Native-range studies on insect herbivores associated with African Lovegrass (*Eragrostis curvula*) in South Africa: Prospects for biological control in Australia

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Abstract:

Eragrostis curvula (Schrad.) Nees. (African Lovegrass) is an African native species of grass that was intentionally introduced for pasture in Australia. It has since escaped cultivation and has become widespread and highly invasive. *Eragrostis curvula* has been recorded in every state and territory in Australia where it has altered fire regimes, disrupted nutrient cycles and can reduce livestock carrying capacity by up to 50%. The Centre for Biological Control at Rhodes University and the New South Wales Department of Primary Industries have been working in collaboration to identify and screen herbivorous insects as biological control agents for *E. curvula* in Australia.

Native-range surveys were conducted between 2021 and 2022 on *E. curvula* at twenty-two sites across South Africa to identify herbivorous natural enemies associated with it. Species accumulation curves were generated to ensure adequate sampling was performed to identify all the insects associated with *E. curvula.* Twenty-nine non-target grass species were surveyed simultaneously to determine the field-host range of the natural enemies associated with the target weed. Herbivorous natural enemies were prioritised as possible biological control agents against *E. curvula* in Australia based on field-host range, predicted efficacy and climatic suitability.

Four insect species were consistently found on *E. curvula*, two of which were herbivorous, as well as a parasitoid and a detritivore. Species accumulation curves show that the insect community was adequately sampled in South Africa. The two herbivorous insects were identified to the lowest taxonomic level using COI barcoding. Both species are undescribed phytophagous wasps in the genus *Tetramesa* (Hymenoptera: Eurytomidae). Because *Tetramesa* species have been shown to be host specific and highly damaging in previous biological control programs for other invasive grass weeds, we assessed their suitability as candidate biological control agents for use on *E. curvula* in Australia.

Both *Tetramesa* species (“sp. 4” and “sp. 5”) were found on several native congeners under field conditions in South Africa. Congeneric South African-native non-target grass species were used as phylogenetic proxies to assess the risk posed to Australian native *Eragrostis* species. This highlighted

three non-target Australian native *Eragrostis* species, namely: *E. parviflora* (R. Br.) Trin., *E. leptocarpa* Benth. fl., and *E. trachycarpa* Benth., that are at risk of being attacked by the two candidate agents based on their phylogenetic proximity to *E. curvula*.

Predicted efficacy trials were conducted at five long-term repeat survey sites and revealed that *Tetramesa* sp. 4 does not reduce the probability of *E. curvula* tiller survival or reproduction, while *Tetramesa* sp. 5 does not reduce the probability of tiller reproduction but does increase the probability of tiller survival. This result was unexpected and may be a plant compensatory response to herbivory. The sites where both *Tetramesa* species were collected in South Africa are climatically similar to the invaded range of *E. curvula* in Australia, and as such, the *Tetramesa* spp. are likely to be suitably adapted to the climate where they would be released in Australia.

These results suggest that both *Tetramesa* species associated with *E. curvula* may have too broad a host range to be used as biological control agents in Australia. However, further quarantine-based host-range assessments on Australian native *Eragrostis* species are recommended to confirm this. The field-based methods used in this study have reduced the number of insect and plant species that host-range assessments will be required to be performed on, thus preventing wasted resources.

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Chapter 1: Introduction

1.1.1. *Invasive alien plants*

Globalisation has given rise to a vast and intricate web of pathways that have allowed invasive alien plants (hereafter 'IAP') to be transported globally at an exceptional rate (Mack et al., 2000). Invasive alien plants can establish outside of their native distribution, where they can threaten native biodiversity, disrupt nutrient cycles, deplete groundwater levels, and decrease local plant diversity (Culliney, 2005; Vilà et al., 2011). Cuthbert et al. (2021) reported that the global costs of aquatic IAP's alone was US$ 20 billion since 1971. Between 1970 and 2021, economic costs of IAP's in Australia were reported as US$ 151.68 billion (Bradshaw et al., 2021). Invasive alien plants are therefore cause for major concern.

The damage caused by IAP's often results in large, costly management programs. For example, in 2012, the annual expenditure on various IAP control and management operations in South Africa reached US$30.5 million (van Wilgen & de Lange, 2011). In 2004, the cost of control of IAP's in Australia was estimated at roughly US$ 87.5 million (Sinden et al., 2004). Common control methods involve mechanical (physical removal), controlled burning, and chemical (herbicide application) methods (Setterfield et al., 2010). These methods can be effective in some circumstances but usually require repeated applications which become labour-intensive and costly (Culliney, 2005; Lake & Minteer, 2018). Often, mechanical and/or chemical control, even when applied together, are insufficient in reducing the degree of an infestation over large spatial scales and plants can develop resistance to herbicides (Culliney, 2005). Mechanical control methods and herbicides also frequently have negative secondary effects including health and safety risks and non-target impacts (Crone et al., 2009). Controlled burning can often lead to increased abundance, seed germination, and seedling establishment of IAP's (Hatcher & Melander, 2003; Setterfield et al., 2010). Current control methods have often proved to be ineffective, expensive, and can degrade the environment further, highlighting the need for classical weed biological control.

1.1.2. *Classical weed biological control*

Classical weed biological control is a method that has been used to control many invasive alien plants worldwide (Hinz et al., 2020). Biological control is defined as making use of populations of natural enemies to reduce populations of pest species to lower densities, either permanently or temporarily (van Driesche et al., 2009). Biological control is a broad term that encompasses several methods including classical, augmentative and conservation biological control, but classical biological control is the most common method used for the control of IAP's. Classical biological control (hereafter, 'biological control') makes use of natural enemies that have a shared evolutionary history with the target (common native ranges), to achieve control (McFadyen, 1998). Biological control is based on the enemy-release hypothesis which states that the establishment and proliferation of an IAP outside of its native distribution is facilitated by its release from specialist natural enemies that regulate its population in the native distribution (Keane & Crawley, 2002). Biological control involves reuniting the IAP with host-specific natural enemies from its native distribution (Müller-Schärer & Schaffner, 2008). It is relatively inexpensive, sustainable, and often highly effective (Paynter et al., 2020; van Wilgen et al., 2020; Zachariades et al., 2017).

There are several benefits of biological control. Established biological control agent populations may self-perpetuate and spread naturally as they respond to the densities of the target weed in the invaded range (Culliney, 2005). The ability of biological control agents (hereafter, 'agents') to spread naturally results in their establishment in areas that are difficult or impossible for humans to access (Culliney, 2005). Although biological control programmes require large financial input during the agent selection, prioritisation and testing phases, established agents require little economic input (Culliney, 2005; van Wilgen & de Lange, 2011; Suckling & Sforza, 2014). Effective biological control programs have high cost-benefit ratios because the economic impacts of uncontrolled IAP's are severe, and biological control requires little continued financial input (Zachariades et al., 2017). Because herbicides are toxic and seldom target-specific, they often result in damage to non-target plant species and can have negative impacts on biodiversity and human health (van Wilgen et al., 2020). Biological control is a non-toxic method of weed control as no hazardous chemicals are used, making it safer for the environment and humans (Culliney, 2005). Furthermore, biological control has stringent protocols in place to minimise the risks of non-target effects and it can be readily used with other control methods for integrated pest management (Greathead, 1995; Culliney, 2005; van Driesche et al., 2009; van Wilgen & de Lange, 2011; Suckling & Sforza, 2014; Zachariades et al., 2017).

1.1.3. Safety and efficacy of weed biological control

As of 2018, 468 species have been intentionally released as agents against 175 species of IAP's in 48 families, across 90 countries (Suckling & Sforza, 2014; Winston et al., 2014; Schwarzländer et al., 2018). Of these 175 IAP species, 25% belong to Asteraceae (44 species), 14% belong to Cactaceae (25 species), 13% belong to Fabaceae (23 species), and ~ 1.5% belong to Poaceae (2 species) (Grevstad et al., 2003; Winston et al., 2014; Moran et al., 2017; Schwarzländer et al., 2018). Despite some concerns over the safety of biological control (e.g., Simberloff & Stiling, 1996; Louda et al., 2003; Havens et al., 2019), biological control has an excellent track record with only 6 agents (1.5% of all agents released) resulting in unpredicted non-target effects (Hinz et al., 2020). With the advancement of biological control science since the 1960's, a decrease in the proportion of non-target impacts has been reported despite an increase in the number of agent releases, which may be attributed to stricter regulations on the release of agents and more stringent host-specificity testing criteria (Hinz et al., 2020). Modern biological control practices can therefore safely and accurately predict non-target impacts.

Of the 468 species of agents released globally, 332 (70.9%) agents established populations, and 115 of the 175 (65.7%) target IAP species are considered to be under some level of permanent control (Schwarzländer et al., 2018). Roughly 25% of global biological control programs have reached complete control of the target weed and between 50% and 70% of them have achieved at least substantial control (Hinz et al., 2020). The success of a biological control program can also be measured on an economic scale using cost:benefit ratios. Cost:benefit ratios indicate the economic return on investment, i.e., the average benefit value per dollar spent on the control program. Barratt et al. (2018) showed that for every dollar the Australian government spent on the biological control of IAP's, the average value of benefits returned was $23.10. Most biological control programs result in a positive return on investment, i.e., they have a cost: benefit ratio greater than one (Hinz et al., 2020). Although biological control seldomly results in eradication of the target weed, it often slows the rate of invasion and substantially reduces the target weed's impact on the environment and economy.

1.1.4. Grasses as invasive weeds

Grasses (Poaceae) consist of roughly 11 000 species and are considered to be the most successful angiosperm family in existence (Linder et al., 2018). They are responsible for approximately 33% of global primary productivity and occupy a third of the global land area

(Tscharntke & Greiler, 1995). It is estimated that grasses originated between 96 and 75 million years ago (Mya) and since they began replacing temperate forests in the early Miocene period (23 – 5.3 Mya) they have been significant in the ecological composition of the modern biosphere (Bouchenak-Khelladi et al., 2010; Owen-Smith, 2013).

Grasses were among the first crops to be cultivated by humans, some 10 000 years ago, and have since become some of the most valuable plants world-wide (Purugganan & Fuller, 2009; Bouchenak-Khelladi et al., 2010). They are cultivated for food, building materials, grazing, lawns, windbreaks, medicinal purposes, and biofuel (Tscharntke & Greiler, 1995). Three of the most consumed species of grass are wheat, maize, and rice, which constitute approximately 50% of the global human caloric intake (Bruinsma, 2017). One of the most frequent uses of grasses is for pasture for grazing by mammalian livestock (Gibson, 2009). Their myriad uses have resulted in deliberate introductions of grasses into many non-native regions on a global scale (Daehler, 1998; Pyšek et al., 2012). Of the 11 000 species of grass, 4807 (~ 44%) have become weeds in non-native habitats worldwide, mostly because of the many deliberate introductions (Randall, 2017). Grasses as IAP's have many negative impacts to both the environment and the economy (Linder et al., 2018). For example, *Cenchrus ciliaris* L. (Buffel grass) threatens native biodiversity in regions of Australia by degrading areas where threatened central Australian animals take refuge (Pucky et al., 2007). The water consumed by *Arundo donax* L. (giant reed) along the Rio Grande River in the USA was estimated to cost the agricultural sector between $98 and 160 million per year in 2010 (Gowda et al., 2011). Their ability to spread without anthropogenic assistance, outcompete native grasses and other plant families, and invade a wide range of habitats, make grasses formidable invaders (D'Antonio & Vitousek, 1992; Grice et al., 2012).

While there are few cases of grass invasions within Africa, many of the global invasive grass species are native to Africa (D'Antonio & Vitousek, 1992; Visser et al., 2016). Grasses in Africa evolved under the pressures of an abundance of large grazing species and harvesting by humans and thus, many species are tolerant to heavy grazing pressure (Cook & Clem, 2000). The ability to tolerate grazing has resulted in many African grass species being viewed as highly valuable pasture species (Williams & Baruch, 2000; Cook & Dias, 2006; Firn, 2009). Their value for pasture has resulted in many intentional introductions of African grasses to multiple regions across the globe (Williams and Baruch, 2000; Cook & Dias, 2006; Firn, 2009; Overholt & Franck, 2017; van Klinken & Friedel, 2017). Usually, IAP's with severe negative impacts are targeted for active control programmes, whether it is chemical, mechanical, or controlled burning (Culliney, 2005; Lake & Minteer, 2018). The geographic extent and scale of exotic grass invasions makes these management practices largely infeasible and expensive (Culliney, 2005; Grice et al., 2012; Quirion et al., 2018). Grasses have been shown to develop resistance to chemical control methods and thrive after fire (Culliney, 2005; Hatcher & Melander, 2003; Powles & Yu, 2010; Setterfield et al., 2010), which often makes traditional control methods less effective or feasible for managing invasive grasses.

At the start of the 20th century, Australian agronomists intentionally introduced more than 2000 species of Poaceae because the native Australian grasses were not sufficient to support large-scale livestock farming, having evolved under little grazing pressure due to the absence of indigenous ungulates in Australia (Cook & Clem, 2000; Houston & Duivenvoorden, 2002; Cook & Dias, 2006; Cook & Grice, 2013). Native Australian species are outcompeted by most introduced African grass species (Cook & Clem, 2000) and many of the introduced African species have now been classified as invasive weeds (van Klinken & Friedel, 2017). Australia has 398 species of non-native plants recorded as noxious weeds and 32 of the noxious weed species have been classified as Weeds of National Significance (WoNS) (Invasive Plants and Animals Committee., 2016). Grasses (Poaceae) make up 12.8% (51 species) and 12.5% (4 species) of the noxious weeds and WoNS lists respectively (Invasive Plants and Animals Committee., 2016).

Among several other IAP's recorded in Australia, there are fifty-one invasive alien grasses (Sutton, 2019). Four significant invasive African grasses in Australia are *Sporobolus pyramidalis* Beauv., *S. natalensis* (Steud.) Th. Dur. and Schinz. (collectively, giant rat's tail), *Andropogon gayanus* (Kunth.) (gamba grass), and *Eragrostis curvula* (Schrad.) Nees. (African lovegrass) (Firn, 2009; Rossiter-Rachor et al., 2009; Sutton et al., 2021). *Sporobolus pyramidalis* and *S. natalensis* have invaded large areas of the Australian east coast where they have been shown to reduce livestock carrying capacity by up to 80%, costing the livestock industry AUS$ 60 million per year (Bray & Officer, 2007). *Andropogon gayanus* has invaded vast regions of northern Australia and has disrupted nutrient cycles, altered fire regimes and has severely impacted native biodiversity (Rossiter-Rachor et al., 2009). The impacts of *E. curvula* in Australia are discussed in section 1.2.2.

1.1.5. Grasses as targets for biological control

Given the factors that render the typical control methods ineffective in the invasive grass context, other methods should be explored for their control. Despite being severe invaders, grasses have historically been overlooked as targets for biological control (Pemberton, 1996; Sutton et al., 2019). There are three main reasons why this has been the case, 1) grasses were not thought to harbour host-specific insects, 2) grasses were considered resistant to herbivory, and 3) economically important closely related crop grasses were thought to be at risk of non-target attack.

Grasses were not thought to host any specialised or damaging insect species that could be exploited in the context of biological control (Evans, 1991; Pemberton, 2002; Sutton et al., 2019). Typically, grasses are not expected to host specialised herbivorous insects because they do not have the same secondary chemical compounds that dicotyledons use to defend themselves against herbivores. These chemical compounds are considered as key drivers of herbivore specialisation (Ehrlich & Raven, 1964; McNnaughton et al., 1985; Kellogg, 2015; Moore & Johnson, 2017), so it was assumed that specialist natural enemies would not have evolved on grasses. This has resulted in grasses being avoided as targets for biological control. For example, because of fears of limited host specificity and the number of valuable native pasture species (*Stipa* spp.) in Australia that are closely related to *Nassella trichotoma* (Nees) Arech. (Serrated Tussock) (an IAP in Australia), biological control was overlooked out of fear of non-target consequences (Wapshere, 1990). Furthermore, grasses tend to be resistant to herbivory because of their co-evolution with grazing mammals and have an ability to regrow from meristems and underground nutrient storage organs (Coughenour, 1985; Tscharntke & Greiler, 1995). As such, it was assumed that herbivores were not expected to inflict the level of damage required to establish control (Coughenour, 1985; Tscharntke & Greiler, 1995).

Although grass biological control was painted in a pessimistic light for many years, biological control is now considered as a suitable method for managing alien invasive species of grass (Sutton et al., 2019). Several biological control programmes against grasses have been undertaken and have shown that there are often suites of host-specific and damaging herbivorous natural enemies associated with grasses that can be used as agents. There are three biological control agents used against *A. donax,* the Arundo wasp *Tetramesa romana* Walker (Hymenoptera: Eurytomidae), the Arundo leaf sheath-miner *Lasioptera donacis* Coutin & Faivre-Amiot (Diptera: Cecidomyiidae), and the Arundo scale *Rhizaspidiotus donacis* (Leonardi) (Homoptera: Diaspididae (Goolsby et al., 2009, 2016, 2017). One insect has been released against *Spartina alterniflora* Loisel. in Willapa Bay, Washington (USA), a planthopper *Prokelisia marginata* (Van Duzee) (Hemiptera: Delphacidae) (Grevstad et al., 2003). Moreover, several other biological control programmes have agents that have been approved for release following host-specificity trials, such as: *Archanara* spp. (Lepidoptera: Noctuidae) approved for release on *Phragmites australis* (Cav.) Trin. ex Steudel (common reed) in the United States of America (Blossey et al., 2020), and *Uromyces pencanus* Arth. & Holw. (Uredinales) (a rust fungus) approved for release on *Nassella neesiana* (Trin. & Rupr.) Barkworth (Chilean needlegrass) in New Zealand (Anderson et al., 2017). Currently, two potential agents, both species in the genus *Tetramesa*, have been sent to Australia to be tested under quarantine conditions for use against two invasive African grass species, *S. pyramidalis* and *S. natalensis,* after being shown to be suitably host specific under native field conditions in South Africa (Sutton et al., 2021).

