subtropical coastal regions of the country such as coastal KwaZulu-Natal and the northern coastal areas of the Eastern Cape (Fig. 1.3; Henderson 2001). Consequently, the government, landowners, conservationists and biological control practitioners are seeking sustainable measures to halt its spread and reduce its negative ecological effects.

Fig. 1.2 The distribution of *Pereskia aculeata* in its native range in South America, the Caribbean, and southern part of North America (after Leuenberger 1986). The black dots represent the localities of *P. aculeata*.

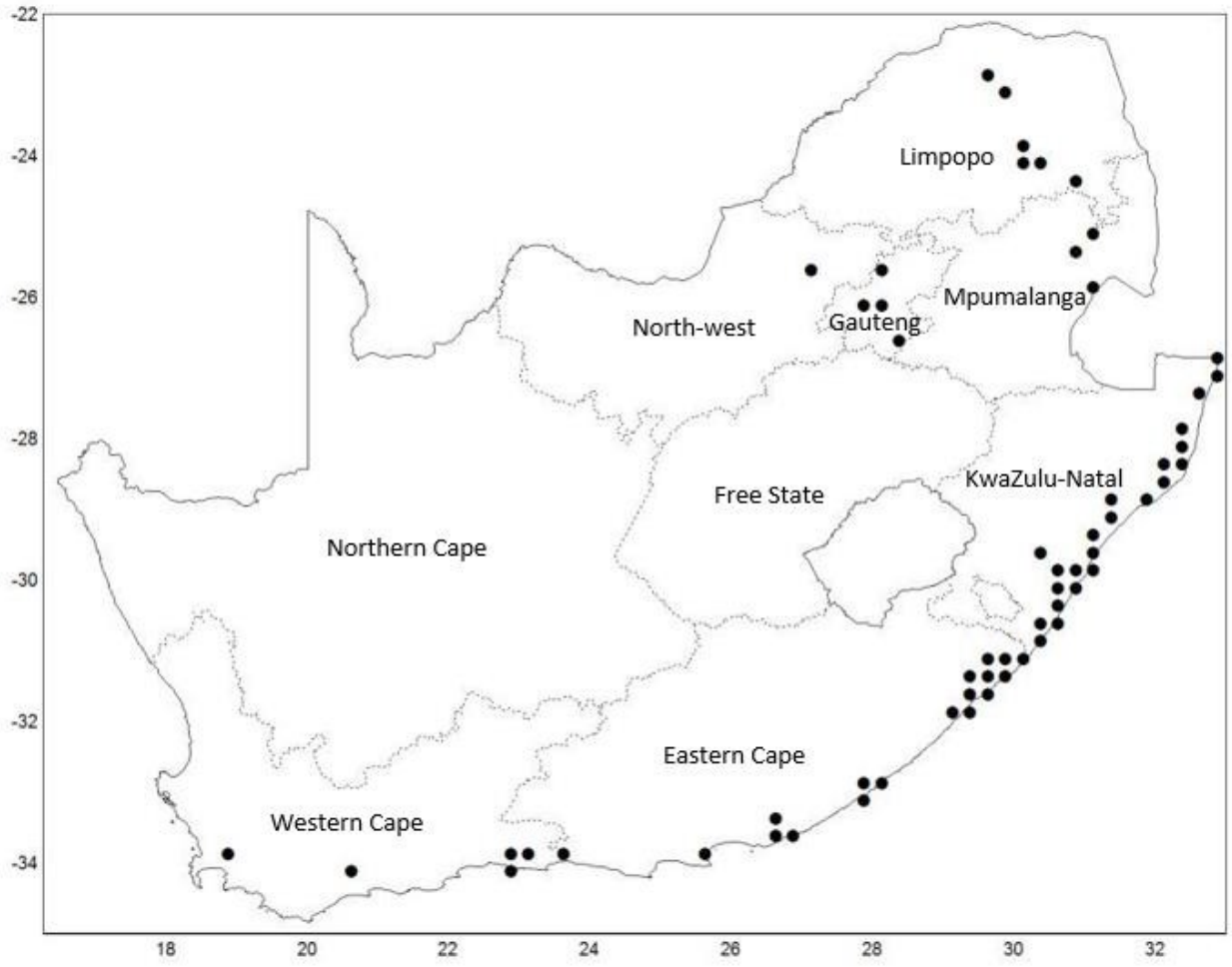

Fig. 1.3 The recorded distribution of *Pereskia aculeata* in South Africa (Modified from L. Henderson). The most invaded provinces are KwaZulu-Natal and Eastern Cape Provinces.

1.2.4 Beneficial properties of *Pereskia*

Pereskia aculeata is commonly known by native inhabitants of Latin America as *grosellero*, *guinda* and *tomate americano*. It is referred to as *ramo de novia* means 'bridal bouquet' in Cuba and *ora pro nobis* 'pray for us' in Brazil. The abundance of indigenous names for *P. aculeata* are evidence of its importance as a beneficial and culturally significant plant. *Pereskia aculeata* is eaten as food in Minas Gerais and Bahia, Brazil, and has various medicinal properties (Leuenberger 1986; Takeiti *et al.* 2009; Zareisedehizadeh *et al.* 2014, Souza *et al.* 2014; Souza *et al.* 2016). In South Africa, it is referred to as *uqwaningi* (isiZulu) or *barbadosstekelbessie*

(Afrikaans) (ISSA 2016). The initial record in Cape Town Botanical gardens (McGibbon 1858) exemplifies its horticultural use in South Africa and there are much earlier accounts of the use of *P. aculeata* as a horticultural plant from other parts of the world, including its presence in Kew Gardens as early as 1696 (Britton & Rose 1919). Its characteristic spines have been used as insect pins by some entomologists. Soldiers were known to have used them for fixing tattered uniforms during wars, securing clothing and stitching wool sacks (Brown *et al.* 2011). Similarly, *P. lynchnidifolia* spines served as needles in Guatemala (Eichlam 1909). The most common use for *P. aculeata* in South Africa is, however, its use as a barrier fence or hedge (Haigh 1979; Bruton 1981; Leuenberger 1986; Klein 1999). *Pereskia aculeata* was also used in making preserves as far back as 1881 in South Africa (De Beer 1988; Moran & Zimmermann 1991).

1.3 STATUS OF *PERESKIA ACULEATA* IN SOUTH AFRICA

In South Africa, *P. aculeata* grows in very high densities covering indigenous plants including large forest trees that often collapse due to the weight of the weed in the canopy (Moran & Zimmermann 1991). Paterson *et al.* (2011b) recorded a reduction in indigenous plant diversity associated with *P. aculeata* infestations, as well as a change in the functional group composition of indigenous plants favouring vines rather than shrubs and trees. The mechanism of this change in indigenous ecosystems is firstly by reducing sunlight to indigenous plants underneath the *P. aculeata*, thus reducing their productivity (e.g., vegetative growth, flowering and fruit production).

Vertebrates play a key role in the dispersal of *P. aculeata* even though as with many cactus species, the spines can be injurious to them (Standley & Williams 1962; Leuenberger 1986). The seeds of *P. aculeata* are dispersed by generalist frugivores such as monkeys, bats and birds (Roosmalen 1985; Leuenberger 1986). This could exacerbate forest infestation with effects on pristine habitats as the animals transfer its seeds far into undisturbed, pristine habitats. This ability to impact pristine environments makes *P. aculeata* a driver of biodiversity loss and not a back-seat driver (MacDougall & Turkington 2005; Bauer 2012).

The effects of *P. aculeata* in infested forests are density-dependent and modulated by invasion histories (Paterson *et al.* 2011b). As its abundance increases, species richness and functional group diversity decline (Paterson *et al.* 2011b). However, Paterson *et al.* (2011b) found that such impacts are reduced at densities below 40% cover, and recommended that to reduce and maintain *P. aculeata* infestations below such density should be the aim of management practices.

The negative impacts of *P. aculeata* are increasing as its spread remains unabated and its density is rising to the detriment of native floras and faunas in South Africa. Forests are particularly prone to invasion by *P. aculeata* (Paterson *et al*. 2011a). The forest biomes in South Africa are relatively rare and the majority of species are endemic (Mucina & Rutherford 2006). *Pereskia aculeata*'s invasion poses an enormous threat that demands urgent attention, especially when the forests are '… home to some 21,137 species of vascular plant', eighty percent of which are endemic (van Wilgen *et al.* 2001). Consequently, adopting appropriate strategies that would preserve ecological integrity and only target *P. aculeata* are required.

At present, South Africa has 534 invasive alien species classified into four categories namely categories (1a, 1b, 2 and 3) (Henderson 2001; CTIN 2014; Department of Environmental Affairs 2014). Species in category 1(a) are prohibited by law and infested properties 'must' be evaluated and monitored by government officials who will assist with their control (Henderson 2001; Department of Environmental Affairs 2014). Species in Category 1b must be contained and 'may' need government assistance although they might already be under government-sponsored management programmes like biological control. Category 2 and category 3 plants may remain within demarcated areas such as gardens, but require permits, which are seldom granted (Henderson 2001; Department of Environmental Affairs 2014). However, when present in ecologically sensitive areas like riparian zones, Category 3 status becomes category 1b for which species containment and eradication must be attempted (Henderson 2001; Department of Environmental Affairs 2014). Given the status of *Pereskia aculeata* in South Africa as a weed in category one (1b), any use of the plant is prohibited by law and it must be contained and controlled (Department of Environmental Affairs 2014).

1.3.2 Management of *Pereskia aculeata*

1.3.2.1 Chemical/Mechanical Control

The initial efforts to manage *P. aculeata* incorporated multiple control techniques such as mechanical and chemical control (Vermuelen *et al.* 1996; Klein 1999). However, these methods are practically difficult to execute against *P. aculeata* for several reasons. Attempts to eradicate invasive alien plants with conventional techniques such as the use of herbicides abound in the literature, but are usually short-term and require several costly episodes of re-applications and have significant non-target effects (Carlson 1962; Vermuelen *et al.* 1996; Klein 1999). Given the cost and negative impacts to the environment (Carlson 1962), and other barriers such as poor management plans, herbicide application would have sufficed if eradication was attainable, but generally, this is not possible, rarely effective and prematurely discontinued in widespread infestations (Vermuelen *et al.* 1996; Klein 1999; Wilson *et al.* 2013; Kraaij *et al*. 2017 and references therein). Triclopyr (butoxy ethyl ester; 480 g/l active ingredient) is registered for the management of *P. aculeata* in South Africa, but the chemical is not translocated within the plant tissue and therefore only kills small sections of the plant stem (Vermuelen *et al.* 1996; Klein 1999). Since triclopyr does not kill the whole plant, its use requires integration of mechanical methods consisting of manual removal and burning to remove plant parts after treatment (Klein 1999). Mechanical removals invariably fail to remove all pieces of living plant fragments and these fragments are capable of regeneration (Klein 1999; Fig. 1.4). In addition, both mechanical and chemical control lack safeguards for non-target effects on indigenous flora because not only would indigenous trees that are interwoven with *P. aculeata* be damaged while removing the vine, they can also be affected by herbicide treatments (Moran & Zimmermann 1991). Thus, mechanical and chemical control are only appropriate for very small infestations of *P. aculeata* in accessible areas where trees canopies have not been covered and tree stands are devoid of severe entanglement with the vines (Moran & Zimmermann 1991).

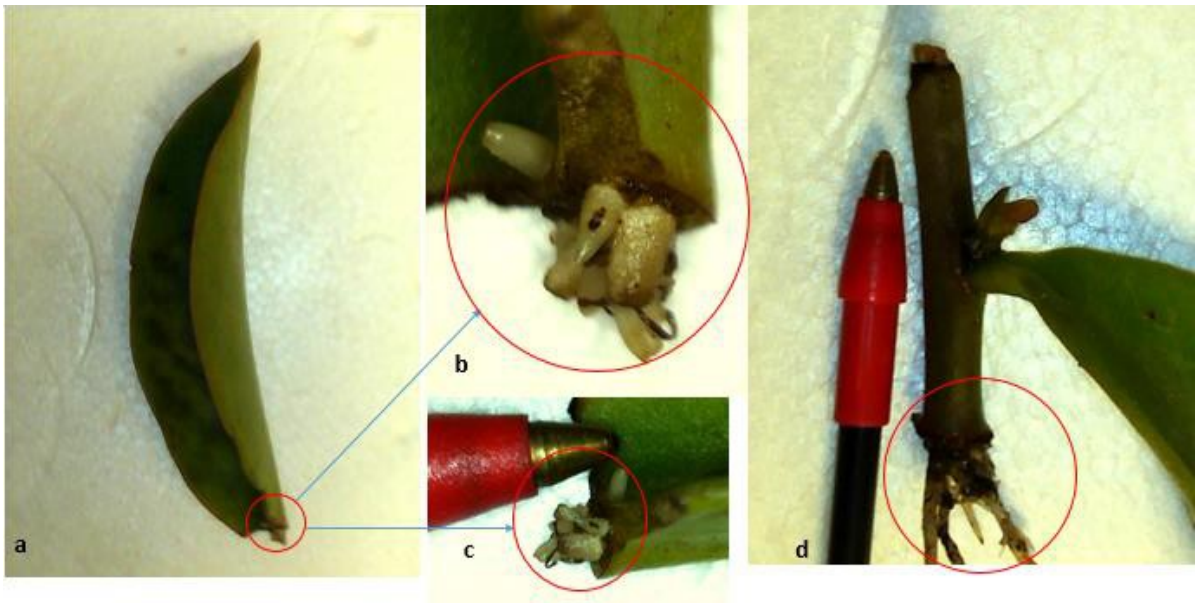

Fig. 1.4 Fragments of *Pereskia aculeata* generating new roots and shoot after being severed from parent plants. a) Leaf growing new roots. b) and c) magnified section of the leaf with root growths and d) A tiny fragment of stem regenerating roots and shoot from the areole. (Image credit: I.N. Egbon a-d).

1.3.2.2 Biological Control of *Pereskia aculeata*

Biological control uses coevolved and target-specific natural enemies to suppress densities of invasive pests to 'a lower average than would occur in their absence' (De Bach 1964). These natural enemies, referred to as biological control agents once released in the introduced range, could be parasites or predators for insect pests but are usually pathogens and arthropod herbivores for weeds like *P. aculeata* (Moran & Zimmermann 1991; Pereira *et al.* 2007; Paterson *et al.* 2014a, b; Hoddle *et al.* 2015). The agents are density-dependent regulators and therefore agent populations will fluctuate depending on the density of the target weed. Total eradication of the target weed is therefore not expected and should not been seen as a goal of biological control programmes. Rather, the host populations would be minimised and maintained at a new equilibrium in which the negative impacts on indigenous species is lowered (De Bach 1964; Paterson *et al.* 2011b). *Pereskia aculeata* should be lowered to below 40% cover in order to minimise impacts on indigenous biodiversity (Paterson *et al.* 2011b).

Many natural enemies have been identified on *P. aculeata* and a number of these are promising potential biological control agents (Klein 1999; Pereira *et al.* 2007; Paterson *et al.* 2014a, b). Phytopathogenic rust fungi (*Uromyces pereskiae* and *Aecidium pereskiae*) have been isolated from *P. aculeata* in Brazil, but these are yet to be considered for biological control (Pereira *et al.* 2007). Thus far, insect herbivores have been prominent in biological control against *P. aculeata.*

The biological control programme for *P. aculeata* began opportunistically along with surveys for agents for other problematic weeds (Moran & Zimmermann 1991; Klein 1999; Olckers 2004). During the 1990's three agents were introduced into quarantine facilities in South Africa, namely: *Loxomorpha* (formerly: *Epipagis*) *cambogialis* (Guenée) (Pyralidae), a leaf-feeding pyralid moth; *Maracaryia chlorisalis* (Walker) (Crambidae), a stem and shoot boring moth; and a flea beetle, *Phenrica guerini* (Chrysomelidae) which feeds externally on leaves and shoots (Klein 1999). Later, in 2014, two more potential agents, a stem boring weevil, *Pereskiophaga brasilensis* Anderson (2015) (Coleoptera: Curculionidae) and, the test insect of this thesis, a shoot-wilting bug *Catorhintha schaffneri* (Paterson *et al.* 2014b) were imported into quarantine as promising potential agents.

Of the first three potential agents that were imported into quarantine in the 1990's only *P. guerini* was released, while the others were either shelved (*M. chlorisalis*) or rejected (*L. cambogialis* (Guenée) (Moran & Zimmermann 1991; Klein 2011 updated 2016: http://www.arc.agric.za/arc-ppri/Documents/Target weed species in South Africa.pdf). *Pereskiophaga brasilensis* is currently under investigation and yet to be released and *C. schaffneri* was released in 2014 (Paterson *et al.* 2014b). There are therefore only two agents that have been released on *P. aculeata* and these are *P. guerini* and *C. schaffneri* (Klein 1999, 2011 updated 2016; Paterson *et al.* 2014a, b).

1.3.2.2.1 *Phenrica guerini* Bechyné

Phenrica guerini was formerly called *Nephrica guerini* Bechyné 1955 and later placed in the genus *Phenrica* (Bechyné 1956). It is a host-specific foliar-feeding beetle that was collected in Brazil and subjected to host specificity testing in quarantine in South Africa (Klein 1999; Paterson *et al.* 2012). It was first released in KwaZulu-Natal Province and

subsequently extended to the Eastern Cape Province in South Africa (Klein 1999). Although the initial release was in 1991, its first record of the establishment was in 1995 (Klein 1999). Releases were carried out using adults reared from a stock population collected from Barra de São João (140 km northeast of Rio de Janeiro), Lagoa de Marapendi and Praia da Barra de Tijuca, all in Brazil (Klein 1999).

The impact of *P. guerini* was negligible and did not reduce the weed density even at sites where the agent's populations became abundant (Paterson *et al.* 2011b). However, no formal post-release evaluation had been conducted to ascertain its present performance. Visual observation of damage inflicted by *P. guerini* at some sites suggest that it may be damaging to *P. aculeata* but a formal post-release evaluation is required before the impact of this agent can be estimated.

1.3.2.2.2 *Catorhintha schaffneri* Brailovsky and Garcia, 1987

Catorhintha schaffneri has been recorded in three states in southern Brazil, Rio Grande Do Sul (Petolas), Rio de Janeiro (Corcovado & Tijuca) and Santa Catarina (Penha and Porto Belo) (Brailovsky & Garcia 1987; Paterson *et al.* 2014b). Paterson *et al.* (2014b) observed the agent at two and three sites in Rio de Janeiro and Santa Catarina states respectively, and the population released into South Africa was collected at the two sites in Santa Catarina (Penha: 26°46′31.29″S, 48°36′04.19″W and Porto Belo: 27°08′08.51″S, 48°31′55.16″W). The introduced population was released in South Africa in 2014 after host specificity testing and approvals from the relevant government agencies (Paterson *et al.* 2014a).

Catorhintha schaffneri is a sap-sucking shoot-feeder, which depends solely on *P. aculeata* for all its nutritional requirements (Paterson *et al.* 2014a, b). Feeding causes severe damage to the shoot tips (apical meristematic region) of its host plant. Consequently, the shoots wilt and split (Fig. 1.5), and these attributes earned it the common names 'stem-wilter' or 'shoot-splitter' (Paterson *et al.* 2014a, b).

Key aspects of the biology and ecology of the biological control agent *C. schaffneri* from development, host range, impacts and eco-climatic distribution have been sufficiently documented (Paterson *et al.* 2014a, b). *Catorhintha schaffneri* is a hemimetabolous insect, which develops through four-instar stages (Fig. 1.6) (Paterson *et al.* 2014a). Eggs are laid in clutches in single or multiple rows that are glued on leaf surfaces, stems, or any dry, firm surface. According to Paterson *et al* (2014a), hatching occurs synchronously after an average of 15 days at 25 °C and after an average of 3.7 days, the nymphs moult into the second instar. The second, third and fourth instars require 6.6, 13.5 and 23.8 days respectively before moulting to adults (Paterson *et al.* 2014a). The adults lived for an average period of 25.8 days (Paterson *et al.* 2014a).

Host specificity testing indicated that 74% of neonates survive to adult on *P. aculeata* as opposed to 3% survival recorded on the closest relative *P. grandifolia* (Paterson *et al.* 2014a). Although it feeds on other cacti species like *Opuntia aurantica* Lindley and *Rhipsalis baccifera* (J.S.Muell.) Stearn, it never manages to surpass the fourth instar stage, which is only reached by a few individuals and after a much longer period than on the host plant (Paterson *et al.* 2014a).

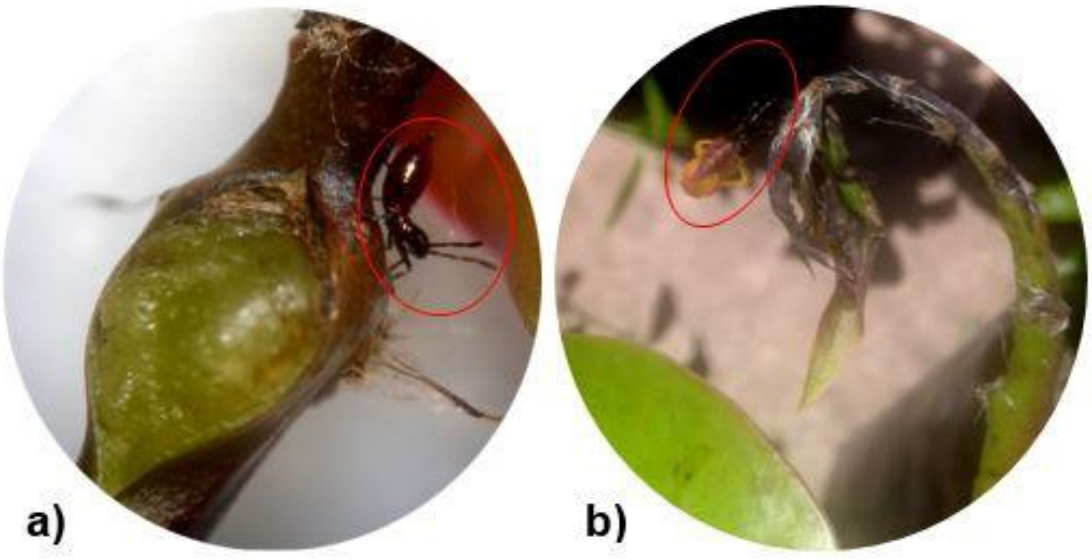

Fig. 1.5 Shoot tips of *Pereskia aculeata* damaged by *Catorhintha schaffneri*: (a) split shoot as induced by nymphs' feeding activities; (b) wilted shoot and a final instar moulting at the apical end of the shoot.

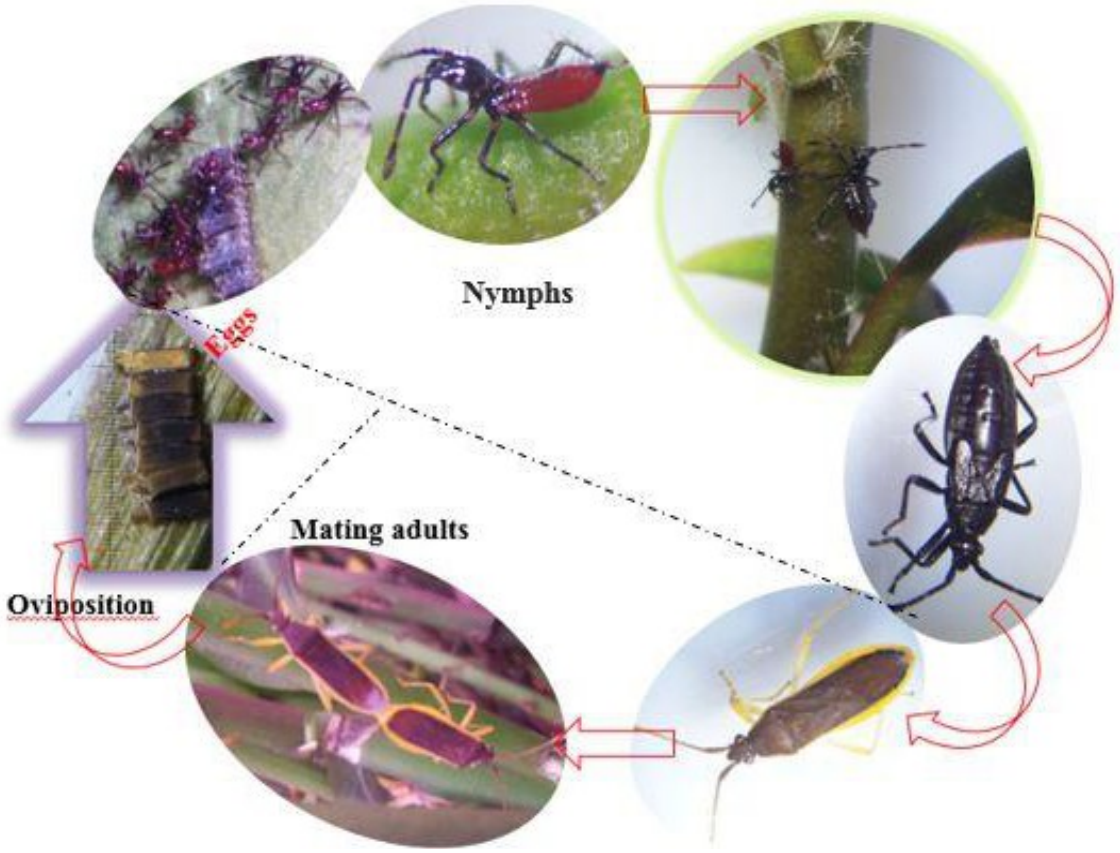

Fig. 1.6 Schematic life cycle of *Catorhintha schaffneri* with nymphal stages on *Pereskia aculeata*. Not drawn to scale and not all stages were demonstrated.

1.4 RATIONALE AND AIM

Different forms, varieties and clones of *P. aculeata* may affect the performance of *C. schaffneri* as a biological control agent. *Catorhintha schaffneri* populations that are now released in South Africa were sourced from a very restricted area in Santa Catarina State, Brazil, and only off two similar genotypes of host plant (Paterson *et al*. 2009; Paterson *et al*. 2014a,b). However, the South African populations of *P. aculeata* originated from a slightly different genotype in a different geographic area in Rio de Janeiro State and have had a long history as a horticultural plant which has changed its genetic profile (Paterson *et al*. 2009). The relationship between the South African genotypes, genotypes in the native distribution and horticultural varieties of the plant are known (Paterson *et al*. 2009). These different genotypes have been documented to have different assemblages of natural enemies in the areas where they occur, which might have shaped their anti-herbivore defences differently through local adaptations to certain herbivore species

(Thompson 2005; Paterson *et al*. 2014b; Hokkanen & Pimentel 1989). If local adaptation exists between *C. schaffneri* and *P. aculeata* at some localities where selection pressures from different herbivore assemblages and abiotic factors might differ, it is plausible that the Santa Catarina population of *C. schaffneri* may not effectively utilise the Rio de Janeiro host plant population, thereby undermining control efforts in South Africa.

The aim of this study was to evaluate the role of intraspecific variation in *P. aculeata* on plant vigour and the performance (fitness, impact, preference and behaviour) of its biological control agent *C. schaffneri*, with a special focus on the weed's and agent's origins. **Chapter 2** seeks to establish whether invasive genotypes of *P. aculeata* grow significantly faster than their native genotypes and whether the agent would inflict greater damage on the former than the latter. **Chapter 3** investigates the agent's fitness on native and invasive genotypes of *P. aculeata* including native genotypes from within the distribution of the agent and native genotypes from areas outside of *C. schaffneri's* distribution. **Chapter 4** seeks to unravel the behaviour (preference patterns) of nymphs of *C. schaffneri* in paired host choices and of adults of *C. schaffneri* in multiple host choices. The general implication of the findings of these studies to the success of the biological control programme against *P. aculeata* and the possible knowledge gaps for further studies are discussed in **Chapter 5**.

CHAPTER 2 Enemy release and evolution of defence against herbivory in *Pereskia aculeata*

2.1 INTRODUCTION

Understanding the invasion process and what makes alien species so successful has long fascinated ecologists because only a few species become invasive when introduced outside of their native environment (Williamson & Fitter 1996; Keane & Crawley 2002; Richardson & Pyšek 2006, 2012). Although some traits such as rapid growth, prolific production of propagules, rapid germination and short life cycle have been associated with successful plant invasion, they do not fully explain invasion success (Baker, 1955; Rejmánek & Richardson 1996; Pyšek & Richardson 2007). For many invasive species it is clear that a release from their natural enemies, such as predators, parasitoids, parasites and pathogens, has given the invasive species a competitive advantage over native species which are exposed to their natural enemies. This has been termed the Enemy Release Hypothesis (Keane & Crawley 2002). Biological control is often viewed as a proof of concept of the Enemy Release Hypothesis because reuniting alien invasive species with their natural enemies often results in the alien species no longer being invasive (Blossey & Notzold 1995; Keane & Crawley 2002)

The enemy release hypothesis suggests that natural enemies, such as specialist herbivores and parasites, suppress plant fitness in the native range as opposed to the invaded range (Keane & Crawley 2002). It assumes that specialists are key regulators of native plants and that these specialists are absent in the introduced range. Upon liberation, the alien plants capitalise on the enemy-free space and become overabundant.

An expansion of the Enemy Release Hypothesis is the Evolution of Increased Competitive Ability (EICA) hypothesis (Blossey & Notzold 1995). This hypothesis suggests that once released from their natural enemies the selective pressures on the plant change because there is no pressure to allocate resources to defence against natural enemies. Resources that were used for defence can therefore be re-allocated to growth and reproduction. There are three key assumptions that can be tested to determine whether the EICA plays a role in the invasion process. First, invasive alien plant species should grow faster than their conspecifics from the native range when propagated in a common environment. Second, the absence of natural enemies, which previously reduced the fitness of the invasive alien plant, encourages a reduction

in defence structures among the invasive species as opposed to their source populations in the native distribution, so defensive traits should be minimised in invasive populations. Third, the invasive populations should be more prone to attack by natural enemies because defences against these enemies have been minimised. Blossey & Notzold (1995) tested these assumptions on purple loosestrife, *Lythrum salicaria* Linneaus (Myrtales: Lythraceae) in which an invasive population from Ithaca (U.S.A.) had higher growth than a native population from Lucelle, Switzerland. While the former allocated more resources to growth and less to defence due to reduced selective pressure from its insect herbivores in the invasive range, the contrary was true for the latter. Although a foliar feeder *Galerucella pusilla* Linnaeus (Coleoptera: Chrysomelidae) performed equally on both populations of *L. salicaria,* a root-feeder *Hylobius transversovittatus* Goeze (Coleoptera: Curculionidae) performed better on the invasive population than on the native ones.

Historically, several studies have tested the predictions of the 'EICA,' and its predictions hold true for some species (Jakobs *et al.* 2004; Joshi & Vrieling 2005; Doorduin & Vrieling 2011; Joshi & Toelbörger 2012; Felker-Quinn *et al.* 2013), but not others (Bossdorff *et al.* 2004; Hull- Sanders *et al.* 2007; Cripps *et al.* 2009; Paterson *et al.* 2012; also see reviews, Colautti *et al.* 2004; Felker-Quinn *et al.* 2013). The reasons for the inconsistencies might be related to differences in plant-herbivore interactions and habitats (Joshi & Vrieling 2005; Hull-Sanders *et al.* 2007; Abhilasha & Joshi 2009; Caño *et al.* 2009; Huang *et al.* 2010). For instance, the agent with the greatest impact against *L. salicaria* in Blossey and Notzold's work was *H. transversovittatus* but *L. salicaria* is also exposed to many other natural enemies whose effects remained the same irrespective of their host origins.

Pereskia aculeata in South Africa is genetically distinct from native populations and has not been exposed to its natural enemies for the last 400 years while the plant was in cultivation as a horticultural variety (Paterson *et al.* 2009). This release of its natural enemies has not changed its susceptibility to the first biological control agent released against the weed, *P. guerini,* (Paterson *et al.* 2012) but whether the new agent, *C. schaffneri* is impacted by the changes to the plant while in the horticultural trade and since introduction to South Africa is not known. *Pereskia aculeata* populations in South Africa may therefore have evolved traits for increased growth and

reproduction at the expense of defensive traits, which may protect it from herbivory by *C. schaffneri* in the native distribution.

The distribution of natural enemies of *P. aculeata* has been assessed in several surveys conducted between the late 1980s and 2014 (Klein 1999; Paterson *et al*. 2014a). The assemblages of insect herbivores that are present in different parts of the native distribution differ significantly in terms of species composition and abundance. Far fewer species and a lower herbivore abundance is present in the northern region of the native distribution than in the southern region. *Phenrica guerini* (Coleoptera: Chrysomelidae) is only present in Rio de Janeiro, *Pereskiophaga brasiliensis* (Coleoptera: Curculionidae) only present in Santa Catarina and the cerambycid, *Acanthodoxus machacalis* Martins and Monné is very rare in Santa Catarina and abundant in Rio de Janeiro. Importantly, *C. schaffneri* is only found in coastal Brazil – and that is where the South African *P. aculeata* originated, although this is complicated by time spent as a horticultural plant and time since introduction in South Africa (Paterson *et al.* 2009; Leuenberger 1986).

Evolution of increased competitive ability may have played a role in the success of *P. aculeata* as an invasive species in South Africa and understanding the mechanism behind the success of *P. aculeata*'s invasion in South Africa would be central to developing an effective management strategy. Thus, this study focuses on *P. aculeata* and *C. schaffneri* within the framework of the EICA hypothesis with a view to establish whether the invasive *P. aculeata* had undergone adaptive changes following its liberation from its co-evolved natural enemies. This may have resulted in South African *P. aculeata* populations allocating more resources in growth and reproduction rather than defence making them better competitors when removed from their natural enemies but also making them more prone to damage by the biological control agent. Furthermore, given the multiple host varieties in the native range and the different assemblages of natural enemies, each variety or genotype may have different local adaptations (Bernays & Chapman 1994). Although the invasive populations are most closely related to the genotypes from Rio de Janeiro, Brazil, the biological control agent, *C. schaffneri*, was sourced from two sites in Santa Catarina State (Brazil) and the plants in Santa Catarina and Rio de Janeiro have been shown to be genetically different from each other (Paterson *et al.* 2009).

The aim of this study was to determine whether enemy release and local adaptations to different herbivore assemblages has resulted in evolutionary change in populations of *P. aculeata* in the native and introduced distribution. The study specifically examined (i) resource allocation to growth between the native and invasive genotypes of *P. aculeata*; (ii) the impact of *C. schaffneri* on *P. aculeata* genotypes, and (iii) the extent of agent's mortality that was induced by the host plants at a fixed level of herbivory.

2.2 MATERIALS AND METHODS

2.2.1 Study system

The study organisms used were *C. schaffneri* and genotypes of *P. aculeata*, which were sourced from the native range in South and Central America, and the invasive range in South Africa. The *C. schaffneri* used in this study were from the same generation sourced from a breeding culture that is maintained under similar conditions, such as light, temperature, food and water regimes, within the Rhodes University's biological control mass rearing facility. The culture was established from progenies of a population of twenty-three adults sourced from Brazil in 2012 (Paterson *et al.* 2014a).

For *P. aculeata*, Paterson *et al.* (2009) established the origin of the invasive populations with DNA sequencing and an Inter-Simple Sequence Repeat (ISSR), which was converted to a genetic distance matrix using Jaccard's Index in assessing 40 locally distinct populations. Using neighbour joining, maximum parsimony and Bayesian analyses, they found that the haplotypes (or subsequently genotypes) of the introduced populations, except for a variegated garden form (SA8), were similar to genotypes from the southern native range, which consist of Brazil, Argentina & Paraguay (Leuenberger 1986; Paterson *et al*. 2009; Fig. 2.1 & 2.2; also see introduction for map: Fig. 1.2). With *P. grandifolia* as an outgroup, *P. aculeata* from the northern native range (Venezuela and Dominican Republic) were grouped together in a closely related group (group 1) with an average genetic distance of 0.28 unlike those within the southern native range which had an average distance of 0.48 (group 3 and group 4) (Paterson *et al*. 2009). The average genetic distance between the two ranges was 0.62 (Paterson *et al*. 2009). The genetic analysis also revealed a high average genetic distances between the introduced genotypes

and the native ones, with an average distance of 0.548 (Paterson *et al*. 2009). The introduced genotypes were grouped with DR1 and SA8, both of which were horticultural plants, and the Rio de Janeiro genotypes (B7 & B8) which are also closely related to Londrina genotypes (B1 & B2) (Fig. 2.1; Paterson *et al*. 2009). The most closely related plants from the native range to the introduced-range genotypes are B7 and B8, followed by B1 & B2 (Fig. 2.1) (Paterson *et al*. 2009). In this current study (and others in this thesis), genotypes from Argentina and Brazil, which were designated as A and B in Paterson *et al.* (2009), were referred to as AR and BR respectively to keep all codes as a two-lettered codes (e.g. A3 is the same as AR3); all others genotypes remain unchanged.