Several studies have shown these insects can be damaging to grasses and can be effective biological control agents. For example, laboratory trials show that *T. romana* reduces the main shoot growth of *A. donax* by up to 90% (Goolsby et al., 2009), while field assessments showed that between 2007 and 2014 (two years before and five years after its release respectively) *T. romana* decreased *A. donax* shoot biomass by 22%, and by 2016, total *A. donax* biomass was reduced by 45% (Goolsby et al., 2016; Moran et al., 2017). The use of the Arundo wasp in the USA against *A. donax* has reduced water loss from the Rio Grande basin and has facilitated an increase in native biodiversity (Moran *et al.* 2017). Furthermore, the cereal crop pest *Aceria tosichella* Keifer (wheat curl mite) (Prostigmata: Eriophyidae) has caused yield losses of up to 30%, and *Eragrostis tef* (Zucc.) Trotter., a crop species in North America, experiences 70% yield losses due to infestations of *Eurytomocharis eragrostidis* Howard (Hymenoptera: Eurytomidae) (McDaniel & Boe, 1990; Harvey et al., 2002). Both examples of effective agents and damaging grass pests show that there are natural enemies of grasses that can cause substantial damage to their hosts. Considering the success of biological control on many other IAP's globally, and the lack of success with current control methods (mechanical and chemical), avoiding biological control on grasses is a wasted opportunity (Sutton et al., 2019).

1.2. *Eragrostis curvula*

Eragrostis curvula is an African perennial, tussock-forming grass that was intentionally introduced for pasture but has escaped cultivation and has become highly invasive and widespread in Australia (Firn, 2009). This species has rapid seed germination and seedling growth and can reproduce asexually via the process of apomixis when conditions aren't favourable for fertilisation to occur or when the plant is stressed (Lazarides, 1997; Roberts et al., 2021). In its native distribution, it germinates mostly during the warmer months between spring and autumn, although it can germinate throughout the year (Firn, 2009; Roberts et al., 2021). *Eragrostis curvula* reaches heights up to 150 cm, and has a branched, open inflorescence that is between 6 and 40 cm long and 5 – 10 cm wide (Fig. 1). The inflorescences open throughout the year but mostly between spring and summer and its roots typically occur within the top 50 cm of the soil (Jessop et al., 2018; Roberts et al., 2021). Each inflorescence can harbour more than a thousand seeds with a high viability rate (Lazarides, 1997). *Eragrostis curvula* also has an estimated seed density of 600 kg/ha (Lazarides, 1997; Csurhes et al., 2016). Seeds are dispersed by water (hydrochory), wind (anemochory), and animals (zoochory) (Parsons & Cuthbertson, 2001).

Figure 1: A) & B) The typical morphology of *Eragrostis curvula*'s open, branched inflorescence harbouring thousands of seeds. C) A healthy, dense monoculture of *E. curvula* in its native range at Coombs Valley in the Eastern Cape province of South Africa. Photos taken by Liam Yell.

1.2.1. Native range and habitat

The native range of *E. curvula* encompasses a large geographic area throughout several African ecosystems from open shrublands to semi-arid subtropical grasslands (Fig. 2) (Parsons & Cuthbertson, 2001; EPPO International, 2020; Roberts et al., 2021). The native range includes Kenya, Tanzania (East Tropical Africa), Mozambique, Zambia, Zimbabwe (South Tropical Africa), Botswana, Namibia, Lesotho, and South Africa (Southern Africa) (Bernal et al., 2015; EPPO International, 2020; Roberts et al., 2021). There is a known regional sampling bias in plant distribution records towards South Africa (Hughes et al., 2021), so there are likely to be many localities missing from countries besides South Africa. Within its native range, *E. curvula* is used for candle, rope, and basket making, ornamental purposes, and is extensively planted for pasture to be grazed by livestock and native herbivores (Scott & Delfosse, 1992; Barkworth et al., 2003; Roberts et al., 2021). Although *E. curvula* is widespread in its native range, it is most prolific in regions where the mean annual precipitation (henceforth, 'MAP') is between 400 and 700 mm and mean daily temperatures fall between 10 and 30°C (Cox et al., 1988).

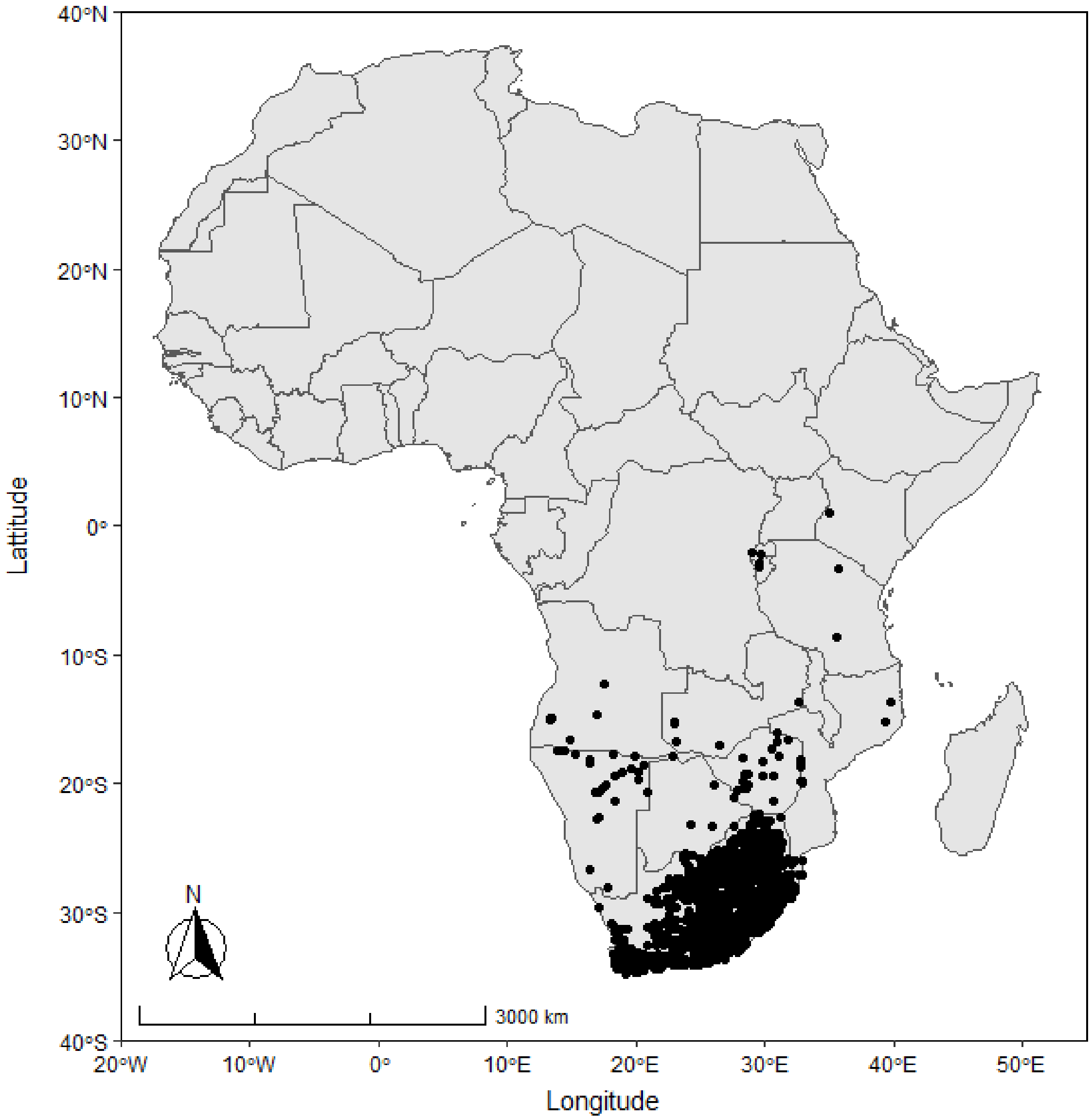

Figure 2: The native range of *Eragrostis curvula*. Species occurrence data were downloaded from the Global Biodiversity Information Facility (GBIF), 05/11/2022.

1.2.2. African lovegrass invasion

As a direct result of intentional introductions, *E. curvula* is present on every continent, barring Antarctica (Roberts et al., 2021). *Eragrostis curvula* is present but not widespread in six European countries, namely: Germany, France, Italy, Belgium, United Kingdom, and Spain (EPPO International, 2020; Roberts et al., 2021). *Eragrostis curvula* is naturalised and widespread in Mexico and in 24 states in the United States of America where it has invaded more than 80 000 ha in Oklahoma and North-West Texas (EPPO International, 2020; Roberts et al., 2021). In South America, *E. curvula* is present and widespread in Bolivia, Argentina, Chile, and Columbia (Cox et al., 1988; EPPO International, 2020; Roberts et al., 2021). In Asia, *E. curvula* is widespread in scattered areas of India, China, and Japan (Lazarides, 1997; Zhu, 2006; EPPO International, 2020). It has been found in every state and territory in Australia but is most prolific in eastern and south Australia where it has invaded in excess of 45 000 ha since being introduced between 1945 and 1953 (Fig. 3) (Campbell, 1983; Cox et al., 1988; EPPO International, 2020; Firn, 2009; Firn et al., 2018).

Eragrostis curvula was introduced intentionally to Australia early in the 1900s as part of the Commonwealth Plant Introduction Scheme to facilitate livestock farming as Australian native grass species were considered too unproductive to support large-scale livestock farming (Firn, 2009). Furthermore, *E. curvula* was introduced to stabilise soils and slow rates of soil erosion (Leigh & Davidson, 1968). Despite being considered a valuable grazing species in its native range, this species has been described as one of the most unpalatable pasture species to be introduced to agriculture in Australia and it contains low levels of crude protein, especially in mature stages of its growth (Firn, 2009). It has outcompeted native species and has altered fire regimes, disrupted nutrient flows, and water cycling in areas with severe infestations (Williams & Baruch, 2000; Firn, 2009; Cook & Grice, 2013). Livestock carrying capacity has been shown to be reduced by up to 50% on land with dense infestations of *E. curvula* (Crosthwaite & Dorrough, 2014). Although *E. curvula* is widely considered as a weed, it has been shown to be useful in pasture if intensive management is regularly applied (Firn, 2009; Roberts et al., 2021). Moreover, ‘Consol’ is an *E. curvula* accession that is highly palatable to livestock and is grown extensively for pasture in Australia, particularly in northern New South Wales (Firn, 2009).

Given the severe economic and environmental consequences of *E. curvula* infestations, several control methods have been attempted to manage the weed (Crosthwaite & Dorrough, 2014). Slashing is the most common form of mechanical control, which includes the use of a mower or high-density rotational grazing which forces the livestock to consume unpalatable species (Crosthwaite & Dorrough, 2014). Roller whipping, otherwise known as whick-whipping, is a common method of herbicide application used on *E. curvula*. The roller is laced with herbicide, towed behind a vehicle, and set at a height that minimises herbicide application to non-target species (Crosthwaite & Dorrough, 2014). However, because of the geographic extent of *E. curvula* invasions, mechanical control of this grass is likely to be impractical, ineffective, and prohibitively expensive (Lake & Minteer, 2018). Herbicides are seldomly target-specific and often have inadvertent effects on non-target species (Lake & Minteer, 2018). Chemical control methods often require repeated applications and are therefore expensive and there is a risk of the target weed developing resistance to these treatments (Lake & Minteer, 2018).

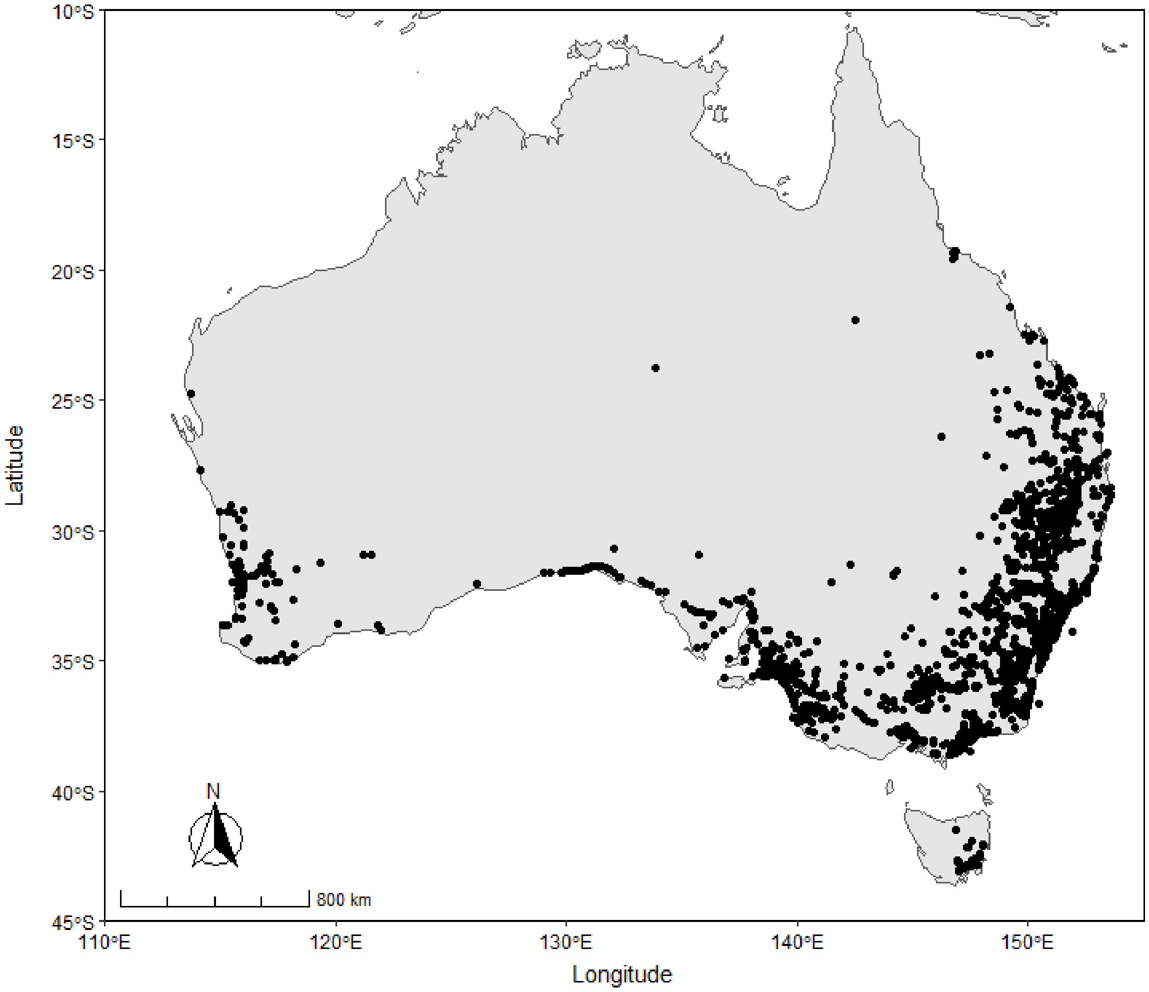

Figure 3: The invaded range of *Eragrostis curvula* in Australia. Species occurrences were downloaded from the Global Biodiversity Information Facility (GBIF), 05/11/2022.

The geographic extent of the invasion coupled with the high costs of current, ineffective control methods call for a new approach to manage the spread of the weed. Biological control is currently being assessed for the management of *E. curvula* in Australia.

1.2.3. Field-based studies in the native range for weed biological control

The first stage in a new biological control program involves compiling a list of natural enemies that could be studied as potential biocontrol agents, which is often done by conducting field surveys in the weed's native range (e.g., Palmer & Pullen, 1995; Paterson et al., 2014; Chari et al., 2020; Reid et al., 2020). However, because of the large number of herbivorous insects typically associated with plants, it would be a lengthy process and impractical, labour intensive, and expensive to perform host-specificity tests on the whole suite of insects found on a target species (Kennedy & Southwood, 1984; Goolsby et al., 2006; Paterson et al., 2014; Sutton et al., 2021). For example, the native range surveys on *L. camara* uncovered 550 insects associated with the plant in its native range (Palmer & Pullen, 1995). Thus, it is desirable to prioritise the insects that exhibit favourable traits for the purposes of biological control (Paterson et al., 2014). Natural enemies that have a narrow field-host range, inflict substantial damage, and are likely to survive the climate in the target weed's invaded range should be prioritised.

There are stringent protocols in place that must be adhered to when selecting an insect or pathogen for the biological control of IAP's (van Klinken, 1999). The most important of these criteria is host specificity (Cullen, 1990). Agents typically need to be host-specific (i.e., attack only the target weed in its invaded range) (Cullen, 1990). However, this depends on the presence and number of plant species that are closely related to the target weed in the region where the agent will be introduced (Cullen, 1990; van Klinken, 1999). For example, generalist cactus herbivores can be used in Africa because there are no native cactus species, with the exception of *Rhipsalis baccifera* Muell (Moran & Zimmermann, 1991). There are a number of tests that can be done to assess the host-specificity of potential biocontrol agents, namely, choice, no-choice, sequential, and multichoice tests (Schaffner et al., 2018). Each of these host-specificity tests are usually done under laboratory conditions by testing the potential agent against the target weed and a number of its close relatives but they can also be performed under native field (open-field) conditions (Cullen, 1990; van Klinken, 1999; Schaffner, 2001; Goolsby et al., 2006; Paterson et al., 2014; Schaffner et al., 2018).

The ability to successfully predict the efficacy of a new agent has been referred to as the 'holy grail" of biological control (McFadyen, 1998). This is because only a proportion of released agents that become established contribute to successful control of the target weed despite being suitably host specific and it is very difficult to predict which ones will be effective prior to release (McClay & Balciunas, 2005). Efficacy refers to the ability of a potential agent to damage the target weed to an extent that inhibits the plant's ability to survive, reproduce, and spread (van Klinken & Raghu, 2006; Sutton et al., 2021). Assessing the efficacy of a potential agent should preferably be done prior to its release, resulting in these assessments being predominantly quarantine-based (Sutton et al., 2021). For example, Balciunas & Smith (2006) performed pre-release efficacy assessments on the agent *Parafreutreta regalis* Munro (Diptera: Tephritidae) to determine if it will reduce the target weed, Cape Ivy (*Delairea odorata* Lemaire). Pre-release efficacy assessments should therefore be performed to give priority to natural enemies that have the potential to inflict the most damage on the target weed.

Recently, several authors have called for more rigorous field-based ecological studies in the weed's native range prior to quarantine-based testing (Egli, 2017; Sutton et al., 2021). Native range assessments can be highly beneficial as a first step towards developing a new agent (Goolsby et al., 2006; van Klinken & Raghu, 2006; Egli, 2017). Identifying the insect communities associated with the target weed, its congeners, sympatric plants, and structural analogues in its native range can reveal potential agents that only occur on the target weed under natural field conditions (van Klinken, 1999; Goolsby et al., 2006). Natural insect behaviour and a range of sensory cues drive host selection and use in the field, which often cannot be replicated under artificial conditions, thus, field-based methods can provide more realistic host-range predictions (Marohasy, 1998; Barton Browne & Withers, 2002). Furthermore, because quarantine space is limited and expensive, field-based host-range assessments and damage surveys can be performed under native field conditions to guide the selection of agents before quarantine-based assessments (Moran et al., 2005; Paterson et al., 2014; Sutton et al., 2021). Therefore, potential agents that are climatically matched to the invaded range, exhibit a suitably narrow field-host range, are sufficiently damaging, and are seasonally abundant are typically considered as high priority candidate agents (van Klinken, 1999; Goolsby et al., 2006; Paterson et al., 2014).

Potential agents that are found in regions of the native range that are climatically similar to that of the invaded range are more likely to survive and become established post-release (Goolsby et al., 2006; Robertson et al., 2008; Paterson et al., 2014). The climatically matched regions in the native range can then be used as sources for biological control agent populations that are likely to be suitably adapted to the climatic conditions in the invaded range, should they be released (Goolsby et al., 2006; Bell et al., 2013).

Eragrostis curvula was nominated as a target for biological control in Australia in 2020. To improve the management and limit the further spread of *E. curvula* in Australia, a biological control program was initiated in 2020 through a collaboration between the Centre for Biological Control (CBC) and New South Wales Department of Primary Industries (NSW-DPI). This represents the first biological control program against *E. curvula* globally. Surveys in South Africa, where *E. curvula* is native, are required to identify and priortise agents based on their field host range, predicted impact and climatic suitability.

1.3. Aims

The aim of this study was to identify potential agents for *E. curvula* and to prioritise which potential agents are most likely to be suitable for release in Australia. The host specificity of the potential agents was inferred from the field host-range of promising species using a phylogenetic approach. The predicted efficacy of the potential agents was determined by assessing how their presence/absence influenced key growth and reproductive structures of the target weed under native field conditions. Areas in the native range of *E. curvula* that are climatically matched to its invaded range were identified using climate matching models. The prospects for successful biological control were then predicted based on the field host-range, level of damage and climatic suitability of potential agents.

Chapter 2: Methods and materials

2.1. Field sites

Field surveys to sample the natural enemy community and to record the list of potential agents associated with *E. curvula* were performed across its native range in South Africa. Field surveys consisted of a mix of once-off surveys across a wide geographic distribution and repeat surveys at a subset of sites that were visited regularly. In general, sites were selected to cover as wide a geographic range as possible.

Five sites were selected for long-term repeated (hereafter, 'LTR') sampling in the Eastern Cape province of South Africa, namely: Coombs Valley, Ferndale Lodge, Penderry Farm, Klipfontein Farm and Alexandria (Fig. 4). The repeated surveys were conducted to assess the level of seasonal variation in insect species richness and abundance as insect communities may differ across seasons (Manners et al., 2011). An additional 17 other sites were surveyed, including 6 in KwaZulu-Natal, 2 in the Northern Cape, 1 in the North-West, 1 in the Free State, 2 in Mpumalanga, 2 in Gauteng, and 3 in the Western Cape provinces of South Africa (Table 1). The long-term and other sites were chosen as they have an abundance of the target weed (*E. curvula*) and several non-target grass species that are either closely related to, or naturally coexist with it. The additional sites were surveyed to determine if there was any variation in insect community assemblages across wide geographic and climatic ranges. The long-term survey sites were sampled once every three months from March 2021 to June 2022. This ensured that three sampling events occurred during each season at LTR sites. The seasons were categorised into Summer (October – March) and Winter (April – November) (van der Walt & Fitchett, 2020).

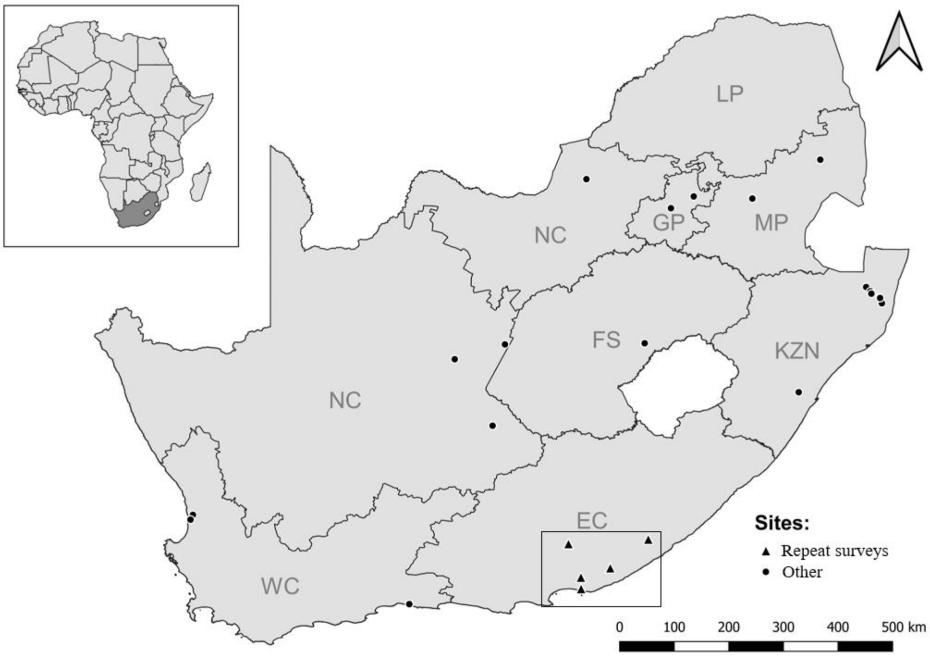

Figure 4: The sites surveyed across the native range of *E. curvula* in South Africa between March 2021 and June 2022, the long-term repeat sites are indicated by triangles and the other sites are indicated by circles.

Table 1: The sites surveyed (both long-term repeat and other) between March 2021 and June 2022 indicating which grass species were surveyed at each site and the number of times they were surveyed in each season.

				Number of times surveyed					
	Site	**Latitude**	**Longitude**	**Summer (n)**	**Winter (n)**	**Total (n)**	**Closest town**	**Habitat**	**Species surveyed**
Repeat surveys	Penderry Farm	32°47'54.2"S	26°01'00.3"E	3	3	6	Bedford	Open grassland	*Eragrostis curvula* *Eragrostis planiculmis* *Eragrostis obtusa* *Sporobolus fimbriatus* *Digitaria sp.*
	Coombs Valley	33°17'07.4"S	26°51'35.7"E	3	3	6	Grahamstown	Roadside	*Eragrostis curvula* *Hyparrhenia hirta* *Chloris gayana* *Eragrostis plana* *Eragrostis capensis*
	Ferndale Lodge	32°42'26.4"S	27°37'39,6"E	3	3	6	Qonce	Open grassland	*Eragrostis curvula* *Eragrostis plana* *Eragrostis chloromelas* *Sporobolus natalensis* *Megathyrsus maximus* *Eragrostis capensis*
	Alexandria	33°41'36.0"S	26°16'36.9"E	3	3	6	Alexandria	Roadside	*Eragrostis curvula* *Eragrostis capensis*
	Klipfontein Farm	33°28'33.7"S	26°16'00.0"E	3	3	6	Alicedale	Open grassland	*Eragrostis curvula* *Hyparrhenia hirta* *Eragrostis capensis* *Melinis repens* *Digitaria eriantha*
	Mkhuze 1	27°37'26.4"S	32°01'33.5"E	0	1	1	Mkuze	Roadside	*Eragrostis trichophora*

Other	Mkhuze 2	27°42'32.9"S	32°06'37.6"E	0	1	1	Mkuze	Roadside	*Eragrostis trichophora*
	Mkhuze 3	27°45'25.4"S	32°07'54.0"E	0	1	1	Mkuze	Roadside	*Eragrostis trichophora*
	Hluhluwe 1	27°57'02.8"S	32°20'36.2"E	0	2	2	Hluhluwe	Roadside	*Eragrostis trichophora* *Eragrostis superba* *Cynodon dactylon* *Megathyrsus maximus*
	Drummond	29°44'36.8"S	30°39'52.0"E	0	1	1	Hillcrest	Roadside	*Sporobolus pyramidalis* *Eustachys paspaloides*
	Phinda 1	27°50'03.9"S	32°18'00.7"E	0	1	1	Hluhluwe	Open grassland	*Hyparrhenia hirta* *Cymbopogon* sp. *Andropogon gayanus* *Heteropogon contortus* *Eragrostis superba* *Sporobolus pyramidalis* *Urochloa mosambicensis* *Setaria* sp. *Themeda triandra* *Megathyrsus maximus* *Sporobolus fimbriatus* *Eragrostis rigidiour*
	Phinda 2	27°50'26.5"S	32°18'17.0"E	0	1	1	Hluhluwe	Open grassland	*Hyparrhenia hirta* *Cymbopogon* sp. *Andropogon gayanus* *Heteropogon contortus* *Eragrostis superba* *Sporobolus pyramidalis* *Urochloa mosambicensis* *Setaria* sp. *Themeda triandra* *Megathyrsus maximus*

								Sporobolus fimbriatus
								Eragrostis rigidiour
Phinda 3	27°50'39.9"S	32°18'24.3"E	0	1	1	Hluhluwe	Open grassland	*Hyparrhenia hirta*
								Cymbopogon sp.
								Andropogon gayanus
								Heteropogon contortus
								Eragrostis superba
								Sporobolus pyramidalis
								Urochloa mosambicensis
								Setaria sp.
								Themeda triandra
								Megathyrsus maximus
								Sporobolus fimbriatus
								Eragrostis curvula
Hazyview	25°03'23.7"S	31°06'00.7"E	1	0	1	Hazyview	Roadside	*Eragrostis curvula*
								Hyparrhenia hirta
								Eragrostis trichophora
Overpass	25°50'25.7"S	29°43'40.5"E	1	0	1		Roadside	*Eragrostis curvula*
N4 Toll Gate	25°47'53.2"S	28°32'43.1"E	1	0	1	Pretoria	Roadside	*Eragrostis curvula*
St. Peters College	26°02'05.4"S	28°05'06.6"E	1	0	1	Sunninghill	Roadside	*Eragrostis curvula*
								Hyparrhenia hirta
Lambertsbaai	32°12'54.4"S	18°26'00.9"E	0	1	1	Lambert's Bay	Roadside	*Eragrostis curvula*
Elandsbaai	32°18'43.0"S	18°23'08.3"E	0	1	1	Elands Bay	Roadside	*Eragrostis curvula*
Sedgefield	34°00'51.7"S	22°48'09.3"E	0	1	1	Sedgefield	Roadside	*Eragrostis curvula*
Kimberly	28°46'47.8"S	24°44'01.9"E	1	0	1	Kimberly	Roadside	*Eragrostis echinochloidea*
								Eragrostis curvula
								Eragrostis superba
Douglas	29°04'44.8"S	23°43'13.1"E	1	0	1	Douglas	Open grassland	*Eragrostis echinochloidea*

Petrusville	30°25'08.1"S	24°29'01.0"E	1	0	1	Petrusville	Roadside	*Eragrostis curvula*
Groot Marico	25°26'57.2"S	26°22'40.3"E	1	0	1	Groot Marico	Open grassland	*Eragrostis curvula* *Megathyrsus maximus* *Hyparrhenia hirta* *Themeda triandra*
Kombuisland	28°45'24,02"S	27°33'17,73"E	5	0	5	Marquard	Open grassland	*Eragrostis curvula* *Eragrostis plana* *Eragrostis tef* *Eragrostis gummiflua* *Arundo donax* *Eragrostis capensis* *Eragrostis biflora*

2.2. Insect sampling

To characterise the natural enemy communities associated with *E. curvula*, approximately ~200 tillers of the target and non-target grass species were collected randomly from each site during the surveys. The grass samples were placed in standard insect emergence chambers with dimensions; height: ~ 800 mm, width: ~ 450 mm, and breadth: ~ 450 mm. Roughly 50 tillers were placed in each chamber. Emerging insects were collected from the cages over a 25-day period. Preliminary data indicated that many of the insects that emerged have life cycles shorter than 25 days (G. F. Sutton, unpublished data). Collections from the emergence chambers were terminated prior to the completion of their life cycles to ensure that emerging adult insects were those that were developing within the grasses when they were collected from the field.

The collected insects were preserved for genetic analysis by immersion in 80% ethanol (Carew et al., 2018). The insects were sorted into morphospecies. The Mitochondrial cytochrome oxidase I (henceforth 'COI') gene from voucher specimens was amplified and sequenced to obtain the lowest possible taxonomic level to ensure accurate identifications. Three legs from each *Tetramesa* specimen were used to perform DNA extractions using a PureLink Genomic DNA Mini Kit (ThermoFisher Scientific, catalogue number K182002) according to the manufacturer's protocols. The forward FWPTF1 (Li et al., 2010) and reverse LepR1 (Hebert et al., 2004) primers were used to obtain COI sequences. Successfully amplified PCR samples were sent to Macrogen Inc. in the Netherlands for post-PCR purification and sequencing in the reverse direction. The COI gene sequences were used to develop a genetic reference bank for the undescribed insect species. The insects have been preserved in 80% ethanol to be sent to the South African National Insect Collection for species identification.

2.3. Field host-range

Open field-choice observations were performed by comparing the insect communities found on the target weed to the insect communities found on non-target grass species in the target weed's native range. These observations were replicated 6 times. Twenty-nine species of grass growing in sympatry with *E. curvula* were surveyed for phytophagous insects. Non-target species were sampled according to the same methods used for *E. curvula* (Section 2.2). Non-target species were prioritised according to the centrifugal phylogenetic model (Wapshere, 1974). Phylogenetic proximity was the primary factor in selecting which non-target species to prioritise because the traits that regulate herbivory are typically phylogenetically conserved (Pearse & Altermatt, 2013).

Sampling effort was first focussed on the *Eragrostis* genus (congeners), then other members of the Chloridoideae sub-family and then on species from other sub-families. Non-target plants included during surveys were also selected based on structural similarity and relative abundance (Sutton et al., 2021). Abundant, sympatric non-target species are more likely to host insects that overlap with the target weed in its native range. Because plant structure plays a role in its defence against herbivory, structurally similar species may be attacked by natural enemies that attack the target weed (Bernays & Graham, 1988).