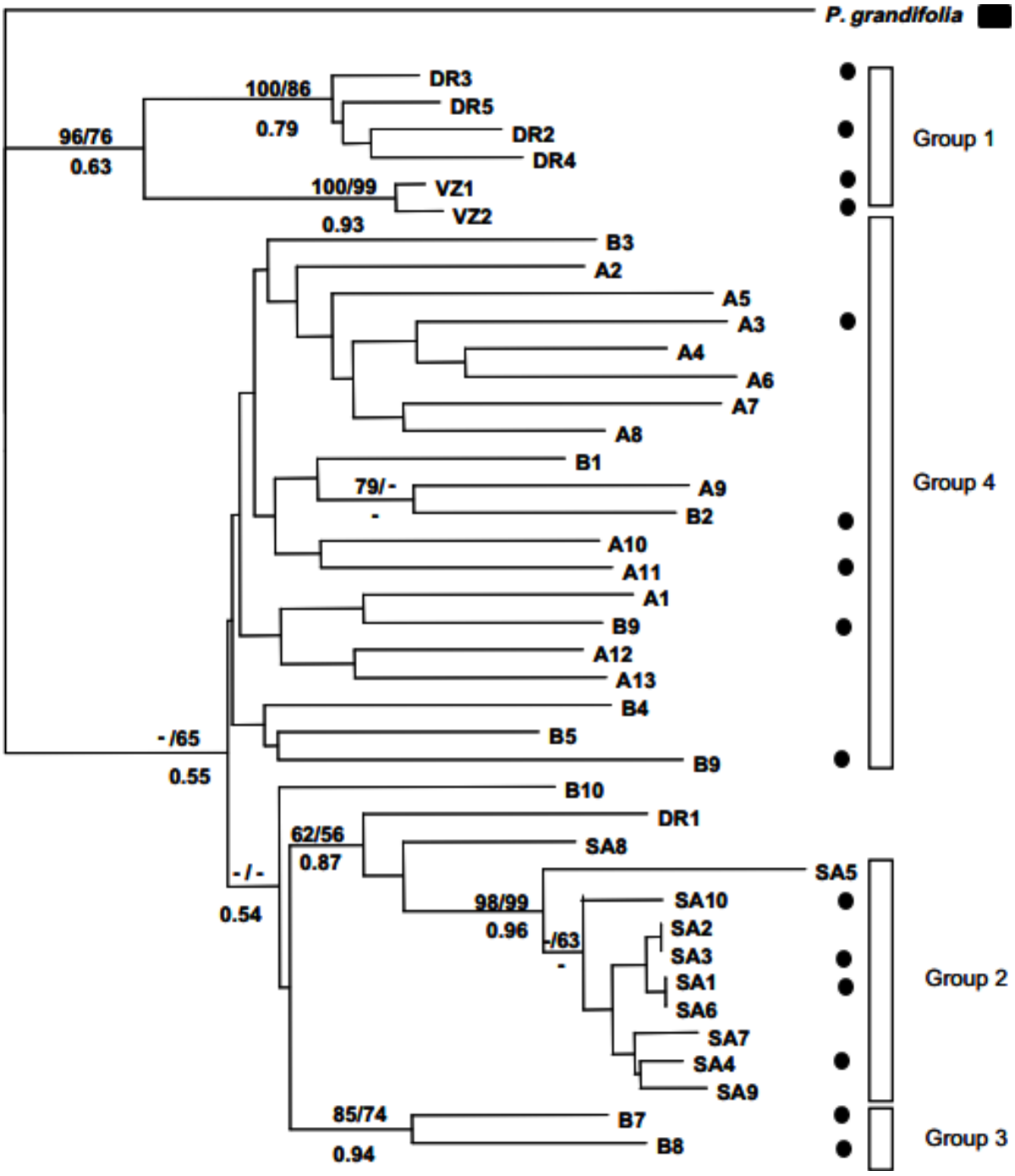

Fig. 2.1 Genetic relationships of *Pereskia aculeata* using neighbour-joining tree constructed from ISSR data excluding bootstrap values and posterior probabilities lower than 0.5. The neighbour joining bootstrap values/parsimony bootstrap values were provided above and the Bayesian posterior probabilities provided below each node (Adapted from Paterson *et al.* 2009). Dots beside the vertical group bars represent the genotypes selected for this study.

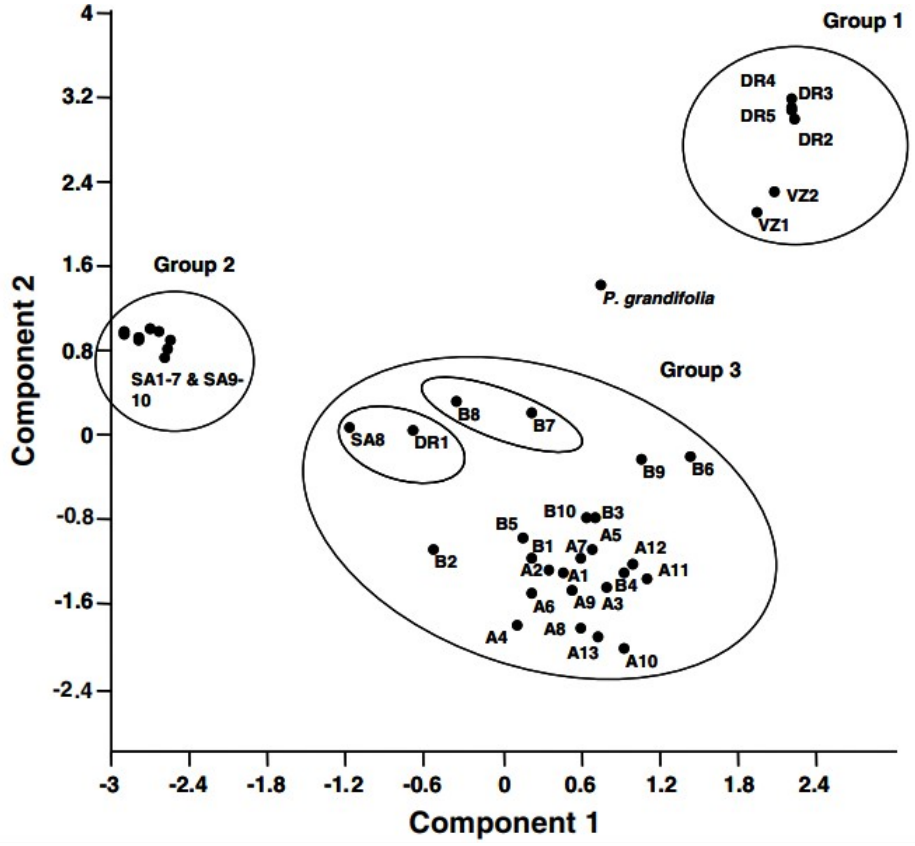

Fig. 2.2 Genetic relatedness of *Pereskia aculeata* using principal components plot drawn from ISSR binary data. Components 1, 2 and 3 explain 21.924%, 19.052% and 6.145% of the variation respectively (Paterson *et al.* 2009).

Of the 40 genotypes in Paterson *et al.* (2009), 15 were selected for this current study (Fig 2.1), of which eleven genotypes of *P. aculeata* were sourced from two distinct native regions (northern and southern ranges), and four from the invasive range. Genotypes from the Dominican Republic and Venezuela were selected from the northern native range; while those from Brazil and Argentina constituted the southern native range. An additional four genotypes were sourced from the invasive range in South Africa (Table 2.1).

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 from Misiones (AR3 & AR11), Argentina (Table 2.1). While five genotypes were obtained from Brazil namely: Londrina (BR2), Santa Catarina (BR6 & BR9), and Rio de Janeiro (BR7 & BR8) – the probable origin of the invasive genotypes (Paterson *et al.* 2009). The invasive genotypes

were collected from Knysna (SA1) in Western Cape Province, Port Alfred (SA3) and Port St. Johns (SA4) in Eastern Cape Province, and Kosi Bay (SA10) in KwaZulu-Natal Province, South Africa (Paterson *et al.* 2009; Table 2.1).

While the native genotypes from the northern native range are from several thousands of kilometres away from the Santa Catarina in Brazil (the province from which the agent was sourced), those from Brazil and Argentina were less than a few hundred kilometres away (Table 2.1). The genotypes BR6 and BR9 are approximately 40 km apart and occur in the same area as *C. schaffneri* as computed using their geographic coordinates that were documented by Paterson *et al.* (2014b). All selected genotypes were genetically unique as illustrated by their individual genetic distance from BR9, which was the closest host (BR6) to the source of the *C. schaffneri* population. Although a few individuals of *C. schaffneri* were sourced about two to four kilometres away from the origin of BR9 (cf. Paterson *et al.* 2014a, b; I.D. Paterson pers. comm.), BR9 cannot be assumed as the same genotype as those on which the few agents were collected.

Table 2.1 Sources of genotypes, relative geographic and genetic distances with reference to BR9, which was collected Santa Catarina Province (Brazil). [1]Country: SA = South Africa, VZ = Venezuela, DR = Dominican Republic, BR = Brazil, AR = Argentina. ♠ With reference to BR9, the distance (km) was measured using Google Earth™ 2015. [†](Source: with permission; adapted from Iain *D.* Paterson's unpublished report; also refer to Paterson *et al.* (2009) for geographic distribution).

						Distances	
P. aculeata[1]	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	Londrina	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

2.2.2 Plant propagation

Each genotype was grown into a large plant from cuttings taken from the field. Multiple cuttings were then taken from the plant in order to replicate each genotype that was used in this experiment. The genotypes were grown in pots under 10% shade cloth. Cuttings of 8-10 cm long were propagated individually, immediately after pruning from their parent plants. Each genotype was replicated twenty times in growth medium of loamy soil and wood chips in 3:1 ratio. Plant bags of dimensions 125 x 100 x 225 mm were filled with the growth medium to approximately three centimetres below the rim. These were watered to saturation three days before the stem cuttings were propagated and fertilised with 5 grams of 3:1:5 slow-release N-P-K Wonder™ fertiliser and $MgSO_4$. Plants were 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 (Table 2.2). All the plants were exposed to identical watering and fertilizing regimes.

Table 2.2 The average (±SE) monthly weather conditions during the study period

Monthly duration	Temperature °C	Relative humidity (%)
19 Dec, 2015 – 18 Jan, 2016	25.2 (± 0.4)	67.4 (± 1.1)
19 Jan, 2016 – 17 Feb, 2016	24.6 (± 0.3)	75.8 (± 1.0)

2.2.3 Growth of *Pereskia aculeata* genotypes

Growth parameters were quantified from shoot lengths (total) and heights 60 days after propagation. Although Blossey and Notzold (1995) used plant height and biomass, the use of biomass was impractical in this study because the same plants were needed for other trials. Shoot length was used here as it is a good parameter for assessing plant fitness (vigour) and correlates with biomass and relates directly to the negative impacts of herbivory on the plants. The use of shoot length as a parameter has been reported in previous studies (Blossey and Notzold 1995; Paterson *et al.* 2014a). 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. Number of leaves were not measured here as 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.

2.2.4 Impact of *Catorhintha schaffneri* on genotypes of *Pereskia aculeata*

Ten genotypes of *P. aculeata* were grown from cuttings under similar conditions and replicated ten times. The replicates were later split into two groups of plants: (i) five plants for control (i.e., herbivore-free) and (ii) five plants for herbivore-inoculation, a method used previously by Paterson *et al.* (2014b). However, in that study, the assessments had been carried out on whole plants, while this assessment was made on a single shoot. A single shoot was used instead of a whole plant to restrict feeding to one shoot for ease of data collection. All plants were pruned so that only a single succulent shoot was left on each plant and the shoots were standardised by marking off the apical parts at ten centimetres using a xylene-free permanent black marker. The test plants were confined to cages of 60 x 40 x 40 cm made from an aluminium wire (diameter = 2 mm) covered in an Organza™ fabric. All cages were set up under a ten percent shade house. A single plant of a known genotype was placed in each cage. On one set of plants, five adults of *C. schaffneri* that emerged within seven days (or less) were introduced (i.e., herbivore-inoculated plants), while on the other set of plants no insects were introduced (i.e., control plants). This procedure was replicated five times per genotype.

Catorhintha schaffneri was introduced as two males (♂) and three females (♀). Since the adult lifespan averages at twenty-five days (Paterson *et al.* 2014a), the age limits were kept under seven days to eliminate the effects of senescence on feeding behaviour, if any. Such that at the

end of the trial, any insect that survives through the entire duration would be seventeen days old or less.

In order to maintain a fixed herbivore pressure on all inoculated plants, the same number of insects was maintained by replacing every dead individual with another individual of the same sex daily. The numbers of leaves above the reference marks on both control and herbivore-inoculated plants were recorded before and after the trials. Given the standardisation, shoot lengths above the marks at the beginning of the trials were uniform (at ten centimetre), but varied after ten days due to either growth (control) or herbivory (inoculated plants). Thus, the lengths after the trials were later recorded while the before-trial lengths were equal (10 cm). The initial number of insects introduced and the replacements made over the trial duration were documented in order to obtain a measure of mortality on each plant genotype.

Dry weights of damaged shoot tissues were recorded after drying (see section 2.2.3 for methods). The damaged shoot tissues included wilted leaves (which were counted) and the wilted and split stems (which formed parts of the shoot lengths as measured above the reference marks). To establish the boundaries between the damage and the undamaged shoot parts of the plant shoot that were exposed to herbivory, pruning was initiated at the visible boundary between the wilted apical portion (which is brown and dead) and the lower part thereof (which still has green shoot tissue). The pruning was continued downward toward the stem (green and healthy parts) until the internal vascular tissue (the central tissues), which is green for healthy plants, no longer revealed any signs of damage namely brown-tinted tissues. All trials were conducted under ambient weather conditions, which were recorded using iButton™.

2.2.5 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 fit GLMM (Gaussian family with log link function; for rationale, see review by Bolker *et al.* 2009). 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 genotypes. The global significance of fitted models

was tested using type III ANOVA. 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 (R Core Team 2017).

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. The association between shoot lengths and dry weights were established using simple linear model after global validation of assumptions (Peña & Slate, 2006; and references therein). For agent's damage (damaged shoots and number of wilted leaves), herbivore load and dry weight/insect ratio 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 (2017). 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.

2.3 RESULTS

2.3.1 Plant vigour

2.3.1.1 Plant heights

At the first level of fixed effects (range), the invasive genotypes grew taller than the native genotypes of *P. aculeata*. As revealed in their shoot heights, which had a significant range effect (difference in geographic localities) with the average heights of 23.47 ± 0.66 cm and 19.04 ± 0.37 cm for the invasive and native genotypes, respectively (t statistic = -2.14, $p < 0.05$; Table 2.3). The genotypes from the invasive range were collectively 23.26% taller than the

average heights of the native-range genotypes (Table 2.3). At the second level of fixed effects (region), invasive plants from South Africa and northern native populations had mean heights of 23.47 ± 0.66 cm and 21.39 ± 0.65 cm respectively, while the southern native populations were 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, but not statistically different from the genotypes from Argentina and Venezuela (F = 145.87, df = 4, $p < 0.001$; Table 2.3). 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 (Fig. 2.3).

2.3.1.2 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 of fixed effects (Table 2.3). The average shoot lengths of invasive and native populations 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), significant regional effects were observed (Table 2.3). 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 (Table 2.3). 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 (Table 2.3). 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 2.3; F = 50.46, df = 4, $p < 0.05$). Generally, the analysis revealed that there was a significant genotypic effect (Fig. 2.4; F = 154.28, df = 14, $p < 0.001$). Consequently, a *posthoc* test with Tukey contrast and Bonferroni adjustment to minimise false positive error revealed that growth among three out of four invasive genotypes

was among the fastest growers in terms of the average total shoot lengths, which also included two other genotypes from Misiones (Argentina), one each from Santa Catarina and Rio de Janeiro (Brazil) (Fig. 2.5).

Table 2.3 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 ±SE	Mean ±SE
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}
- statistic		145.87***	50.46***

Groups with similar letters within the same column are not significantly different ($p < 0.05$). Significance codes: *** Significant at $p < 0.0001$; ** $p < 0.001$; *$p < 0.05$; ^{ns} $p > 0.05$.

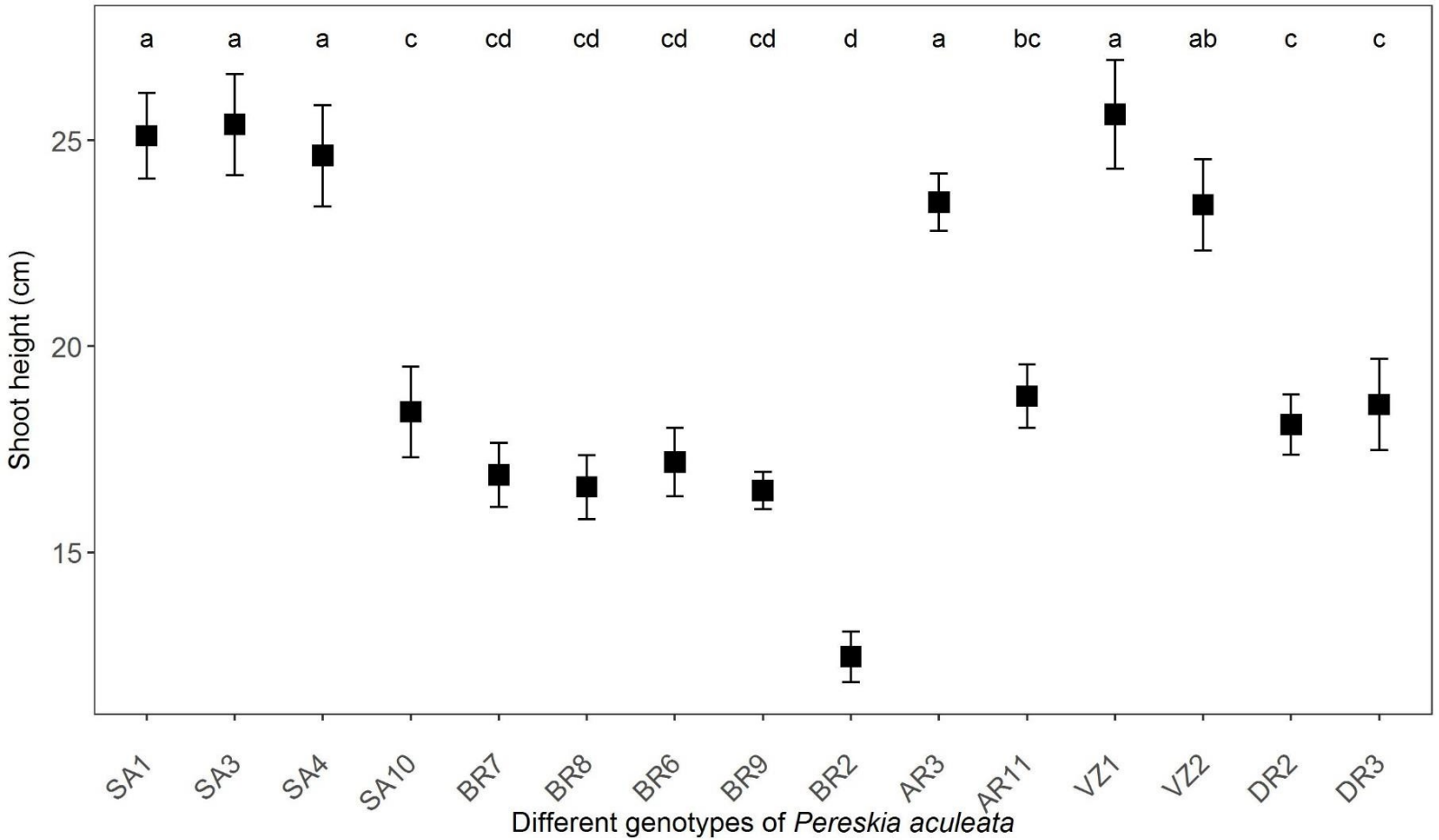

Fig. 2.3 Means of plant heights of different genotypes of *Pereskia aculeata* sourced from both invasive and native ranges. The data were collected sixty days after cultivation under similar ambient weather condition and watering regimes. Bars represent standard errors. The first two letters in the codes for genotypes signify the countries from which they were obtained, the same as codes in Paterson *et al.* (2009). Significant differences are represented by the letters above each genotype.

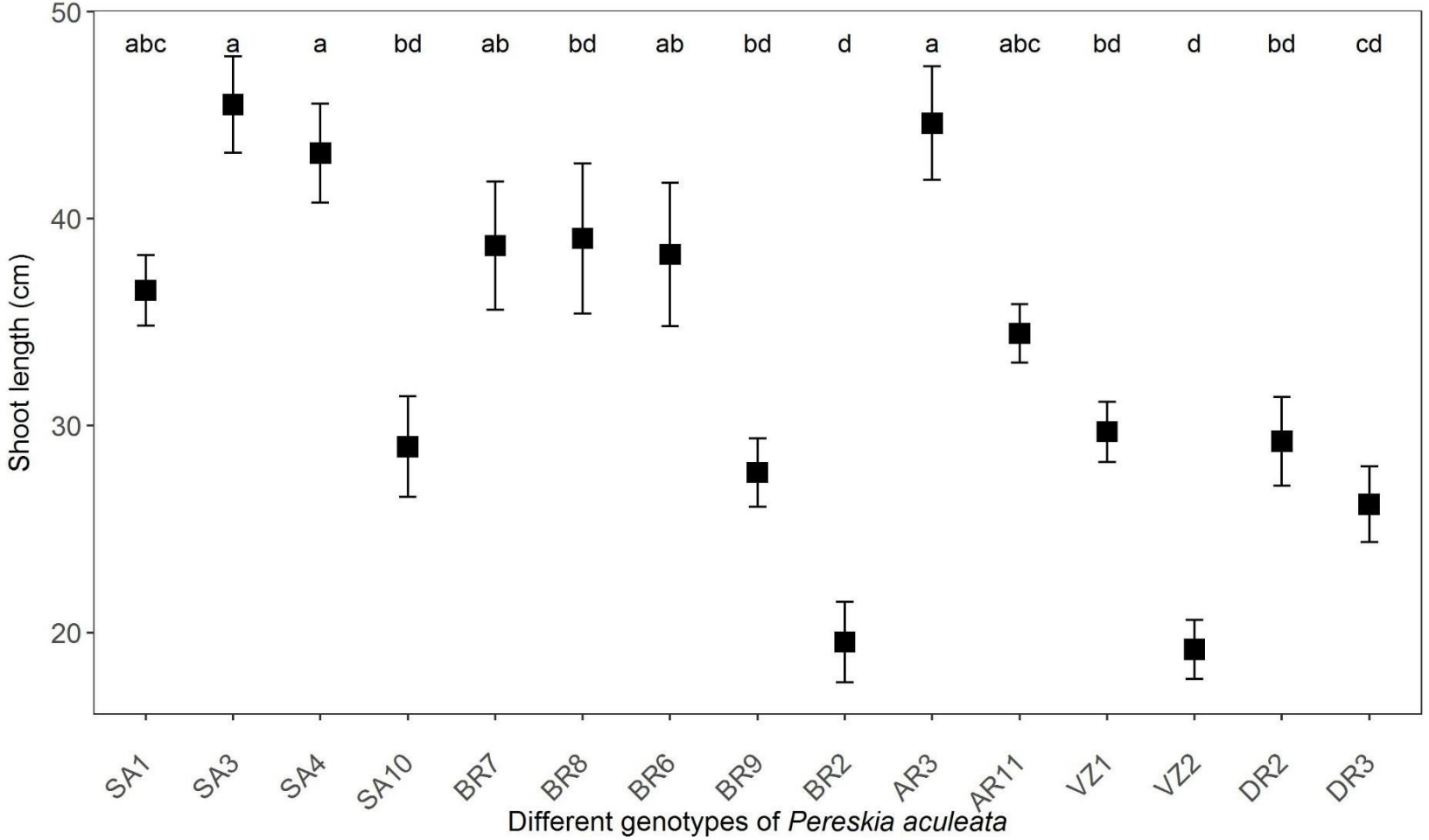

Fig. 2.4 Means of total shoot lengths of different genotypes of *Pereskia aculeata*, sourced from five countries, which include its invasive and native distributions, after sixty days under similar ambient weather condition and watering regimes. Bars represent standard errors and significant differences are represented by the letters above each genotype.

2.3.1.3 Association between shoot lengths and shoot biomass

The shoot length (cm) of *P. aculeata* is a predictive tool for biomass (dry weight) (for rationale, see section 2.2.3). Across all genotypes (Table 2.4), significant regression equations were found, e.g., AR11, (F = 83.82, df = 8, $p < 0.001$) had an adjusted R^2 of 0.81. Shoot length predicted dry weight for AR11 was equal to − 0.2777 + 0.1063 (shoot length) grams when the length is measured in centimetres as explained by 81% of the variance. For each unit gained in length, dry weight of AR11 increased 0.1063 g. Similarly, other genotypes had highly significant regression equations. However, the least predictive model was found in BR2 from Londrina in Brazil and dry weight increased with 0.0534 g for each unit growth as explained by 24% of the data set (R^2 = 0.24; $p < 0.05$).

Table 2.4 Simple linear regression of shoot length as a function of the dry weight of the different genotypes of *P. aculeata* obtained from different countries, planted in a common garden under similar conditions. Assumptions of linear models were examined before accepting the final predictive models

Genotypes	Coefficient	Intercept	Adj. R^2	df	*p*-value
AR11	0.1063	−0.2777	0.8134	18	***
AR3	0.0858	−0.2074	0.9654	21	***
BR2	0.0534	0.6931	0.2412	17	*
BR6	0.0927	−0.4221	0.8326	36	***
BR7	0.1116	−0.5761	0.6961	19	***
BR8	0.0816	0.0083	0.6483	19	***
BR9	0.0823	0.0275	0.4818	18	***
DR2	0.0683	−0.0337	0.9230	16	***
DR3	0.1071	−0.5789	0.9111	29	***
SA1	0.1272	−0.8628	0.8572	20	***
SA3	0.8035	−0.4245	0.8035	28	***
SA4	0.0992	−0.4698	0.8030	19	***
SA10	0.0898	−0.7435	0.8157	19	***
VZ1	0.1123	−0.0628	0.6949	22	***
VZ2	0.1354	−0.1282	0.8775	18	***

***significant at $p < 0.001$; **significant at $p < 0.01$; *significant at $p < 0.05$; ^{ns}not significant at $p > 0.05$

2.3.2 Impact of *Catorhintha schaffneri*

2.3.2.1 Damage and impact on shoot length

Damage was defined by difference between the before and after herbivore-induced changes in shoot length on treated plants only, and impact was the differences between damaged plants and their respective control plants [above the pre-defined reference mark (see section 2.2.4.). Generally, although all the ten genotypes exposed to *C. schaffneri* were damaged within ten days, only four were significantly more damaged than the other six genotypes (Fig. 2.5). The results showed that the least damaged shoot was 2% of the predefined apical portion of the shoot on AR3 from Misiones (Argentina), while as high as 95% damage of similar portion was observed on BR6 from Santa Catarina (Brazil) (Fig. 2.5 & 2.6).

For damage, all ten genotypes were damaged by *C. schaffneri*, however, only four genotypes incurred significantly ($p < 0.05$) more damage than the other six genotypes as illustrated by a 'one-sample Wilcoxon Signed-Rank test'. These genotypes 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, $p = 0.03$). The other six genotypes incurred 2% to 31% damage but did not change significantly from their respective initial shoot lengths (Fig. 2.5 & Fig. 2.6). The effects of herbivory on all herbivore-inoculated plants (comparative damage) as demonstrated using a one-way Kruskal-Wallis *H* test shows that the native genotype BR6 from Santa Catarina was significantly more damaged than the Argentina genotype (AR3) from Misiones, and also than another genotype from Santa Catarina (BR9). Nonetheless, BR6 did not incur significantly more damage compared with other genotypes ($H_9 = 27.43$, $p = 0.001$; Fig. 2.5).

For impact, Mann-Whitney *U* test revealed a significant halt in growth for each herbivore-challenged plant (for all genotype; $U = 25$; $p < 0.05$). There was a significant impact on four genotypes namely SA1, BR8, BR6 and SA4 (ANOVA: $F_{(9,\ 40)} = 3.48$, $p = 0.003$; Fig 2.7). Although the genotype BR8 from Rio de Janeiro was not significantly more impacted than BR7, which is also from Rio de Janeiro, the latter (BR7) was significantly less impacted than an invasive genotype from Knysna (SA1) and then, than a native genotype from Santa Catarina (BR6) ($F_{(9,40)} = 3.48$, $p = 0.003$) as shown from a Fishers' LSD *posthoc* test. The native genotype, BR7 from Rio de Janeiro (Brazil), was impacted less compared to an invasive genotype, SA1 from Knysna, but other invasive genotypes SA4 and SA10 were more or less similar to BR7 respectively (Fig 2.7). The least impacted genotypes were the

invasive genotype (SA10), the native genotypes outside the agent's natural range (DR3 and AR3) [i.e., non-local host plants], and interestingly a native genotype within the agent's natural range of Santa Catarina (BR9).

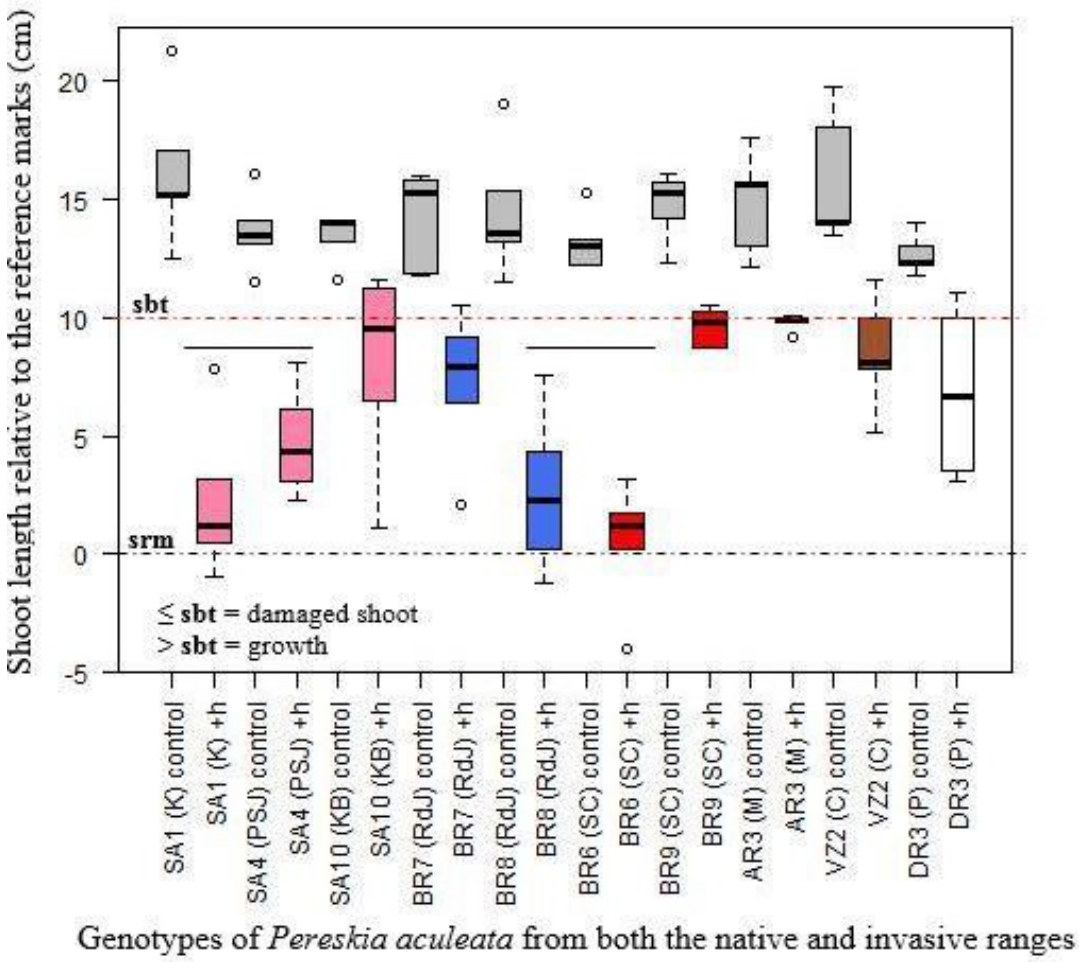

Fig. 2.5 Damage of *Catorhintha schaffneri* on shoot lengths of *Pereskia aculeata* at a fixed level of herbivory (2♂:3♀). Control plants are the grey boxes and the mixed coloured boxes are herbivore-inoculated plants (h) Line 'sbt' is the height of shoot tips at ten centimetre above the standardised reference marks that is, line 'srm'. The boxplots depict medians, 25th and 75th percentiles and minimum and maximum values. The bars above the 'boxplots SA1 and SA4, BR8 and BR6 signify significant damage relative to the sbt. Codes in parenthesis are the parent plants' localities: C = Caracas, K = Knysna, KB = Kosi Bay, M = Misiones, P = Pedernales, PSJ = Port Saint Johns, SC = Santa Catarina, RdJ = Rio de Janeiro (see Table 2.1).

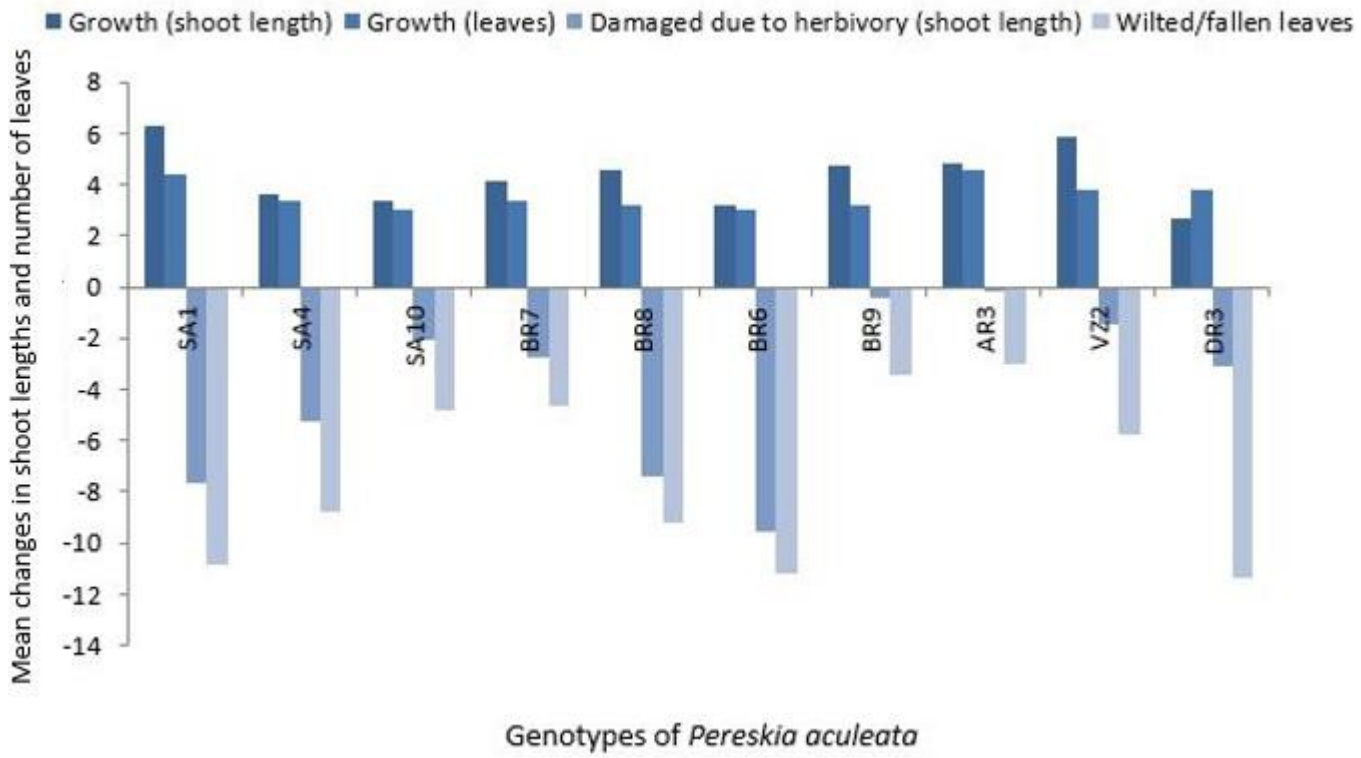

Fig. 2.6 General outlook of the changes in shoot lengths and number of leaves on different genotypes of *P. aculeata*. Bars above zero were data from the control plants without *Catorhintha schaffneri*, whereas those below zero were herbivore-inoculated plants after a ten-day period. The statistical analyses are shown in the other charts (Fig 2.5, 2.7-2.9).