To prioritise candidate agents based on the level of host specificity, a phylogenetic approach was used to make predictions on which non-target plant species in the invaded range are likely to be at risk, without them being present in South Africa (Sutton et al., 2021). Because the traits that regulate herbivory are phylogenetically conserved, the phylogenetic approach assumes that the main driver of herbivore host-range is the phylogenetic distance between plant species (Pearse & Altermatt, 2013). Barrett et al. (2020) produced a molecular phylogeny for *Eragrostis* (Poaceae: Chloridoideae: Eragrostideae) and current molecular work (van Steenderen, C. unpublished data) has allowed phylogenetic relationships between native African and Australian *Eragrostis* species to be determined. There are 90 native African (Fish et al., 2015) and 73 native Australian *Eragrostis* species (Palmer et al., 2005; Simon & Alfonso, 2011). Following Sutton et al. (2021), native African *Eragrostis* species that were surveyed for insects were defined as 'phylogenetic proxies' and were used to infer any risk posed by potential agents to native Australian *Eragrostis* species. Candidate agents that were associated with any phylogenetic proxy during native-range surveys were considered to pose a threat to native Australian species in the same phylogenetic clade, and clades that were more closely related in phylogenetic proximity to the clade containing *E. curvula* (Sutton et al., 2021).

For example, a candidate agent associated with only *E. curvula* would be given high priority as a potential agent as its predicted host range does not include Australian native *Eragrostis* species (Fig. 5, Hypothetical sp. 1). If a candidate agent was found associated with *E. curvula* and *E. trichophora* Coss. & Durieu., it would also be given high priority as an agent because *E. trichophora* is an African native species that is more closely related to *E. curvula* than any Australian *Eragrostis* species (Fig. 5, Hypothetical sp. 2). However, a candidate agent associated with *E. curvula* and any phylogenetic proxy such as *E. lehmanniana* Nees. or *E. planiculmis* Nees. would be given low priority as an agent (Fig. 5, Hypothetical sp. 3). Because *E. lehmanniana* and *E. planiculmis* are more closely related to the Australian native *Eragrostis* species; *E. leptocarpa* Benth. Fl., *E. trachycarpa* Benth. and *E. parviflora* (R. Br.) Trin. than to *E. curvula,* it is hypothesised that this candidate agent would include the Australian native species in its host range.

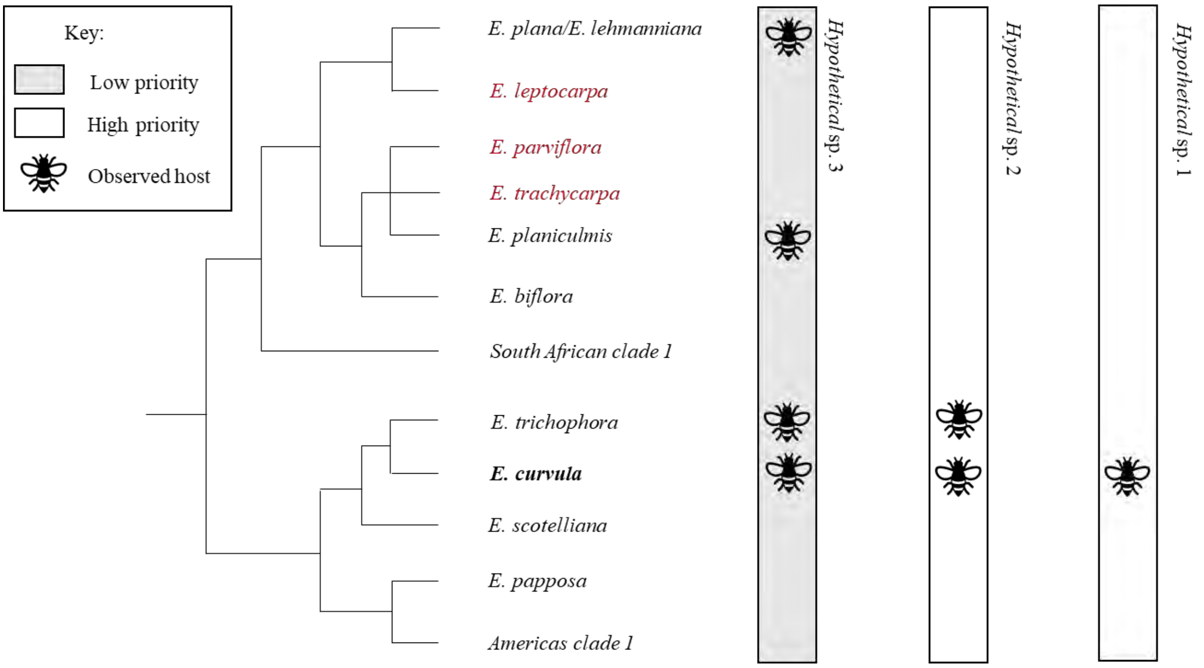

Figure 5: A schematic overview of how candidate agents were prioritised based on their predicted host range using a phylogenetic approach. Any African native *Eragrostis* species other than *E. curvula* that was surveyed was defined as a 'phylogenetic proxy' and was used to infer the potential risk posed by prospective agents to Australian native *Eragrostis* species (indicated by the red text in the figure). 'South African clade 1' and 'Americas clade 1' represent several species of *Eragrostis* native to each region.

2.4. Predicted efficacy

The efficacy of each prioritised herbivore was inferred from assessments of damage at the long-term study sites. Twenty-five tillers of *E. curvula* were randomly selected from each long-term site during each visit (Moran & Goolsby, 2014). The presence or absence of each herbivore was recorded per tiller and the tillers were scored as alive or dead ("tiller survival") by visual inspection to determine whether either of the prioritised herbivore species influenced tiller survival (Sutton et al., 2021). The presence or absence of seeds on the inflorescence ("tiller reproduction") was documented. Tiller reproduction was used to determine how the insects may affect the reproduction of *E. curvula* (Sutton et al., 2021). Each tiller was dissected and examined under magnification for endophagous insects. Insects found inside the tillers were preserved in 80% ethanol for genetic analysis (Carew et al., 2018). Adults were identified to morphospecies level and larvae were assigned to a morphospecies using genetic COI barcoding. These data were used to determine the potential efficacy of promising candidate agents.

2.5. Statistical analyses

2.5.1. Insect species richness

To determine if insect species richness differed between the long-term repeat sites and between seasons, a generalised linear mixed model (GLMM) (Bolker et al., 2009) with a negative-binomial distribution was fitted using the '*glmmTMB*' function from the '*glmmTMB*' package (Brooks et al., 2017). Insect species richness (number of unique insect species) recorded per survey was fitted as a continuous integer response variable, while site and season were specified as categorical predictor variables. An interaction term between site and season was specified to investigate whether any possible seasonal effects were dependent on which site was surveyed. Model diagnostics were run to ensure the most appropriate statistical distribution was used for the analyses using the '*simulateResiduals*' function from the '*DHARMa*' package (Hartig, 2022). A negative-binomial distribution was selected because model diagnostics results showed that it was a better fit than Poisson or Gaussian distributions (Appendix 1). A Likelihood Ratio Test (with type III sum-of-squares), henceforth 'LRT', was performed to test for fixed effect parameter significance using the '*Anova*' function from the '*car*' package (Fox & Weisberg, 2019). Pairwise comparisons between sites and seasons were performed on the marginal means, following Tukey adjustment to account for multiple comparisons, using the '*emmeans*' package (Lenth, 2022). All modelling and statistical analyses above were conducted using 'R' version 4.0.3. (R Core Team, 2022).

2.5.2. Insect species abundance

To determine if the insect species abundance differed between long-term repeat sites and between seasons, a GLMM with a negative-binomial distribution was fitted using the '*glmmTMB*' function from the '*glmmTMB*' package (Brooks et al., 2017). Insect species abundance (number of individuals of unique insect species) recorded per survey was specified as a continuous integer response variable while site and season were specified as categorical predictor variables. An interaction term between site and season was specified to investigate whether any possible seasonal effects were dependant on which site was surveyed. Model diagnostics were run to ensure the most appropriate statistical distribution was used for the analyses using the '*simulateResiduals*' function from the '*DHARMa*' package (Hartig, 2022). A negative-binomial distribution was selected because model diagnostics results showed that it was a better fit than Poisson or Gaussian distributions (Appendix 1). An LRT (with type III sum-of-squares) was performed to test for significance using the '*Anova*' function from the '*car*' package (Fox & Weisberg, 2019). Pairwise comparisons between sites and seasons were performed on the marginal means, following Tukey adjustment to account for multiple comparisons, using the '*emmeans*' package (Lenth, 2022).

2.5.3. Prioritised natural enemy abundance per site per season

The two prioritised natural enemies found during this study showed the highest degree of host specificity (section 3.2. below). The abundances of the two species were compared per site and per season by fitting a GLMM with a negative-binomial distribution using the '*glmmTMB*' function from the '*glmmTMB*' package (Brooks et al., 2017). Species abundance was specified as a continuous integer response variable and site and season were specified as categorical predictor variables. Model diagnostics were run using the '*simulateResiduals*' function from the '*DHARMa*' package (Hartig, 2022). A negative-binomial distribution was chosen over Gaussian distributions as it was shown to be of better fit during model diagnostics (Appendix 1). To test for significant differences in the abundances across the sites and seasons, a type III LRT was performed using the '*Anova*' function from the '*car*' package (Fox & Weisberg, 2019). Pairwise comparisons between sites and seasons were performed on the marginal means, following a Tukey adjustment to account for multiple comparisons, using the '*emmeans*' package (Lenth, 2022).

2.5.4. Sampling effort

Total species richness estimates and species accumulation curves were generated to determine if sufficient sampling effort was performed to identify the entire phytophagous insect assemblage associated with *E. curvula*. Species accumulation curves plotted the observed insect species richness against the number of surveys performed. Three species richness estimators were used to calculate estimated species richness, (1): The bootstrap estimator ('boot'), (2) second-order jackknife ('jack'), and (3) Chao 2 ('chao2'), following Bell et al. (2013). If the observed species richness approached the same asymptote as the estimated species richness, sampling effort was considered sufficient (Longino et al., 2002; Sutton et al., 2021). Uncertainty was shown in the species accumulation curves by calculating 95% confidence intervals around the fitted model using the '*poolaccum*' function from the '*vegan*' package in R (Oksanen et al., 2020).

2.5.5. Predicted efficacy

To determine the magnitude of the effect each prioritised natural enemy had on key productivity parameters (i.e., tiller survival and tiller reproduction), GLMM's were used. The probability that an individual tiller was alive or dead (tiller survival) and the probability that an individual tiller was seeding/not seeding (tiller reproduction) (dependent variables) were modelled against the presence/absence of the two potentially host-specific insects, following Sutton et al. (2021). The presence/absence of each natural enemy species was fitted as a categorical fixed effect. The GLMM's made use of maximum likelihood estimation and were specified with a binomial distribution and a logit link function. Site was modelled as a random effect to account for any potential spatial and temporal non-independence of the data (Bolker et al., 2009). To ensure goodness-of-fit, model diagnostics were run using the '*simulateResiduals*' function from the '*DHARMa*' R package (Hartig, 2022).

2.6. Climate matching

Regions in the native range of *E. curvula* in South Africa that are climatically similar to its invaded range in Australia were identified using the Maximum Entropy Species Distribution Model (henceforth, 'MaxEnt') version 3.4.3 (Phillips et al., 2006; Phillips & Dudík, 2008). Climatically matched regions are considered to be high priority regions to survey for agents because natural enemies found here are predicted to be capable of surviving the climate of the invaded range (Sutton & Martin, 2022). Climate matching was done to ensure that any potential agents would be able to survive the climate in the invaded range of *E. curvula* in Australia.

MaxEnt was implemented using the '*dismo*' package (version 1.3-9) in R (Hijmans et al., 2022). MaxEnt works by using environmental predictor layers and species occurrence points (GPS records) to predict the potential geographic distribution of a species (Phillips et al., 2006). The environmental predictors used by MaxEnt are the 19 standard bioclimatic variables at a 2.5 min resolution in the WorldClim database (version 2.1) (Fick & Hijmans, 2017) (data available at: www.worldclim.org/download.html). These variables have been used to model invasive grasses and identify high priority weed biological control survey areas before (Sutton, 2019; Sutton & Martin, 2022). Invaded range species occurrences were downloaded from the Global Biodiversity Information facility (GBIF) (accessed on 28/10/2022) using the '*spocc*' R package (Chamberlain, 2021). Online repositories such as GBIF often display spatial biases which may affect species distribution models, whereby the bias is typically that GPS records are concentrated around human settlements, roads, and other easy-to-access areas (Phillips et al., 2009; Veloz, 2009). Spatial autocorrelation can lead to inaccurate model predictions (Veloz, 2009). As such, duplicate occurrences were removed, and spatial autocorrelation analyses were performed to improve model quality (Veloz, 2009). The '*ecospat*' R package was used to perform spatial autocorrelation analyses (Broennimann et al., 2022).

Model calibration for MaxEnt requires the user to define the geographic background where the climate is sampled from grid cells where the target weed is assumed to be absent (as it is a presence/pseudo-absence modelling algorithm) (VanDerWal et al., 2009; Hill and Terblanche, 2014). The Köppen-Geiger climate classification system (available at: http://koeppen-geiger.vu-wien.ac.at) was used to define the model background (Webber et al., 2011). Only Köppen-Geiger zones with at least one GPS point were selected to make up the background area using the '*raster*' package in R (Hijmans, 2022).

To reduce the risk of calibrating overfit models, multicollinearity between climatic variables was assessed by computing Pearson's correlation coefficients for each pair of environmental predictors (Appendix 2) (Capinha & Anastácio, 2011). Highly correlated predictors ($|r| > 0.75$) were not included in the final set of predictors (Capinha & Anastácio, 2011). The final set of environmental predictors consisted of eight climatic variables, namely: mean annual temperature (bio1), mean diurnal temperature range (bio2), isothermality (bio3), maximum temperature of the warmest month (bio5), mean temperature of the wettest quarter (bio8), mean temperature of the driest quarter (bio9), mean annual precipitation (bio12), and precipitation seasonality (bio15) (Fick & Hijmans, 2017) (Appendix 3).

Several studies have shown that MaxEnt models specified with default settings are typically overfit and perform poorly, particularly when predictions are made into novel geographic regions (Phillips et al., 2006; Merow et al., 2013). To remedy this, MaxEnt allows users to specify different combinations of regularization multipliers (hereafter, RM) and feature classes (hereafter, FC) (Merow et al., 2013). FC allows the shape and complexity of the species-environment functions (linear – 'L', hinge – 'H', quadratic – 'Q', and product – 'P') to be controlled and RM balances the model fit and model complexity using 'L1' regularization, which removes climate variables that don't explain enough variation, penalising unnecessarily complex models (Merow et al., 2013). The optimal FC and RM were selected using model tuning experiments (Shcheglovitova and Anderson, 2013; Boria et al., 2014; Galante et al., 2018). Models were scored according to their goodness-of-fit and their complexity simultaneously by the Akaike Information Criterion corrected for small sample sizes (AICc) criterion, whereby the model with the lowest AICc was selected to determine optimal model settings (Muscarella et al., 2014; Galante et al., 2018). Model tuning experiments and AICc calculations were performed using the '*ENMeval'* package in R (Kass et al., 2021). Model tuning experiments tested RM values between 1 and 8 and five different FC combinations for each RM (FC combinations: H, L, LQ, LQH, and LQHP) (Table 2). The final MaxEnt model was specified using the following settings: 10 replications, RM = 1, FC = "LQ", and the 'fade by clamping' argument was set to "true" to allow the model to extrapolate predictions in non-analogous climate space.

To determine whether the MaxEnt models were extrapolating or interpolating, Multivariate Environmental Similarity Surfaces (hereafter, 'MESS') were computed (Elith et al., 2010). MESS maps are used as a measure of caution against inferences in extrapolation space (Elith et al., 2010). MESS analyses compare the degree of climatic similarity of any given point to a set of reference points, whereby the reference points were defined as the species presence points that were used to calibrate the models (Kriticos et al., 2014). The MESS map that was used to assess interpolation/extrapolation was generated using the '*dismo*' R package (Hijmans et al., 2022). Extrapolation space (represented by negative MESS values, MESS-) indicate geographic regions that are outside of the range of climatic variables used to calibrate the models. MESS values between 0 and 100 (interpolation space/ MESS+) show geographic areas that are within the range of climatic variables used to calibrate the models (Elith et al., 2010).

Table 2: The results from the model tuning analyses performed using the 'ENMeval' package in R. The bold row shows the model configuration with the lowest AICc value that was used. 'auc.val' = area under the curve (auc.val ± std. dev.), 'auc.diff' = area area under the curve difference (auc.diff, ± std. dev.) 'OR10' = tenth percentile omission rate, 'ΔAICc' = change in Akaike Information Criterion, and 'ncoef' = model complexity.