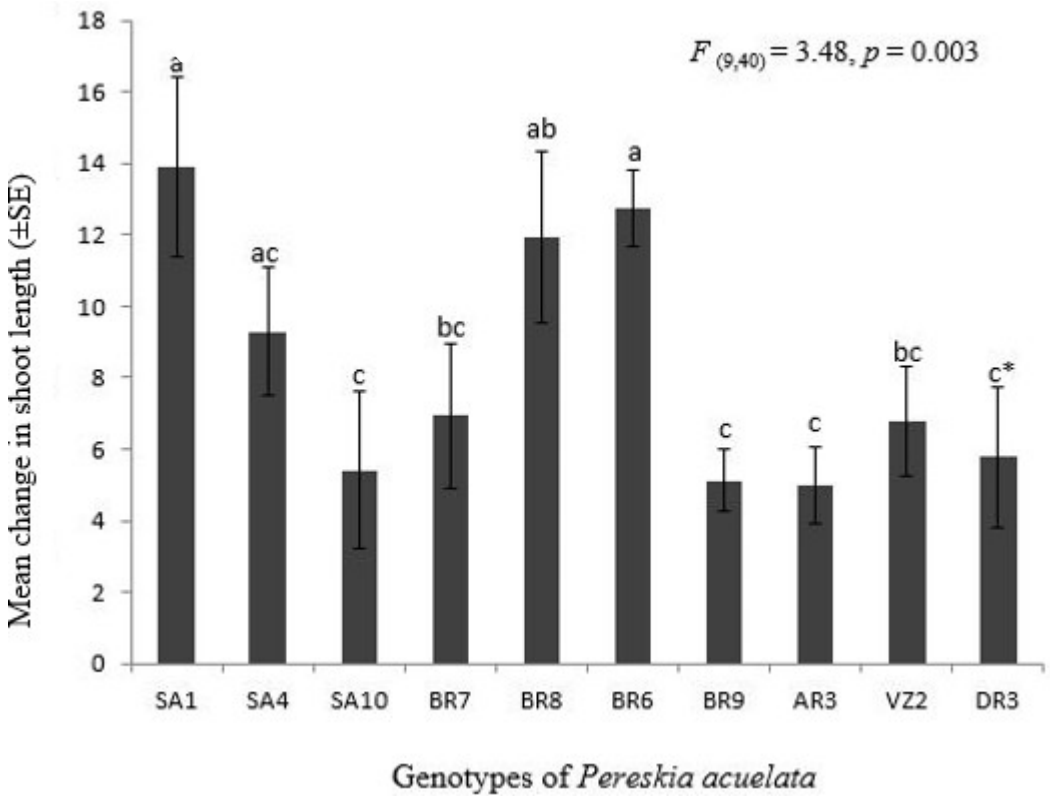

Fig. 2.7 Impact of *Catorhintha schaffneri* on the apical shoot of genotypes of *Pereskia aculeata*. This was represented as the differences between controls and inoculated plants. Notes: *DR3 was drooping, pale green shoots, unlike others wherein top-down wilting were observed along with several 'shoot splitting.'

2.3.2.2 Damage and impact on apical leaves

Damage and impact had been defined in section 2.2.3.1. All the herbivore-inoculated plants incurred varied extents of loss of apical leaves while the control plants gained additional leaves over the ten-day 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 rate of leaves gained of 42% on a native genotype VZ2 from Caracas, Venezuela. The herbivore-inoculated plants lost as few as 22% of their apical leaves on the Argentina genotype (AR3) unlike the greatest loss of 93% encountered on the native genotypes from Santa Catarina, Brazil (BR6) (Fig. 2.8).

Although Mann-Whitney *U* tests did not show any significant differences in the leaf counts between the control and inoculated plants prior to treatment, the contrary holds true for all

genotypes after ten days of herbivory (Fig. 2.8). Comparison with pre-trial leaf counts on the herbivore-inoculated plants using a one-sample Wilcoxon Signed-Rank test showed that all the plants that were exposed to herbivory had statistically (p = 0.03) fewer leaves, except for SA10 from Kosi Bay (p = 0.10). Kruskal-Wallis test among all the treated plants showed that significantly more leaves of BR6 were wilted than BR9 and AR3, but it did not rank differently from any other genotypes (H_9 = 28.89, p = 0.001). Fig. 2.10 shows the impact of herbivory on wilted leaves as analysed using analysis of variance followed by Fisher's Least Significant Difference (LSD). Although the loss of apical leaves was largely similar between the other genotypes, DR3 [see caveat (Fig. 2.9) where droopy and not necessarily wilted leaves were counted], SA1, BR6, and BR8 were statistically more impacted than BR9 (ANOVA: $F_{(9,40)}$ = 2.27, p = 0.037).

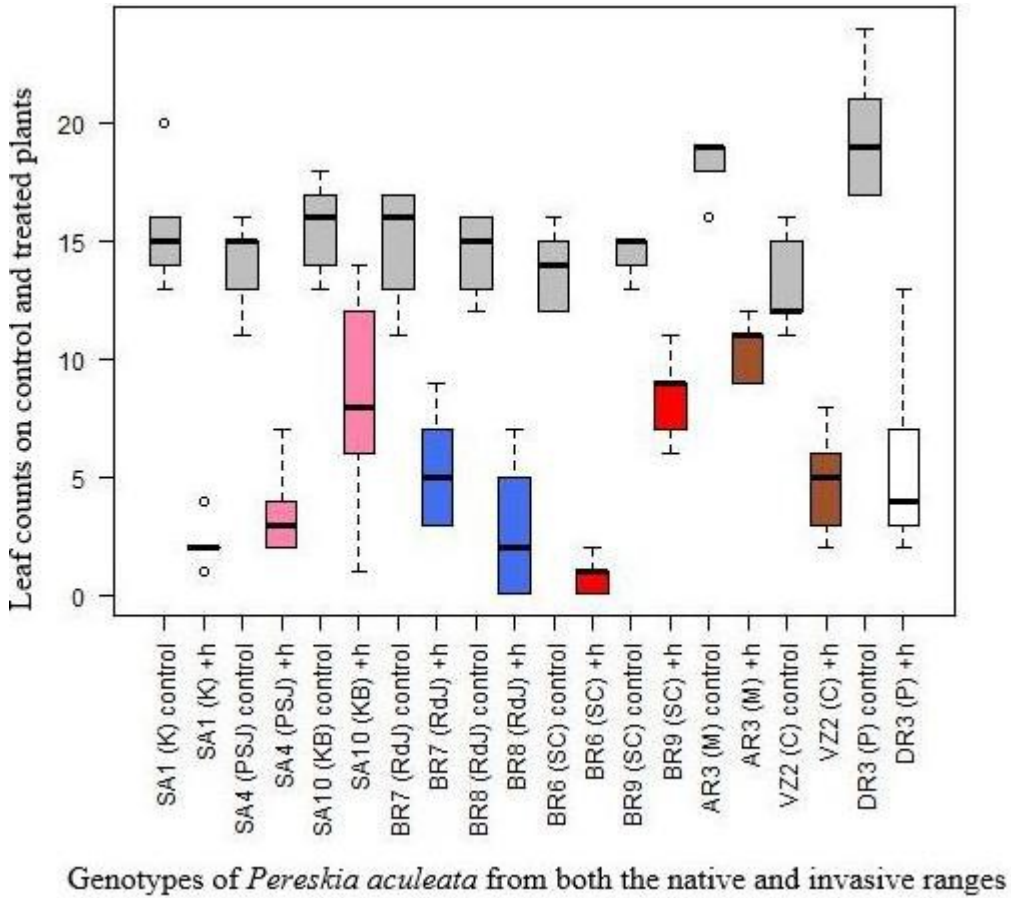

Fig. 2.8 Damage of *Catorhintha schaffneri* on a number of apical leaves of *Pereskia aculeata* after the fixed period and level of herbivory (2♂:3♀). Control plants are the grey boxes and the mixed coloured boxes are herbivore-inoculated plants (h). The boxplots depict medians, 25th and 75th percentiles and minimum and maximum values. The bars above the 'boxplots SA1 and SA4, BR8 and BR6 signify significant damage relative to the sbt. Codes in parenthesis are the parent plants' localities: C = Caracas, K = Knysna, KB = Kosi Bay, M = Misiones, P = Pedernales, PSJ = Port Saint Johns, SC = Santa Catarina, RdJ = Rio de Janeiro.

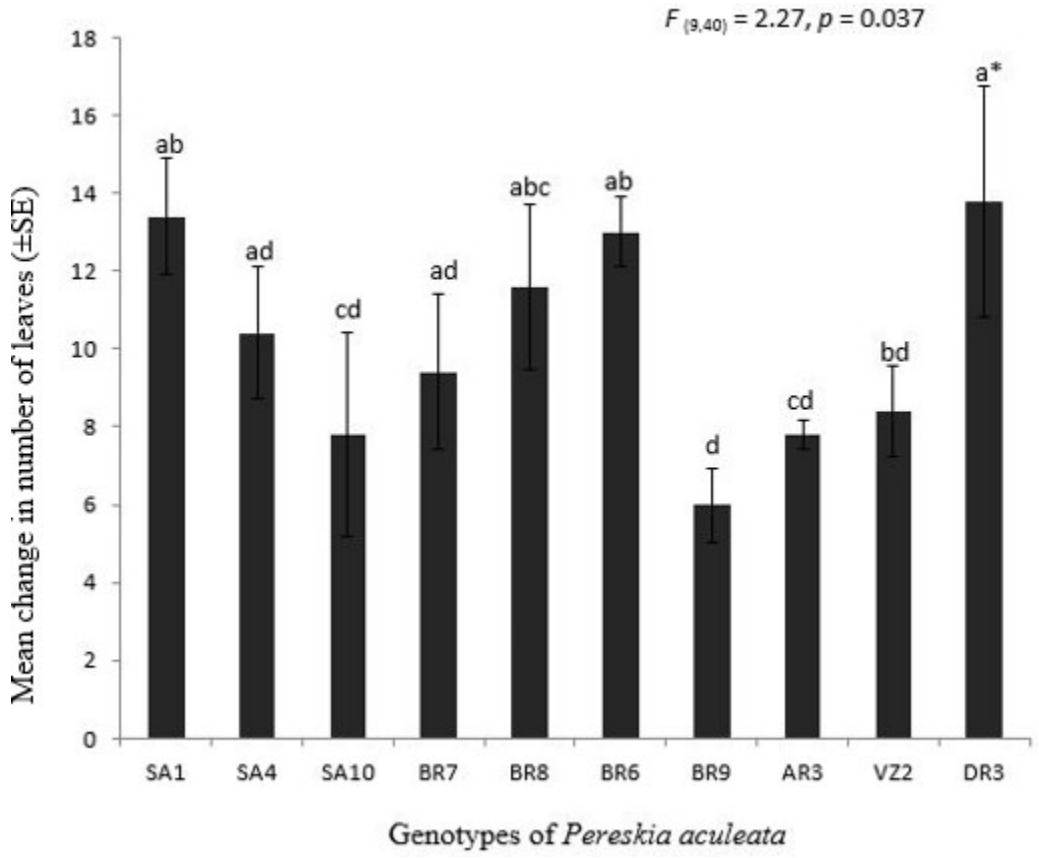

Fig. 2.9 Impact of *Catorhintha schaffneri* on leaves among genotypes of *Pereskia aculeata* as differences between control and inoculated plants after ten days herbivory. Note: *The value for DR3 must be interpreted with caution as most leaves were not wilted, but droopy and pale green and they remained attached to the shoot, unlike the others on which leaves were completely wilted or had fallen off.

2.3.2.3 Dry weight of damaged shoot matter

The effect of the fixed pressure of herbivory as revealed in dry weight (biomass) of damaged tissues (shoot stalk and leaves; see section 2.4) was high in three out of the ten genotypes of *P. aculeata* (Fig. 2.10). BR6 genotype had the greatest dry weight of damaged tissues as opposed to AR3, which had the lowest. Kruskal-Wallis rank sum test showed that the observed differences were significant. A *posthoc* test showed that BR6 and SA1 had significantly higher amount of damaged apical tissues than AR3 ($H_9 = 26.07$, $p = 0.002$), but no significant difference was found between the former (BR6 and SA1) and the other genotypes.

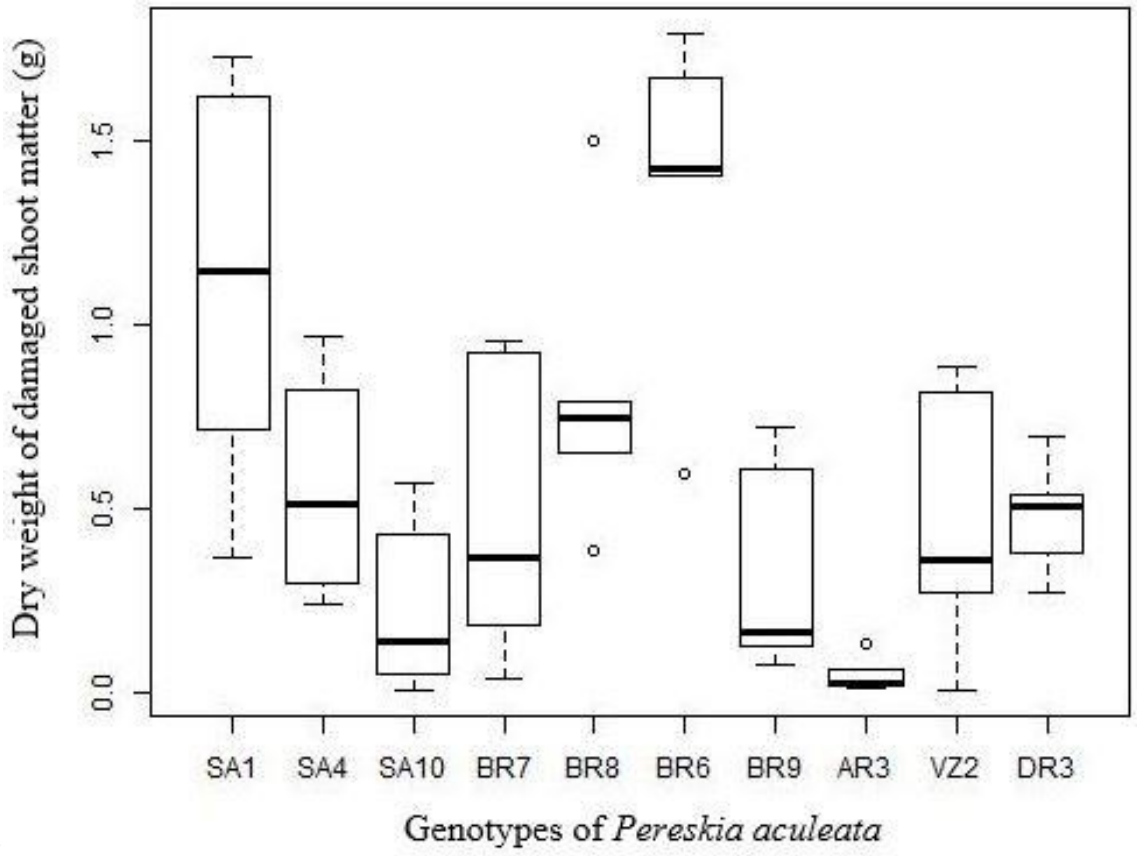

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Fig. 2.10 The biomass of damaged apical tissues per genotypes of *P. aculeata* due to induced herbivory by *Catorhintha schaffneri* after ten days. The boxplot depicts medians, 25th and 75th percentiles and minimum and maximum values.

2.3.3 Effect of host on agent and plant loss

2.3.3.1 Mortality on each genotype

Generally, lower agent mortality was observed on the invasive genotypes and three genotypes from southern Brazil (BR6, BR7 & BR8: from Santa Catarina and Rio de Janeiro; Fig 2.11) than other native genotypes. The invasive genotype SA1 (from Knysna) had the lowest mortality contrary to SA10 (from Kosi Bay), which had the highest mortality (Fig 2.11). *Catorhintha schaffneri* had the lowest mortality on one genotype from Santa Catarina (BR6) and two genotypes from Rio de Janeiro (BR7 & BR8). A one-way Kruskal-Wallis rank sum test showed differences among the test plants and thus, a Kruskal-Wallis multiple comparison *posthoc* test was conducted. Significantly fewer adults died on the invasive genotype SA1 than on SA10 and AR3 ($H_9 = 27.21$, $p = 0.001$; Fig. 2.11), but there were no statistical differences in the median mortality of *C. schaffneri* between SA1 and the other host genotypes.

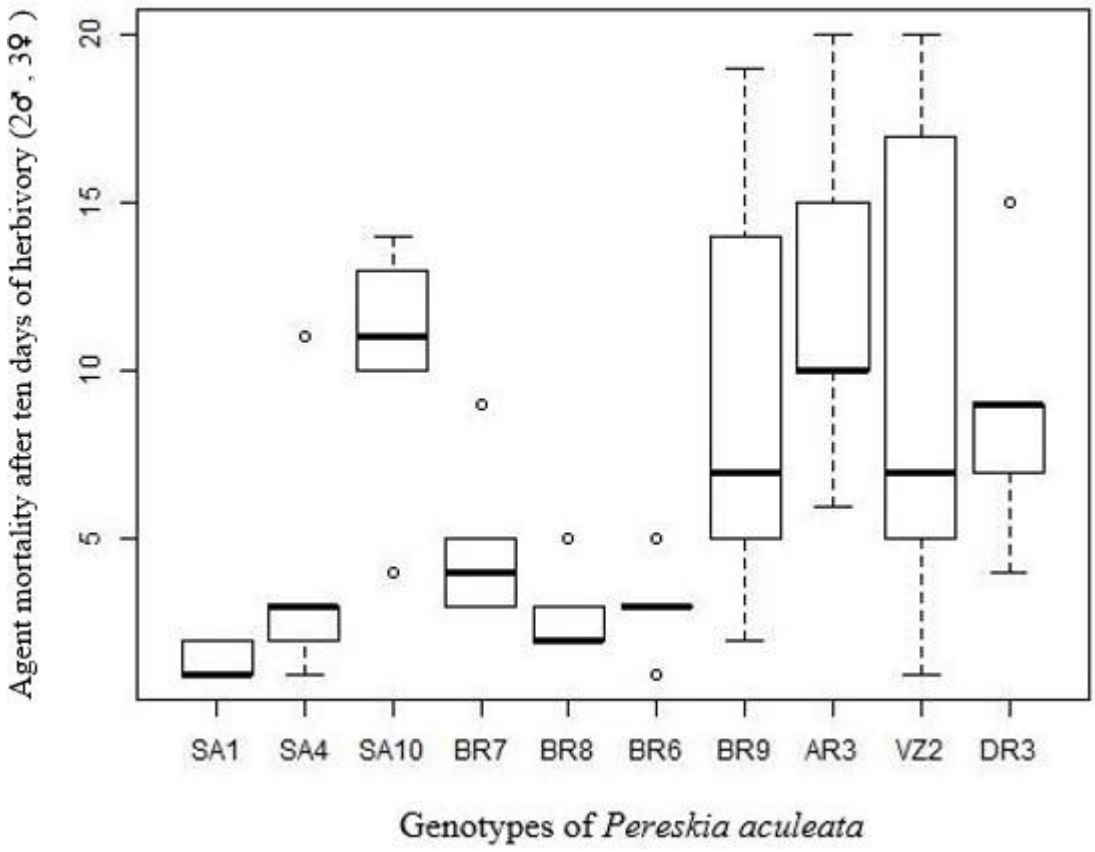

Fig. 2.11 Mortality of *Catorhintha schaffneri* on *Pereskia aculeata* over a ten-day period of fixed herbivory. The boxplot depicts medians, 25th and 75th percentiles and minimum and maximum values. The small circles represent outliers.

2.3.3.2 Ratio of damaged tissue to agent mortality

The BR6, SA1 and BR8 genotypes had significantly more damaged tissue (median dry weight) in relation to the extent of mortality incurred by the agent which was lower than the SA10 genotype from Kosi Bay, South Africa and the AR3 genotype from Misiones, Argentina (H_9 = 31.93, $p < 0.001$) (Fig. 2.12). Thus, more tissue (dry weight) was damaged by a few herbivores, and less mortality was inflicted, on both native and invasive genotypes (e.g., BR6 and SA1) than most of the native genotypes on which higher median deaths. Those genotypes that cause higher deaths than other also recorded less damage to host tissue, even when herbivory was constantly maintained (e.g., SA10, AR3, BR9; Fig. 2.12).

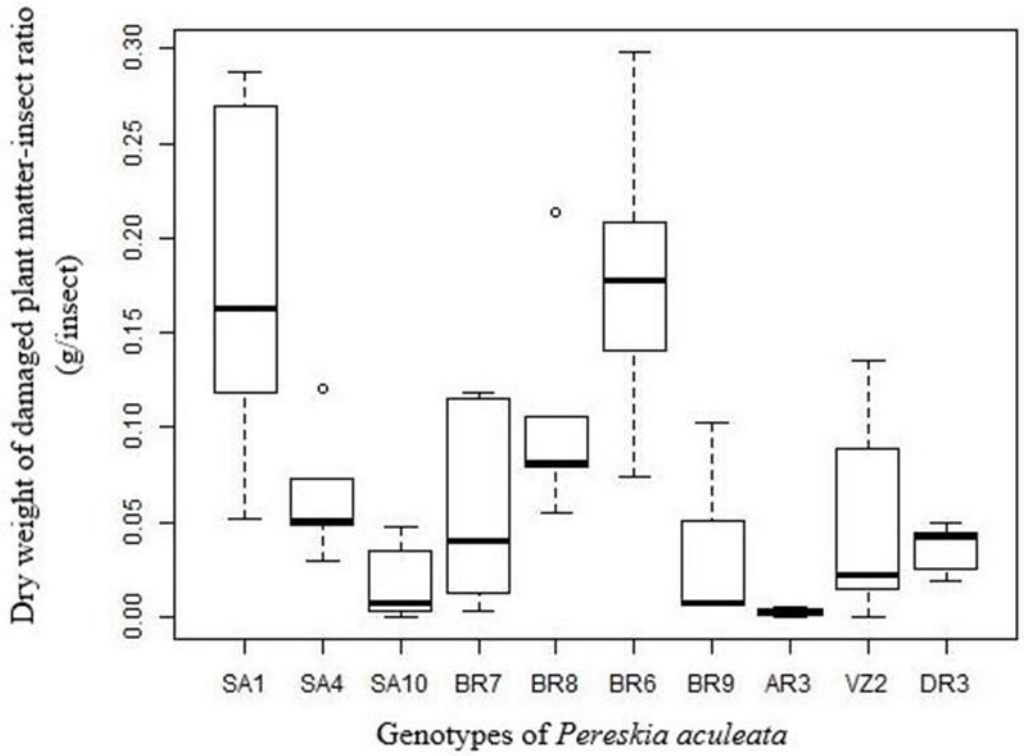

Fig. 2.12 The ratio of tissue dry weight to an agent of *Pereskia aculeata* over ten days of herbivory. Boxplot depicts medians, 25th and 75th percentiles and minimum and maximum values.

2.3.3.3 Correlation of parameters

The average dry weight of tissues damaged by feeding activities of *C. schaffneri* (the dataset in Fig. 2.10), correlated significantly and negatively with the average mortality of agents that caused the damage (from the dataset in Fig. 2.11). Plants with the highest amount of tissue damage had lesser deleterious effects on the agents ($R^2 = 0.7216$; $p < 0.01$; Fig. 2.13). On the other hand, although a correlation between the extent of damage (dry weight of damage tissues) and plant vigour was tested, there was no significant relationship. Nonetheless, the relationship tended to indicate that the vigorously growing genotypes suffered more damage than the slow-growing ones. However, the observed association was weak and was not significant ($R^2 = 0.0329$; $p > 0.05$; Fig 2.14).

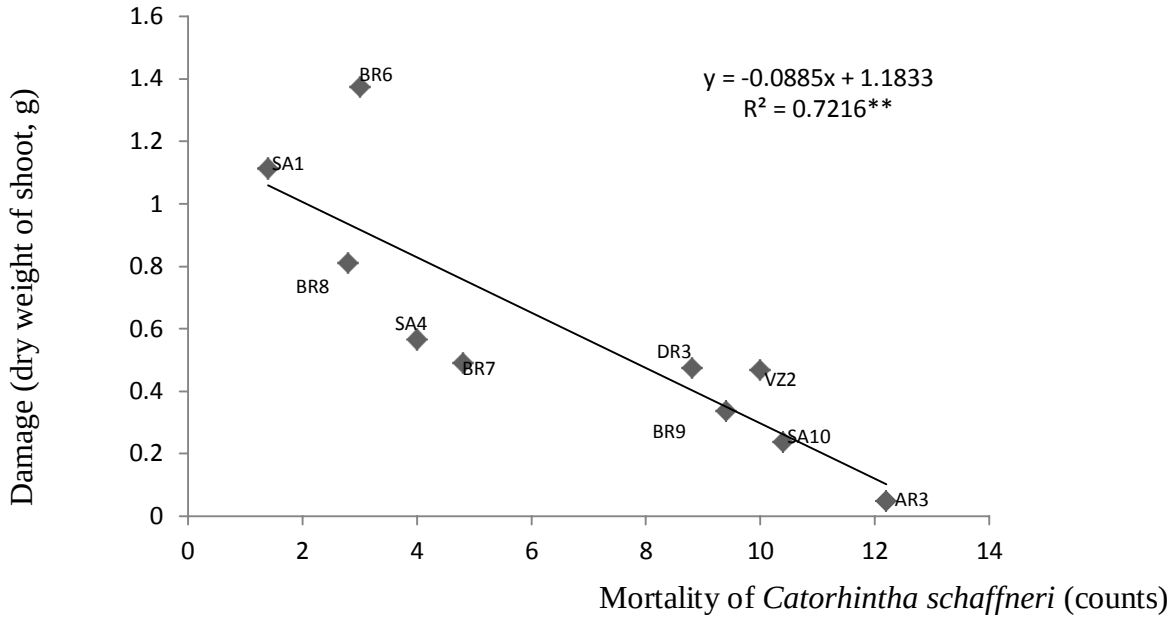

Fig. 2.13 Correlation of parameters between the weights of damaged apical tissues and the observed mortality of adult *Catorhintha schaffneri* during the ten days impact trials on different genotypes of *Pereskia aculeata*.

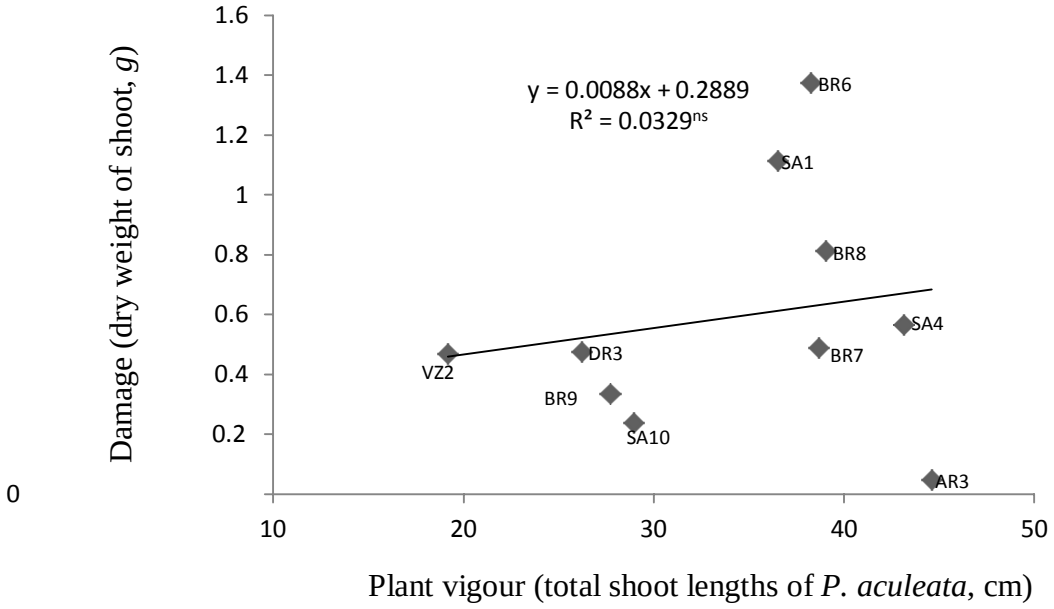

Fig. 2.14 Correlation of parameters between the weights of damaged apical tissues and plant vigour of *Pereskia aculeata.*

2.4 DISCUSSION

This study examined two predictions of enemy release as assumed in the Evolution of Increased Competitive Ability (EICA) hypothesis namely that common garden invasive alien plant genotypes grow more vigorously, and are more susceptible to natural enemies due to reduced resource allocation to defence, than the native-range genotypes (Blossey & Notzold 1995). The assumptions presuppose that the invasive genotypes of *P. aculeata* would be defended poorly against natural enemies (Blossey & Notzold 1995), having been released from its natural enemies hundreds of years ago (McGibbon 1858; Paterson *et al.* 2009). The expectations were that the selected genotypes of *P. aculeata* from four invaded sites in South Africa would: (i) grow taller and faster, and (ii) incur more damage from, and be more impacted by, *C. schaffneri* than the eleven native-range genotypes from South and Central America.

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, the total shoot lengths (a

measure of growth) were not always greater among the invasive genotypes than the native genotypes. The taller plants among the invasive genotypes support one of the predictions of EICA, as they were different to the native plants from Brazil. The Brazilian genotypes, especially those from Rio de Janeiro, are of particular interest for comparison because genetic evidence suggests they are the closest relatives of the invasive genotypes (Paterson *et al.* 2009). The invasive genotypes of *P. aculeata* had slightly greater shoot lengths than the natives, but there was a weak range or regional effect, which reflects a considerable variation within genotypes and between genotypes from the same ranges and regions. The implication is that range and regional differences could not explain the differences in shoot lengths between genotypes but did explain increased heights among the invasive genotypes. The invasive-range genotypes grew taller than the native ones and are therefore likely to creep easily into neighbouring trees and damage indigenous vegetation. Plants that can grow quickly taller 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. Unlike the South American genotypes, which have never been reported to have aggressive traits over other native flora (Paterson *et al.* 2014b), the invasive genotypes do have an advantage (in shoot height) over the native-range genotypes and EICA may explain these changes in their growth forms.

In several studies, 'enemy release' has largely been inferred from perceived adaptive changes in plant traits (enhanced height, biomass, and growth) that result in 'the evolution of increased competitive ability', for instance in the *Lythrum salicaria* example (Blossey and Notzold 1995). Adaptive changes lead to an increase in competitive ability of invasion alien plants within the introduced range, thus reflecting an adaptation to the essentially herbivore-free environment and also from an increased susceptibility to specialist herbivores that might be released as biological control agents (Blossey & Notzold 1995; Joshi & Tielbörger 2012). Enhanced fitness in enemy free conditions arises from resource optimisation, when metabolic and structural investments towards anti-herbivore defence (such as strengthening in cell walls or production of trichomes) are down-regulated (Feng *et al.* 2009; Heshula & Hill 2011). Defence is costly and usually traded for growth and reproduction when the need to defend drops and ultimately culminates in range expansion and increases in abundance in the adventive ranges (Blossey & Notzold, 1995; Runyon & Birdsall 2016). Reduced anti-herbivore defence can enhance tissue palatability and digestibility which would result in a greater quantity of plants matter being consumed by

herbivores. Less tissue is consumed from native plant populations because of a heightened level of tissue protection due to the greater need for these defence mechanisms (structurally: Feng *et al.* 2009; Heshula & Hill 2011, or chemically: Ngxande-Koza *et al.* 2017). Thus, native genotypes, like those of *P. aculeata*, are less likely to be more palatable to their natural enemies than invasive ones (Blossey & Notzold 1995; Joshi & Tielbörger 2012).

Several attempts at unravelling the mechanisms responsible for invasion success have generated ambiguous findings, possibly because different taxa and habitats respond differently (Blossey & Notzold, 1995; Thompson 2013), meaning that each plant-herbivore system is unique. For *P. aculeata*, for which increased fitness was detected, there was however insufficient evidence to suggest that the invasive genotypes were more impacted by herbivory from *C. schaffneri* 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 after a longer exposure time to the agent with multiple defoliation events over many seasons. The adaptation time of of the herbivore to other environmental factors and not necessarily the less defended host may also impede the agent chances of inflicting more damage on the invasive genotypes than the native ones. For the records, an increase in the number of replicates per genotype may reduce experimental error, but cannot invalidate the findings of this study, given that *C. schaffneri* feeds on a limited portion of the apical region of its host plant, which would not change greatly regardless of the sample sizes.

The extent of impact inflicted by *C. schaffneri* could have been influenced by inherent traits of the different hosts, because biochemical pathways and resource allocation to chemical defences may have genetic links. The less-impacted genotypes, like SA10, BR9, and others, were not palatable to *C. schaffneri* and this may have been due to phytochemical differences. *Pereskia aculeata* and its sister species *P. grandifolia* are rich in several flavonoids, terpenoids and alkaloids (Zareisedehizadeh *et al.* 2014; Souza *et al.* 2014; Souza *et al.* 2016). In Souza *et al.*'s (2014) assessment of oil extracts, 81% and 40% of thirty-seven chemical constituents were identified as unique to either *P. aculeata* or *P. grandifolia* respectively. They had 21% of the chemicals in common including the compound β-ionone, which is inducible in canola *Brassica napus* to deter a flea beetle *Phyllotreta cruciferae* (Goeze) (Coleoptera: Chrysomelidae) (Gruber *et al.* 2009; Cáceres *et al.* 2016). Meanwhile, in synergy with terpenoids like myricene, pinene,

and linalool, different effects can arise from being a deterrent (*Phyllotreta cruciferae*: Gruber *et al.* 2009) to being an attractant (in females rootworm, *Diabrotica virgifera virgifera* LeConte (Coleoptera: Chrysomelidae) (Hammack 2001). Although the role of β-ionone and other chemical compounds in *P. aculeata* remain unknown, it is plausible that *C. schaffneri* would be impacted by the concentrations of these chemicals and that differences between genotypes may occur.

Structural components are a plant first-line of defences, which range from a macroscopic scale (e.g., waxy cuticles, spines, setae, trichomes), to a microscopic scale (e.g., sclerophylly, suberization and lignification of cell walls) (War *et al.* 2012; Feng *et al.* 2009). These traits were not directly investigated in this study and there are no noticeable differences in macroscopic traits between genotypes of *P. aculeata* that could be related to defence. It is, however, possible that microscopic defence traits between the genotypes exist.

The results of this study can be compared with a similar study that was conducted using the other biological control agent for *P. aculeata*, the flea-beetle, *P. guerini* (Paterson *et al.* 2012). In that study, no variable impact on, or suitability of, invasive and native genotypes was detected (Paterson *et al.* 2012), and as such it was inferred that *P. aculeata* has not evolved differences in susceptibility to the leaf-chewing insect. Although *C. schaffneri* is a sap sucker, the results are similar, as damage incurred by the invasive genotypes was similar to those incurred by the native Brazilian genotypes with few exceptions.

Given the observed between-genotype variation in *P. aculeata*, validating or refuting the EICA's assumption that the herbivore impact would be higher on the invasive genotypes than the native genotypes is challenging but these findings do emphasise the importance of examining multiple forms of alien plants while testing EICA. For instance, 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 support the EICA's prediction, whereas the latter would not. Consequently, these findings suggest that the ecological outcomes of *P. aculeata* and *C. schaffneri* interactions are genotype dependent and that no simplistic model (like the EICA hypothesis) can broadly predict the impact of *C. schaffneri* on its polytypic host. *Catorhintha schaffneri* is not present across all its host's native range and where it does occur, relative abundance varies, as higher densities were found in the coastal sites of Porto Belo than in Penha, Brazil (see Paterson *et al.* 2014a, b). Thus,

the predictions of change in native-range plants only relate to native areas of Santa Catarina and Rio de Janeiro, where the insect, *C. schaffneri*, is present relative to the invasive *P. aculeata*. Furthermore, it is interesting to note that even the plants from within the insect range differ from those found outside the agent's distribution.

In addition, *C. schaffneri* has the potential to reduce *P. aculeata*'s growth regardless of the host variation. The ability to halt growth arises presumably from the effect of the agent's non-selective, exploratory feeding behaviour on all genotypes, thus damaging the shoots regardless of genotype. After this exploratory feeding the growth is inhibited as the meristematic tissue architecture becomes altered. In many cases, high *C. schaffneri* mortality was recorded on plants that were not significantly damaged, thus supporting the hypothesis cited above about chemical concentration on the genotypes. For instance, on the less impacted genotypes (e.g. SA10), where less tissue was damaged, the agent's mortality was high. Although damage was ubiquitous, some plants suffered little impact to suggest genotype-dependent susceptibility. For instance, *C. schaffneri* was very damaging to the BR8 genotype from Rio de Janeiro and BR6 genotype from Santa Catarina (both in Brazil) and the agent occurs in both localities (see Paterson *et al.* 2014b), yet BR7 and BR9 were less damaged compared to BR8 and BR6 despite being from similar areas within the insect natural range. Meanwhile, the impacts were not higher on BR8 and BR6 than the invasive genotypes, contrary to EICA assumptions. Also, an invasive genotype, SA10, from Kosi Bay in South Africa suffered very low impact, even though invasive genotypes were predicted to be more susceptible according to EICA. The patterns of susceptibility of the plant genotypes to the agent can therefore not be fully explained by the origin of each genotype but significant variability between genotypes was found.

The association between vigour and damage did not correlate and as such did not suggest that fast-growing genotypes were more damaged than slow-growing plants (cf. Price 2000), but the contrary was true for damage incurred and agent mortality. The latter suggests that the more susceptible hosts were simply more suitable for feeding and implies that more agents would be needed to suppress deleterious invasive genotypes like SA10 than the more susceptible ones like SA1 and SA4. Although all genotypes were impacted to some extent, some genotypes were clearly more impacted than others were, and mortality of insects differed between genotypes. Generally, the findings of this study beg the question whether *C. schaffneri* could successfully develop to adulthood on all genotypes, given that some of the tested plants were less impacted

suggesting that they might be an inferior food source.

In summary, invasive genotypes of *P. aculeata* do appear to have acquired traits that will increase invasive potential, but this has not resulted in the invasive genotypes being more susceptible to either of the biological control agents. There is strong evidence for variability in susceptibility to herbivory between genotypes but this variability cannot be explained by the genetic relationship between the plant genotypes or their geographic origin. *Catorhintha schaffneri* is damaging to all invasive genotypes, despite having relatively poor performance of genotype SA10, so the biological control programme should not be hampered by agent-plant incompatibility.

CHAPTER 3 Performance of *Catorhintha schaffneri* on polytypic *Pereskia aculeata*: Do invasive plant populations provide better hosts?

3.1 INTRODUCTION

Insects and plants interact in many ways, but the most frequent interaction is herbivory, where insects feed on plants. A noticeable fitness cost for host plants arises from insect herbivory, which creates selection pressure on the plants that leads to the evolution of different anti-herbivore defences that are either constitutive (usually present) or induced (produced in response to the insects) (Herms & Mattson 1992; Stamp 2003; Orians & Ward 2010). Some insects are generalist herbivores, whereas others are specialists that feed on just one or a few closely related plants. The specialised herbivores are often able to utilise their host, regardless of the plant defences, which may result in an arms race between specialist insects and their host plants (Stamp 2003; Thompson 2005; Karban & Agrawal 2002; Runyon & Birdsall 2016; Myers & Sarfraz 2017).

In plants, anti-herbivore defences are costly and can alter host quality to the detriment of their natural enemies. For example, anti-herbivore defences in the invasive houndstongue, *Cynoglossum officinale* Linnaeus (Boraginales: Boraginaceae), exhibited fitness costs as demonstrated using simulated herbivory in which tested plants became stunted, had fewer and smaller size leaves and nutlets, but had more trichomes than undamaged plants (Runyon & Birdsall 2016). The absence of herbivores, in a study conducted on an invasive alien plant by Feng *et al.* (2009), facilitated a reduction in the use of nitrogen to thicken cell walls. Thick cell walls, similarly to trichomes, are a structural defence against herbivores.

Different populations of host plants can differ in their defence structures (quantitatively and qualitatively) depending on their exposure histories to herbivore attacks, which may ultimately have implications for biological control programmes (Cipollini & Lieurance 2012; Gruntman *et al.* 2017). An example is *Lythrum salicaria* and its leaf-feeder and root-feeder that both fed on an invasive host variety from Ithaca (US) and a native variety from Lucelle (Switzerland), but performed differently on plants from each population (Blossey & Notzold 1995; see chapter 2 for details). Differences in agent performance manifested themselves in fitness metrics such as survival, development time, and body weight; which remained unaffected for *Galerucella pusilla* on both host populations. However, the same was not true for *Hylobius transversovittatus*, which benefited more from the invasive population (Blossey & Notzold 1995). In another study, Manrique *et al.* (2008) found similar results in the

performance of three introduced specialist agents on two host genotypes of *Schinus terebinthifolius* Raddi (Sapindales: Anacardiaceae). While one agent, *Episimus utilis* Zimmerman (Lepidoptera: Tortricidae), was unaffected by host variants, two other agents, *Pseudophilothrips ichini* Hood and *Pseudophilothrips* sp near *ichini* (Thysanoptera: Phlaeothripidae) had uneven fitness. The implication of not evaluating individual species against the different forms of their interacting host (or agent as the case maybe), may result in population decline for the specialist agent, if any of such host species is harmful to the agent, and as such undermine the biological control efforts.

In weed biological control, exotic weeds are managed using introduced specialist natural enemies (or biological control agents), which may be sensitive to intraspecific host variation either as evident in host phenotypes or genotypes. Variation in host plants can influence host suitability to agents, as food quality remains a key determinant of herbivore demography and poor quality food would negatively affect the agents' impacts and fitness to the detriment of weed biological control (e.g., Price 2000). There is substantial evidence of the effects of host plant variation against different specialist agents in the biological control literature (e.g., Hanks & Denno 1994; Dray *et al.* 2004; Simelane 2006). However, in other studies such negative effects from host variation were not reported. For example, *P. guerini* can feed on many different genotypes and forms of *P. aculeata* without any fitness cost (Paterson *et al.* 2012) and varietal differences in *Chromolaena odorata* (Linnaeus) King and Robinson (Asterales: Asteraceae) did not affect its specialist agent, *Pareuchaetes insulata* (Walker) (Lepidoptera: Erebidae) (Uyi *et al*. 2014). Such contrasting evidence suggests that an agent's ability or inability to utilise host variants can be context-specific and depend on the insect-plant system, especially for different host populations or genotypes of a clonal species, which differ in traits responsible for nutrient uptake, photosynthesis, vigour and chemistry (Chapin & Oechel 1983; Blossey & Notzold 1995; Thompson 2005; Flood *et al*. 2011). Therefore, in managing exotic weeds, an agent's response to such host plant variation would determine the fate of the weed biological control programme (Price 2000; Awmack & Leather 2002; Manrique *et al.* 2008; Bottega *et al.* 2017).

The performance of specialist agents can be locally structured along the geographic origin of its host genotypes, as environment-associated factors may influence separate populations differently (Thompson 2005; Goolsby *et al*. 2006; Simberloff & Rejmanek 2011). When host and agent populations interact at subpopulation levels, local adaptation can arise (Thompson 1994, 2005; Simberloff & Rejmanek 2011). Such adaptation may be common among

specialists if there is low exchange of genetic material between geographically separate populations (Kawecki & Ebert 2004; Thompson 1994, 2005). Local adaption becomes evident when a species (e.g., specialist agent) performs better or is fitter on genotypes with which it has co-evolved. Natural enemies from the origin of the invasive plant population are therefore most likely to be the most effective biological control agents. It is for this reason that genetic matching studies have become almost routine in biological control programmes (Goolsby *et al.* 2006; Paterson *et al.* 2009; Gaskin *et al.* 2011). The influence of local adaptation on host fitness has been detected in some plants (Joshi *et al.* 2001; Leimu & Fischer 2008; Leger *et al.* 2009), but not others (Hamann *et al.* 2017). Leimu and Fischer's (2008) meta-analysis emphasised that it may be rarer than previously thought, nonetheless, 45% of a thousand-paired plants exhibit some form of local adaptation.

Although rarely evaluated among invasive alien plants, local adaptation between herbivores and the host plants can influence agents performance, but not always. For example, Goolsby *et al*. (2006) explored the potential link between distinct populations of an invasive alien weed, *Lygodium microphyllum* Brown (Schizeales: Lygodiaceae), which has multiple-location-specific forms along its native distribution, and its specialist natural enemy, a phytophagous mite, *Floracarus perrepae* Knihinicki & Boczek (Arachnida: Prostigmata: Eriophyidae). By focusing on gall-inducibility from leaf rolling as a reproductive fitness metric, they show that *F. perrepae* from different locations as their host forms were less able to induce leaf roll galls than the mites from the same locality as their host population. Similarly, Karban (1989) showed a finely tuned association between thrips populations of *Apterothrips secticornis* Trybom (Thysanoptera: Thripidae) and its host variants of *Erigeron glaucus* Ker Gawl (Astreales: Asteraceae). In contrast, evidence of local maladaptation in which local herbivore populations underperformed on their local hosts unlike on their nonlocal host, has also been seen (Kaltz *et al.* 1999). These results demonstrate that agents with no prior experience with a host plant (new association) may, or may not, be better equipped to use a non-local host plant, which lacks the evolutionary tool to defend itself, than the local herbivores (old associations) (Hokkanen & Pimentel 1984; 1989).

The leaf-chewing beetle *Pherica geurini* has been shown to feed equally on several different genotypes and forms of *P. aculeata* from the northern and southern native ranges as well as the intrdocued range (Paterson *et al*. 2012). But agents of different feeding guilds can perform differently on different populations of a host species (Hanks & Denno 1994;

Manrique *et al.* 2008; Blossey & Notzold 1995; Goolsby *et al*. 2006) so it is possible that the stem-wilter, *C. schaffneri*, will respond differently to the various *P. aculeata* genotypes.

The populations of *P. aculeata* introduced into South Africa is genetically depauperate (Paterson *et al*. 2009), but has recently been subjected to a biological control agent, *C.schaffneri*, which was sourced from an origin known to be different from the origin of its new host population in Rio de Janeiro, Brazil (Leuenberger 1986; Paterson *et al.* 2009; Chapter 1). Although *C. schaffneri* has been recorded at Rio de Janeiro, it is possible that the agent population in South Africa has acquired a 'finely tuned' evolutionary relationship (local adaptation) with its actual source population in Santa Catarina. Distance is a key factor that can facilitate the attrition of any local adaption as populations that are far apart and have less ability to disperse would have low chances of gene mixing, flow and genetic drift, thus possessing high chances of a finer scale adaptation (e.g., Hanks & Denno 1994; Hufbauer & Roderick 2005; Goolsby *et al*. 2006). Whether the populations of *C. schaffneri* at both Rio de Janeiro and Santa Catarina exchange genetic materials sufficient for a single source population to utilise any of the host plants from another locality remains unclear and difficult to test. If the agent had become locally adapted on its populations in Santa Catarina (local host) before its introduction in South Africa for biological control of *P. aculeata*, it would be fitter on its local host than on the host populations from other localities (nonlocal hosts). This may have important implications for biological control because the agent may be less suited to the invasive genotypes of the target weed than those at the origin of the agent population in the native range.

3.2 MATERIALS AND METHODS

3.2.1 Study organisms: *Pereskia aculeata* and *Catorhintha schaffneri*

The performance of *C. schaffneri* was evaluated against ten genotypes of *P. aculeata* (Table 3.1). The genetic relationships between these plant genotypes are known from a genetic matching study conducted by Paterson *et al*. (2009) (see Fig 2.1 & Fig. 2.2 in Chapter 2 for more details). The genotypes included two native genotypes from Rio de Janeiro, which are the closest native relatives to the South African genotypes (BR7 and BR8) (Table 3.1). *Catorhintha schaffneri* has been recorded in Rio de Janeiro (Brailovsky & Garcia 1987; Paterson *et al*. 2014b), but the population of the biological control agent was not collected from these sites (Fig. 3.1-3.2). These two invasive plant genotypes (SA1 and SA4) are, therefore, likely to have been exposed to *C. schaffneri* herbivory in the past, but not necessarily to this same population of *C. schaffneri*.

Three other genotypes from Brazil that were also included are a single genotype from Londrina, Paraná Province, where *C. schaffneri* does not occur (BR2), and two genotypes from Santa Catarina Province (BR6 and BR9) from within the distribution of *C. schaffneri* (Table 3.1). While BR2 was genetically closer to the two genotypes (BR6 and BR9) from Santa Catarina, the Rio de Janeiro genotypes were genetically different (group 3: Paterson *et al*. 2009; Table 3.1). These three Brazilian host plants (BR2, BR6 and BR9) are also closely related to the South African weed populations, but are not as genetically close a match as those from Rio de Janeiro (Paterson *et al*. 2009). The biological control agent was sourced within the same locality as the BR6 and BR9 genotypes (Table 3.1). With Santa Catarina being the origin of the both BR6 and BR9 and the agent population, their relationships could be regarded as an old association, unlike against BR2 and any other native genotypes from outside the agents' native distribution, which should be considered new associations (Hokkanen & Pimentel 1984; 1989).

Other host genotypes that were included are two genotypes from the northern native range of *P. aculeata* and one more genotype from the southern native range in Argentina (Fig. 3.1). Firstly, a genotype was sourced from the northernmost extreme of the native distribution in Pedernales, Dominican Republic (DR3) and another from Caracas, Venezuela (VZ2). Both genotypes were genetically closer to each other (as group 1) than to the other previously mentioned genotypes (in group 3 and 4, Paterson *et al*. 2009; Table 3.1). None of these genotypes had ever been reported to play host to either the population of *C. schaffneri* used

here or any other population (Paterson *et al*. 2009). Hence, the genotypes (VZ2 and DR3) are both nonlocal hosts to *C. schaffneri* and their relationships with the agent are a new association. The last genotype sourced from the southern native range was AR3 from Misiones in Argentina and it aligns in the same genetic grouping (4) as the Santa Catarina populations. However, it is not a local host to *C. schaffneri* because the agent has never been recoded anywhere is Argentina (Paterson *et al*. 2014a; Table 3.1; Fig. 3.2).

The genotypes from the invasive range in South Africa were one each from Knysna, Western Cape Province (SA1) and Port St. Johns, Eastern Cape Provinces (SA4) (Fig. 3.1). Their genetic relationship has been highlighted above (Table 3.1). In relation to the biological control agent, these genotypes were grouped as new association. The origin of these genotypes is Rio de Janeiro, which is within the distribution of *C. schaffneri*, but is not the source of the agent population used in this study. In addition to this, the time as a horticultural plant has resulted in the South African genotypes being genetically very different to their closest relatives in the native range (Paterson *et al*. 2009).

Table 3.1 Origin of genotypes from native and invasive ranges of *Pereskia aculeata*, and the status of exposure to the population of *Catorhintha schaffneri* released in South Africa. Country codes: SA = South Africa, BR = Brazil, AR = Argentina, DR = Dominican Republic, VZ = Venezuela. References: a = McGibbon (1858); b = Paterson *et al.* (2014b); c = Britton and Rose (1919), d = De Beer (1988)

Sample	Locality	Relationship between the biological control agent in South Africa and the tested genotypes of its host plant	Status of agent in the locality	Phylogenetic groupings	References
SA1	Knysna	New association: Invasive genotypes have differentiated from all indigenous genotypes due to artificial selection in the horticultural trade (it is a new host)	recently released	2	a, b, c, d
SA4	Port St. Johns	``	recently released	2	a, b, c, d
BR2	Londrina	New association: This genotype is genetically similar to plants where *C. schaffneri* was collected; but *C. schaffneri* does not occur in the region (it is a nonlocal host).	not found	4	a, b
BR6	Santa Catarina	Old association: Agent population was collected from a site, which is about 10 km from this plant genotype (it is likely a local host).	highly abundant	'?'4	b
BR9	Santa Catarina	``	low abundance	4	b
BR7	Rio de Janeiro	New association: Although the agent population was collected from outside this region, it is within the agent's native distribution. Nonlocal host to *C. schaffneri* (it is a nonlocal host)	low abundance	3	b
BR8	Rio de Janeiro	``	low abundance	3	b
AR3	Misiones	New association: Plants are genetically distinct from plants on which *C. schaffneri* was collected and are found outside of the distribution of the agent (it is a nonlocal host)	not found	4	b
DR3	Pedernales	``	not found	1	b
VZ2	Caracas	``	not found	1	b

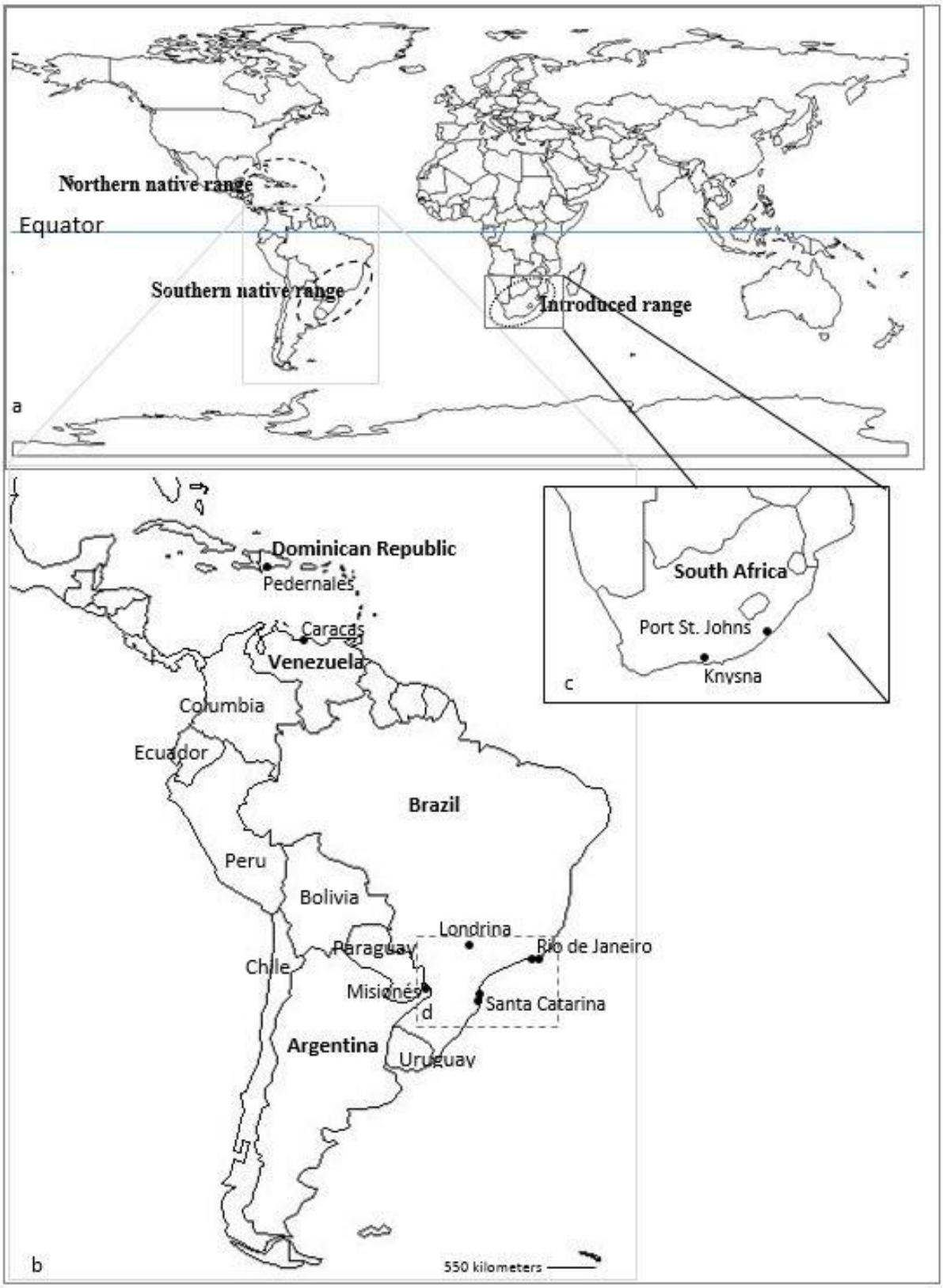

Fig. 3.1 The distribution of *Pereskia aculeata* in its (a) Northern and Southern native ranges, and the introduced range and (b) localities within the native; (c) introduced ranges where plants were sourced and (d) origins of both plants and agents. Regions and countries relevant to this study are in boldface. Map templates source: South (2011).

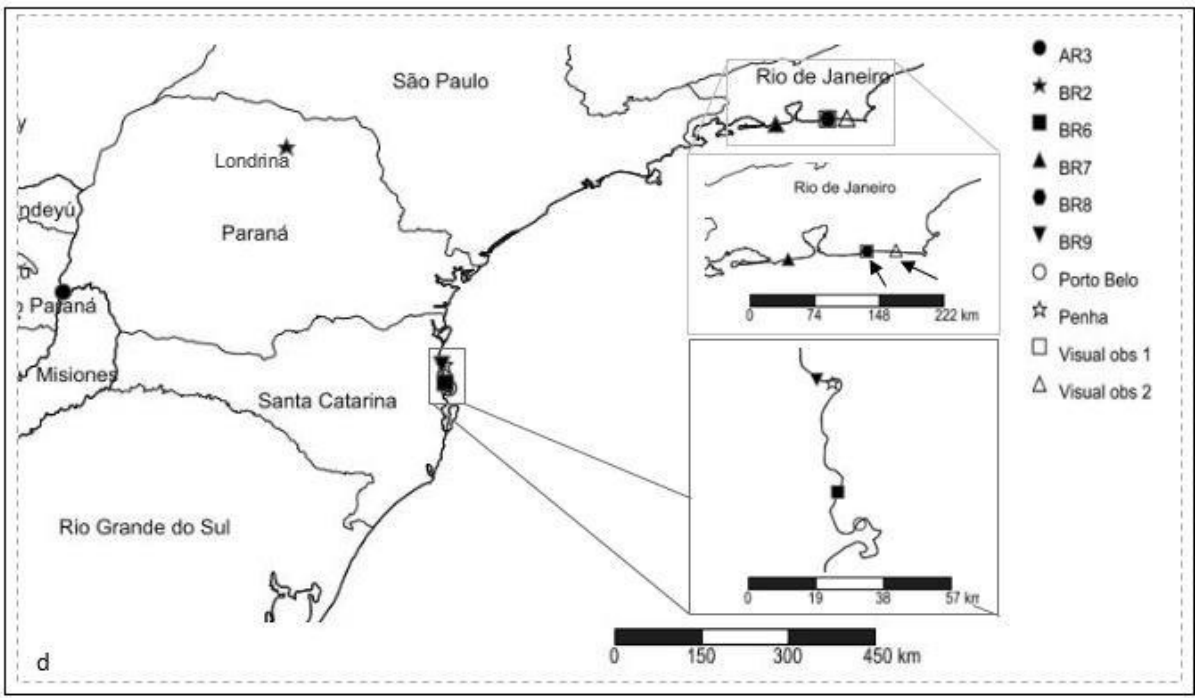

Fig. 3.2 The distribution of the source genotypes of *Pereskia aculeata* in Brazil and Argentina, and the origin of *Catorhintha schaffneri* in Brazil. In the upper expanded map, the two arrows show sites where *C. schaffneri* has been visually observed while feeding on the *P. aculeata*. The open square in the key overlaps with the hexagon shape (as indicated by the left arrow) for a site where BR8 was sourced (I. Paterson, unpublished records). Map templates source: Shorthouse (2010).

Test Plant. Although collected at different times, the stock plants used in this study had sufficient time in a common greenhouse to shed any non-genetic effects caused by handling and housing (Kawecki and Ebert 2004). Stem cuttings of about ten to twelve cms in length and ten mms thick were sourced from the stock plants and propagated with 6 cm of the cuttings inserted into a standardised 50-50 (loam soil-compost) growth medium. These media were contained in plant bags of dimensions 125 x 100 x 225 mm, and fertilised with five grams of 3-1-5 (N-P-K) Wonder™ commercial fertilisers at the onset. The fertiliser was reapplied six weeks thereafter (following manufacturer's recommendation) until they were due for use in the trials. Plants were watered uniformly as needed and upon commencement.

Agent. The eggs of *C. schaffneri* used here were sourced from adult females from a breeding culture of the same generation of agent reared under similar conditions, feeding and water regimes. The breeding culture has been sustained over multiple generations from the twenty-three individuals imported into South Africa (Paterson *et al.* 2014b; also see Chapter 2). From those eggs, unfed newly hatched nymphs were collected using a camel-hair brush

immediately after hatching and placed onto the growing shoots of different genotypes of *P. aculeata* that were enclosed in cages of dimensions 40 x 40 x 60 cm. The shoots of the test plants inoculated with *C. schaffneri* were sprayed with a fine mist of water every evening using a handheld sprayer.

3.2.2 Experimental design

Groups of six newly hatched and unfed nymphs were placed on each genotype of live potted plants encloded in cages. The insects developed simultaneously on ten selected genotypes, and the experiment was repeated five times. The stage of development of nymphs was recorded at 19:00hr daily, but frequent inspections were upheld towards adult emergence to collect adults shortly after emergence for their body weight assessments. The presence of exuviae was used to determine whether an insect had moulted or not. Any exuvia found on the plants or within the cages (usually beneath the shoot where they were feeding) were removed to prevent recounting of moulting episodes. Test plants were replaced when plants were damaged (usually every four days) to replenish food supplies and avoid food being a limiting factor. All trials were conducted in a temperature-controlled room at 25 °C to maintain stable conditions and the humidity and temperature were recorded using 1-Wire® Hygrochron™ iButtons (Model DS 1923#F5; Maxim Integrated Products, San José, CA, USA). Photoperiodicity was fully automated using standard growth lamps regulated at 14 hr days and 10 hr nights.

3.2.3 Survival, developmental biology and damage incurred by plants

Survival and duration of development to each ecdysis event was recorded as well as the level of damage inflicted to the plant. The total developmental time, survival rate and the quantified damage were used to calculate Maw's (1976) host suitability index (HSI), while Dobie's (1974) host susceptibility index (DSI) was calculated from developmental time and survival rate. While the focus of HSI is agent-oriented, the DSI is host plant-oriented (Dobie 1974; Maw 1976).

Maw's host suitability index (HSI) was used previously to integrate fitness parameters in *Cassida hemisphaerica* Herbst (Coleoptera: Chrysomelidae) (Maw 1976). Aspects that were sensitive to host variation (pupal mass, percentage pupation and development time) were combined, with 'Host suitability index = (pupal mass) x (percentage pupation)/developmental time' (Maw 1976,).

In this study instead of pupal weight, the body weight of newly emerged adult was acquired, as *C. schaffneri* is a hemimetabolous insect. The weight was measured using a sensitive weighing balance (± 0.0001 mg), and the percentage pupation was taken as the proportion of successful adult emergence relative to the initial number that was introduced on each host plant. Thus HSI was calculated as (body weight*percentage emergence)/ development time.

Dobie's susceptibility index (DSI) was developed to assess host susceptibility to *Sitophilus zeamais* Motsch. (Coleoptera: Curculionidae) (Dobie 1974). It uses the number of surviving F_1 progeny and developmental time, with 'Susceptibility Index = ($\log_e$ F_1) x 100/developmental time'. In this current study, an adjustment was made; instead of numbers F_1 progeny, the number of ecloded adults was used. Damage to the plants was quantified using the total length of wilted shoots and the total number of leaves wilted. The length of wilted shoot (that is, the dry stalk generated by insect feeding) was measured to the nearest centimetre, while the numbers of wilted leaves were counts of wilted leaves on plants before replacements with healthy plants.

3.2.4 Statistical analysis

Shapiro-Wilk's (*W*) and Levene's tests were used to affirm assumptions of parametric tests. Data for percentage survival to the adult stage and developmental periods for the nymphs violated the assumptions of parametric tests so a non-parametric Kruskal-Wallis *H* test was adopted. A *post hoc* Kruskal-Wallis multiple comparison tests for significant differences were conducted using *dunnTest* function in FSA package (Ogle 2016) followed by Bonferroni-adjusted *p*-value to control for False Discovery Rate (Mangiafico 2015). Samples with unequal sizes such as body weight and total developmental period were unbalanced and analysed using Welch's *t*-test/ Welch's ANOVA followed by a post hoc test Games-Howell multiple comparisons when significant differences arose to avoid a false positive type 1 error (Games & Howell 1976; Newsom 2006; McDonald, 2014; Mangiafico, 2015). The extent of damage (wilted leaves and shoots) met parametric assumptions (as visualised in quantile-quantile plots and confirmed with Shapiro-Wilk and Levene's tests); thus a one-way ANOVA was conducted and differences separated using Tukey's Honest Significant Difference *posthoc* test (*glht* function in multcomp package). Simple linear regression was used to establish the relationship between wilted leaves and wilted shoots after conducting a global statistic (*gvlma*) and outlier (*outlierTest*) tests. Outliers with a significant effect on the

model (as seen in three cases) were excluded. The null expectations were an equal agent's performance on all genotypes. All data were analysed using R 3.3.3 (R Core Team 2017) and its associated packages, except for Kaplan-Meier survivorship calculated in Excel™.

3.3 RESULTS

3.3.1 Survival, development and body weight

The survival rate of *C. schaffneri* on different host plant genotypes ranged from 0% to 80% (Fig. 3.3 and Table 3.3). Three genotypes namely AR3, DR3 and BR9 did not support complete development of nymphs to the adult stage and they were genotypes from Argentina, the Dominican Republic and Brazil, respectively. The other genotypes, from Venezuela (VZ2), Brazil (BR6, BR7, BR8 and BR2), and South Africa (SA1 and SA4), did support development to the adult stage. Among these, the lowest survival rate was 50% on VZ2 whereas the highest was 80% of SA1. Kruskal-Wallis tests confirmed significant differences in survival rates between genotypes that did and did not support maturation, but not among the genotypes where the agent developed to maturity ($H_{(9)} = 35.47$, $p < 0.001$). Although there were significant differences between genotypes there is no clear pattern of suitability of the agent to genotypes from a specific area or any clear indication as to whether 'new' or 'old' associations are most suitable. *Catorhintha schaffneri* had higher survival on the Brazilian genotypes (BR8 and BR6) (which were on the average 46.5% higher) than the 50% survival on VZ2. Although an invasive South African genotype (SA4) had similar percentage survival (73.3%) as BR6 and BR8, it was 8% less supportive for *C. schaffneri* development compared to SA1 on which the highest survival (80%) was recorded (Fig. 3.3; Table 3.3). None of the percentage survival of the host genotypes that support complete development differed significantly (after a posthoc Dunn Test with a Bonferroni adjusted *p*-value). Kaplan-Meier survivorship curves showed two broad patterns of survivorship: (i) a set of three genotypes with unsuccessful development that culminated in zero probability after twenty-five days (i.e., BR9, AR3 and DR3) and (ii) another set, which supported successful development (Fig. 3.3).

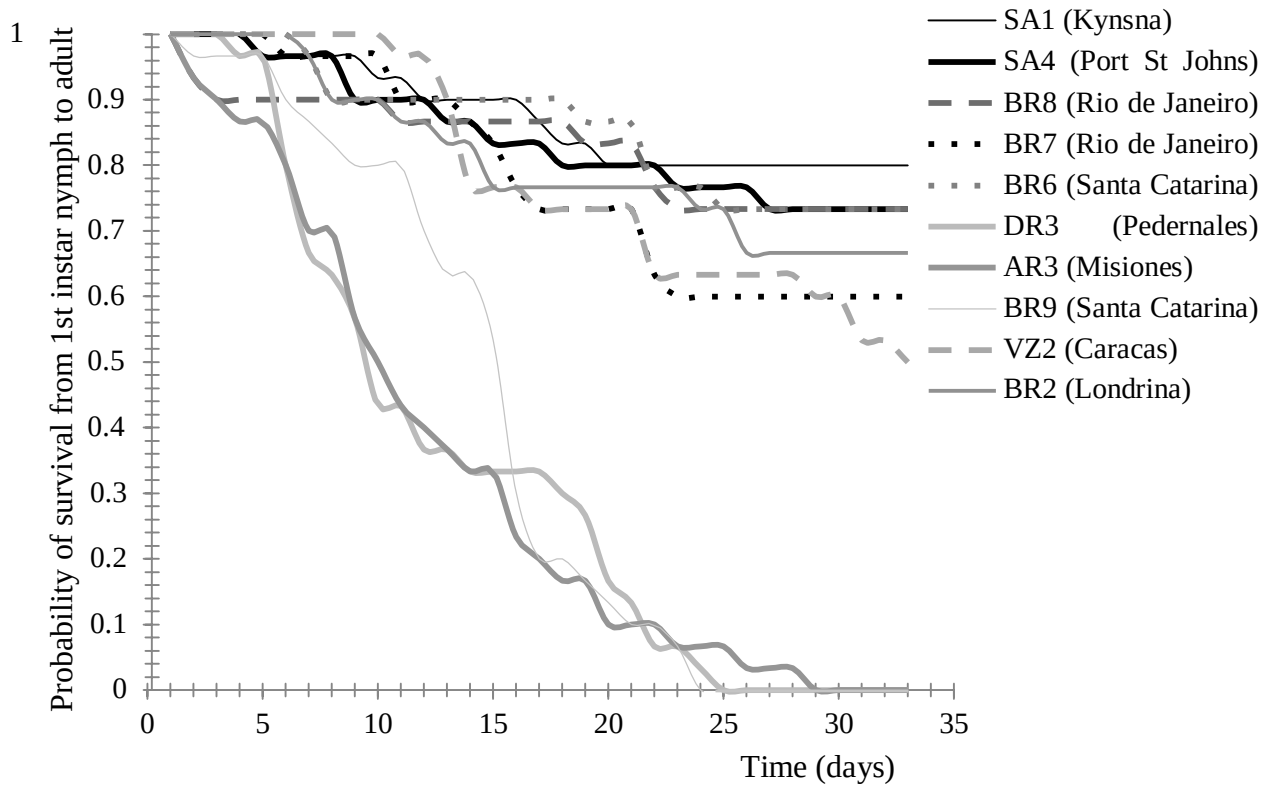

Fig. 3.3 Survival probability of nymphs of *C. schaffneri* from the first instar to adult eclosion. Data for each genotype was an aggregate of thirty unfed newly hatched first instar nymphs.

3.3.1.1 Stage-specific development of nymphs

Over the study period, a five-instar developmental pattern was observed prior to maturation (Table 3.2).

First Instars. The developmental period of first instar nymphs varied between 4.20 days to 5.07 days on different host genotypes. The first instar varied significantly on different genotypes ($H_9 = 34.34$, $p < 0.001$), *C. schaffneri* had a significantly shorter duration on two genotypes from Brazil (BR2 at 4.33 ± 0.10 days and on BR9 at 4.20 ± 0.14 days) than on the genotype from Venezuela (VZ2 at 5.07 ± 0.13 days before ecdysis). Observed durations on other genotypes did not differ from VZ2 nor BR2 and BR9 (Table 3.2).