RM	FC	auc.val	auc.diff	OR10	ΔAICc	ncoef
1	H	0,92 ± 0,07	0,06 ± 0,06	0,24 ± 0,25	5435,75	46
1	L	0,84 ± 0,16	0,05 ± 0,03	0,14 ± 0,15	4944,99	8
1	**LQ**	**0,89 ± 0,10**	**0,04 ± 0,02**	**0,9 ± 0,9**	**0,00**	**13**
1	LQH	0,92 ± 0,10	0,06 ± 0,06	0,24 ± 0,25	5499,81	43
1	LQHP	0,91 ± 0,07	0,07 ± 0,06	0,24 ± 0,25	6568,98	41
2	H	0,93 ± 0,05	0,05 ± 0,04	0,22 ± 0,26	931,85	30
2	L	0,85 ± 0,15	0,05 ± 0,03	0,13 ± 0,14	4744,98	6
2	LQ	0,87 ± 0,13	0,03 ± 0,02	0,09 ± 0,08	615,49	10
2	LQH	0,93 ± 0,05	0,05 ± 0,03	0,21 ± 0,24	913,49	28
2	LQHP	0,93 ± 0,05	0,05 ± 0,04	0,21 ± 0,24	1103,69	29
3	H	0,93 ± 0,04	0,05 ± 0,03	0,19 ± 0,25	331,85	18
3	L	0,85 ± 0,15	0,05 ± 0,03	0,13 ± 0,13	4525,65	6
3	LQ	0,87 ± 0,13	0,03 ± 0,02	0,19 ± 0,19	1140,56	9
3	LQH	0,93 ± 0,05	0,05 ± 0,03	0,19 ± 0,25	367,53	21
3	LQHP	0,93 ± 0,05	0,05 ± 0,03	0,19 ± 0,24	451,85	19
4	H	0,93 ± 0,04	0,05 ± 0,03	0,18 ± 0,25	229,45	17
4	L	0,85 ± 0,14	0,05 ± 0,03	0,13 ± 0,13	4331,08	6
4	LQ	0,87 ± 0,14	0,03 ± 0,02	0,10 ± 0,09	1593,60	9
4	LQH	0,93 ± 0,05	0,05 ± 0,03	0,17 ± 0,24	225,71	17
4	LQHP	0,95 ± 0,05	0,05 ± 0,03	0,17 ± 0,24	247,79	17
5	H	0,95 ± 0,02	0,04 ± 0,01	0,05 ± 0,07	213,64	17
5	L	0,85 ± 0,14	0,05 ± 0,04	0,13 ± 0,13	4160,50	6
5	LQ	0,86 ± 0,14	0,04 ± 0,03	0,10 ± 0,08	2043,43	8
5	LQH	0,93 ± 0,05	0,05 ± 0,03	0,16 ± 0,24	205,73	19
5	LQHP	0,93 ± 0,05	0,05 ± 0,03	0,16 ± 0,24	206,86	20
6	H	0,95 ± 0,02	0,03 ± 0,01	0,04 ± 0,06	221,66	13
6	L	0,86 ± 0,13	0,05 ± 0,04	0,13 ± 0,12	4009,28	6
6	LQ	0,86 ± 0,14	0,04 ± 0,04	0,11 ± 0,09	2503,95	8
6	LQH	0,93 ± 0,04	0,05 ± 0,03	0,16 ± 0,24	218,27	14
6	LQHP	0,93 ± 0,05	0,05 ± 0,03	0,16 ± 0,24	226,75	15
7	H	0,95 ± 0,02	0,03 ± 0,01	0,04 ± 0,07	327,89	12
7	L	0,86 ± 0,13	0,05 ± 0,04	0,12 ± 0,12	3872,63	6
7	LQ	0,86 ± 0,13	0,04 ± 0,04	0,11 ± 0,09	2735,46	6
7	LQH	0,93 ± 0,04	0,05 ± 0,03	0,16 ± 0,24	308,67	12
7	LQHP	0,93 ± 0,05	0,05 ± 0,03	0,16 ± 0,23	323,08	13
8	H	0,93 ± 0,04	0,05 ± 0,03	0,17 ± 0,26	427,81	11
8	L	0,86 ± 0,13	0,05 ± 0,04	0,12 ± 0,11	3752,72	5
8	LQ	0,86 ± 0,13	0,04 ± 0,04	0,11 ± 0,08	2824,07	5
8	LQH	0,93 ± 0,05	0,05 ± 0,03	0,16 ± 0,24	405,68	12
8	LQHP	0,93 ± 0,05	0,05 ± 0,03	0,15 ± 0,23	415,23	13

Chapter 3: Results

3.1. Insect community structure on *E. curvula*

A total of 25 sites were sampled during 51 sampling events across eight provinces in South Africa (Table 1). Surveys found a total of 373 individual insects from 40 insect morphospecies (hereafter, 'species') occurring across all grass species studied. Twenty-eight insect species in total were found on *E. curvula* from all sites (both long-term repeat and other sites). Following Sutton et al. (2021), after excluding insects where fewer than ten individuals were found in total and insects that were only recorded on one occasion, or from one site, only four insects were consistently associated with *E. curvula* in South Africa (Table 4). The coverage (proportion of sites each insect was found at) and incidence (proportion of surveys each insect was recorded in) was calculated for each of these species.

Of the four species associated with *E. curvula,* two were herbivorous; *Tetramesa* sp. 4 and sp. 5 (Hymenoptera: Eurytomidae) (Table 4). One was a parasitoid of *Tetramesa* (Sutton et al., unpublished data), *Pediobius* sp. 30 (Hymenoptera: Eulophidae), and the last was a fungus gnat (Diptera: Sciaridae) which feeds on fungus, not on the host plant directly (Table 4.). *Tetramesa* sp. 4 and sp. 5 were the most widespread insects recorded on *E. curvula* being recorded at 53% and 60% of the total number of sites respectively with an incidence of 26% and 30% respectively (Table 4). *Pediobius* sp. 30 had a coverage of 46% and an incidence of 20% and Sciarid sp. 30 had a coverage of 26% and an incidence of 13% (Table 4).

The *Tetramesa* wasps found in this study are endophagous herbivores during their larval stage (Fig. 9c & 10). The adult female, after mating, oviposits on fresh green tillers. The eggs hatch and the larvae burrow into the stem where they form feeding galleries (Fig. 9b.) in which they will consume the vascular tissue until they pupate and chew neat circular 'exit holes' in the wall of the tiller (Fig. 9a). Hymenopterans make up ~ 90% of the total occurrences of insects associated with *E. curvula* in South Africa. *Tetramesa* sp. 4 constituted approximately 30.5% of occurrences and *Tetramesa* sp. 5 constituted approximately 53.5% of occurrences.

There was a statistically significant interaction between site and season explaining insect species richness ($X^2 = 11.29$, df = 4, $P < 0.01$). A seasonal effect was found at the Ferndale site near East London only ($t = 2.77$, df = 115, $P < 0.01$), where insect species richness was significantly higher during summer (1.83 ± 1.53) than it was during winter (0.08 ± 0.28) (Fig. 6).

There was a statistically significant interaction between site and season explaining insect species abundance ($X^2 = 9.54$, df = 4, $P < 0.05$). A seasonal effect was found within the Ferndale site ($t = 2.88$, df = 116, $P < 0.01$), where species abundance was significantly higher during summer (5.5 ± 5.35) than it was during winter (0.08 ± 0.29) (Fig. 7). There was a significant difference in species abundance between the Coombs (3.1 ± 4.9) and Penderry (0.2 ± 0.6) sites ($t = 3.06$, df = 116, $P < 0.05$).

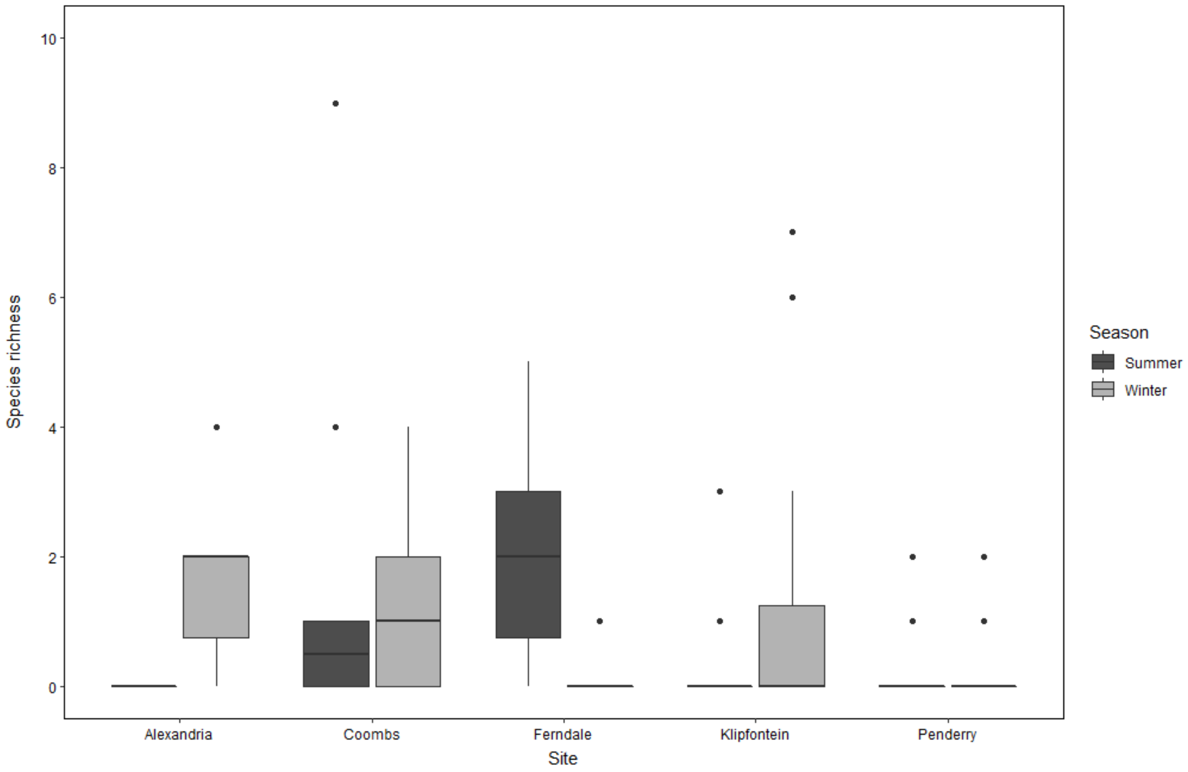

Figure 6: Insect species richness per site per season. Each site was sampled on 6 occasions (n = 3 sampling events in winter and in summer). Each sampling event consisted of allowing insect emergence from 200 tillers of each species of grass from each site for a period of 28 days. All insects were identified to morphospecies. The points above the bars represent extreme values (outliers) in the data.

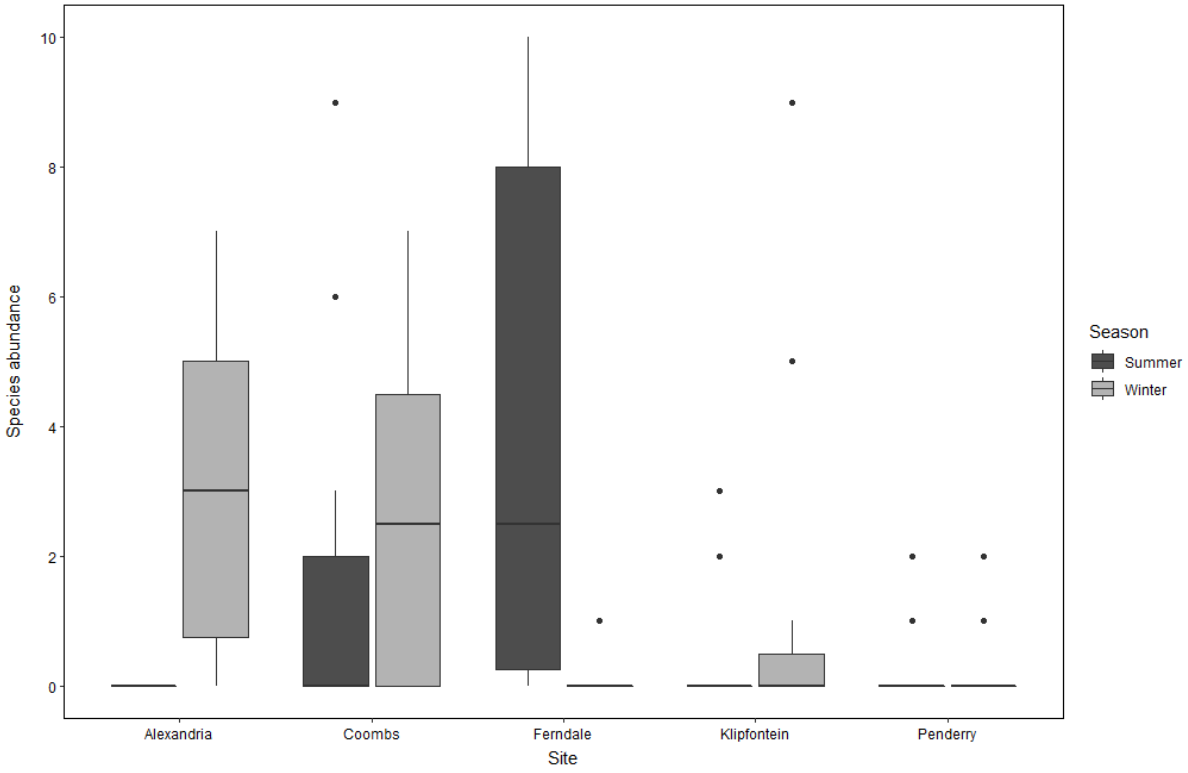

Figure 7: Species abundance per site per season. Each site was sampled on 6 occasions (n = 3 sampling events in winter and in summer). Each sampling event consisted of allowing insect emergence from 200 tillers of each species of grass from each site for a period of 28 days. All insects were identified to morphospecies. The points above the bars represent extreme values (outliers) in the data.

3.2. Sampling effort

Species accumulation curves showed that the insect community associated with *E. curvula* was sampled sufficiently (i.e., the entire insect assemblage was recorded, including both phytophagous species and all others). The confidence intervals of the expected species richness for two of the three species richness estimators (‘*boot*’, and ‘*jack2*’) converged on observed species richness for the insect assemblage associated with *E. curvula* (Fig. 8). This provides strong support that no additional insects are likely to be found on the target weed in South Africa, should more sampling occur. However, the confidence intervals for the ‘*Chao2*’ species richness estimator did not converge on the observed species richness (Fig. 8).

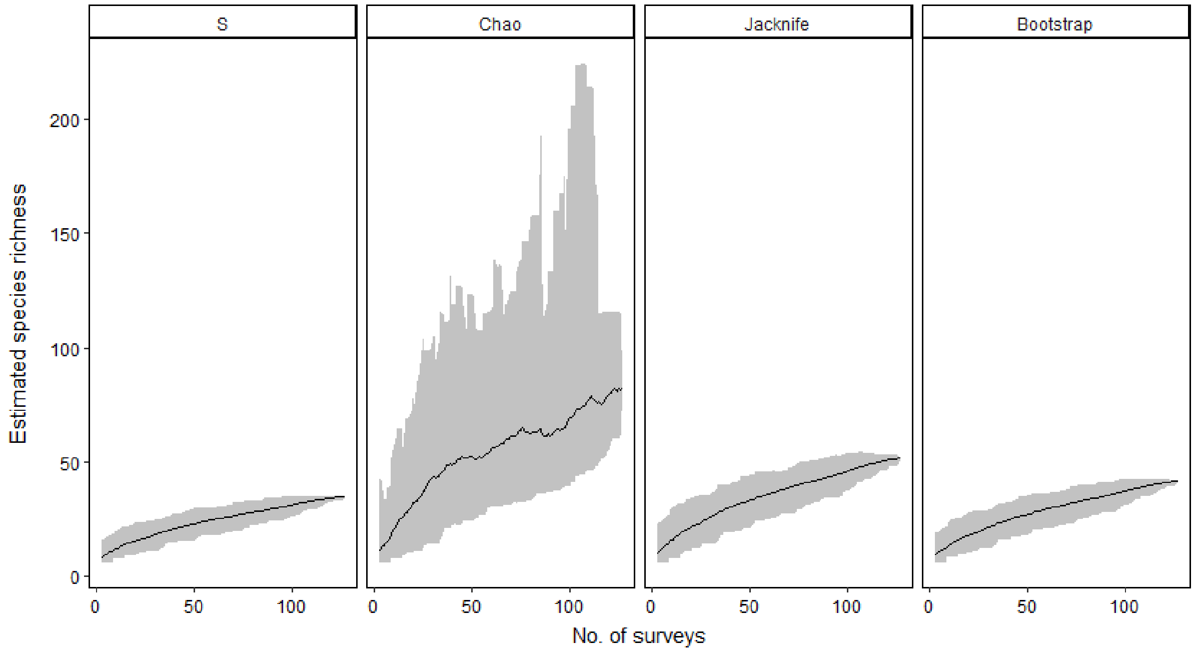

Figure 8: Species accumulation curves showing the observed (S) versus expected species richness for insects found on *Eragrostis curvula* in South Africa according to three species richness estimators. The solid black line represents the observed (‘S’) or predicted (‘*boot*’, ‘*chao2*’, and ‘*jack2*’) insect species richness. The grey shaded area around the solid black line indicates the 95% confidence intervals around the fitted species richness estimators.