Second Instars. The intermoult periods of the second instars of *C. schaffneri* on different genotypes ranged from 3.14 to 5.78 days. Their shortest durations were encountered on SA1 at 3.14 ± 0.12 days. Although duration was significantly shorter on SA1 than on AR3 at 5.78 ± 0.43 days, it did not differ statistically from the durations on VZ2, SA1, BR6 and BR2 ($H_9 = 62.31$, $p < 0.001$; Table 3.2).

Third Instars. Intermoult periods of the third instar nymphs varied from 3.60 to 6.60 days on BR8 and AR3 respectively (Table 3.2). On the majority of the Brazilian genotype, the 3rd instars spent significantly shorter duration as seen on BR2 (4.08 ± 0.17 days) from Londrina, BR6 from Santa Catarina at 3.88 ± 0.19 days, and on BR8 from Rio de Janeiro at 3.60 ± 0.14 days, when compared to the genotype from Misiones, AR3 on which 6.60 ± 0.40 days were spent ($H_9 = 37.88$, $p < 0.001$). Nymphs on other genotypes such as SA1 and SA4 were similar to either AR3 or BR8.

Fourth Instars. The duration of development ranged from 3.0 days on DR3 from Dominican Republic to 6.58 days on VZ2 from Venezuela (Table 3.2). Nymph's duration on DR3 was atypically and significantly shorter than durations on other genotypes but it did not survive into the next developmental stage, whereas those on BR9 had all died ($H_{(8)} = 32.58$, $p < 0.001$).

Fifth Instars. A range of 6.78 to 9.07 days were spent during the fifth stadium before moulting to adults (Table 3.2). Although nymphs that developed on the invasive genotypes SA1, SA4 and a native genotype BR6 had statistically similar durations of 6.78 ± 0.30 days, 7.27 ± 0.30 days and 7.09 ± 0.15 days respectively, they were significantly shorter than on VZ2 where 9.07 ± 0.43 days was recorded ($H_{(6)} = 20.09$, $p = 0.002$).

3.3.1.2 Total developmental duration

Nymphs to adults (both sexes combined). Of the ten genotypes screened, three did not support complete development. None of the genotypes that did not support development were from the invasive range, but interestingly the Brazilian BR9 coexists with *C. schaffneri* while the others were obtained from Argentina and Dominican Republic; outside the agent's distribution. The quickest total development time of *C. schaffneri* was on plants from the origin (BR6: 23.7 ± 0.56 days) and the slowest was from the most distantly related plant (VZ2: 29.1 ± 0.90 days). Although development on BR6 was significantly shorter than on VZ2 (Welch $F = 4.78$, $p < 0.001$; Table 3.3), it was not significantly shorter than the average total development time of *C. schaffneri* on any of the other native and invasive genotypes ($p > 0.05$). These other genotypes were BR2, BR7, BR8, SA1 and SA4 on which a range of 24.1 to 25.3 days of development time was recorded, but the average development times of *C. schaffneri* on SA4 and BR2 were not statistically less than the average time that *C. schaffneri* expended from first instar nymph to adult stage on VZ2 ($p > 0.05$).

Nymphs to adults by sexes. A two sample Welch *t*-test between sexes (♀/♂) showed that adults generally ecloded after the same duration of development regardless of sex ($p > 0.05$), but there was an exception for BR7, in which the females emerged significantly later than males (Table 3.3).

Table 3.2 Stage-specific development of *Catorhintha schaffneri* on genotypes of *Pereskia aculeata*. Although presented as means and standard errors, analyses were based on the ranked median as dictated by Kruskal-Wallis ranked sum test and Dunn *post hoc* Test (*dunnTest*) with Bonferroni adjusted *p* values. Stages with unsuccessful developments were statistically excluded. Values followed by the same letters within the same column were not significantly different.

Genotypes	Stages and durations of development and (Mean ± SE, days)				
	First	Second	Third	Fourth	Fifth
BR2	4.33 ± 0.10^{a}	3.33 ± 0.17^{bcd}	4.08 ± 0.17^{bc}	5.46 ± 0.15^{ab}	7.70 ± 0.24^{ab}
BR6	4.29 ± 0.16^{ab}	$3.59 \pm 0.12^{b\text{-}e}$	3.88 ± 0.19^{bc}	5.10 ± 0.19^{a}	7.09 ± 0.15^{a}
BR7	4.89 ± 0.11^{b}	$3.52 \pm 0.09^{b\text{-}e}$	4.32 ± 0.17^{abc}	5.42 ± 0.31^{ab}	7.47 ± 0.38^{ab}
BR8	4.56 ± 0.13^{ab}	3.93 ± 0.16^{abce}	3.60 ± 0.14^{b}	4.68 ± 0.12^{a}	7.59 ± 0.15^{ab}
BR9	4.20 ± 0.14^{a}	4.57 ± 0.34^{abe}	5.69 ± 0.67^{ac}	nil	Nil
AR3	4.95 ± 0.27^{ab}	5.78 ± 0.43^{a}	6.60 ± 0.40^{a}	4.07 ± 0.67^{ab}	Nil
DR3	4.80 ± 0.15^{ab}	5.25 ± 0.59^{ae}	5.63 ± 0.73^{ac}	3.00 ± 0.00^{ab}	Nil
SA1	4.69 ± 0.10^{ab}	3.54 ± 0.17^{bcd}	4.79 ± 0.34^{abc}	4.76 ± 0.27^{a}	6.78 ± 0.30^{a}
SA4	4.86 ± 0.12^{ab}	3.14 ± 0.12^{d}	4.29 ± 0.14^{abc}	5.00 ± 0.17^{a}	7.27 ± 0.30^{a}
VZ2	5.07 ± 0.13^{b}	3.31 ± 0.17^{d}	4.88 ± 0.27^{ac}	6.58 ± 0.33^{b}	9.07 ± 0.43^{b}
H statistic	34.34^{***}	62.31^{***}	37.88^{***}	32.58^{***}	20.09^{**}

Significance codes: $^{***}p < 0.001$; $^{**}p < 0.01$; $^{*}p < 0.05$; ^{ns}not significant ($p > 0.05$)

Table 3.3 Total developmental periods and survival of *Catorhintha schaffneri* on different genotypes of *Pereskia aculeata.* All data were analysed using Welch's ANOVA, except for percentage survival in which Kruskal-Wallis *H* test was adopted to examine variation in the pattern of mortality across the test genotypes including those that could not complete development. Values followed by the same letters (or ^{ns}) in the same columns are not significantly different ($p > 0.05$). †Unsuccessful development to maturation (also see Kaplan-Meier survivorship curve for patterns, Fig. 3.2)

Genotypes	Adults (from 1st instar to adult emergence, days)			Comparison by sex		Survival (%)
	Both sexes (♂♀)	Male (♂)	Female (♀)	*t* statistic	*p*-value	
BR2	24.1 ± 0.74^{ab}	22.6 ± 1.69^{ns} (5)	24.5 ± 0.80^{ns} (15)	-1.03	0.34	66.7^{a}
BR6	23.7 ± 0.56^{a}	23.4 ± 0.87^{ns} (10)	23.9 ± 0.75^{ns} (12)	-0.42	0.68	73.3^{a}
BR7	25.3 ± 0.64^{ab}	23.9 ± 0.66^{ns} (9)	26.7 ± 0.89^{ns} (9)	-2.50	0.02*	60.0^{a}
BR8	24.0 ± 0.55^{a}	21.8 ± 1.65^{ns} (6)	24.4 ± 0.53^{ns} (16)	-1.55	0.20	73.3^{a}
SA1	24.3 ± 0.44^{a}	24.1 ± 0.68^{ns} (14)	24.6 ± 0.57^{ns} (10)	-0.56	0.58	80.0^{a}
SA4	24.8 ± 0.55^{ab}	24.9 ± 0.77^{ns} (8)	24.7 ± 0.76^{ns} (14)	0.15	0.88	73.3^{a}
VZ2	29.1 ± 0.90^{b}	29.0 ± 1.20^{ns} (9)	29.2 ± 1.59^{ns} (6)	-0.08	0.92	50.0^{a}
BR9†	-	-	-	-	-	0.00^{b}
AR3†	-	-	-	-	-	0.00^{b}
DR3†	-	-	-	-	-	0.00^{b}
Welch *F* statistics	4.800^{***}	2.882^{*}	2.235^{ns}	n/a	n/a	n/a

Significance codes: ***$p < 0.001$; ** $p < 0.01$; *$p < 0.05$; ^{ns}not significant ($p > 0.05$). n/a = not applicable

3.3.1.3 Body weights

Both sexes combined. Upon emergence, the body weights of *C. schaffneri* varied from 26.0 ± 1.14 mg to 35.75 ± 1.27 mg for adults that fed on Venezuelan (VZ2) and Brazilian (BR6) genotypes respectively. Adults which had developed on BR7 (29.7 ± 1.33 mg) were statistically neither heavier than those on VZ2 nor lighter than the other genotypes. The body weight of adults that emerged from VZ2 was significantly less compared to those that emerged from SA1 (33.7 ± 1.23 mg), SA4 (35.8 ± 1.27 mg), BR6 (35.5 ± 1.21 mg), BR8 (35.2 ± 1.10 mg) and BR2 (33.79 ± 0.81 mg) (Welch $F = 9.159$, $p < 0.0001$).

By Sexes. All females were significantly ($p < 0.05$) heavier than males that emerged from the same host genotypes (Fig. 3.4; Welch *t*-test: Table 3.4). Males of *C. schaffneri* had body weights which ranged from an average of 23.8 mg (±0.74) for adults that fed on VZ2 from (Venezuela) to 32.4 mg (±1.66) for adults that fed on BR6 from Santa Catarina (Brazil). The body weights of female adults varied from the lightest weight at 29.3 mg (±2.02) on VZ2 to the heaviest weight of 38.8 mg (±1.87) on SA4 from Port St Johns in South Africa. For both males and females, *C schaffneri* were lightest on the Venezuelan genotypes.

Although comparisons among genotypes showed that females had significantly different weights between genotypes (Welch $F = 3.47$; $p = 0.011$), no differences were detected after the *posthoc* test. The body weights of the males were statistically different (Welch $F = 12.85$, $p < 0.001$), and a pair-wise *post hoc* test revealed that males' weights on VZ2 were lighter relative to those that fed on BR8 from Rio de Janeiro ($t = 6.66$, $p = 0.016$) and males that fed on SA1 from Knysna ($t = 5.70$, $p = 0.004$), but not statistically different from the others.

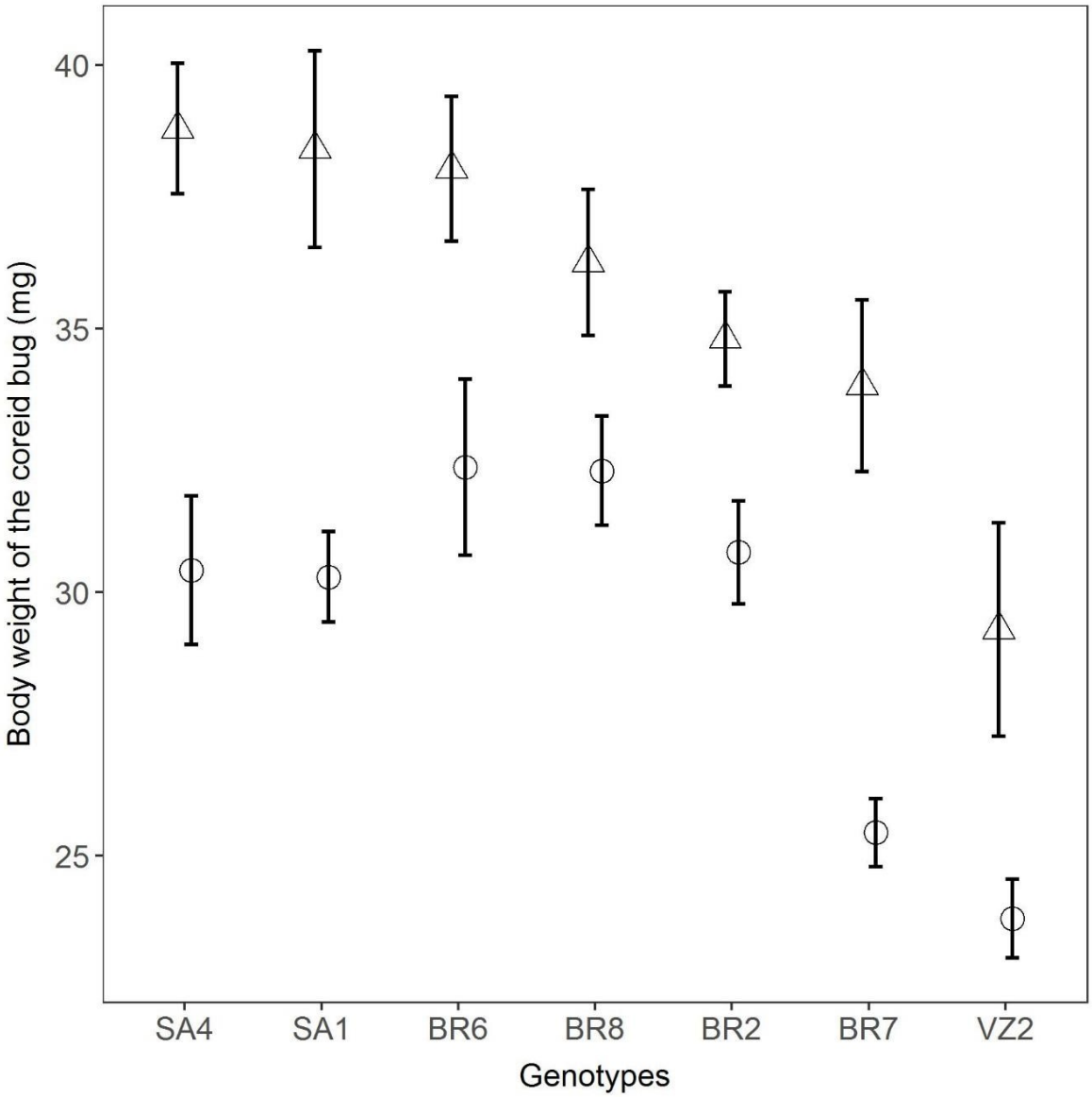

Fig. 3.4 Mean body weight of *Catorhintha schaffneri* (mg). The triangular symbol represent females, while the circular symbols represent males. Bars represent standard errors (S.E.).

Table 3.4 Welch's *t*-test (unequal samples) for body weights of males and females *Catorhintha schaffneri* that fed, from first instar nymphs to adult emergence, on seven genotypes of *P. aculeata* (see Fig. 3).

Genotypes	**SA1**	**SA4**	**BR2**	**BR6**	**BR7**	**BR8**	**VZ2**
t statistics	3.95	4.46	3.05	2.62	4.84	2.28	2.54
p-values	0.002	0.001	0.01	0.01	0.001	0.03	0.04

3.3.1.4 Extent of damaged tissues

Number of wilted leaves. Wilted apical leaves varied from an average of 7.0 ± 1.4 on AR3 from Misiones, Argentina to 94.8 ± 31.1 on BR6 from Santa Catarina, Brazil (Fig 3.5). The number of wilted leaves was statistically different among the various genotypes ($F_{(9,\ 40)}$ = 12.61, $p < 0.001$). Some native genotypes e.g., BR7, BR9, DR3, and AR3 had significantly fewer wilted leaves than the invasive genotypes (SA1 and SA4) and a few native genotypes such as BR6 & BR8 (Fig. 3.5). The native genotype, BR6, had the highest number of wilted leaves, but it was not significantly higher than the number recorded on SA1, SA4 and BR8. In relation to BR6, there was moderate levels of wilted leaves on VZ2, BR7 and BR2 which were similar to losses incurred by BR8, SA1 and SA4, but not BR6 (Fig. 3.5). The number of wilted leaves from BR9 genotypes was significantly lower than that recorded from BR6. Between BR7 and BR8 (both from Rio de Janeiro), the number of wilted leaves were significantly different. However, for both the invasive genotypes (SA1 and SA4) from South Africa, the number of leaves that were wilted was similar ($p > 0.05$).

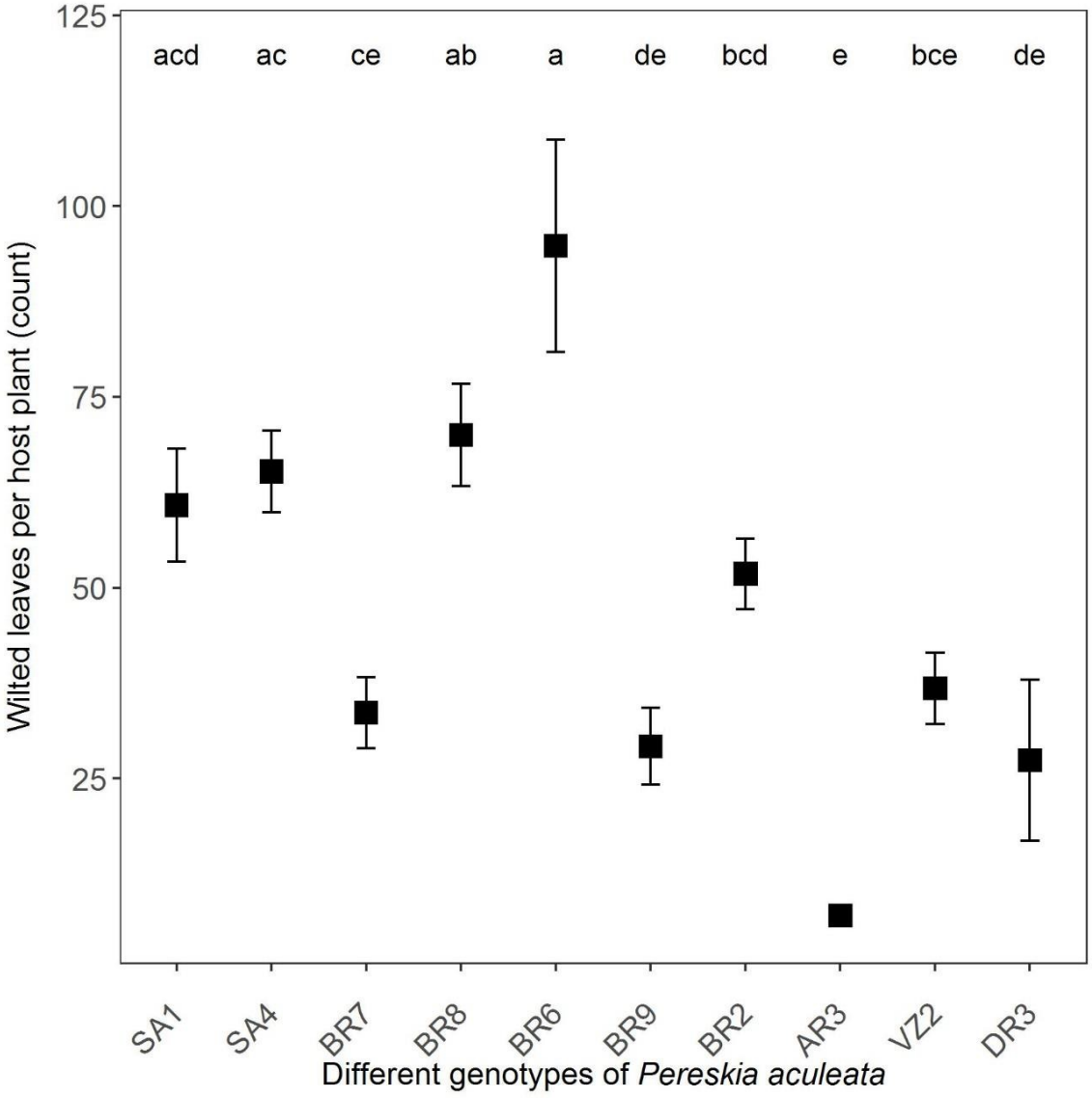

Fig. 3.5 Aggregate numbers of apical leaves wilted due to herbivory from *Catorhintha schaffneri* over their development periods. Bars represent standard error (S.E.). Boxes with the same letter(s) are not significantly different ($p > 0.05$).

3.3.1.5 Wilted apical shoots

Feeding by *C. schaffneri* resulted in varying total lengths of wilted shoots, which ranged from a minimum of 1.7 cm on AR3 from Misiones in Argentina to a maximum length of 59.1 cm on BR6 from Santa Catarina in Brazil (Fig. 3.6). Although AR3, DR3, BR9, and BR7 were all from the native region, relatively small portions of the host shoots were wilted and the total length of wilted parts were significantly shorter than those on SA1, SA4, BR8 and BR6 ($F_{(9,\ 40)}$ = 14.99, p < 0.001), which originated from two native regions and two invasive regions.

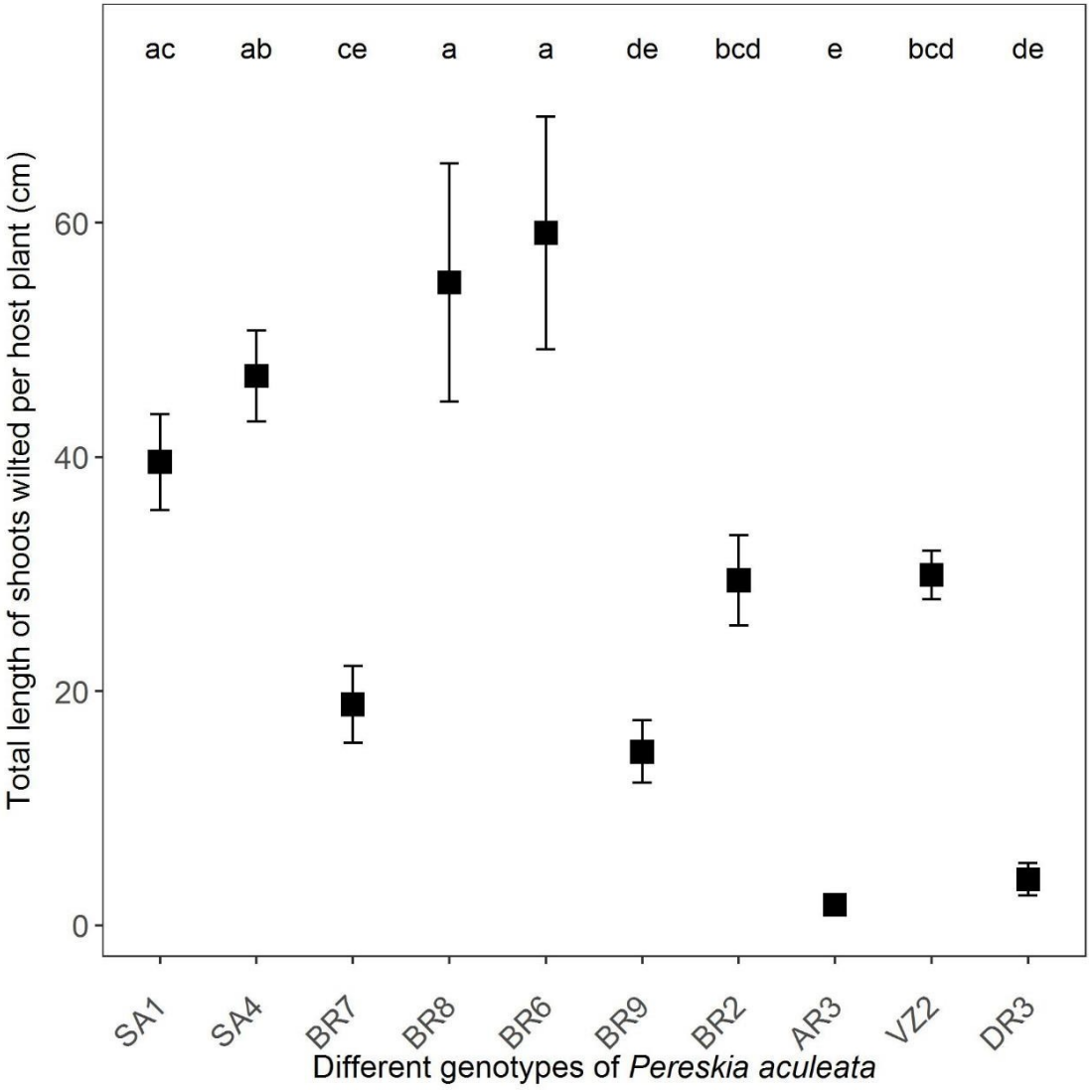

Fig. 3.6 Aggregate lengths of shoots wilted due to herbivory from *Catorhintha schaffneri*. Bars represent standard errors (S.E.). Boxes with the same letter(s) are not significantly difference (p > 0.05).

3.3.1.6 Association between lengths of wilted shoots and number of wilted leaves

The linear relationship between wilted shoot lengths with the number of wilted leaves was variable, with R^2 ranging from 0.540 to 0.884 ($p < 0.001$) (Table 3.5). Brazilian genotype BR6 had a significant regression equation ($F_{(1,94)} = 385.9$, $p < 0.001$), with an R^2 of 0.804 indicating that the predicted length of the wilted shoot was equal to 2.818 + 0.813 leaves per centimetre. Therefore, shoot deterioration increased by a factor of 0.813 centimetres for every leaf lost on genotype BR6 due to the wilting caused by *C. schaffneri* (Table 3.5).

Although other genotypes had similar significant associations (Table 3.5), there was no evident of a significant relationship between the wilted shoot lengths and the number of wilted leaves in the Argentina genotype, AR3, with $R^2 = 0.003$ ($F_{(1,16)} = 0.047$, $p = 0.8314$).

Table 3.5 Correlates of the two parameters of damage (wilted leaves and wilted shoots) after herbivore-induced injuries caused by nymphs of *Catorhintha schaffneri*.

Genotypes	Regression analysis of wilted leaves against wilted shoots [model: y ~ c + coefficient(shoot)]					
	R^2	Coefficients		*F*	Degree of freedom	*p* - values
		Intercept (c)	Shoots			
BR6	0.804	2.818	0.813	385.9	1, 94	< 0.001
BR8	0.540	2.661	0.667	81.1	69	< 0.001
SA4	0.802	2.373	0.917	259.8	64	< 0.001
SA1	0.698	2.898	0.819	120.4	52	< 0.001
BR2	0.688	3.289	0.746	99.22	45	< 0.001
VZ2	0.752	2.405	0.720	130.4	43	< 0.001
BR7	0.735	2.486	0.679	105.3	38	< 0.001
BR9	0.884	2.041	0.896	182.2	24	< 0.001
DR3	0.617	3.129	2.123	41.79	26	< 0.001
AR3	0.003	2.252	0.048	0.047	16	0.8314

3.3.1.7 Host suitability and susceptibility

Maw's host suitability index varied widely from zero to 111.4, while the adapted Dobie's susceptibility index also varied from zero to 118.2 (Table 3.6). Average values from both indices revealed that the invasive genotypes and two of the native genotypes were highly suitable and susceptible ($p < 0.05$). On the contrary, AR3 from Misiones, Argentina and DR3 from Pedernales, Dominican Republic were not susceptible to damage by the agent or suitable hosts for the agent. The first four ranked genotypes with an average index greater than one hundred were considered suitable, unlike the moderately suitable genotypes (e.g., BR2 and BR7 with $\geq 50 < 100$), while AR3 and DR3 were not favourable to *C. schaffneri*'s development (Table 3.6).

Table 3.6 Host suitability and susceptibility indices of different genotypes of *Pereskia aculeata* to *Catorhintha schaffneri* that were ranked from the highly suitable host to the less suitable or more resistant hosts. Ratings are rank averages for both indices. HIS = Maw's host suitability index (Maw, 1976) and DSI = an adapted Dobie's susceptibility index (Dobie 1974).

	Indices[†]		Ranks
Genotypes	HSI	DSI	
SA1	111.4 ± 9.3^{a}	118.2 ± 8.6^{a}	1
BR6	110.9 ± 11.8^{a}	112.0 ± 10.0^{a}	2
BR8	109.0 ± 9.6^{ab}	111.2 ± 10.24^{a}	3
SA4	106.9 ± 6.8^{ab}	108.8 ± 6.5^{a}	4
BR2	95.4 ± 24.8^{ab}	90.3 ± 25.7^{ab}	5
BR7	70.9 ± 15.5^{bc}	78.7 ± 20.0^{ab}	6
VZ2	51.9 ± 8.04^{c}	42.3 ± 19.9^{b}	7
BR9	0.0	0.0	8
AR3	0.0	0.0	8
DR3	0.0	0.0	8
$F_{6,28}$	2.994^{*}	2.518^{*}	

3.4 DISCUSSION

This study addressed the fitness of *C. schaffneri* on ten genotypes of *P. aculeata* from the plant's native and introduced ranges to provide insights on the influence of previous host-agent interactions on agent performance under similar conditions. Agent development patterns, survivorship, body mass, host suitability and susceptibility indices were monitored.

Successful development of unfed first instar nymphs of *C. schaffneri* to adult stage was encountered on seven out of ten tested genotypes. Of these seven, 71% (n = 5) were native-range plants (from Brazil and Venezuela) and 29% (n = 2) invasive-range plants (SA1 and SA4). Agent success was genotype-specific as no pattern of suitability could be explained by the geographic location of the plant genotypes, the genetic relationship of the genotypes or whether the genotype was considered a 'new' or 'old association'. The good hosts were a local host genotype (BR6) (from the same area in which the agent was sourced) in Santa Catarina and two non-local but native host genotypes of *P. aculeata* (BR7 and BR8) from Rio de Janeiro, Brazil, where the agent is present but not the source of the agent population that has been released in South Africa (see Table 3.1). A nonlocal host genotype (VZ2) from Caracas in Venezuela was also a good host to *C. schaffneri*, based on the survival outcomes (but not in development time). By contrast, nonlocal host genotypes (AR3) from Argentina and another nonlocal genotype (DR3) from the Dominican Republic were poor hosts, as *C. schaffneri* could not complete its development on them. Interestingly, a single local host genotype (BR9) from Santa Catarina, Brazil, which was sourced from the same state as the biological control agent population, could not support complete development of *C. schaffneri*. Thus, *C. schaffneri* successfully utilised both local and nonlocal hosts. For example, it utilised the genotypes of *P. aculeata* from Santa Catarina in Brazil (BR6: a local host and old association), Caracas in Venezuela (VZ2: nonlocal host plant and new association) and Rio de Janeiro in Brazil (BR7 and BR8: nonlocal host plants and new association). This shows the agent's ability to exploit hosts from both old associations (with local hosts) and new associations (with nonlocal hosts). The overall survival rates of *C. schaffneri* on the invasive and native genotypes in this study are similar to the 74% survival that was observed by Paterson *et al.* (2014b). Meanwhile, there was a much higher rate (80 %) on SA1 from Knysna, South Africa, which was 1.6 folds higher than the lowest rate on VZ2 genotype, from Caracas, Venezuela.

The developmental time of *C. schaffneri* was longer on the nonlocal host (VZ2) than on the other invasive and native-range genotypes, which were both local and nonlocal hosts relative to the agent's origin (Paterson *et al.* 2014a). The nonlocal host genotypes from Argentina, the Dominican Republic, and a local genotype (BR9) from Santa Catarina were poor hosts for the agent as no nymph completed its development on them. Although the core reason for these outcomes remains unclear, perhaps the enzymes required to nullify the harmful host chemistries are absent in the agent's population given that they are from different localities (Manrique *et al.* 2008). The fitness cost of utilising nonlocal hosts ranges from incomplete development to delayed development, high mortality and low body mass amongst other fitness parameters like reduced fecundity and fertility (e.g., Karban 1989; Hanks & Denno 1994; Goolsby *et al*. 2006). Such fitness costs become even more apparent when nonlocal hosts have little or no genetic similarity to the host at the origin due to restricted gene flow between local (sympatric) host and nonlocal (allopatric) populations (e.g., Karban 1989; Hanks & Denno 1994; Goolsby *et al*. 2006). The key conclusion from the results is that the agent is able to develop on both nonlocal and local host genotypes as well as those with a confirmed history of co-existence. The promise of *C. schaffneri* for biological control remains high given its success rate (with successful development on 70% of the tested genotypes). *Catorhintha schaffneri* was able to develop equally on at least six host genotypes –two of which were invasive in South Africa and four from the southern native range in Brazil. This outcome is understandable given that the Brazilian populations were the source of invasive South African populations (Paterson *et al.* 2009).

The inability of *C. schaffneri* to develop on BR9 from Penha in Santa Catarina Province may be explained by its genetic closeness to the Argentina genotypes (Paterson *et al.* 2009), which were also unsuitable for *C. schaffneri*. The geographic closeness of two local host populations (BR6 and BR9), with one suitable and the other unsuitable, shows that provincial proximity did not necessarily translate to equal fitness of the agent. This may be because coevolved host-herbivore interactions can differ spatially and unpredictably in virulence (severity), even over short distances (Thompson 2005; Goolsby *et al*. 2006; King *et al.* 2009; Hanifin *et al.* 2008; Toju 2009). Although the dispersal potentials of *C. schaffneri* remains unclear, dispersal is a key component that facilitates genetic mixing in a spatially structured host environment like that of *P. aculeata* in Central and South America (Bonte & Marshall 2017; Bonte & Dahirel 2017). The source of BR9, Penha is about 40 km away from Porto Belo, where the biological control

agent population was collected. As of yet, there is no account of the dispersal potential of *C. schaffneri*; so whether this is a distance over which gene flow would be restricted or not is unknown. Meanwhile, the host plant mainly reproduces asexually within a given area and clones are expected to occur at each site. Hence, maladaptation may explain the agent's inability to develop on BR9 as minor genetic alteration even in a single nucleotide can confer resistance of a species against another, as seen in diamondback moth *Plutella xylostella* (Linnaeus) against four *Bacillus thuringiensis* toxins (Tabashnik *et al.* 1997). The variable performance of *C. schaffneri* on different host genotypes may have been dictated by a combination of factors such as sclerophylly, waxiness and secondary metabolites like alkaloids and flavonoids (Feng *et al.* 2009; Heshula & Hill 2011; Caceres, 2011; Soto *et al.* 2014).

Alternatively, the inability of *C. schaffneri* to use BR9 suggest that the twenty-three survivors off transit to South Africa (Paterson *et al.* 2014a), may have been those from Porto Belo only, and not from Penha, because it was reported that both localities contributed to biological control population in South Africa. In fact, only a few individuals of *C. schaffneri* were sourced from Penha (the origin of BR9) (I.D. Paterson, personal communication). Thirty individuals were collected from Port Belo whereas about four were collected from Penha (I.D. Paterson, personal communication). Considering that 23 survivors got to South Africa, at least 11 individuals died in transit and these may have included all four insects collected from Penha or the individuals from Penha may not have reproduced. Hence, the genetic contribution of the Penha population of *C. schaffneri* could have been lost in transit or due to inability to reproduce or other bottleneck effects (Hufbauer & Roderick 2005; Estoup *et al*. 2016). For the records, as a non expected result, it should be noted that there was no possibility that the material BR9 was confused with another during the experimental design. In addition, the inability of *C. schaffneri* to develop on AR3 and DR3 began to manifest as nymphs spent slightly longer developmental periods and had higher mortality progressively. The outcomes were unlike the patterns observed on VZ2 and BR7 for which developmental time was similar to all other genotypes.

Not only was the equal performance on both native and the invasive genotypes inconsistent with the assumption that the biocontrol agent would perform better on a local host than the nonlocal host (local adaption), it also does not support the notion that an invasive host would be more susceptible to biological control agents than the native hosts of *P. aculeata* (Blossey & Notzold

1995; Keane & Crawley 2002; Hanks & Denno 1994; Goolsby *et al*. 2006). Therefore, there was neither evidence for local adaptation nor was there evidence in favour of 'evolution of increased competitive ability' (Blossey & Notzold 1995; Keane & Crawley 2002).