3.3. Field host-range

The field host-range for the two herbivorous species is reported on because there were only 2 herbivorous insects consistently found on *E. curvula* and therefore the only possible candidates for biological control. Full *Tetramesa* sp. 4 larval development (and consequently the adult emergence) was recorded from at least six non-target *Eragrostis* species under native field conditions (Table 3). *Tetramesa* sp. 4 completed larval development on *Eragrostis capensis* (Thunb.) Trin., *E. plana* Nees., *E. gummiflua* Nees., *E. biflora* Hack. ex Schinz., *E. rigidiour* Pilg., and *E. trichophora* Coss. & Durieu. *Eragrostis plana* and *E. biflora* are key phylogenetic proxies for native Australian *Eragrostis* species (Fig. 11). *Tetramesa* sp. 4 therefore poses a potential risk to the following native Australian species: *E. parviflora*, *E. leptocarpa,* and *E. trachycarpa*; as such, it was given low priority as a potential agent for the biological control of *E. curvula* in Australia (Fig. 11).

Tetramesa sp. 5 was recorded on three non-target *Eragrostis* species under native field conditions (Table 4). *Tetramesa* sp. 5 completed larval development on *Eragrostis curvula, E. plana, E. gummiflua*, and *E. rigidiour. Eragrostis plana* and *E. biflora* are key phylogenetic proxies for native Australian *Eragrostis* species (Fig. 11). *Tetramesa* sp. 5 therefore also poses a potential risk to the following native Australian species: *E. parviflora*, *E. leptocarpa*, and *E. trachycarpa*; as such, *Tetramesa* sp. 5 was given low priority as a potential agent for the biological control of *E. curvula* in Australia (Fig. 11).

Figure 9: A) Exit holes made by emerging adult *Tetramesa* on *Eragrostis curvula*. B) Feeding galleries inside the tiller of *E. curvula* made by *Tetramesa* larvae. C) *Tetramesa* sp. 5 in the feeding gallery it made inside an *E. curvula* tiller. Photos taken by Liam Yell.

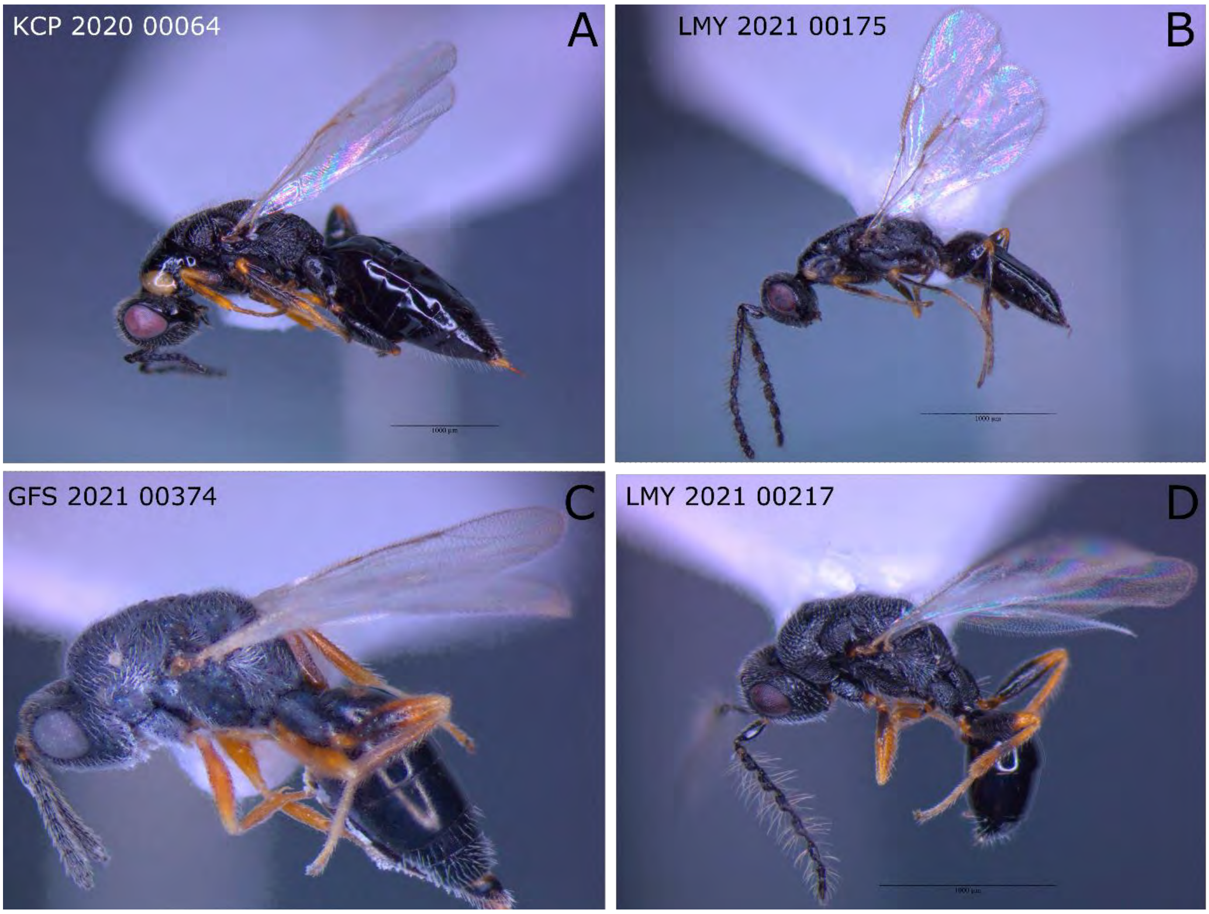

Figure 10: Photographs of pinned specimens. A) Female *Tetramesa* sp. 4 (collected by Dr. Kim Canavan). B) Male *Tetramesa* sp. 4 (collected by Liam Yell). C) Female *Tetramesa* sp. 5 (collected by Dr. Guy Sutton). D) Male *Tetramesa* sp. 5 (collected by Liam Yell). Photos taken by Clarke van Steenderen.

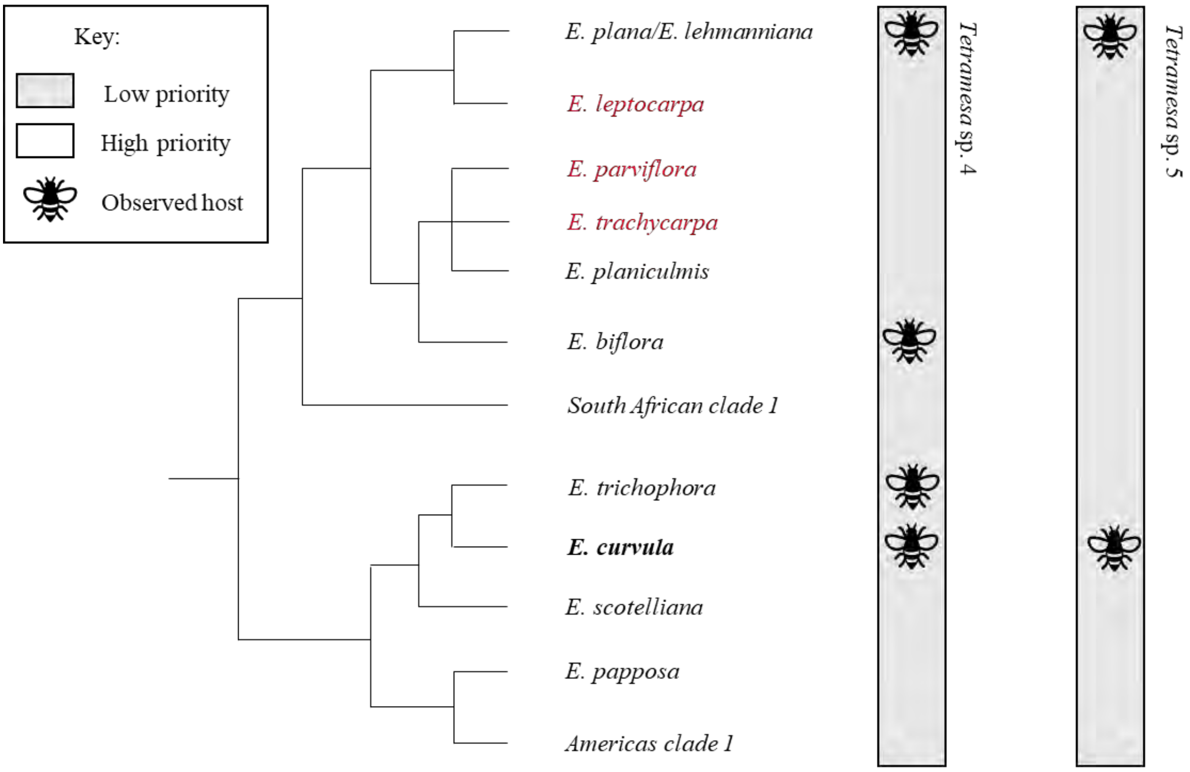

Figure 11: A schematic overview of how the two candidate agents (*Tetramesa* sp. 4 and sp. 5) were prioritised based on their predicted host range using a phylogenetic approach. Any African native *Eragrostis* species was defined as a 'phylogenetic proxy' and was used to infer the potential risk posed by prospective agents to Australian native *Eragrostis* species (indicated by red text in the figure). 'South African clade 1' and 'Americas clade 1' represent several species of *Eragrostis* native to each region.

Table 3: The grass species surveyed during this study, the reason/s they were included in the surveys, the number of sites they were surveyed in, and indication of the presence/absence of either of the *Tetramesa* species under native, field conditions in South Africa.

Plant species	Reason Tested					Field surveys		
	Target weed	Congener	Sympatry	Abundance	Structural analogue	N (sites)	*Tetramesa* sp. 4	*Tetramesa* sp. 5
Eragrostis curvula	**					13	**Yes**	**Yes**
Eragrostis capensis		**				5	**Yes**	No
Eragrostis planiculmis		**	**		**	2	No	No
Eragrostis plana		**	**		**	3	**Yes**	**Yes**
Eragrostis gummiflua		**	**			1	**Yes**	**Yes**
Eragrostis biflora		**	**	**		1	**Yes**	No
Eragrostis lehmanniana		**	**		**	3	No	No
Eragrostis rigidiour		**	**	**		2	**Yes**	**Yes**
Eragrostis tef		**	**	**		1	No	No
Eragrostis trichophora		**	**	**		3	**Yes**	No
Eragrostis chloromelas		**			**	1	No	No
Eragrostis superba		**	**			4	No	No
Sporobolus pyramidalis			**			4	No	No
Sporobolus fimbriatus			**			4	No	No
Sporobolus natalensis			**			1	No	No
Digitaria sp. 1			**			1	No	No
Hyparrhenia hirta			**	**		5	No	No
Chloris gayana			**	**		1	No	No
Megathyrsus maximus			**	**		5	No	No
Melinis repens			**			1	No	No
Digitaria eriantha			**			1	No	No
Cynodon dactylon			**			1	No	No
Eustachys paspaloides			**			1	No	No
Cymbopogon sp. 1			**			3	No	No
Andropogon gayanus			**			3	No	No

Heteropogon contortis	**	**	3	No	No
Urochloa mozambicensis	**	**	3	No	No
Setaria sp. 1	**		3	No	No
Themeda triandra	**	**	4	No	No
Arundo donax		**	1	No	No

Table 4: Summary of the insects associated with *Eragrostis curvula* in South Africa. *Coverage = proportion of sites each insect was recorded in. **Incidence = proportion of sampling events each insect was recorded in. Host range is only indicated for herbivorous insects.

Insect taxa			Feeding biology	Coverage*	Incidence**	Host range	Potential agent
Order	Family	Species					
Hymenoptera	Eurytomidae	*Tetramesa* sp. 4	Herbivore (Endophagous)	0.53	0.26	Oligophagous	Yes
	Eurytomidae	*Tetramesa* sp. 5	Herbivore (Endophagous)	0.6	0.3	Oligophagous	Yes
	Eulophidae	*Pediobius* sp. 30	Predator (Parasitoid)	0.46	0.2	-	No
Diptera	Sciaridae	Sciarid sp. 30	Non-herbivore (Detritivore)	0.26	0.13	-	No

3.4. *Tetramesa* spp. abundance on *E. curvula*

Overall, *Tetramesa* sp. 5 was more abundant (0.67 ± 1.81) than *Tetramesa* sp. 4 (0.5 ± 1.3) across all seasons and sites. *Tetramesa* species abundance differed significantly across the 5 sites ($X^2 = 22.47$, df = 4, $P < 0.01$) (Fig. 12). The post-hoc comparison showed that the significant difference occured between the Coombs (0.9 ± 1.6) and Penderry (0.04 ± 0.2) sites ($t = 3.95$, df = 242, $P < 0.01$). *Tetramesa* sp. 4 abundance was highest on average at the Coombs site (0.99 ± 1.5) and lowest at the Penderry site (0.04 ± 0.2). *Tetramesa* sp. 5 abundance was highest on average at the Ferndale site (1.5 ± 2.5) and lowest at the Penderry site (0.04 ± 0.2). There was no significant difference in *Tetramesa* species abundance across the seasons ($X^2 = 0.04$, df = 1, $P = 0.83$).

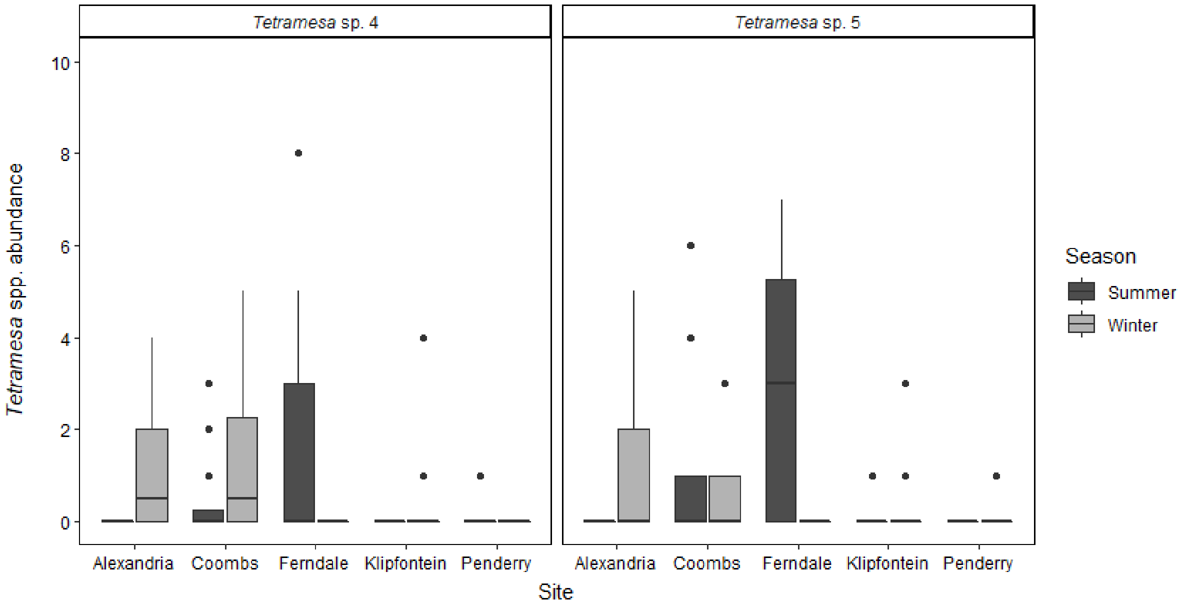

Figure 12: *Tetramesa* sp. 4 and sp. 5 abundances at each site across the seasons. Each site was sampled on 6 occasions (n = 3 sampling events in winter and in summer). Each sampling event consisted of allowing insect emergence from 200 tillers of *E. curvula* from each site for a duration of 28 days. *Tetramesa* spp. were identified to sp. 4 and sp. 5 morphospecies. The points above the bars represent extreme values (outliers) in the data.

3.5. Predicted efficacy

In total, 30 efficacy assessments were performed, 15 in winter and 15 in summer (six at each long-term repeat site). Only the *Tetramesa* species were included in the efficacy assessments because they were the two herbivorous species with potential as agents. There was no evidence for *Tetramesa* sp. 4 having any statistically significant effect on tiller survival ($X^2 = 0.11$, df = 1, $P >$

0.05) (Fig. 13a). The mean estimated probability of tiller survival ('P(surv)') when *Tetramesa* sp. 4 was present was 63% (95% CI: 0.61 – 0.71) and was 66% (95% CI: 0.44 - 0.79) when it was absent. *Tetramesa* sp. 5 had a statistically significant effect on tiller survival ($X^2 = 5.9$, df = 1, $P <$ 0.05) (Fig. 13b). *Tetramesa* sp. 5 presence was associated with an increased probability of tiller survival (Fig. 13b), whereby the P(surv) when *Tetramesa* sp. 5 was present was 76% (95% CI: 0.66 – 0.83) and was 64% (95% CI: 0.58 – 0.69) when it was absent. Although the statistical test results showed that *Tetramesa* sp. 5 presence may increase the probability of tiller survival, the confidence intervals for P(surv) when *Tetramesa* sp. 5 is present versus absent were largely overlapping which suggests a low degree of support for the significant result (Fig. 13).