The body weights of newly emerged adults was low on the nonlocal native hosts (areas far from the native distribution of *C. schaffneri*) and high on the invasive host plants, but there were no statistically significant differences due to the high variability within groups. Insect's body mass is an essential fitness component that sometimes correlates with reproductive output (e.g., heavier females of *Gratiana spadicea* Klug (Coleoptera:Chrysomelidae) were found to be more fecund than the lighter females: Czypionka & Hill 2007). If that holds true for *C. schaffneri*, the agents that emerged from the invasive genotypes may be better contributors to subsequent generation of the agents than the lightweight females. In addition, agents with greater body (mass) may have higher energy reserves to fuel dispersal.

For a polytypic and geographically varied target weed like *P. aculeata* (Leuenberger 1986; Paterson *et al.* 2009), host genotype–agent compatibility is crucial for successful biological control. Although it has now been established in this study that host variation is unlikely to undermine agent's success in the introduced range, laboratory outcomes may not necessarily translate to in-field success. For example, Lym and Carlson (2002) examined different root-feeding *Aphthona* species (Chrysomelidae) on seven genotypes of leafy spurge *Euphorbia esula* L. (Euphorbiaceae) and found contrasting preference outcomes in short-term free choice laboratory trials compared with field based studies. No evidence of inter-genotype preference was found in laboratory based studies, while some evidence that the native genotype was preferred by the biological control agent was found in field based studies (Lym & Carlson 2002). However, such a situation is highly unlikely for *C. schaffneri* in that it was able to utilise a wide range of its native hosts including local and nonlocal hosts.

While host genotype–agent incompatibility may be inconsequential for some agents it may be detrimental to others. For instance, the same genotypes of *Schinus terebinthifolius* on which *Episimus utilis* was unaffected, sibling species of *Pseudophilothrips ichini* and *Pseudophilothrips* sp near *ichini* displayed different responses to host genotypes (Manrique *et al.* 2008). In Paterson *et al.* (2012), *P. guerini* fed on six genotypes of *P. aculeata* from the Dominican Republic, Brazil and South Africa without fitness cost, but in this current study, fitness metrics were uneven for *C. schaffneri* on all genotypes tested. Not only was, host plants from more countries evaluated here than in the

previous study on *P. guerini* (Paterson *et al.* 2012), *C. schaffneri* was also more sensitive to host variation than *P. guerini* as some of the genotypes that negatively affected *C. schaffneri* were similar to those that had no effects on *P. guerini*. Thus, this study revealed the importance of assessing agent performance across different populations of host plants whether or not there is evidences of local adaptation from studies of other agents.

One key discrepancy of this study, relative to previous work by Paterson *et al.* (2014b) and Muskett (2017), is that five nymphal stages were recorded here rather than four in those studies. The difference begs the question whether stress (e.g., biotic or abiotic stress) may have been responsible. Although the average developmental period on a single population form Port Alfred in Paterson *et al.* (2014b) was 24 days at 23-25 ℃, the time spent for agents that emerged from six host genotypes were relative similar. However, the numbers of instar was different as the former recorded four and this current study observed five. The average development times in this study ranged from 24 days on six good hosts (BR2, BR6, BR7, BR8, SA1 and SA4) to 29 days, at 25 ℃, on a moderate host (VZ2). Thermal and water stress coupled with other factors like nutrients can modify insect development (Zhao & Jones 2012). Insects in this study were reared under more natural, but variable, conditions. Daily variation in thermal conditions in the laboratory varied between a maximum of about 30 ℃ at mid-day and 20 ℃ at night. Whether this dynamic could have influenced instar number requires further study, but it should be noted that coreids generally undergo five nymphal stages rather than four (Rodrigues & Moreira 2005; Egonyu *et al.* 2013; Doh *et al.* 2016).

An agent's ability to use its host (using a suitability index) and to inflict damage (using a susceptibility index) had been used separately in previous studies (Hill & Hulley 1995; Egbon *et al.* 2012; Paterson *et al.* 2012). In Hill and Hulley (1995), Maw's HSI of *Solanum sisymbriifolium* Lamarck (Solanales: Solanaceae), *S. acanthoideum* Drège ex Dunal and *S. melongena* Linnaeus (eggplant) for a candidate agent *Gratiana spadicea*, were 60.86, 22.81 and 12.42 respectively. The high score for *S. sisymbriifolium* indicates a higher suitability. Similarly, Egbon *et al.* (2012) used susceptibility index for a stored-product pest on varieties of cowpea and sorghum and had DSI scores of 20.7 and 19.7 respectively, which were higher than maize (16.6), showing that cowpea and sorghum were more susceptible to *Sitophilus oryzae* than maize. In this current

study, two invasive genotypes (SA1 and SA4) and two native genotypes, BR6 and BR8, which are both local and nonlocal host respectively, ranked high for both HSI and DSI indices and as such were equally suitable and susceptible to *C. schaffneri*. However, a nonlocal host from Rio de Janeiro, BR7 genotype, ranked lower than SA1, SA4, BR6 and BR8, while a distant nonlocal host genotype (VZ2) had the lowest score. Such low scores indicate poor host suitability, even if *C. schaffneri* did succeed in developing on them.

Finally, the *C. schaffneri* released on *P. aculeata* in South Africa is sensitive to host variation but develops on the invasive genotypes equally well as it would on some native genotypes. The general outcome suggests that the agent's success is genotype-specific and was neither explained by the geographic location (origin) of the plant genotypes in relation to the origin of the agent; the genetic relationship among the genotypes; nor the 'new' or 'old association' approach. If all environmental and biological factors (such as natural enemies) remain favourable to *C. schaffneri*, its potential as an effective biocontrol agent on the invasive host genotypes is high. More generally, host-agent compatibility assessments remain crucial in biological control programmes and one should not assume that suitability or susceptibility would always be related to geographic origin or the relationship between invasive populations and those in the native distribution.

CHAPTER 4 Preference of *Catorhintha schaffneri* to *Pereskia aculeata* genotypes

4.1 INTRODUCTION

The diet breaths of herbivores are difficult to define because preference and performance may or may not correlate across different populations of the same herbivore species and their host plants (Rausher 1979; Valladares & Lawton 1991; Bernays & Chapman 1994; Gripenberg *et al.* 2010; Benda *et al.* 2011; Marchioro & Foerster, 2014). For example, in three neighbouring states in the United States of America (Utah, Arizona and New Mexico), the Colorado potato beetle *Leptinotarsa decemlineata* Say (Coleoptera: Chrysomelidae), which is oligophagous at the plant species level, fed exclusively *in situ* on potato in Utah, but fed on *Solanum elaeagnifolium* Cavanilles (Solanale: Solanaceae) in Arizona, and *S. rostratum* Dunal in New Mexico (Bernays & Chapman, 1994).

An insect capacity to choose host plants in the wild can sometimes be inconsistent. For example, the oviposition behaviours of some adults show their inability to choose a superior host for their offspring amid suitable but inferior hosts (Hufnagel *et al.* 2017; Jaenike 1978; Gripenberg *et al.* 2010). Such poor choices may be inevitable and could leave monophagous insects, including those typically used in biological control, with contrastingly unpredictable fates. They could either orient to a different host and live (antixenosis: aversion) or stay and incur reduced fitness and high mortality due to antagonistic effects from host plants (antibiosis) (Dethier 1954; Smith 1989; Jayaraj & Uthamasamy 1991; Cardona *et al.* 2004; Hufnagel *et al.* 2017). Some authors have attributed these sub-optimal choices to a risk-spreading (or bet-hedging) strategy, where unpredictable conditions (inclement weather, the presence of predators, etc.) drive their choices (Olofsson *et al.* 2009; Hufnagel *et al.* 2017). In such situations, short-term compromises in fitness are employed to maximise long-term fitness when favourable conditions are subsequently guaranteed (Olofsson *et al.* 2009; Hufnagel *et al.* 2017).

An agent's ability to select preferred hosts (for nutrition or reproduction) can improve performance and ensure quick attainment of the large population that is needed in biological control of invasive alien plants. Several cases where superior hosts are chosen by adult females exist (Rausher 1979; Craig *et al.* 1989; Minkenberg & Fredrix 1989), but in many studies insect herbivores select inferior hosts resulting in reduced performance (Rausher 1979; Karban &

Courtney 1987; Futuyma & Moreno 1988; Auerbach & Simberloff 1989; Valladares & Lawton 1991; Benda *et al.* 2011; Marchioro & Foerster 2014). Such inability to choose precisely can undermine an agent establishment, especially among specialist herbivores that feed on fewer hosts than generalists do. For example, a field study by Valladares and Lawton (1991) showed that the females of a specialist leaf miner *Phytomyza ilicis* Curtis (Diptera: Agromyzidae) which feeds on *Ilex aquifolium* Linnaeus (Aquifoliales: Aquifoliaceae) preferred host plants on which larval mortality was subsequently high to other hosts on which mortality was low. In another study, the eggs of a specialist moth *Heliothis subflexa* Guenée (Lepidoptera: Noctuidae) were laid two times out of ten on plants that were not suitable for development (Benda *et al.* 2011). Nonetheless, when the right host *Physalis* sp. (Solanaceae) was chosen, oviposition occurred on leaves instead of the preferred feeding site (fruits), leaving noticeable effects on the offspring survival, as the hatchlings were more predisposed to predation on the bad host than on a good host (Benda *et al.* 2011).

In weed biological control, specialist herbivores are often reared from a few individuals of their native hosts, which may be different from the invasive weed populations which in turn may have variable forms. Subjecting an agent to new genotypes may or may not alter the behaviour of the natural enemy. Previous studies have shown that the variable genotypes of *P. aculeata* affect its biological control agent, *C. schaffneri* differently (Chapter 2 & 3). What remains unclear is how intraspecific variation within *P. aculeata* would affect the behaviour (choices or preference) of *C. schaffneri*. Many insects demonstrate preference for different hosts even within the same host-plant species (Bernays & Chapman 1994). If an agent prefers a host genotype, poor host genotypes would be rejected, but rejecting the inferior host in search of a better host may come at a price to both hatchlings and adults if the superior host is not easily found. If an adult oviposits on poor host, the hatchlings may orient away from the poor host; thereby increasing their chances of mortality (Dethier 1954; Valladares & Lawton 1991; Benda *et al.* 2011; Ågren *et al.* 2012). Alternatively, accepting a poor host would result in demographic consequences, like longer developmental times, higher mortality, and reduced fecundity (Rausher & Papaj 1983; Gripenberg *et al.* 2010), which in turn can lead to a long-term population decline and a failure of biological control.

Some genotypes of *P. aculeata* are good hosts to *C. schaffneri* (Chapter 2 & 3). The behavioural response (preference) of *C. schaffneri*, to different host plant variation, is crucial to understand how the insect would select and accept its host in the introduced range. Differences in cues or sign stimuli from the different plant genotypes could cause the agent to respond differently. Thus, the aim of this study was to evaluate how *C. schaffneri* chooses its host amid conspecific host variation within *P. aculeata*.

4.2 MATERIALS AND METHODS

4.2.1 Study systems

In this study, agent preferences in paired and multiple-choice trials were examined using nymphs and adults. Newly hatched, unfed nymphs of *C. schaffneri* from the same generation were used for the paired-choice trials while both sexes of adults were used for the multiple–choice trials. Both the paired and multiple-choices experiments were conducted under ambient conditions within the biological control research facility at Waainek, Rhodes University, Grahamstown. While the paired choices were conducted under similar laboratory conditions, the multiple choices were conducted in a walk-in cage within a greenhouse facility, where directional air flow was restricted.

4.2.2 Paired–choice trials

In the paired-choice tests, a succulent (healthy and growing) shoot of two different host plants (genotypes) were paired by crossing them (in an X-shape pattern). The point of contact of the two shoots was at ten centimetres below their respective shoot tips. The shoots of both test plants were held together using cotton thread and a plastic clip. The plastic clips thereafter served as the neutral arena on which the newly hatched nymphs were introduced onto the paired plants. In each case of paired choice, the paired plants were replicated eight times. The paired plants were mainly between those from Santa Catarina (the province where the insects originated: BR6 & BR9) against those from the native and invasive ranges. BR6 and BR9 were chosen because they are both known to be the native local hosts and also because the former was highly suitable and susceptible (good host) while the latter was neither suitable nor susceptible (thus signifying a poor host) (Chapter 2 & 3). For each pair of plants, six newly hatched and unfed nymphs were introduced onto the plastic clip. The nymphs were allowed to choose their host undisturbed. The

number of nymphs on each shoot was counted at specific time intervals of 1, 2, 3, 4, 6, and 24 hours after the start of the experiments and then daily until the 10th day when the trials were discontinued. The paired shoots were sprayed three times daily with a fine mist of water. Further details of the paired plants are shown in Table 4.1 (also see, Chapter 2: Table 2.1). This trial was conducted in 40 cm x 40 cm x 60 cm cages under room conditions at an average of 25 °C with a light regime of 14:10 L:D photoperiod.

Table 4.1 Seventeen paired choices for the assessment of preference among newly hatched nymphs of *Catorhintha schaffneri* for the first ten days post-hatching. The codes of the plants are similar to those in the previous chapter (2 and 3) and the first two letters represent the countries from which they were sourced.

Number	Genotypes paired with		Other
	BR6	BR9	pairings
1	SA4	SA4	BR7 x VZ2
2	VZ1	VZ1	SA1 x SA3
3	BR8	BR8	BR8 x SA10
4	BR9	BR2	
5	SA10	VZ2	
6	AR11	AR3	
7	DR2	BR7	

4.2.3 Multiple–choice trials

The multiple-choice trials were conducted in a 'walk–in' cage of dimensions 3 x 3 x 1.2 m (Fig. 4.1). The roof was lifted higher at the centre than the edges at a height of 1.7 m to form an inverted 'V' shape. The cage was made out of a netting material (Organza™) and setup in a greenhouse facility. A total of eight plants were used in the multiple choice arena and these were six genotypes of *P. aculeata*, one closest relative of *P. aculeata*, *P. grandifolia*, and one non–cactus plant that is native to South Africa, *Crassula ovata* (Miller) Druce (Crassulaceae). *Pereskia grandifolia* and *C. ovata* were used as out–groups to ascertain the herbivore's choice amidst non–host plants. The selected genotypes of *P. aculeata* were BR6 from Santa Catarina; BR7 from Rio de Janeiro; SA1 from Knysna; DR3 from Pedernales; VZ2 from Caracas, and AR3 from Argentina. The groups of eight plants were arranged on separate elevated platforms made of inverted plastic pots (Fig. 4.1), to maintain similar levels of the plant shoots because plants had different heights. The potted plants were placed on the elevated platforms to prevent the agents from climbing directly from the ground, so insects had to fly to select a host plant. Because they flew to the plants (while relying on visual and/or possibly chemical cues), any

positional bias towards a given plant was minimised with this design. Additionally, all plants were randomly positioned for each replication such that no plant maintained the same position for each trial and only one trial was conducted at a time. For each trial, twenty agents (10♂, 10♀) were introduced from the breeding culture.

These insects were sourced randomly from the culture and none had prior exposure to any of the genotypes used in the trial, to eliminate any bias that may occur from learning. The inverted plastic pots were positioned along a circular pattern with a semi-circular perimeter of a radius of 35 cm and neighbouring pots were spaced equally. In a similar multiple preference trial, Shaw and Cock (2017) used non-simultaneous replications and maintained the plants randomly at similar heights. This method made data collection easier, as it was impractical to conduct all ten replicates at the same time. Agents were released into the multiple-choice arena (the walk-in cage) using a plastic bowl that was elevated at ten–centimetre above the ground, which was situated at the centre of the semicircle. Counts were recorded every five minutes for the first sixty minutes, then hourly at 2, 3, 4 hours, and daily until the third day (that is, 72 hours after commencement) when the trials were terminated.

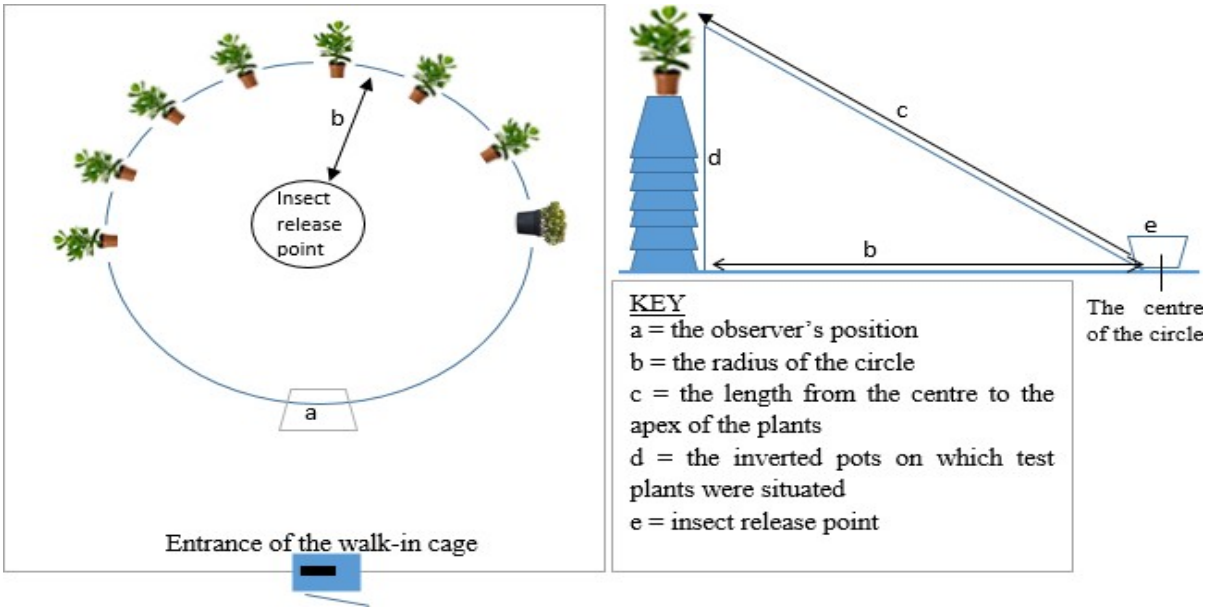

Fig. 4.1 Multiple–choice arena in which the preferences of *Catorhintha schaffneri* adults for different genotypes of *Pereskia aculeata* were examined. Six test plants were sourced from both the native and invasive ranges and two other species were used as outgroups. The entire set up was confined to a working space devoid of directional airflow and within a walk-in cage that was set up in a greenhouse (Caveat: the distance from 'a' varied from as high as 70 cm to as low as 20 cm for better insect counts).

4.2.4 Statistical analysis

Over the trial periods for both the paired and multiple-choice trials, the preference parameter was discrete count data, which were analysed without transformation (O'Hara & Kotze 2010). A generalised linear model (GLM: Poisson family with log link) was used to analyse each dataset at the separate time interval for the paired tests. For the entire ten-day period, a Zero-Inflated Poisson generalised linear mixed effect model, in the *glmmTMB* package (R 2017), was adopted after different diagnostics and comparisons with other models were made (GLMM: Poisson and negative binomial families with no adjustment for excess zeros). The *glmmTMB* was used because of the repeated sampling coupled with extra-zeros arising from the longitudinal dataset and accounted for in the repetitive structure (of time) as a random effect (Bolker *et al.* 2009; Brooks *et al.* 2017), while also taking the structural zero component and over-dispersion into account (Harrison 2014; Brooks *et al.* 2017). In multiple-choice tests, a generalised linear mixed model with random effects activated for zero–inflation with the family set as Poisson (ZIPGLMM) was run from the *glmmADMB* package in R. Differences were examined using the *posthoc* multiple comparison tests in *multcomp* package in a 'tukey' matrix adjusted with Bonferroni correction factors to minimise chances of type I errors. Cases where a statistically significant average number of agents were found at specific days and at the end of the trial were adjudged to have exhibited preference on that day and at the end of the trials, respectively.

4.3 RESULTS

4.3.1 Paired–choice trials

In general, the nymphs rarely change their position after settling on one of the two genotypes. From the seven paired choices in which other genotypes were paired with BR6 (from Santa Catarina), 57.1% of the *C. schaffneri* preferred the other genotypes. These preferred genotypes were an invasive genotype SA4 from South Africa and native genotypes BR9, VZ1 and DR2 from Brazil, Venezuela and the Dominican Republic, respectively. *Catorhintha schaffneri* preferred BR6 when paired with SA10 (an invasive genotype from Kosi Bay) and BR8 (a native genotype from Rio de Janeiro in Brazil), and no preference was shown between BR6 and AR11 (Table 4.2).

For paired choices with BR9, a genotype that is also from Santa Catarina, in 43% of the trials BR9 was preferred as opposed to BR7 (from Rio de Janeiro, Brazil), AR3 (from Misiones, Argentina) and VZ2 (Venezuela) (Table 4.2). Nonetheless, the BR9 genotype was less preferred than VZ1 (Venezuela) and SA4 (Port St. Johns, South Africa). Meanwhile, no preference was seen when BR was paired with BR8 (from Rio de Janeiro, Brazil) and BR2 (from Londrina in Paraná Province, Brazil) (Table 4.2).

For the other three paired choices: (i) no preference was established between BR8 (from Rio de Janeiro) and SA10 (from Kosi Bay), whereas (ii) BR7 (Rio de Janeiro) was preferred to VZ1 (Venezuela). Lastly, no preference was observed between two invasive genotypes SA1 from Knysna and SA3 from Port Alfred (South Africa) (Table 4.2).

Table 4.2 The preferences of *Catorhintha schaffneri* in paired–choice trials after ten days of exposure to two genotypes of *Pereskia aculeata*.

S/No.	Plant pairs (X x Y)	Preference for plant		Model estimates	z scores
		X	Y		
1	BR6 x SA10	+	–	–1.02	-7.27^{***}
2	BR6 x SA4	–	+	0.20	2.34^{*}
3	BR6 x DR2	–	+	0.36	4.03^{***}
4	BR6 x BR9	–	+	0.25	2.66^{**}
5	BR6 x BR8	+	–	–0.30	-3.12^{**}
6	BR6 x AR11	0	0	0.08	1.07^{ns}
7	BR6 x VZ1	–	+	0.20	2.30^{*}
8	SA1 x SA3	0	0	–0.01	-0.09^{ns}
9	BR9 x SA4	–	+	0.70	6.41^{***}
10	BR9 x BR7	+	–	0.32	3.76^{***}
11	BR9 x BR8	0	0	–0.08	-1.03^{ns}
12	BR9 x BR2	0	0	0.13	1.40^{ns}
13	BR9 x AR3	+	–	0.48	5.11^{***}
14	BR9 x VZ2	+	–	–0.27	-2.97^{**}
15	BR9 x VZ1	–	+	0.19	2.22^{**}
16	BR8 x SA10	0	0	–0.04	-0.42^{ns}
17	BR7 x VZ2	+	–	–0.18	-2.18^{*}

$^{***}p < 0.001$; $^{**}p < 0.01$; $^{*}p < 0.05$; $^{ns}p > 0.05$ (not significant). Positive symbols signify preference, whereas the negative symbols signify less preferred. The other alternative is zero, which signifies lack of preference. All plant codes are the same across chapters.

Although preference was shown for BR6 in the BR6-SA10 paired choice test (Table 4.2), some nymphs of *C. schaffneri* remained on SA10, but later declined to near zero from day 3 onward (Fig. 4.2a). For the BR6-SA4 paired choice, though more nymphs were on SA4, preference was not statistically different except at twenty-four hours (Fig. 4.2b). In the BR6-BR8 paired choice, high numbers of *C. schaffneri* were initially present on BR6 but declined gradually with a corresponding increase on BR8 that led to a significant preference for BR8 on day seven onwards (Fig. 4.2c). Nymphs significantly preferred BR9 to BR6, but later at day seven shifted to BR6 thereby losing the significant difference, which was measured earlier (Fig. 4.2d). For the BR6-AR11 pair, the observed patterns were characterised by continuous host shifting, which rose slightly in favour of BR6 as opposed to AR11 (Fig. 4.2e). In addition, for the BR6-VZ1 paired choice, a similar pattern was observed on both genotypes although from the fifth to the seventh day, the nymphs significantly ($p < 0.05$) shifted away from BR6 to VZ1 (Fig. 4.2f). For the BR6- DR2 paired choice, the nymphs significantly preferred DR2 to BR6 most of the time (Fig. 4.2g). Besides comparisons with BR6, a paired choice involving two invasive genotypes SA1-SA3 showed that none of the genotypes was significantly preferred except for the two episodic but inconsistent peaks at the '3 hour' and 'six-day' periods (Fig. 4.2h).

For BR9-SA4 paired choice, there was a steady increase of nymphs that preferred SA4 to BR9, and this culminated significantly in favour of SA4 (Fig. 4.2i). In BR9-BR7 paired choice, the nymphs of *C. schaffneri* significantly preferred BR9 to BR7 initially but declined gradually after the fifth day with no significant ($p > 0.05$) differences between both plants (Fig. 4.2j). In Fig. 4.2k for BR8-BR9 paired choice, occasionally had a significant bias against BR8, but in 73% of the 15 sampling times, no preference was established for BR8. In BR9-BR2 pair choices, 86% of the sampling times the BR9 was preferred to BR2 genotype (Fig. 4.2l). For BR9-AR3 paired choice, BR9 had a gradual acceptance from the onset until the third day when it was markedly preferred to AR3 (Fig. 4.2m). No significant differences were observed between BR9 and VZ2 over most of their exposure time to *C. schaffneri*, except on the eighth and ninth days in which VZ2 was significantly preferred to BR9 genotype (Fig. 4.2n). In BR9-VZ1 paired choice, a sustained increase in the number of nymphs was observed at the early stage on VZ1, which attained significant preference from the third day till onward, except for a subtle decline on the

fifth day (Fig. 4.2o). Among the other paired choices, there was no preference to any genotype throughout the observation period in BR7-VZ2 pair (Fig. 4.2p), whereas in the BR8-SA10, pairs significantly preference to BR8 was registered from the seventh day onward (Fig. 4.2q).

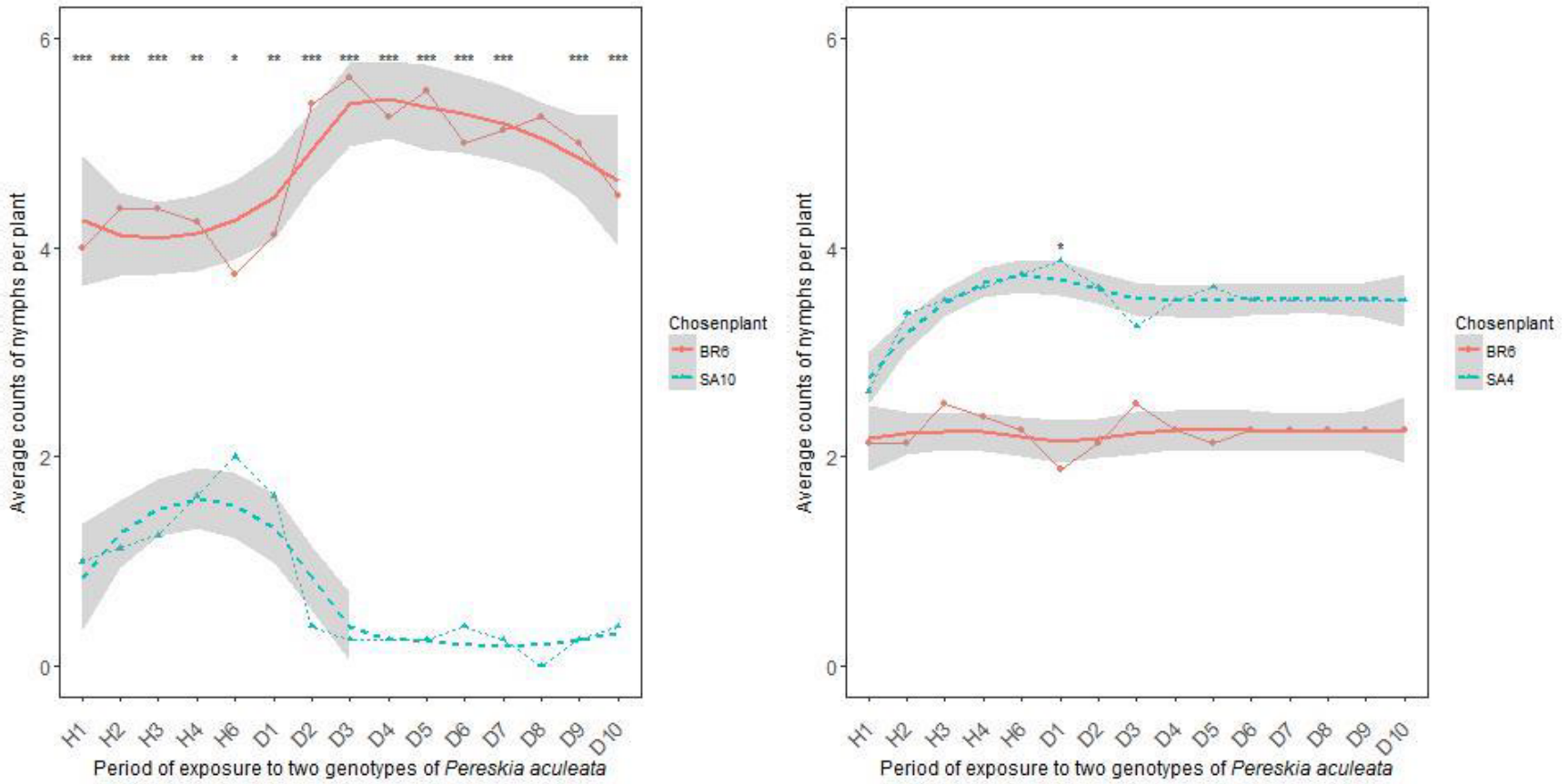

Fig. 4.2 Changes over time in the numbers of nymphs on paired genotypes of *Pereskia aculeata*: [on the left] (a) BR6 (red) x SA10 (blue) and [on the right]; (b) BR6 (red) x SA4 (blue). The smoothed lines were fitted with a local regression approach called loess. On the x-axes, the letter H stands for hour and the corresponding number stands for the specific hours from the time of commencement and the same applies to the letter D for day while the number stands for a specific day.

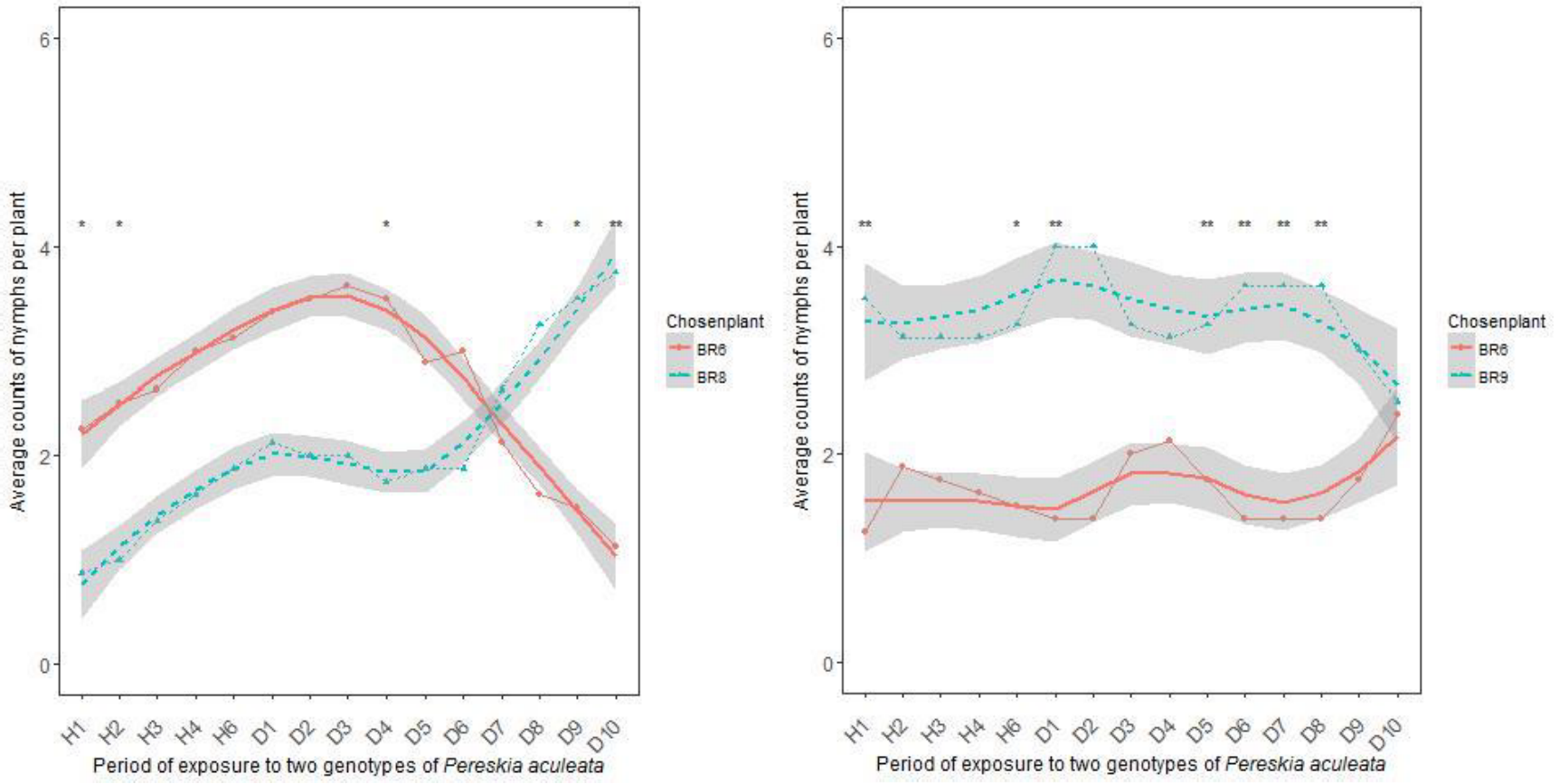

Fig. 4.2 (Continued… i) Changes over time in the numbers of nymphs on paired genotypes of *Pereskia aculeata*: [on the left] (c) BR6 (red) x BR8 (blue) and [on the right]; (d) BR6 (red) x BR9 (blue).

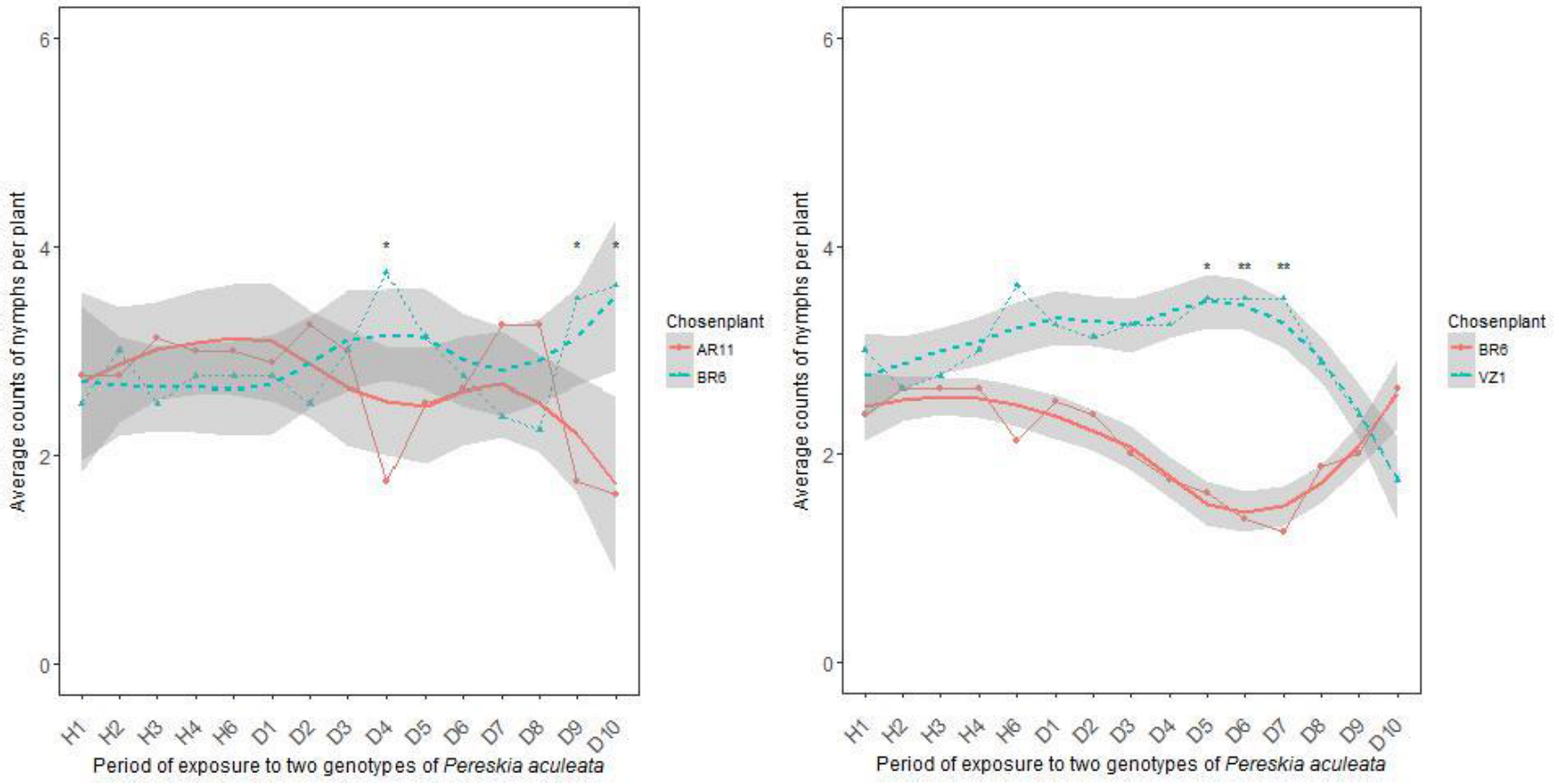

Fig. 4.2 (Continued… ii) Changes over time in the numbers of nymphs on paired genotypes of *Pereskia aculeata*: [on the left] (e) BR6 (blue) x AR11 (red) and [on the right]; (f) BR6 (red) x VZ1 (blue).