There was no evidence for *Tetramesa* sp. 4 having any statistically significant effect on tiller reproduction ($X^2 = 1.2$, df = 1, $P > 0.05$) (Fig. 14a). The mean estimated probability of tiller reproduction ('P(seed)') when *Tetramesa* sp. 4 was present was 83% (95% CI: 0.65 – 0.93) and was 74% (95% CI: 0.70 – 0.77) when it was absent. There was also no evidence for *Tetramesa* sp. 5 having any statistically significant effect on tiller reproduction ($X^2 = 1.7$, df = 1, $P > 0.05$) (Figs. 14a, 14b). The P(seed) when *Tetramesa* sp. 5 was present was 79% (95% CI: 0.71 – 0.85) and was 73% (95% CI: 0.70 – 0.77) when it was absent. The confidence intervals are large, suggesting that the accuracy of predicting the effects of each species' presence/absence on tiller survival/reproduction is low. To determine if there was a cumulative effect of both *Tetramesa* species presences/absences on *E. curvula* tiller survival and reproduction, a two-way interaction term was attempted, but this model was rank deficient. Thus, the sample size was insufficient to fit this model.

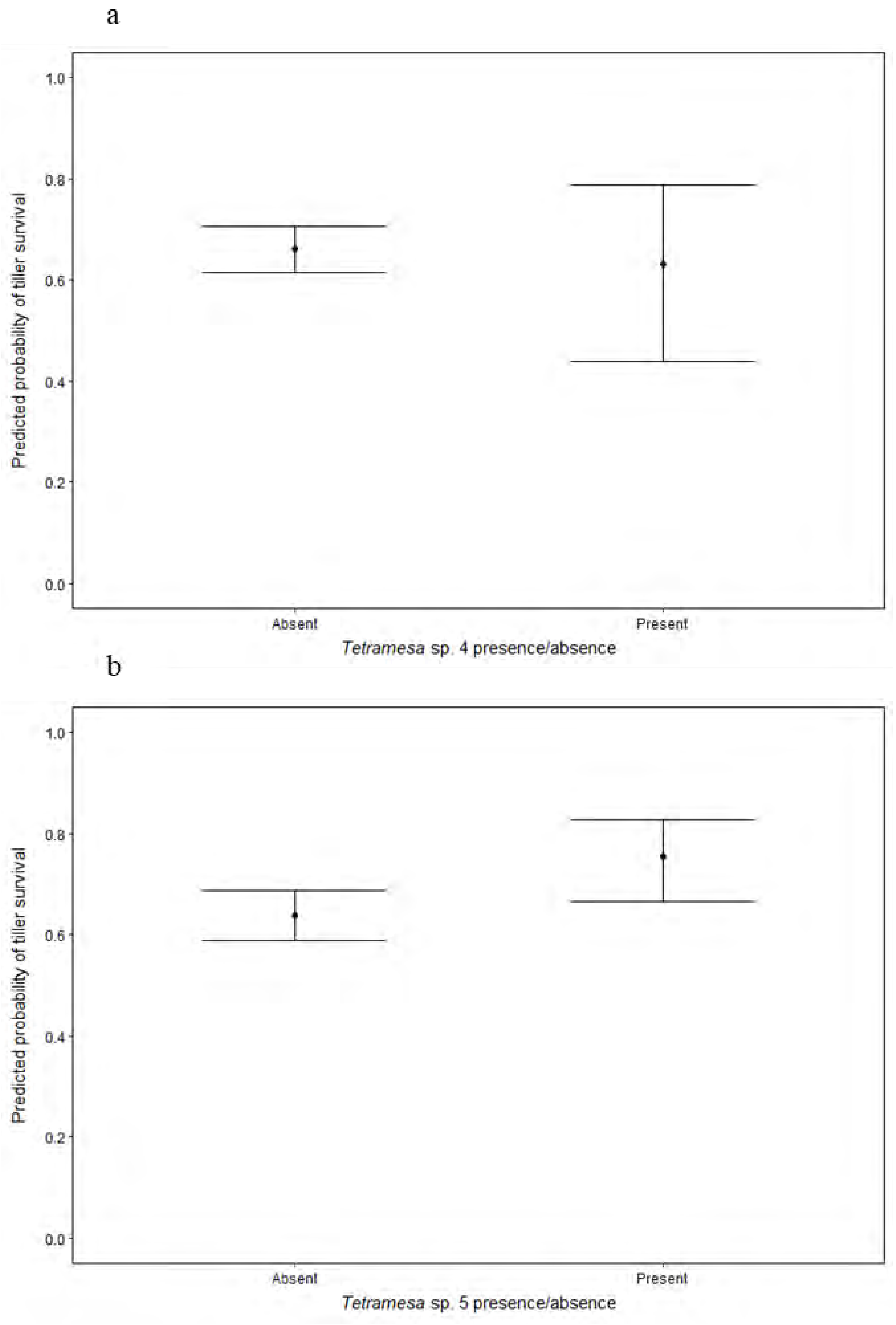

Figure 13: The probability of *Eragrostis curvula* tiller survival with respect to the presence/absence of the endophagous insects (a) *Tetramesa* sp. 4 and (b) *Tetramesa* sp. 5. The error bars represent 95% confidence intervals of the mean.

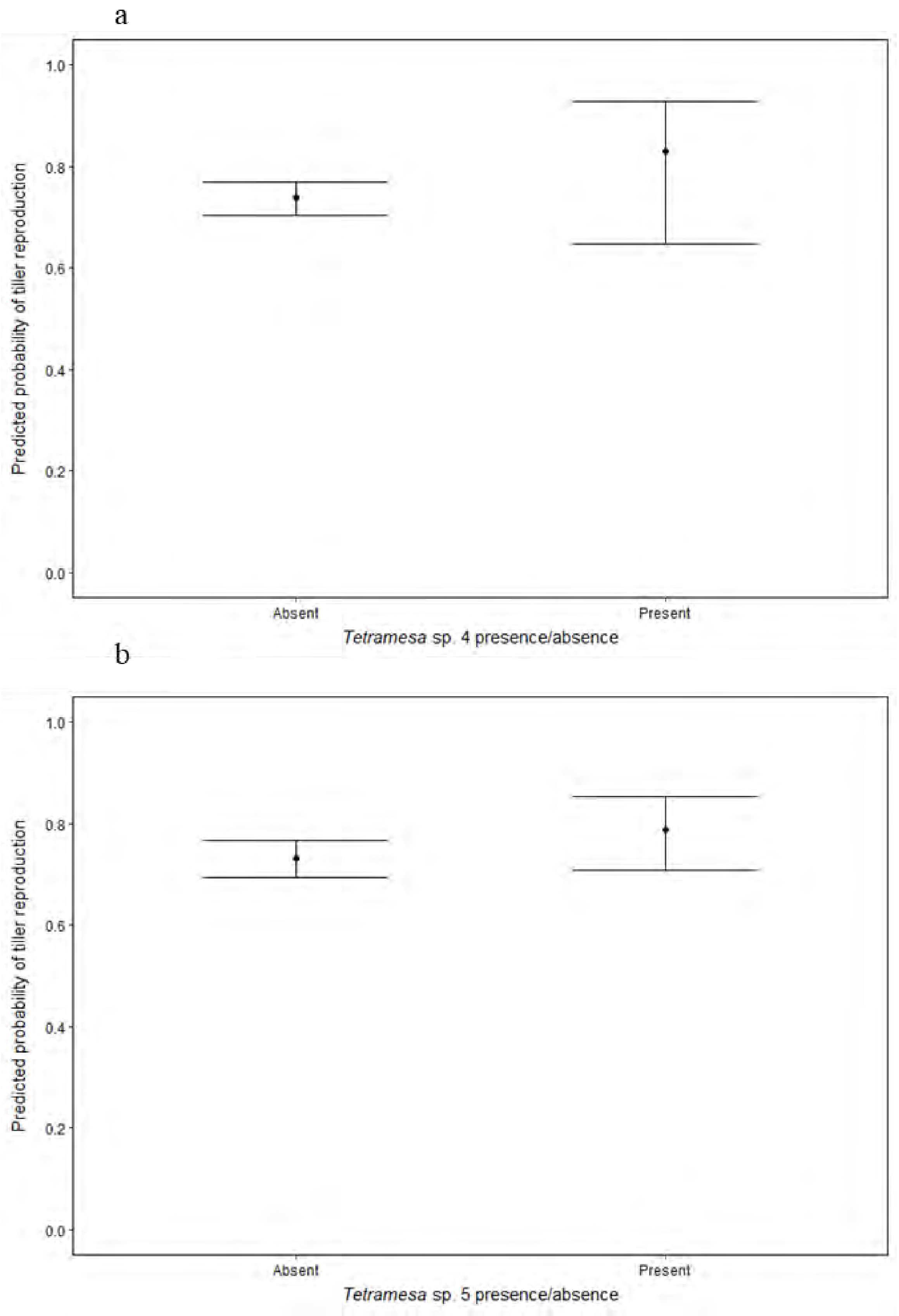

Figure 14: The probability of *Eragrostis curvula* tiller reproduction with respect to the presence of the endophagous insects a) *Tetramesa* sp. 4 and b) *Tetramesa* sp. 5. The error bars represent 95% confidence intervals of the mean.

3.6. Ecological niche modelling

MESS analyses showed that the MaxEnt models were extrapolating (MESS-) for a large portion of South Africa (Fig. 15a). The highest priority regions in South Africa were identified in areas where the MaxEnt models were extrapolating. MESS analyses for Africa also showed that MaxEnt was extrapolating for most of the continent, including the areas in south-west Kenya with the highest climatic similarity scores (Fig. 16a).

High priority regions (regions with the highest suitability scores) from which to collect climatically suitable prioritised insects were predicted in numerous geographic areas across the target weed's native range (Fig. 15b). The coastal regions of the Southern Cape (roughly from George to Nature's Valley) and of the western Eastern Cape (Storm's River to Cape St. Francis) had the highest climatic similarity (~ 0.7 – 0.75). Most of the Eastern Cape and KwaZulu-Natal have a climatic similarity of roughly 0.65 - 0.75. Eastern Mpumalanga and scattered regions of central Limpopo have similarity scores of 0.65 – 0.75 (Fig. 15b). Outside of South Africa, the regions with the highest climatic similarity to the invaded range of *E. curvula* was south-west Kenya (~ 0.75 – 1.0) (Fig. 16b) and south-west Uganda (~ 0.75 – 1.0) (Fig. 16b).

The field surveys reported on in section 2.2. were conducted primarily in highly climatically suitable areas so that any potential agents found would be expected to survive the climate in the plant's invaded range as they are climatically matched. For example, the five long-term repeat survey sites that were sampled every two months were set up in the Albany region of the Eastern Cape which is a highly suitable region (0.7 - 0.75).

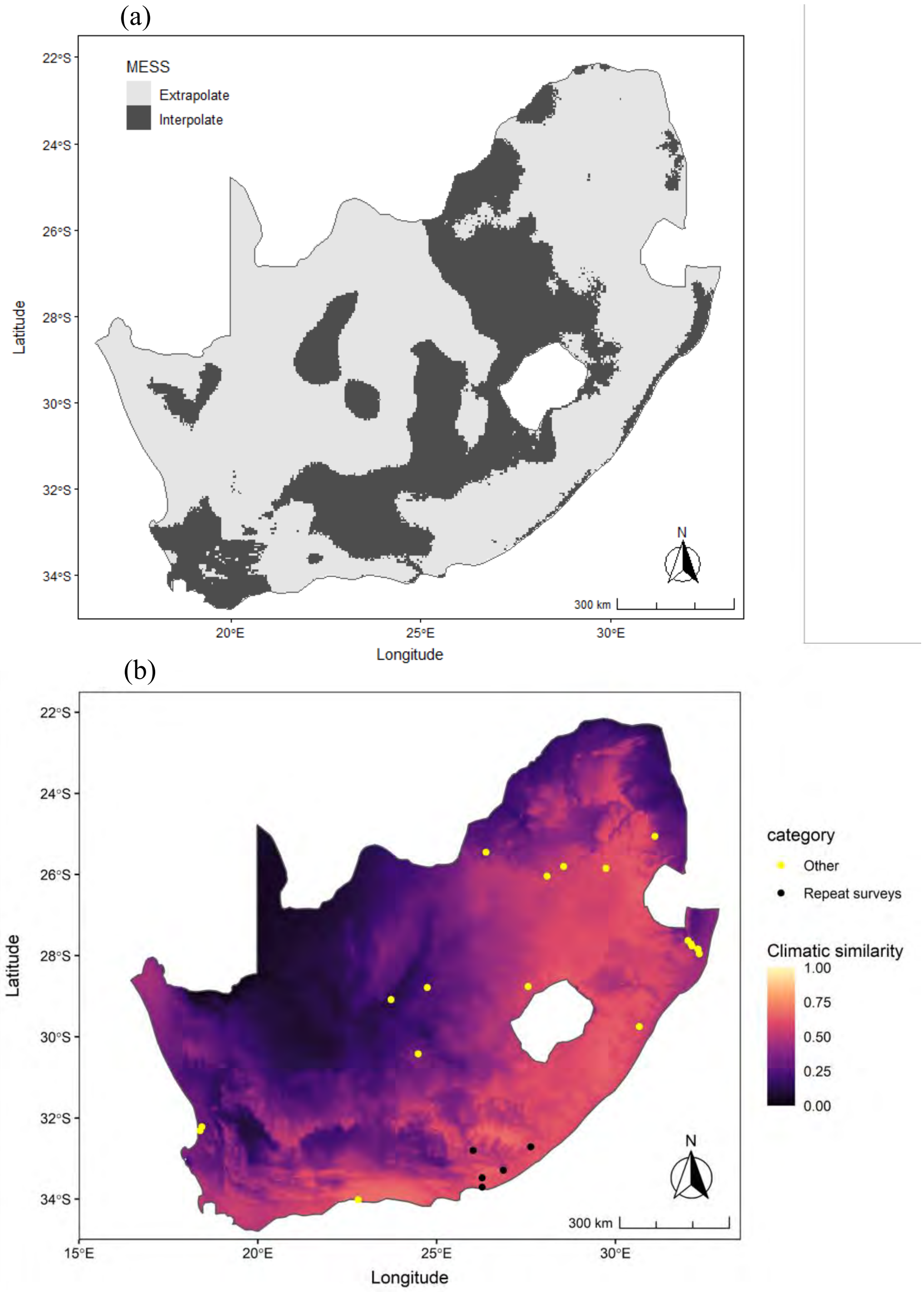

Figure 15: a) The Multivariate Environmental Similarity Surfaces (MESS) map that shows where the MaxEnt models were extrapolating/interpolating to produce climatic similarity scores in South Africa. b) Climatic similarity (models calibrated with invaded range occurrences) of the native range of *Eragrostis curvula,* GPS localities of the sites where surveys were conducted, black dots represent the five long term repeat survey sites and the yellow dots represent the other sites surveyed during this study. Warmer colours indicate geographic regions of higher priority (higher climatic similarity scores).

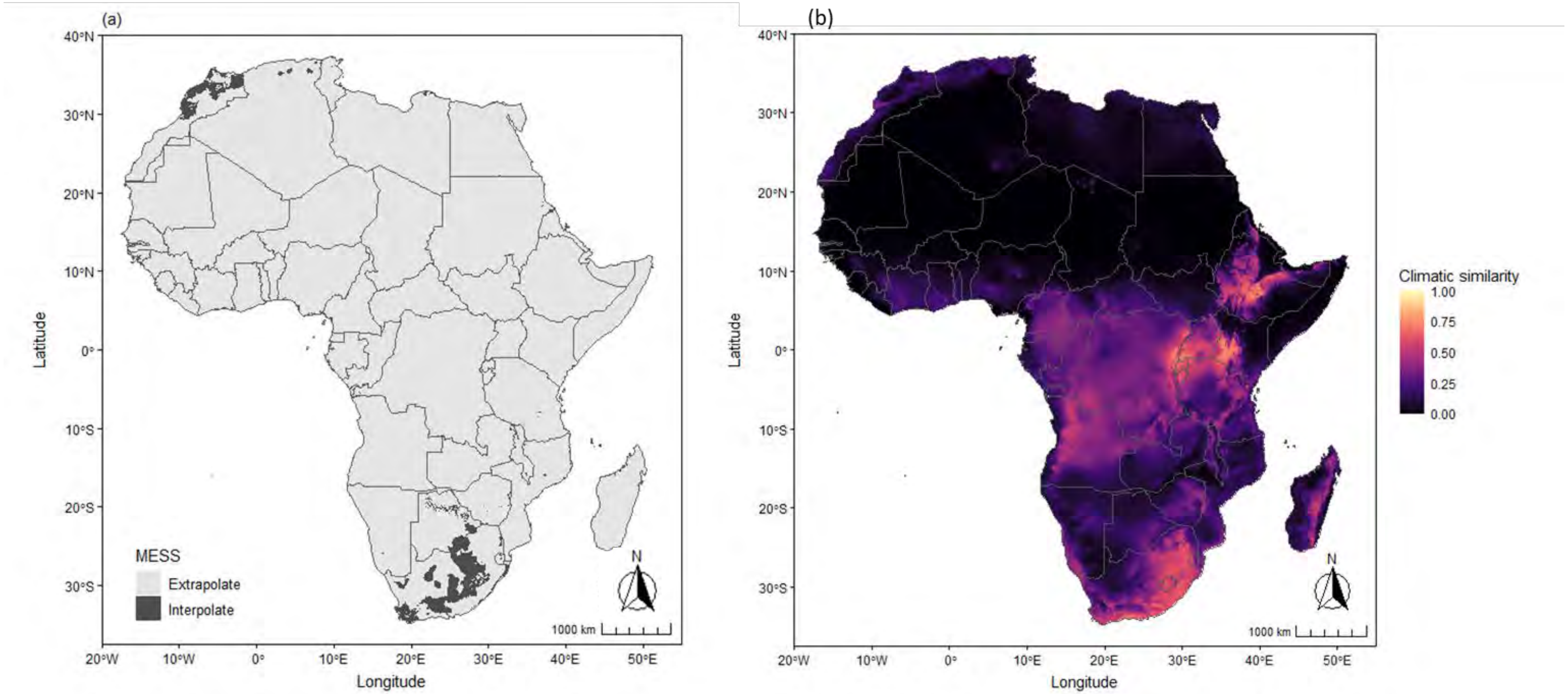

Figure 16: a) The Multivariate Environmental Similarity Surfaces (MESS) map that shows where the MaxEnt models were extrapolating/interpolating to produce climatic similarity scores across Africa. b) Climatic similarity (models calibrated with invaded range occurrences) of the native range of *Eragrostis curvula,* warmer colours indicate geographic regions of higher priority (higher climatic similarity scores).