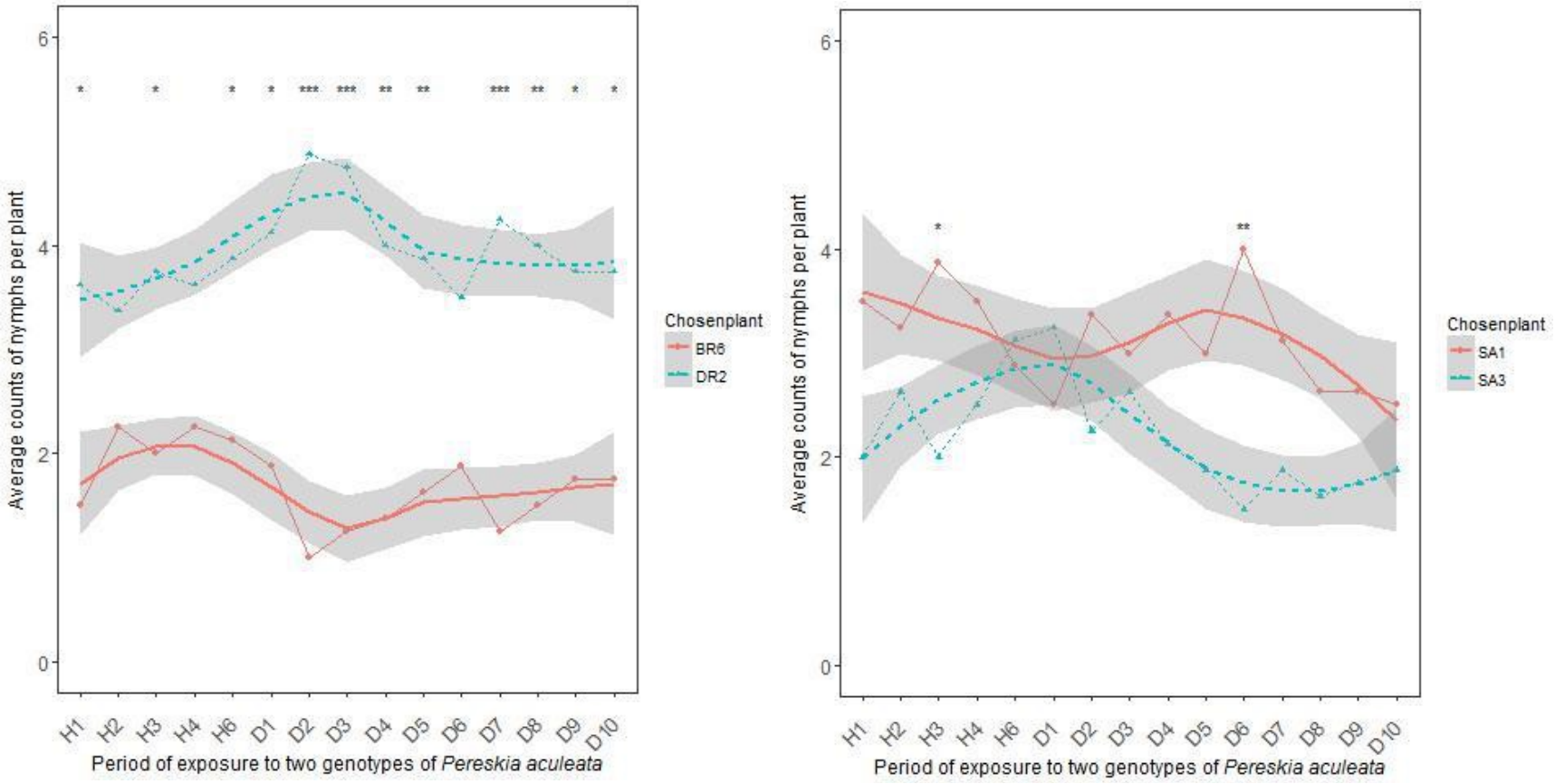

Fig. 4.2 (Continued… iii) Changes over time in the numbers of nymphs on paired genotypes of *Pereskia aculeata*: [on the left] (g) BR6 (red) x DR2 (blue) and [on the right]; (h) SA1 (red) x SA3 (blue).

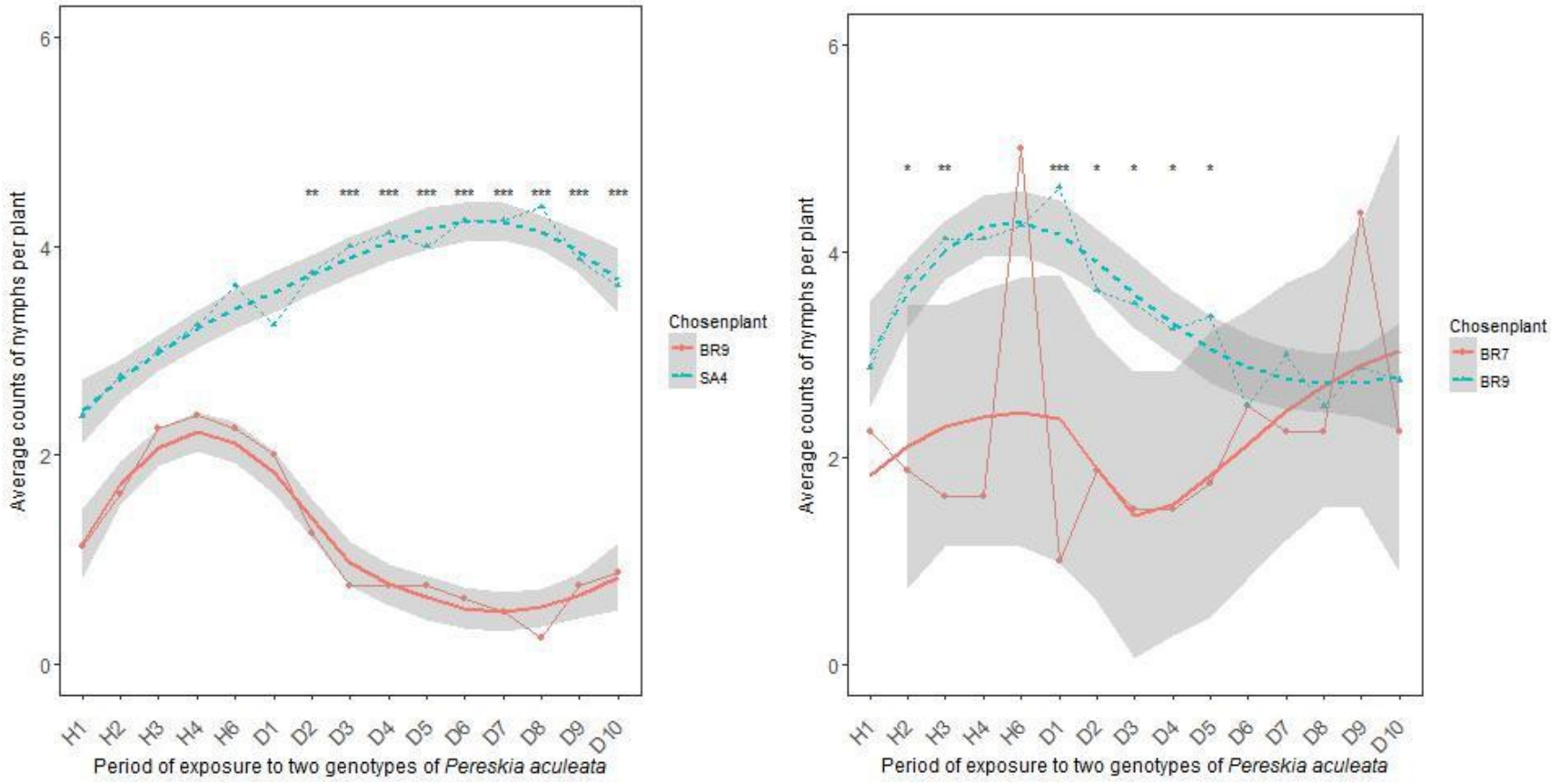

Fig. 4.2 (Continued… iv) Changes over time in the numbers of nymphs on paired genotypes of *Pereskia aculeata*: [on the left] (i) BR9 (red) x SA4 (blue) and [on the right]; (j) BR7 (red) x BR9 (blue).

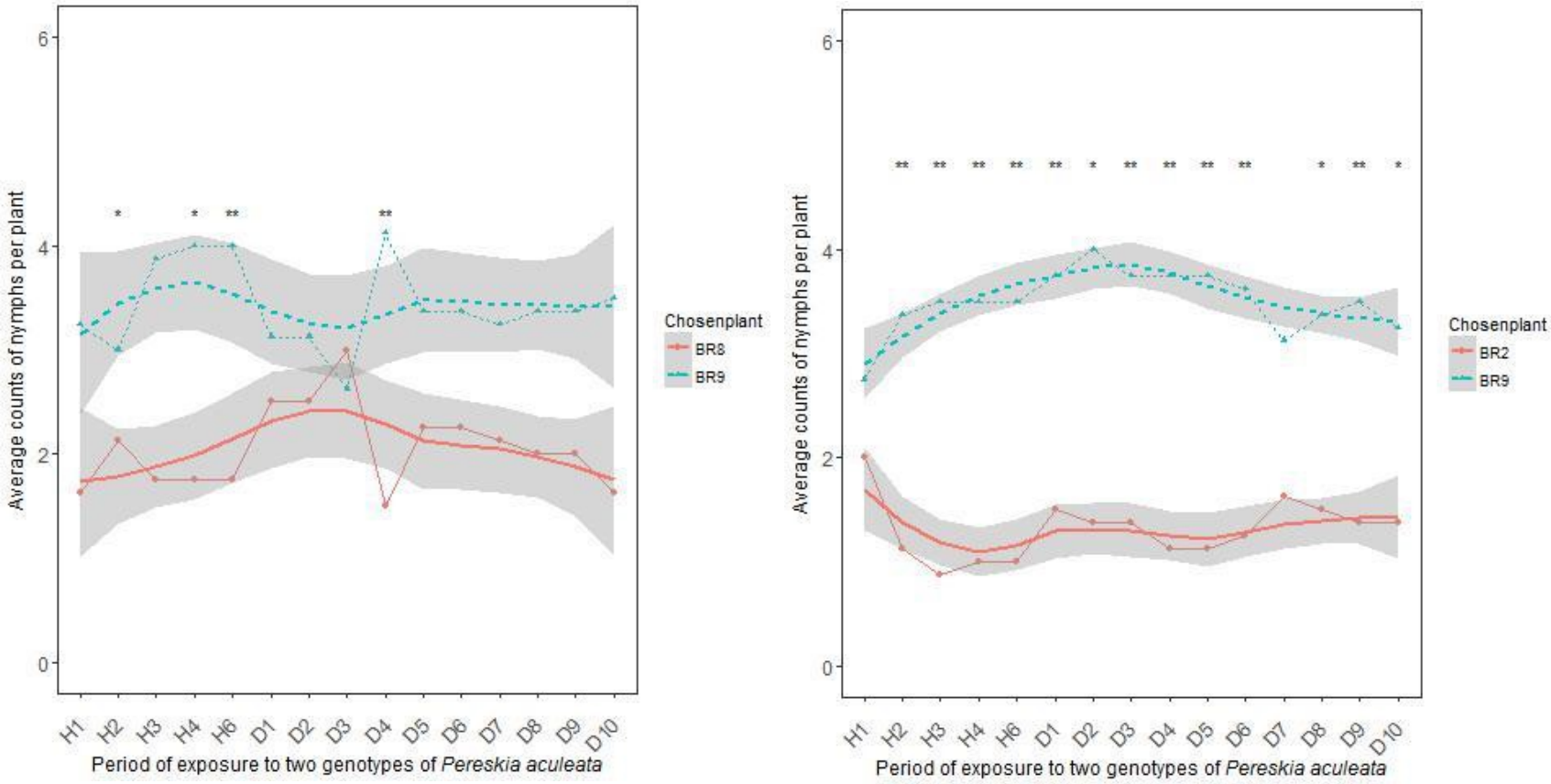

Fig. 4.2 (Continued… v) Changes over time in the numbers of nymphs on paired genotypes of *Pereskia aculeata*: [on the left] (k) BR8 (red) x BR9 (blue) and [on the right]; (l) BR2 (red) x BR9 (blue).

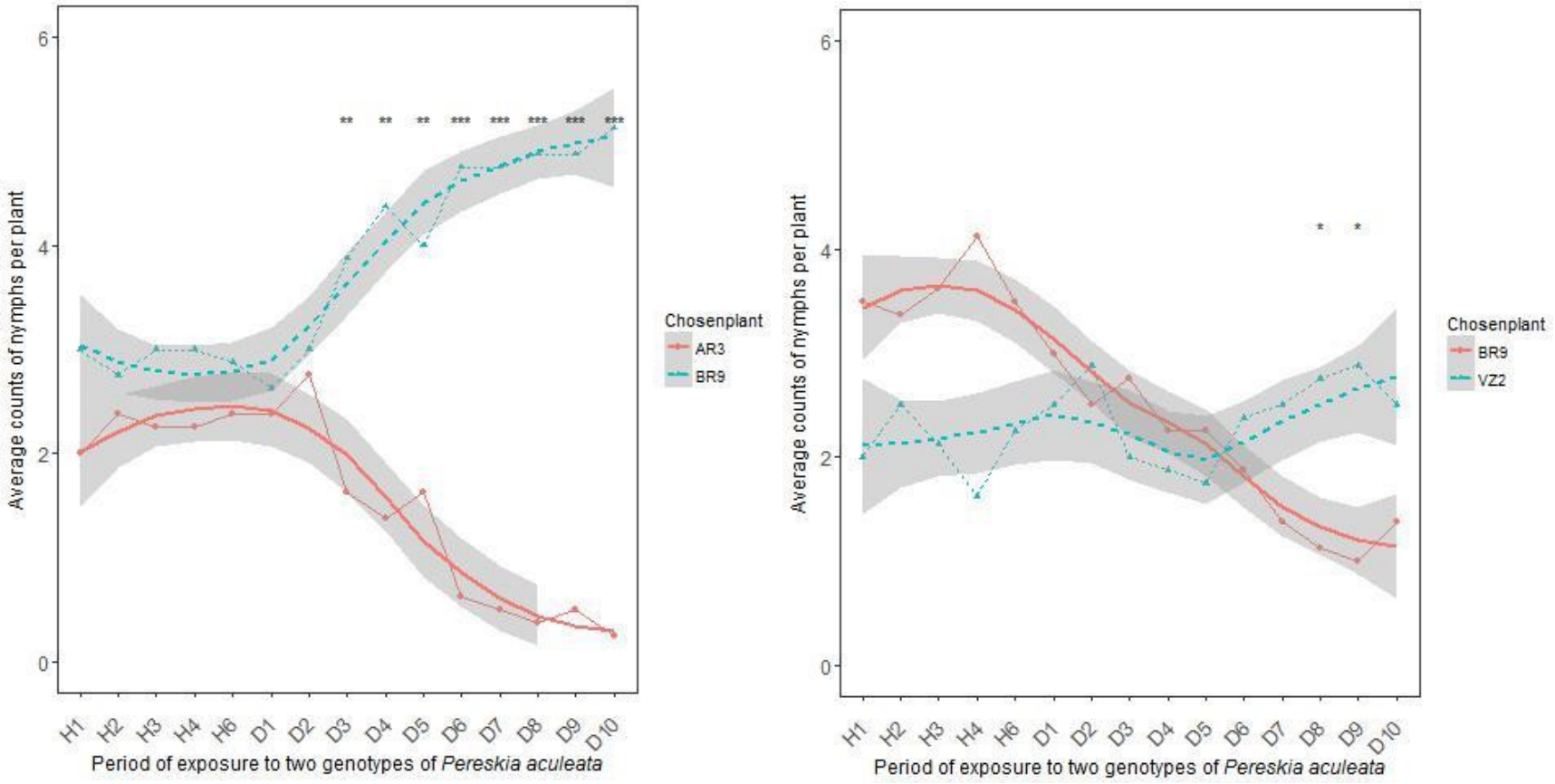

Fig. 4.2 (Continued… vi) Changes over time in the numbers of nymphs on paired genotypes of *Pereskia aculeata*: [on the left] (m) BR9 (blue) x AR3 (red) and [on the right]; (n) BR9 (red) x VZ2 (blue)

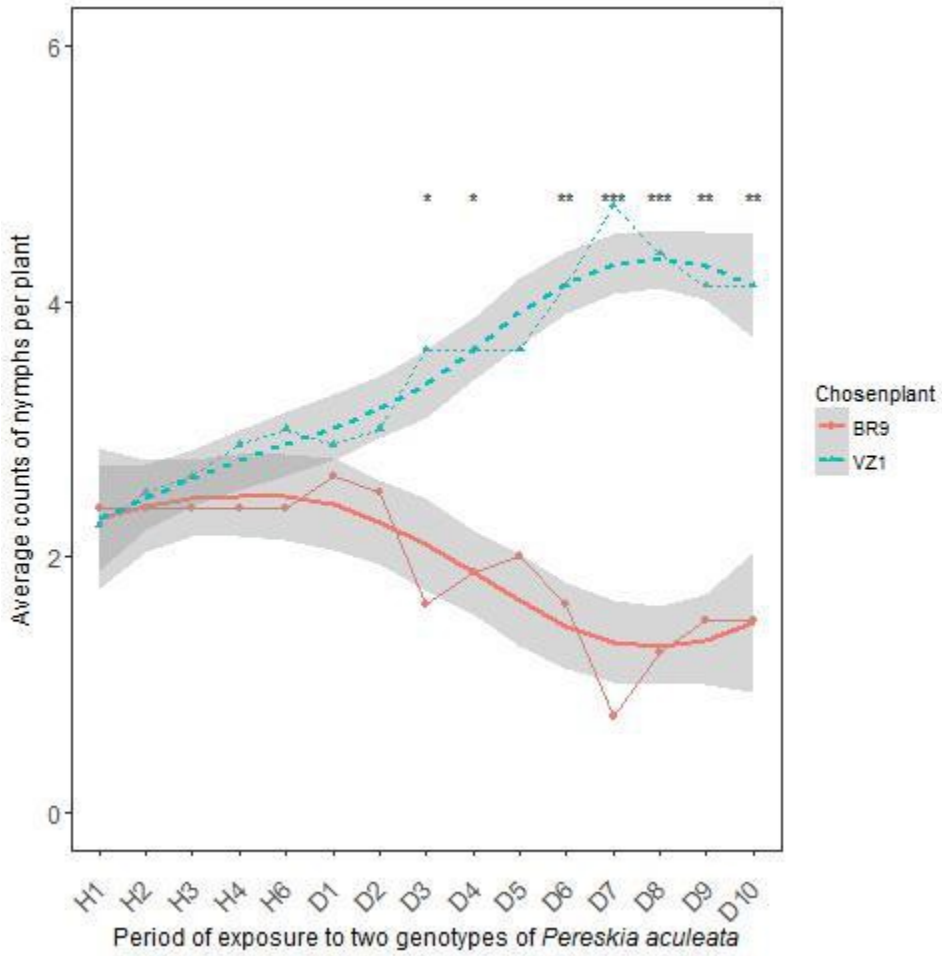

Fig. 4.2 (Continued… vii) Changes over time in the numbers of nymphs on paired genotypes of *Pereskia aculeata*: (o) BR9 (red) x VZ1 (blue).

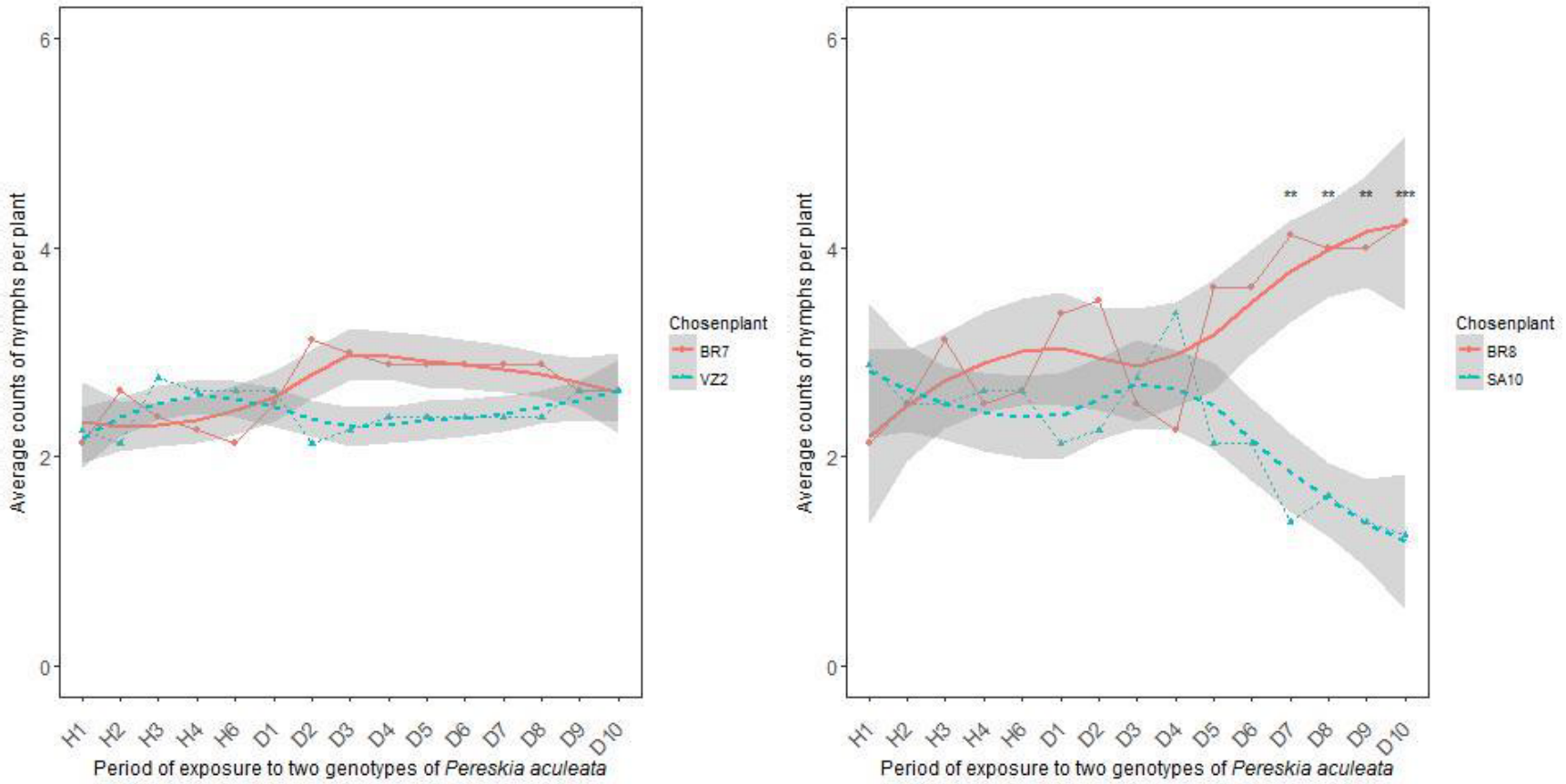

Fig. 4.2 (Continued… viii) Changes over time in the numbers of nymphs on paired genotypes of *Pereskia aculeata*: [on the left] (p) BR7 (red) x VZ2 (blue) and [on the right]; (q) BR8 (red) x SA10 (blue).

4.3.2 Multiple–choice trials

Adult *C. schaffneri* showed a wide range of host choices among the genotypes of *P. aculeata* and the outgroup plants (Fig. 4.3). There was a significant difference in preference (ZIPGLMM ANOVA type II: $\chi^2 = 513.11$, df = 7; $p < 0.001$). A *posthoc* multiple comparison test showed that statistically more insects chose the genotype BR6 from Santa Catarina (Brazil) than BR7 genotype from Rio de Janeiro, Brazil and DR3 genotype from Dominican Republic (Fig. 4.3). However, the number of adults that chose BR6 was not significantly higher than the number that chose SA1 genotype from Knysna, South Africa, AR3 genotype from Misiones, Argentina and VZ2 genotype from Venezuela.

Nonetheless, the numbers of agents that were on all the pereskia plants were significantly higher than the number of agents on *P. grandifolia* and *C. ovata* after 72 hours. The periodic changes in the choice pattern for each test plant (Fig. 4.4) showed that *C. schaffneri* sustained a progressive preference to BR6 and SA1 genotypes until the end of the trial. Although the BR7 and VZ2 genotypes attracted fewer numbers of adult relative to BR6 and SA1, they also showed a similar pattern of acceptance with the BR6 and SA1 genotypes. In addition, a different host acceptance pattern that culminated in host rejection at 3 h and 48 h after exposure to AR3 and DR3 genotypes was seen respectively. *Pereskia grandifolia* and *C. ovata* were less attractive to *C. schaffneri* than the genotypes of *P. aculeata* put together (Fig. 4.4). However, not all the (twenty) agents that were released into the walk-in cage flew to the various plants at the end of the trial. The initial number that flew onto the plants that was recorded in first five minutes at 4%, whereas at the end of the trial (that is, 72 hours later) only 58% were on the plants; further details of the trend are shown in Fig. 4.5.

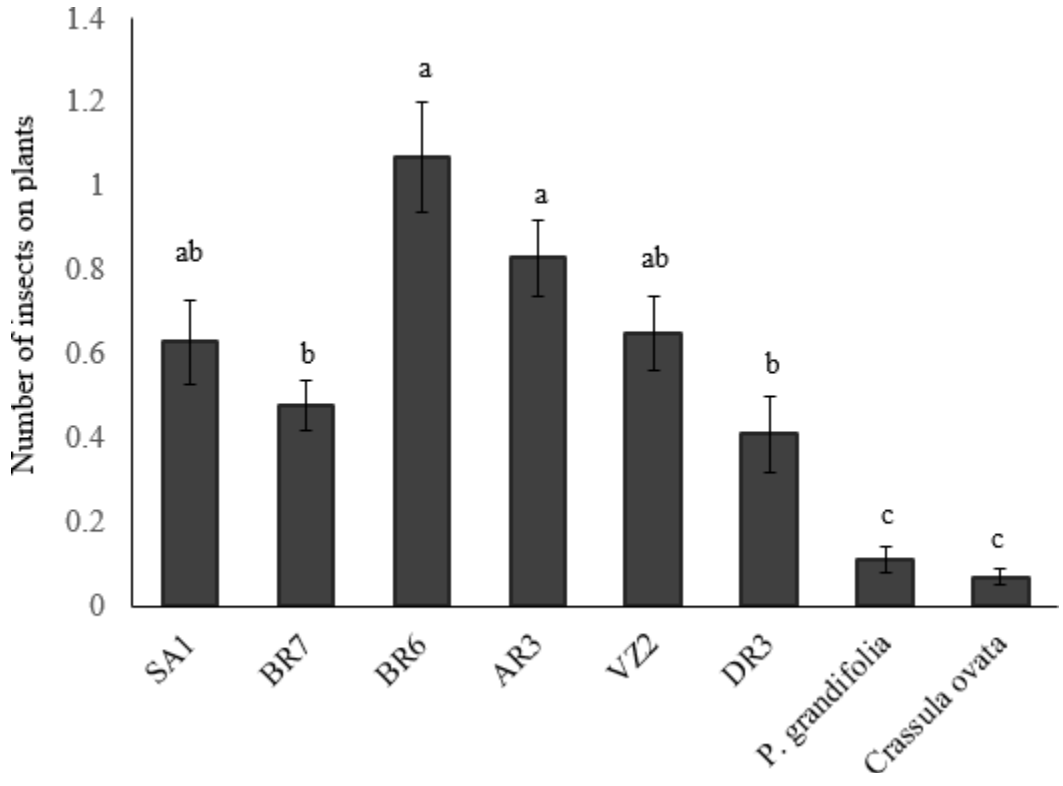

Fig. 4.3 The preference of *Catorhintha schaffneri* in multiple-choice arena after a seventy–two hours of exposure to both host and non-host plants. Bars represent standard errors (S.E.). Genotypes were ordered from the invasive genotype (SA1) to the native genotypes of *Pereskia aculeata* (BR7–DR3), and then the out-group plants. Columns with the same associated letter were not significantly different from each other.

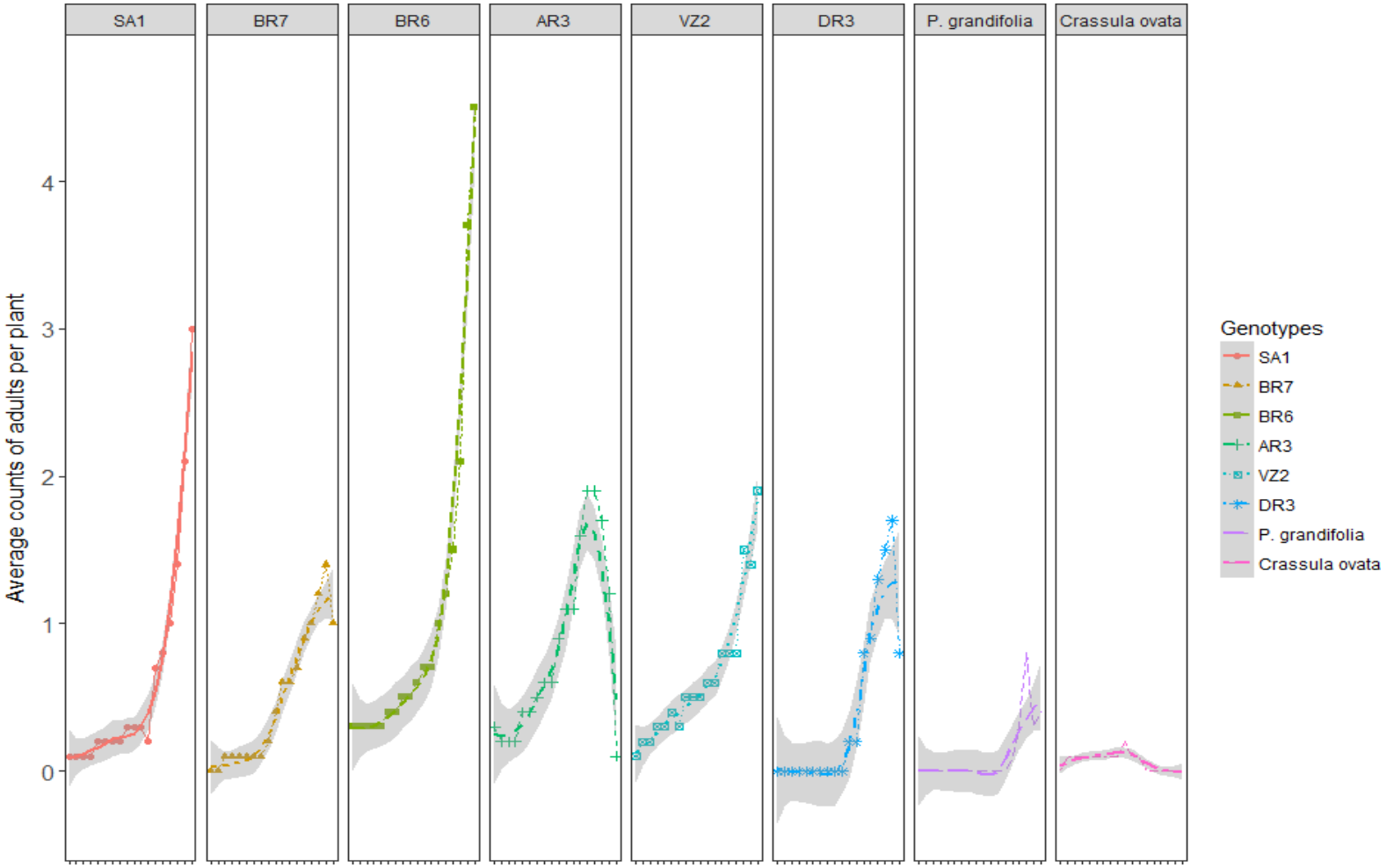

Fig. 4.4 Genotype specific plot of multiple-choice patterns of twenty adult *Catorhintha schaffneri* (10♂ and 10♀) over eighteen observation periods. The six plant genotypes for *P. aculeata* and two names for out groups are shown above each faceted plot. Plants were arranged from the invasive genotype to the native genotypes from Brazil, followed by Argentina, Venezuela and the Dominican Republic genotypes before the outgroup plants. The x-axes are not shown due to excessive clumping of time values but every data point on the graph correspond to the designated time intervals for every 5 minutes for the first 60 minutes, then in 2 h, 3 h, 4 h, 24 h, 48 h and 72 h; where m = minute and h = hour).

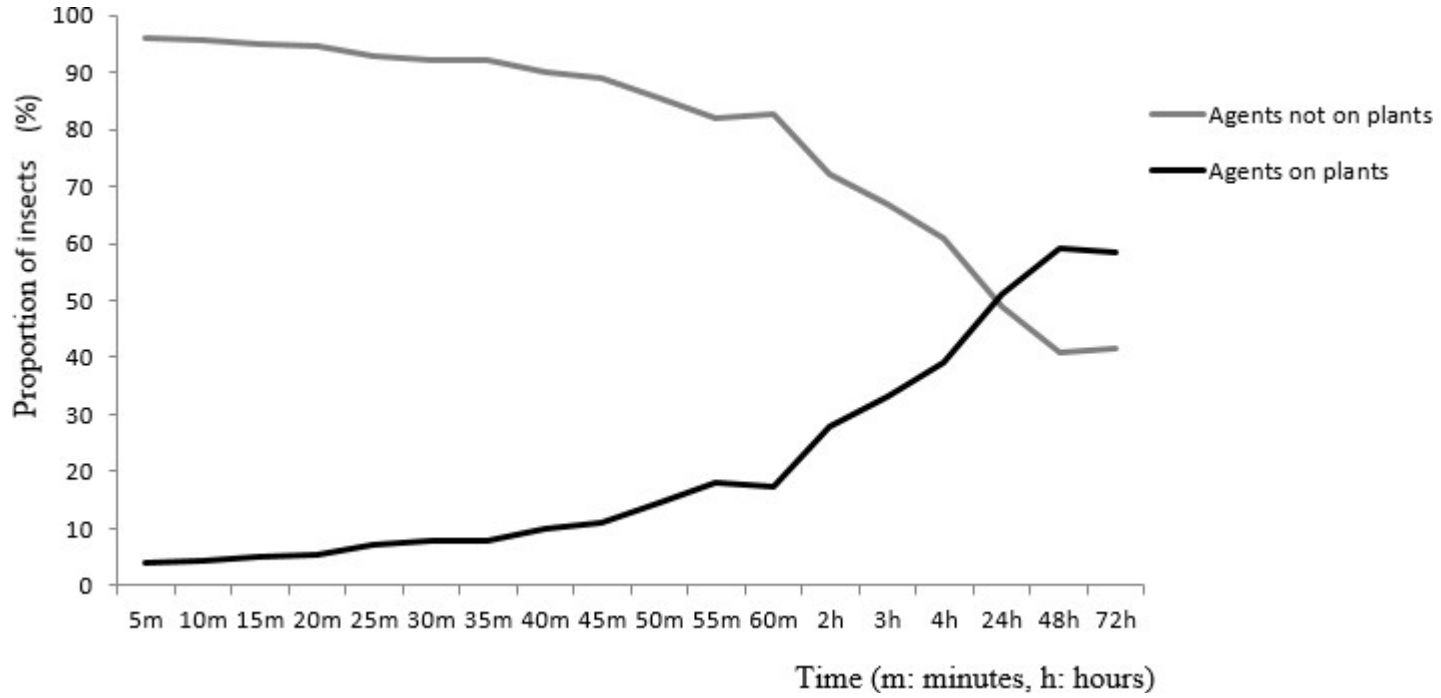

Fig. 4.5 The proportion of *Catorhintha schaffneri* (♂+♀) found on any of the test plant species over time. Test plants were a combination of selected genotypes of *Pereskia aculeata*, and the outgroup plants *Pereskia grandifolia* and *Crassula ovata*.

4.4 DISCUSSION

This study examined whether *C. schaffneri* has acquired any preference for intraspecific variants, which were sourced from different regions of the introduced and native distribution. It also examined their behaviour over the exposure time to understand whether choices are altered due to post-ingestion feedbacks given some plants' abilities to negatively alter their structures and chemistries in response to insect herbivory. The expectations were that the BR6 genotype of *P. aculeata* would be consistently preferred to other genotypes that were ranked as less suitable (Chapter 3) or that had incurred less damage (Chapter 2). In contrast to BR6, BR9 was a less suitable host and was therefore expected to be preferred less than more suitable genotypes including BR6. This study was also another test of the EICA hypothesis which would be supported if the invasive genotypes were preferred over native genotypes (Keane & Crawley 2002; Blossey & Notzold 1995; Paterson *et al.* 2009).

The expectation was that there would be a preference in favour of one genotype over the other, however, *C. schaffneri*'s exposure to pairs of genotypes of *P. aculeata* did not suggest that they consistently choose where to settle. Of the seventeen paired choices, 53% had a relatively stable pattern on the chosen plant, while in 47% of the trials the nymphs vacillated between the two hosts after settling. With regard to the two Santa Catarina genotypes, no consistent choices were seen as BR6 was less preferred (28.6% of the seven paired choices), while 57% chose the other genotypes, which consisted of an invasive genotype (SA4) and three native genotypes namely DR2, VZ1, and BR9. For trials against BR6 and BR9, all possible outcomes – acceptance, rejection and no preference– were observed; thus making it difficult to interpret. Most probably, *C. schaffneri* nymphs may accept any succulent shoot of any host genotype offered to them upon hatching. For instance, the SA10 genotype deemed to be rejected in BR6-SA10 pair was equally accepted when paired with a Rio de Janeiro genotype (BR8). Meanwhile, the BR8 genotype was markedly rejected in a BR6-BR8 pair but equally accepted in a BR9-BR8 pair. Given that BR9 was preferred to BR6, which in turn was preferred to BR8, the expectations, therefore, would have been a preference for BR9 when paired with BR8. However, both genotypes were equally accepted. It is, therefore, unlikely that *C. schaffneri* exhibits any actual preference other than a random settling on any host shoots on a first-encounter-first-use basis. This suggests that the agent will feed on all the different plant genotypes that are present in South Africa and no

genotype mismatching issues between the agent and the invasive plant populations in the country are expected. It could also mean that the agent has not evolved the cues to perceive intraspecific differences among the genotypes of *P. aculeata* or that their basic needs are sufficiently met at an early stage of development to not warrant further search after encountering *P. aculeata*. This is interesting because some plant genotypes were not suitable hosts (Chapter 2 & 3).

The indiscriminate acceptance of different genotypes of *P. aculeata* in paired choice tests may indicate that *C. schaffneri* has the ability to accept its hosts regardless of the genotype. They may select a good shoot from a bad shoot, but not to seek out good genotypes from bad genotypes because under field conditions there is very little or no within-site genetic variability (because of vegetative growth). Although *C. schaffneri* nymphs could not survive to maturity on BR9 and DR3 (Chapter 3), the former and a close relative of the latter (DR2) were chosen in the paired choice experiments. Similarly, a close relative of VZ2 genotype (that is, VZ1) on which the lowest body weight of *C. schaffneri* was recorded (Chapter 3), was preferred to BR6, which originated from Santa Catarina, near the agent's locality. This may imply that the agent cannot actively gage intraspecific variation in the host plant. In a few cases, however, pairing the native BR6 and BR9 genotypes against invasive genotype (SA4) showed a preference for the invasive genotype, whereas BR6-SA10 pair favoured the native genotype BR6 making the results difficult to interpret. Nonetheless, how the agent's rejection of SA10 when paired with the native host from the agent's local habitat would translate to acceptance in the field remains unclear because even at impact level (Chapter 2), SA10 incurred less damage similar to that of other poor hosts. The SA10 genotype could therefore be an exceptional case for acceptance and damage from *C. schaffneri* with possible implications for biological control of that genotype in South Africa.

A few cases in which further movement occurred after settling may be connected to a decline in quality of host shoots or to changes in phytochemistry, as deployed against insect herbivores by the plant. Host-switching behaviour (or acceptance of less preferred hosts) evolved to compensate for poor food quality as seen through diet selection, altered consumption and post-ingestion compensation (Simpson & Simpson 1990; Lavoie & Oberhauser 2004; Pan *et al.* 2015). Other factors like relative succulence, different nutrients and phytochemicals may have influenced food preferences. It is plausible that between any two shoots in the choice arena, one shoot could

be more succulent than the other or have more nutrients (nitrogen/carbon content) with less defence chemicals or even structural defence than the other. These factors are difficult to tease apart within the context of this experiment, because there is no appropriate method to determine the shoot succulence without damaging the same shoot needed for the paired choices. How succulent the plant is and how much nutrients there is in it, could also be genetically determined and therefore be dependent on the plant genotype (Feng *et al.* 2009). The outcome of the paired choice study with closely placed genotypes with potentially different chemical and tactile cues, suggests that *C. schaffneri* has not evolved highly specific short-range cues for identifying a superior host among genotypes of *P. aculeata*.

The multi-choice experiment, conducted using adults, tested whether *C. schaffneri* can distinguish and select suitable host genotypes using a combination of visual cues and chemical cues from a greater distance. There was a clear attraction to *P. aculeata* rather than the other *Pereskia* (*P. grandifolia*) and *C. ovata*. Thus, it is evident that the agent can select its host plant species at this scale (using chemical or visual cues). The findings from this study further suggest that *C. schaffneri* would perceive any *P. aculeata* as a good host to initiate feeding. After exploring these hosts, they may reject inferior hosts for further scouting for good hosts. For instance, the choice of genotypes like AR3 and DR3 demonstrates that adult *C. schaffneri* are also not behaviourally equipped to detect intraspecific differences in the variant of *P. aculeata*, which corroborates the outcomes of paired choice experiments using nymphs. Such an attribute has both positive and negative implications for biological control. While the agent will initiate feeding on all *P. aculeata* genotypes in South Africa, choosing suboptimal hosts could reduce the fitness of the agent population. A reduction in fitness may arise after the agent had imbibed nutrients laden with toxins from the unsuitable hosts during exploratory feeding or from feeding on a host plant with insufficient nutrients.

Generally, intraspecific variation in plants can limit the success of insects, including biological control agents (Day & Neser 2000; Simelane 2006). The effects of host variation on herbivorous insects have been documented in *Latana camara* (Simelane 2006), tomato plants (Minkenberg & Fredrix 1989) and many other plants. For biological control, Sands and Harley (1980) cautioned against sourcing highly target–specific agents for hosts with varied traits as it may account for

agents' failures. For example, *L. camara* has several wild and horticultural varieties in both native and invasive ranges that had confounded biological control attempts as no single agent provided control for all the variants (Day & Neser 2000). *Pereskia aculeata* in the introduced range in South Africa is both morphologically and genetically uniform when compared with *L. camara* and, given that the agent, *C. schaffneri*, will initiate feeding and complete its development on all the genotypes, there is no expectation that the agent will be hampered by incompatibility with certain genotypes of the weed.

The majority of the invasive genotypes that were tested, with the possible exception of SA10 from Kosi Bay, were suitable hosts that are relatively susceptible to the agent, thus it is unlikely that the biological control programme will be negatively impacted by intraspecific variation of the plant in South Africa. Intraspecific variation among South African populations was not expected to be a limiting factor in this biological control programme because all the South African plants appear to be very similar genetically and are likely to have been the progeny of a single introduction from Rio de Janeiro in southern Brazil (Paterson *et al.* 2009).

Some specialists have evolved strategies such as aggregation behaviour to reduce host defences and predatory risk in order to maximise feeding (Wood 1982; Raffa & Berryman 1983; Robins & Reid 1997; Aukema & Raffa 2004; Desurmont *et al*. 2014). *Catorhintha schaffneri* habitually aggregates while feeding and this habit may contribute to its successful shoot colonization across several host genotypes of *P. aculeata* including those that proved to be harmful to their developments and limited their magnitude of damage (Chapter 2 and 3), but this remains anecdotal until proved otherwise. That *C. schaffneri* was sourced outside the origin of the South African invasive *P. aculeata* populations does not appear to have influence its acceptance of the invasive host genotypes, except for the genotypes (SA10) from Kosi Bay, which was less preferred, less damaged and less impacted compared to the other invasive genotypes (Chapter 2). The multiple-choice test (which showed the same preference for AR3 and DR3 as for BR6 and SA1) confirms that the agent does not possess finely tuned receptors to delineate differences among its host genotypes. This inability to discriminate among host genotypes has consequences for biocontrol of *P. aculeata* as *C. schaffneri* is not averse to any genotypes of *P. aculeata*. The gradual increase in the number that selected BR6 and SA1 over time suggested that *C. schaffneri* would gradually move onto these hosts after some individuals have initiated exploratory

feedings, while slow and diminishing patterns were observed on others. Nonetheless, it is difficult to determine whether subsequent arrivals of agents on a host genotype are newcomers from the release points or those that had visited other genotypes prior to arriving on the ones on which they were recorded. Although the highest preference was expressed for BR6 with about 50% more agents at 72 hours than SA1, both were not markedly different due to large variation within groups. The total number that flew onto all plants only reached 58% at the end of the trial and was spread across both the good and harmful genotypes, thus showing the agent's lack of choosiness when offered multiple *P. aculeata* genotypes.

The nymphs and adults of *C. schaffneri* readily accept any host genotype of *P. aculeata* that they encounter including those on which complete development was stalled and mortality was high. The implication for the biological control programme is that the agent would not reject the invasive genotypes of *P. aculeata* in the field, and given that South African genotypes were generally good host and are the only host plants available in the introduced range of South Africa, they are expected to suppress the invasive weed populations in the country.

CHAPTER 5 GENERAL DISCUSSION

The influence of host plant variation on the impact and performance of introduced biological control agents has been studied in many agent-weed systems. The *P. aculeata* in South Africa has a wide distribution in its native range of South and Central America, and has considerable genetic and morphological variation within that range (Leuenberger 1986; Paterson *et al.* 2009). The key questions in this thesis investigated whether the different populations of *P. aculeata* may have evolved along separate evolutionary trajectories due to different selection pressures from their diverse and seldom uniform natural herbivore assemblages at local scales in the native range. Whether the geographic and genetic origin of *P. aculeata* genotypes (native and invasive) cause any fitness cost for the biological control agent, *C. schaffneri*, was interrogated in light of relevant theories such as the Evolution of Increased Competitive Ability Hypothesis (Blossey & Notzold 1995), as well as the use of 'new' vs. 'old' associations in weed biological control (Hokkanen & Pimentel 1984; 1989; Thompson 2005; Goolsby *et al*. 2006; Hufbauer & Roderick 2005).

Differences in natural enemy assemblages could create variations in intensity of interspecific interactions at a site and this could result in genetic changes to the plant or natural enemy populations if exchange in genetic materials is confined by little or no gene flow (Thompson 2013; Kawecki & Ebert 2004). If an agent population has become adapted to a specific host population at a local scale in the native range, and if that plant population is different to those in the introduced range, then the biological control agent may be ineffective due to incompatibility with the target weed populations. In the studies reported in this thesis, agent fitness on various host genotypes was different, but the differences were not explained by geographic origin, evolutionary history of the plant genotypes with the agent or the genotypes genetic relationship with other plant genotypes. The general results neither follow the 'new' or 'old' associations approach, nor fully support the Evolution of Increase Competitive Ability Hypothesis.

This concluding chapter discusses the theories in light of the findings of the three experimental chapters of this thesis. The host plants' effects on the agent's impacts, performance (fitness) and preferences are also examined and discussed. The implications of these findings to the biological control programme against *P. aculeata* and for biological control in general are explored.

5.1 EVALUATING THE EVIDENCE OF EVOLUTION OF INCREASED COMPETITIVE ABILITY

Whether invasive alien plants generally undergo adaptive changes in vigour (growth) following their liberation from natural enemies or not, remains a question in invasion ecology (Blossey & Notzold 1995; Keane & Crawley 2002; Felker-Quinn *et al*. 2013). There are two conceptually similar hypotheses which deal with this issue, the Enemy Release Hypothesis and the Evolution of Increased Competitive Ability Hypothesis. Blossey and Notzold (1995) examined plant fitness parameters (plant biomass and height) on both an invasive and native population of purple loosestrife, *Lythrum salicaria*. The invasive population had substantially greater growth (*improved fitness*) and suffered greater herbivory (*increase susceptibility*) from a specialist natural enemy than the native populations (Blossey & Notzold 1995). This was due to the absence of natural enemies in the introduced range (enemy release: Keane & Crawley 2002) and an associated loss of traits related to defence from herbivory (*reduced defence*) (Blossey & Notzold 1995). Consequently, the three predictions of the EICA hypothesis predicted that invasive alien plants will grow faster, will be less defended against specialist herbivores, and will therefore suffer more damage from herbivory than the counterpart native-range population.

Pereskia aculeata has natural enemies, including pathogens and insect herbivores, in its native range (Pereira *et al.* 2007; Paterson *et al.* 2014b), unlike the invasive population on which none existed except for the recent introduction of two biological control agents (Klein 1999; Paterson *et al*. 2014b). Thus, 'enemy release' could have played a role in the ecology of the invasive *P. aculeata*, but whether that translates directly to evolutionary release as advanced in the EICA hypothesis was unclear until now. In this study, the predictions of EICA in terms of impact of herbivory from *C. schaffneri* (Chapter 2), the agent's performance and host suitability (Chapter 3) and preference (Chapter 4), largely did not support the theoretical concept of EICA hypothesis except for growth and vigour of the plant in the introduced range.

The general results suggest that the invasive genotypes, with an exception of one genotype (SA10), grew faster (greater heights and shoot lengths), than the native genotypes. Thus, lending credence to the first prediction of increased vigour as hypothesised by Blossey and Notzold (1995). However, these increased investments in growth did not translate to greater damage by *C. schaffneri* on the invasive genotypes than the native genotypes (Chapter 2). Although

invasive-range genotypes suffered more impact than some native genotypes, they incurred similar impacts with genotypes from Rio de Janeiro and Santa Catarina, where the invasive host populations and the agent were sourced, respectively (Paterson *et al.* 2009; Paterson *et al.* 2014b). The observed patterns indicated a genotype-dependent susceptibility that could not be explained by whether or not they were invasive or native, and this was inconsistent with EICA's predictions. Thus, the findings here only conform to Blossey and Notzold's prediction on vigour, which explains alien plant invasion success, and not host susceptibility to *C. schaffneri*. In terms of the agent's performance, shorter development time, higher percentage survival and greater body weight when reared on the invasive genotypes would have conformed to the EICA prediction, but the agent's fitness parameters were similar on plants from the native and introduced ranges with the variability being between genotypes rather than regions. Thus, the EICA hypothesis was partially supported (as regards plant's fitness), but not in the context of the invasive genotypes of *P. aculeata* becoming highly susceptible to herbivory.

Studies like Gruntman *et al.* (2017) evaluated post-invasion changes in resistance and allelopathy of (native and exotic) *Impatiens glandulifera* Royle (Ericalea: Balsaminaceae) along a time gradient since enemy release. They found greater host resistance to natural enemies among the native and older invasive populations than the young invasive ones. Gruntman and colleagues suggested that anti-herbivore defence declines after enemy release, but recovers with increase in recruitments of local enemies. Besides the two biocontrol agents on invasive *P. aculeata* released thus far (Klein 1999; Paterson *et al*. 2014b), there are no other herbivores that are known to have colonised the plant in South Africa. The post-invasion defence-recovery hypothesis of Gruntman *et al.* (2017) therefore does not apply to *P. aculeata*. Down-regulating anti-herbivore defence in *P. aculeata* may be a continuing process that is yet to reach its tipping point where the invasive populations would be more susceptible to biological control agents than the native counterparts. However, the residence time for invasive population of *P. aculeata* is over 150 years (McGibbon 1858), while the plant has been removed from its natural enemies as a horticultural plant for over 400 years (Britton & Rose 1919; Leuenberger 1986), which is a very long period of time compared to many, but not all, invasive plant species. Although complete support for the hypothesis is lacking, it cannot be ruled out that the invasive populations do outgrow their native populations. Support for all the predictions in the hypothesis remains largely agent-host specific, and thus the generality of the EICA concept remains elusive (Felker-Quinn *et al*. 2013;

Gruntman *et al.* 2017). Even on *L. salicaria*, the predictions only favoured a root-feeder, *Hylobius transversovittatus* (belowground herbivory) and not a defoliator *Galerucella pusilla* (aboveground herbivory) (Blossey & Notzold 1995).

The expectation of increased fitness of *C. schaffneri* on the invasive-range rather than native-range genotypes was not met. Although plants from different areas ranked differently in host suitability, the differences did not reflect a simple invasive-native range dichotomy. Low survival rates, longer development and lower body weights are indicative of poor quality host plants (Manrique *et al.* 2008; Gripenberg *et al.* 2010; De Lestang & Miller 2010; Egbon *et al.* 2012). Survival rate of *C. schaffneri* on the invasive genotypes (77%) and the southern native genotypes (68%) were similar to the 74% survival that was previously documented on a Port Alfred population of *P. aculeata* in South Africa (see Paterson *et al.* 2014b). Contrastingly, a low rate of 50% survival was recorded on VZ2 and had a longer development time of five days for adults to emerge than any other genotype. The genotype is not a suitable host to *C. schaffneri*, similarly to the Argentinean and the Dominican Republic genotypes, but fortunately, these genotypes have not become established in South Africa and the invasive genotypes were equally suitable as the native-range host genotypes from Brazil, except one (SA10) from Kosi Bay, South Africa. It is, however, possible that more genotypes in South Africa may be suboptimal hosts, but the majority are likely to be suitable.

Female *C. schaffneri* were generally heavier than their male counterparts that developed on similar hosts. Surprisingly, adult *C. schaffneri* emerged from fourth instars (Paterson *et al.* 2014b) as well as from fifth instars (Chapter 3). This is the first time that such outcome has been reported on this insects, although other coreids exhibited five nymphal stages (Rodrigues & Moreira 2005; Egonyu *et al.* 2013; Doh *et al.* 2016), it is also possible for adults to emerge from third instars (e.g., *Mozena obtusa*: van Klinken 1999) or fourth instars (*C. schaffneri*: Paterson *et al.* 2014a). Thus, it is not novel for members of the family Coreidae to vacillate between four and five nymphal stages. Females also had larger body mass, which suggests that they could be more influenced by poor food quality than males as more food (quality and quantity) is required to garner higher body mass and meet the reproductive task of egg laying. Although during development mortality by sexes were not monitored directly (as the sexes of immature stages are difficult to identify), a sex ratio of nearly 1:1 was observed among insects that fed

on and emerged from most native- and invasive-range genotypes. A different pattern of male-female sex ratio of 3:2 was seen from agents that emerged on the Venezuelan genotype. The Venezuelan genotype may had favoured more males relative to females as the latter require more resources for reproductive investment, which a low-quality host cannot support, thus dying earlier than the males (Price 2000; Awmack & Leather 2002). Such nutrition deficits could facilitate early female mortality, thus accounting for the higher male:female ratio of *C. schaffneri* on VZ2. Pupal and adult body weights often correlate with fecundity with smaller females laying fewer eggs and *vice versa* (Czypionka & Hill 2007; Uyi *et al.* 2014).

The findings on preference (Chapter 4) suggest that both nymphs and adults cannot distinguish a good (or more palatable) host from a poor host (Chapter 3). Thus, the genotypes of *P. aculeata* are perceptually similar to those genotypes (populations) that *C. schaffneri* had coevolved with. Some insect neonates display strong negative geotaxis and positive phototaxis upon hatching (Wigglesworth 1972) and this was observed in *C. schaffneri*. Such behaviours allow shoot feeding bugs to quickly climb towards shoot tips and then remain settled upon arrival. The apparent inability of neonates to detect harmful genotypes might be by-passed under natural conditions, if ovipositing females lay on the preferred host plants where the neonates would develop. Thus, the hatchlings may not have evolved genotype-specific cues needed to discriminate among hosts rather rely on their mothers, which may 'know better', but not necessarily 'know best' as choosy insect females sometimes prefer to lay their eggs on suboptimal hosts (Cunningham 2012; Hufnagel *et al.* 2017). Although whether 'mother-knows-best' was not directly tested in this study, as both male and female were used (in multiple-choice trials, Chapter 4), the parent (adult *C. schaffneri*) did not know better than the nymphs. They showed a low ability to discriminate among the genotypes of *P. aculeata*. *Catorhintha schaffneri*'s inability to avoid various genotypes of *P. aculeata* that are known to be unsuitable hosts suggests that they have no preference amongst the different genotypes offered. In practical terms, such non-preference implies that the agents might alight on a poor quality host without any short- term rejection only to imbibe deleterious chemical compounds or poor quality food from an unsuitable host at a fitness cost of high mortality (Chapter 2 and 3). Given that the invasive populations are from a single source (Paterson *et al.* 2009), and that they are suitable and susceptible hosts (Chapter 2 & 3), the need to choose amongst variable host forms by *C.*

schaffneri does not arise and would not be problematic for the biological control programme against *P. aculeata* using *C. schaffneri* in South Africa.

5.2 NEW AND OLD ASSOCIATIONS VS. LOCAL ADAPTATION

A 'new association' in weed biological control is an agent that has not co-evolved with the target weed populations; while an 'old association' is one that has a long history with the target weed (Hokkanen & Pimentel 1984; 1989). The concept originally referred to natural enemies with primary host plants that were different species to the target weed, but close enough relatives for the agent to feed on them. It is thought that a lack of co-adaptation between the plant and the agent could result in the plant being more vulnerable to damage due to a lack of defences to that herbivore species (Hokkanen & Pimentel 1984; 1989). For *P. aculeata*, the same concept could apply to agents collected off different varieties, forms or genotypes of the weed (Paterson *et al*. 2012). The concept of 'new associations' is not widely accepted, with most biological control practitioners opting for 'old associations' whenever possible (Gooslby *et al.* 2006; Paterson *et al*. 2009; Gaskin *et al*. 2011). For examples, while contemplating the role of local adaptation between the phytophagous mite, *Floracarus perrepae*, and its host plant, *Lygodium microphyllum* for possible biological control programme in the New World, where the plant is exotic, Goolsby *et al*. (2006) examined different interacting populations in the native range. They discovered that the different populations of *L. microphyllum* from several localities were genetically distinct as well as their natural enemy populations. Interestingly, using the agent's leaf rolling ability, which correlates positively with reproductive success in *F. perrepae*, as local adaptation metric, they observed that while some populations with similar geographic ranges as their hosts were partially able to roll fern leaves, a remote population was very 'catholic' on its local host and had the strongest rolling effects on that host rather than on others (Price 2000; Goolsby *et al*. 2006).

Whether the two biological control agents that have been released against *P. aculeata* should be considered 'new' or 'old' associations is a matter of debate. *Phenrica guerini* was collected from the source of the invasive populations in Rio de Janeiro, which is the closest possible native relative to invasive populations in South Africa (Paterson *et al.* 2009; Klein 1999). It could therefore be considered an 'old association' but could also be considered a 'new association' because the South African population is very different to the source population genetically due to

years as a horticultural plant (Paterson *at al.* 2009). For *C. schaffneri*, one could make a similar argument, but while the source of the invasive populations in Rio de Janeiro are within the native distribution of the agent, the population used for biological control was sourced from other populations that is geographically and genetically different to those at the source. This study did not support either approach. While there were differences in suitability between many of the different genotypes that were tested in this study, there was no correlation with the geographic origin or genetic relationship. Some genotypes are just better hosts than others.

Although *C. schaffneri* was affected by intraspecific variation as seen with some good and bad host plant genotypes, the observed performance, impacts and preference of *C. schaffneri* did not follow any of the presumed theoretical lines be it local adaptation, new versus old associations or the evolution of increased competitive ability. The findings in this study largely conforms to Paterson *et al.*'s (2012) study on the leaf-chewing beetle, *P. guerini*. Although fewer genotypes were monitored in their study, negative effects (fitness costs) of host genotypes eluded observations (Paterson *et al.* 2012). Nonetheless, *C. schaffneri* incurred remarkable setbacks on some native host plants like the BR9, DR3 and AR3 unlike *P. guerini* that did not show any intraspecific host effects (Chapter 2 and Chapter 3). Despites these outcomes, *C. schaffneri* still cannot distinguish between the various host genotypes whether they were harmful (e.g., AR3 and DR3) or harmless (e.g., BR6) (Chapter 4), when paired with specific genotypes with known fitness cost on the agent (Chapter 2 and 3).

The differences between the plant genotypes could be due to chance, rather than evolutionary history, but it is possible that a complicated evolutionary history with changing exposures to different assemblages of natural enemies led to the pattern that was observed. The communities of natural enemies at these localities in the native range vary widely (Paterson *et al.* 2014b); meaning the separate native populations had been under different selective pressures that change over time.

In the case of *P. aculeata* and *C. schaffneri*, there is no evidence to suggest that the single population of the agent was fitter on a local host than a nonlocal host as both produced largely similar on survival, development time, body mass upon emergence, and suitability rankings. Nonetheless, there is a non-significant trend according to which the findings on the body weights of adult *C. schaffneri* that emerged on the invasive host (nonlocal in source origin to the agent

population) were higher than others were (e.g., genotypes from Rio de Janeiro, Brazil and Caracas in Venezuela), though not significantly different. Thus, the heavier insects could be more fecund and lay more eggs than lighter insects. In addition, heavier body weight may imply a greater amount of reserved fuel, which could become readily deployed for dispersal, such that an insects with more fuel could fly longer distances and also reproduce more. However, if the energy budget of a female insect is expended in long-distant flights it is intuitive that less would be available for the reproductive outputs.

Although no clear evidence for 'new' or 'old associations' was found in this study, it is important to note that *C. schaffneri* performed poorly on the host genotypes that were sourced from the northern native range of *P. aculeata* and, at best could only be considered as a poor host on VZ2 even though development could be completed on it, and could only be considered a very poor host for DR3 and AR3. All of these genotypes are from outside the native distribution of *C. schaffneri* and are unlikely to have ever been exposed to *C. schaffneri* in their evolutionary history. So there are hints in the data which suggests that evolutionary history may play a role in the performance of the agent on different plant genotypes. *Catorhintha schaffneri* showed a widespread ability to reduce growth on its host plant genotypes regardless of their origins, but impact varied largely with no specific ties to any old or new association. The agent was able to inflict similar damage and impacts on both local and nonlocal hosts of *P. aculeata* from Brazil, but a few exceptions exist within and beyond Brazil. Fortunately, the exceptional genotypes are not present in South Africa.

5.3 *BIOLOGICAL CONTROL OF* PERESKIA ACULEATA

The general implications of these findings for the biological control of *P. aculeata* and other similar invasive alien plant species in South Africa and elsewhere are numerous. Firstly, the extent of variability in an invasive alien plant needs careful consideration in post-release studies, given the difficulties of conducting such studies during pre-release trials. Biological control practitioners should not assume that an absence of negative effects of intraspecific variation in a host plant against a specific agent could translate into similar results with another agent. For example, the fitness of *Phenrica guerini* was demonstrably independent of host variation in *P. aculeata* (Paterson *et al.* 2012), but there were differences between the same host plant

genotypes for *C. schaffneri*. Similar examples (e.g., Blossey & Notzold 1995; Manrique *et al.* 2008) also support the need to screen every agent in light of the probable factors that may hinder an agent's success upon release against its target pests. In practical terms, to screen every candidate agent upon collection would be labour intensive and would make biological control much more expensive in the initial phases. Fortunately, the genotypes of *P. aculeata* with the higher fitness cost to *C. schaffneri* are not among the invasive genotypes invading South Africa. Secondly, while some agents may not be sensitive to host plant genotype differences, *C. schaffneri* is genotype-sensitive, with implications on its ability to develop, acquire high body mass, and inflict significant damage on its host.

Despite the genotype-specific performance of *C. schaffneri*, the biological control programme of *P. aculeata* in South Africa is very promising as the agent can inflict similar injuries to the majority of invasive genotypes that would culminate in shoot diebacks and as such restrict the ever-increasing growth of the weed. For instance, a single lengthy shoot of *P. aculeata* would be reduced to multiple short shoots, which are continually susceptible to attack from *C. schaffneri*. When establishment is achieved on all its invasive host forms in South Africa, the capacity to damage and suppress new growth should not be compromised by the genetic differences of the host across the invaded range. The low genetic diversity in South Africa (Paterson *et al.* 2009) may be an important reason why most of the invasive genotypes are suitable, susceptible and supportive of complete development of *C. schaffneri* (Chapter 2, 3 and 4). Taken together, the apparent inability of *C. schaffneri* to discriminate among its hosts based on their suitability (Chapter 3, Chapter 4) is beneficial to the biological control of *P. aculeata*. Nonetheless, there must be some species-specific cues that *C. schaffneri* relies on that are present in most (if not all) genotypes, even among the deleterious hosts (Chapter 2 and 3), which makes it impossible for it to discriminate between good and poor host plant genotypes (Chapter 3 and 4; Cunningham 2012; Hufnagel *et al.* 2017).

To realise the full potential of biocontrol against *P. aculeata*, using multiple agents might be better than a reliance on the two previously released agents, *Phenrica guerini* and *C. schaffneri*. *Catorhintha schaffneri* can halt further apical growth of the invasive host population (Chapter 2), but because *P. aculeata* proliferates by several means (seeds, stem and leaves: Leuenberger 1986; Chapter 1), it would be ineffective against the already accumulated leaves and stems that

took the weed several years to acquire prior to the advent of the biological control programme. Sustained damage, over many years, may however result in the death of these plant parts that are not suitable for consumption by *C. schaffneri*. Increasing herbivore pressure can be achieved by introducing other biological control agents that are capable of damaging other plants parts (like the stems and roots), while sustaining existing ones. In that the leaf chewing beetle, *P. guerini*, and shoot-wilting bug, *C. schaffneri*, had been released to target the leaves (young or old) and shoots (young and succulent) respectively, agents capable of utilising the stems, which at the moment largely elude the reach of both agents, are recommended.

Other potential agents that would complement previous agents should be considered against the backdrop of intraspecific variability in *P. aculeata*, the agent distribution and its dispersal ability to minimise the trappings of local adaptation. Fifteen phytophagous insects associated with *P. aculeata* in its native range have been documented; 13% (2) of which damage shoots (*C. schaffneri* included), 47% (7) damage leaves, 7% (1) damage fruit and 33% (5) damage the stems (Paterson *et al*. 2014b). In that the weed's shoots and leaves are under the influence of the current agents, *C. schaffneri* and *P. guerini* (Paterson *et al*. 2014b; Klein 2011 updated 2016), targeting other aspects of the weed such as the fruits and stems would be apt in order to increase the burden of herbivory on the weed.

The fruit feeder, *Asphondylia* sp. (Cecidomyidae) was found in most regions of the southern native distribution, including Rio de Janeiro where the South Africa invasive populations originated, so it is a suitable candidate in terms of local adaptations (Paterson *et al*.2014b). Although, the stems feeders like *Xyleborus affinis* (Scolytidae) and *Adetus analis* (Cerambycidae) were present in Santa Catarina, they were not host-specific, and therefore not suitable for biological control. It is possible that a stem-mining curculionid which was preliminarily referred to as *Cryptorhynchus* sp. in previous studies (Paterson *et al*. 2014b), and is now formally described as *Pereskiophaga brasiliensis* (Anderson 2015), may be a promising candidate. This species is not present at Rio de Janeiro but it was abundant at seven sites in Santa Catarina, Brazil. Given the similar genetic relationship between the Rio de Janeiro and Santa Catarina populations of *P. aculeata*, the *P. brasiliensis* in Santa Catarina is likely to be able to utilise the South African populations. This candidate should have been considered if suitably host specific. It is currently under evaluation at Rhodes University; but the agent has also been found

to utilise *Anredera* sp. in Brazil, South America (Vitorino M.D. personal communication 2018).

A second cerambycid, *Acanthodoxus machacalis,* is a promising potential candidate. Little is known about its host specificity but it has never been recorded feeding on any other plant species (Paterson *et al.* 2014b). *Acanthodoxus machacalis* was present in high abundance in Rio de Janeiro State where the South African invasive populations originated from (Paterson *et al.* 2009; Paterson *et al*. 2014b). It was also present in Santa Catarina, but at very low densities, and was absent from all other parts of the native range of *P. aculeata* (Paterson *et al.* 2014b). *Acanthodoxus machacalis* should be considered as a potential biological control agent and should therefore be collected and imported into quarantine for host specificity testing. If suitably host specific, this agent would most likely be effective against the invasive population in South Africa invasive population of *P. aculeata* because it could be collected at the population's origin.

In retrospect, given that *P. guerini* was released on *P. aculeata* years earlier than *C. schaffneri*, some of the invasive genotypes on which the former had established could have regained their anti-herbivore defences. If so, detecting differing performance of *C. schaffneri* between the native and the invasive forms (*sensu* Blossey & Notzold 1995 or Hokkanen & Pimentel 1989) would be difficult. Of the twelve sites of *P. aculeata* on which *P. guerini* was released in KwaZulu Natal and Eastern Cape Provinces in South Africa (Klein 1999), only one genotype from Port Alfred (SA3 -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 fit despite the fact that one had been exposed to *P. guerini*. Unfortunately, such comparison is impossible for SA1 (from Knysna) and SA4 (from Port St. Johns) in performance and impact trials in which SA3 was not included. However, even though *P. guerini* was absent in Knysna and Port St. Johns (Klein 1999), there was no sufficient evidence to suggest that these genotypes were more impacted, or that the agent performed better on them, than the native genotypes from Rio de Janeiro and Santa Catarina. Thus, previous exposure to *P. guerini* seems immaterial to the performance of *C. schaffneri* on the different genotypes of *P. aculeata*.

5.4 CONCLUSION

The studies reported in this thesis highlight the significance of genetic variation in host plant populations relative to an agent's fitness and the extent of impact inflicted on the target weed. The difference in suitability of the different genotypes of *P. aculeata* to the shoot-wilting coreid *C. schaffneri* suggests that intraspecific host assessments should be a component part of all studies that define the future use and priority of any biological control agent. The studies also suggest that intraspecific host variation can strongly influences agent fitness, but not necessarily an agent's preference behaviour. Because host variation can play a role in an agent's fitness and impact, it can also undermine the expected outcomes of the agent's ability to provide control. Finally, detailed assessments of intraspecific variation across the wide range of the native region of an invasive alien plant are important for the success any of biological control programme against invasive alien plants and these differences may be genotype specific, rather than the geographic or genetic origin of the plant genotypes in the introduced range.

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