Chapter 4: Discussion

There are very few biological control programmes against grasses relative to other weedy taxa. Globally, there are many invasive grass species threatening biodiversity and causing economic damage. In Australia, African grasses are prolific invaders, one of which is *Eragrostis curvula.* This thesis is the result of the first biological control programme against *E. curvula* globally. Field-based methods were used to compile a list of the herbivorous natural enemies associated with it in its native range and prioritise which of them should be developed as agents. In this chapter, the insects identified in this study and their potential as candidate agents to be used against *E. curvula* in Australia are discussed. Moreover, the importance of field-based host-specificity and predicted efficacy assessments and use of climate matching is highlighted.

4.1.1. Insect community structure

This study determined four insect species associated with the target weed, *E. curvula,* in its native range. Insect inventories associated with other weeds targeted for biological control are typically substantially larger. For example, Palmer and Pullen, (1995) reported 550 phytophagous insects associated with *L. camara* during field surveys in the weed's native range. Brazillian pepper (*Schinus terebinthifolius* Raddi; Anacardiaceae) has more than 150 phytophagous insects associated with it in its native range and *Calotropis procera* Aiton. (Apocynaceae) has 65 insects associated with it in its native range (Bennett et al., 1989; Dhileepan, 2014). Furthermore, the grass species *Phragmites australis* (Cav.) Trin. ex Steaudel (Poaceae) has 171 phytophagous insects associated with it in its native range (Tewksbury et al., 2002). Native range surveys can therefore yield large insect assemblages associated with the target weed, although, this isn't always the case.

Aside from *P. australis,* several grasses have yielded comparatively fewer insect species associated with them. Grasses targeted for biological control support relatively low numbers of herbivorous insects. For example, *A. donax* supports 20 phytophagous insects and *Calamagrostis epigeios* (L.) Roth (wood small-reed) has only 10 phytophagous insects associated with it (Dubbert et al., 1998; Tracy & DeLoach, 1998; Goolsby & Moran, 2009; Goolsby et al., 2009). Furthermore, native range surveys in Australia found a total of six herbivorous insects associated with *Hymenachne acutigluma* (Steud.) Guilliland, only four of which were native to Australia (Bell et al., 2011). *Sporobolis pyramidalis* and *S. natalensis* support a combined total of 15 natural enemies in their native range and two of which (both are *Tetramesa* species) were shown to be suitably host specific (Sutton et al., 2021). It is therefore not uncommon for grasses to support relatively small numbers of insects. However, a small number of phytophagous insects is not necessarily correlated to a lack of host-specific insects. For example, a total of 15 herbivores associated with *Pereskia aculeata* (Cactaceae) were found during native range field surveys yet it has two agents released against it in South Africa (Paterson et al., 2014).

Several studies have shown that there is a positive correlation between the architecture of the plant and insect species richness, whereby increased structural complexity of the plant results in increased species richness of associated insects (e.g., Strong et al., 1984; Tscharntke and Greiler, 1995; Forbes et al., 2017). This is known as the plant architecture hypothesis (Lawton, 1983). However, *A. donax* is arguably more structurally complex and larger than *P. australis* yet *A. donax* has significantly fewer insects associated with it (Tracy & DeLoach, 1998; Tewksbury et al., 2002). This suggests that there are several other factors that contribute to phytophagous insect species richness over and above plant size and architecture. Moreover, chemically defended plants are expected to support more specialised insect species (Cornell, 1989). Grasses are typically not heavily chemically defended and are therefore less likely to be associated with large insect assemblages (McNaughton et al., 1985). While *E. curvula* is substantially smaller than *A. donax,* it still demonstrates structural complexity as it forms large tussocks that are often dense with leaves and tillers in several stages of growth. Furthermore, each segment of the tiller is encased in a leaf sheath that extends into a leaf hanging from the tiller, much like *A. donax*. Thus, despite the structural complexity of large dense tussocks that *E. curvula* forms, there is a small insect assemblage associated with it. This might be attributed to its lack of chemical defences against herbivory and other abiotic factors.

Although the number of herbivores associated with *E. curvula* in its native range was small, this was not due to insufficient sampling. The completeness of sampling for species inventories can be defined as one of three categories: reasonable (>50% of total estimated richness), comprehensive (70-80%), and exhaustive (90-100%) (Cardoso et al., 2009). The sampling completeness of the insect community associated with *E. curvula* in South Africa was exhaustive (~ >90%). Two of the three non-parametric species richness estimators ('*boot*' and '*jack2*') indicated that the entire insect assemblage associated with *E. curvula* in South Africa was likely sampled. The third species richness estimator, '*chao2'* did not indicate that the sampling was exhaustive, however, the Chao index frequently overestimates species richness as it is more robust to undersampling (Bell et al., 2013). Typically, most insect inventories in search of potential agents in a weed's native range yield lower completeness values (30-70%) (Heard and Pettit, 2005; Bell et al., 2013). Sutton et al., (2021) also reported exhaustive sampling completeness values while searching for potential agents in the native range of other African grass species, *S. natalensis* and *S. pyramidalis*. The higher completeness values determined while sampling insects associated with African grass species may be attributed to the smaller herbivorous insect communities, relative to other plant species that have been targeted for biological control (Sutton et al., 2021).

While the insect community associated with *E. curvula* in South Africa is well sampled and further surveys in South Africa are unlikely to uncover new species, the native range for *E. curvula* extends through several other African countries. Sampling the entire assemblage of insect herbivores associated with the target weed in its native range is imperative for selecting effective agents (van Klinken & Raghu, 2006). Surveys in the native range outside of South Africa may produce some new species, especially considering that monophagous herbivores often occupy a narrower geographic range than oligo- or polyphagous herbivores and therefore may only occur in sporadic areas within the weed's native range (Strong et al., 1984). For example, Paterson et al., (2014) recorded *Phenrica guernini* Bechyné (Coleoptera: Chrysomelidae), a leaf-feeding flea-beetle, at only 31% of *P. aculeata* sites in one province in Brazil, despite extensive surveys across its native range across four provinces in Brazil and four countries in South America. Additionally, *Pereskiophaga brasiliensis* Anderson (Curculionidae), a stem-boring weevil, was also only recorded from one province in Brazil during the same surveys (Paterson et al., 2014). Despite the large geographic native range of *P. aculeata,* there was no overlap in the geographic ranges of *P. guerini* and *P. brasiliensis*. Furthermore, geographically isolated populations of *Parkinsonia aculeata* L. (Fabaceae) in its native range each supported significantly different insect assemblages (Bell et al., 2013). Additional surveys in other high priority areas (i.e., climatically matched areas) in African countries outside of South Africa are therefore recommended to ensure that the entire suite of possible agents is sampled. Ecological niche modelling (section 4.1.6) highlighted Uganda and Kenya as climatically-matched regions of high priority outside of South Africa in which to conduct future surveys. However, there are very few records of *E. curvula* in these regions which may make surveying these regions difficult. This additional sampling may increase the number of insect herbivores associated with *E. curvula* and potentially add to the list of candidate agents.

4.1.2. Candidate agent host-range

Host-range testing is comprised of determining both the fundamental host range and the realised host range of a natural enemy (Hinz et al., 2014; Schaffner et al., 2018; Hinz et al., 2019). The fundamental host range is composed of every plant species that a natural enemy can complete its life cycle on while the realised host range is composed of the plant species that the natural enemy actually uses in its life cycle under natural field conditions (Schaffner, 2001). No-choice tests are used to determine the fundamental host range of a natural enemy, wherein the natural enemy is exposed to only one plant species at a time (Schaffner, 2001; Hinz et al., 2014). Typically, no-choice tests are conducted under controlled laboratory conditions, under which the natural enemy is unable to exhibit its natural host selection behaviour (Schaffner, 2001; Hinz et al., 2014). The realised host range is determined under natural field conditions, wherein the natural enemy selects its hosts, exhibiting its natural host selection behaviour, from several possible host plants including the target weed (Hinz et al., 2014; Schaffner et al., 2018). It is necessary to assess the fundamental host range to determine whether the potential agents will attack non target species in the absence of its primary host. Traditionally, these assessments are performed under quarantine conditions, but quarantine space is limited, and time and space in quarantine is expensive (Paynter et al., 2015). This study made use of field-based methods to determine the realised host ranges of phytophagous insects associated with *E. curvula* to prioritise which should be subjected to fundamental host range testing.

The realised host-range of an insect is limited by environmental, ecological, and abiotic factors in addition to the fundamental host-range and is therefore more restricted than the fundamental host range (Fowler et al., 2012). Host-range tests can lead to 'false negatives' and 'false positives' (McFadyen et al., 2002). A 'false negative' is the result of underestimating the realised host range, i.e., released agents result in non-target attack in the introduced range that was not predicted (Marohasy, 1998). A 'false positive' is the result of overestimating the realised host range, i.e., predicting non-target attack in the agent's introduced range that does not actually occur (Marohasy, 1998). Many safe and effective agents would have been rejected if only the fundamental host range of potential agents was used in risk assessments due to 'false positives' (Hinz et al., 2014). Contrarily, a 'false negtaive' could result in the release of agents that are not safe (Marohasy, 1998). Including field-based methods to understand the realised host range of potential agents is beneficial when used alongside fundamental host range assessments to avoid both false negatives and false positives. However, environmental, and ecological factors can vary between the native and introduced ranges of the potential agent (Sutton et al., 2021). Thus, there is a possibility that the potential agent's realised host ranges may also differ, as such, traditional no-choice tests should be conducted to assess the fundamental host range of the potential agents (Sutton et al., 2021). The information gained from the field-based methods may reduce the number of test plants used in the traditional no-choice experiments, as only 'at-risk' plants need be tested (Lesieur et al., 2020; Sutton et al., 2021). In this study, novel candidate agents were ranked using field-host range data and molecular phylogenies to identify only those that have a high probability of meeting the host-specificity requirements to reduce the number of plants required for testing in quarantine.

In this study, field-based methods were used to determine the host range of herbivorous natural enemies associated with *E. curvula* within its native range in South Africa. Two herbivorous species, *Tetramesa* sp. 4, and *Tetramesa* sp. 5 were recorded on *E. curvula* but both species were recorded on multiple key phylogenetic proxies under native field conditions in South Africa. *Tetramesa* sp. 4 was found on 6 congeners, including: *Eragrostis capensis, E. plana, E. gummiflua, E. biflora, E. rigidiour,* and *E. trichophora*. *Tetramesa* sp. 5 was found on *E. plana, E. gummiflua*, and *E. rigidiour*. Neither *Tetramesa* species was found on grasses outside of the genus *Eragrostis.*

There are more than 70 Australian native species of *Eragrostis* (Palmer & Pullen, 1995; Simon & Alfonso, 2011). The phylogenetic relationships between the native African *Eragrostis* species were used to make host-use predictions using a phylogenetic approach. Three native Australian *Eragrostis* species are predicted to be at risk of attack by both *Tetramesa* species identified in this study: *E. parviflora, E. leptocarpa,* and *E. trachycarpa.* These three Australian grass species should be given priority in quarantine tests when testing the fundamental host range of the *Tetramesa* species identified in this study. Several studies have shown that the realised host range of a herbivore is smaller than its fundamental host range (Wapshere, 1989; Blossey et al., 2001; McFadyen et al., 2002; Paynter et al., 2004; Dudley & Kazmer, 2005; Center et al., 2007; Pratt et al., 2009; Medal & Cuda, 2010; Cristafaro et al., 2013). This does not, however, necessarily mean that native Australian *Eragrostis* species will definitely be attacked by *Tetramesa* sp. 4 and sp. 5 if they are released. While these predictions were made based on the premise that plant phylogeny is a primary driver of herbivore host-use (Wapshere, 1974; Gilbert & Webb, 2007; Gilbert et al., 2012), there are several other factors that may determine herbivore host-range. Plant structural traits, secondary chemical defences, relative host plant abundance, and presence of predators and parasitoids are all factors that may contribute to the host range of phytophagous insects (Bernays & Graham, 1988; Beccerra, 1997; Moffatt et al., 2013; Cripps et al., 2016). There is a risk posed to the three Australian native *Eragrostis* species that are closely related to the non - target species used by *Tetramesa* sp. 4 and sp. 5 in South Africa, however, the structural similarity of these non-targets to the target weed suggests that Australian plants that are structurally similar to *E. curvula* should also be prioritised for no-choice tests. Traditional no-choice testing can determine the full array of Australian native *Eragrostis* species that are included in the fundamental host range of *Tetramesa* sp. 4 and sp. 5, as such, the phylogenetic approach was intended only to prioritise which potential agents should be subjected to detailed host-range assessments.

The majority of the described > 200 species of *Tetramesa* are host specific, using a single grass species as a host (Dawah, 1987; Lotfalizadeh et al., 2020). *Tetramesa romana,* has been released in the USA for the biological control of *A. donax* and *T. amica* is currently being investigated as a biological control agent to be used against *Taeniatherum caput-medusae* (L.) Nevski (medusahead) as a result of their host specificity. However, it is not uncommon that

Tetramesa species will use two or more congeneric grasses as hosts and in very few cases *Tetramesa* may use closely related genera as hosts (Dawah, 1987; Lotfalizadeh et al., 2020). For example, *T. hyalipennis* (Walker.) uses *Elymus repens* (L.) as well as *E. farctus* (Viv.) as hosts and *T. eximia* (Giraud.) uses *Calamagrostis epigejos* (L.) Roth. and *Ammophila arenaria* (L.) Link. as hosts (Dawah, 1987). The undescribed *Tetramesa* species identified in this study appear to use only *E. curvula* and congeneric plants as hosts. This suggests that the fundamental and realised host range for both *Tetramesa* species may include Australian native *Eragrostis* species if they were released in Australia.

An abundance of structurally similar, closely related, or congeneric native/economically important species in the target weed's invaded range can make finding suitably host-specific natural enemies difficult. The more native congeners and closely related economically important plant species there are in the region where a biological control programme is intended to be implemented, the more restricted the host range of the agent needs to be. For example, an oligophagous moth, *Secusio extensa* (Butler) (Lepidoptera: Arctiidae) was safe to release against *Senecio madagascariensis* Poir. (Asteraceae) in Hawaii because the other Asteraceae present are also exotic (Egli, 2017). The same moth (*S. extensa*) could not be released in Australia because there are many Australian native *Senecio* species (McFadyen & Morin, 2012). Furthermore, the presence of several native congeners and economically important species in South Africa resulted in the rejection of most of the potential agents tested for use against *Solanum mauritianum* Scop. (Solanaceae), except *Gargaphia decoris* Drake (Hemiptera: Tingidae) and *Anthonomus santacruzi* Hustache (Coleoptera: Curculionidae) (Olckers, 2000; Venter at al., 2021). Contrarily, oligophagous agents are suitable for release against cacti in South Africa because there are no native Cactaceae present, except for *R. baccifera* (Paterson et al., 2011). Because of the presence of more than 70 native *Eragrostis* species in the invaded range of *E. curvula* (Australia), it is likely that only monophagous natural enemies would be acceptable to be released as biocontrol agents. *Eragrostis curvula* was therefore a target with a relatively low chance of finding a suitably host specific agent based on the number of congeners present in Australia, and the evidence from this study suggests that there are likely no natural enemies available in South Africa that are suitably host specific.

4.1.3. Predicted efficacy

Seasonal abundance information for a potential agent in its native range can indicate their potential efficacy in the introduced range and be beneficial for facilitating collections (Singh et al., 2022). In this study, insect species abundance was relatively conserved throughout the year, although the insect species abundance between the Coombs and Ferndale sites was significantly different. Both *Tetramesa* species in this study were prevalent in summer and in winter. This is not unusual as two species of *Tetramesa* associated with *S. pyramidalis* and *S. natalensis* were also found to be prevalent throughout the year in South Africa (Sutton et al., unpublished data). Like the *Tetramesa* species associated with *S. pyramidalis* and *S. natalensis*, the larval and pre-adult life stages of the *Tetramesa* species associated with *E. curvula* overwinter inside the tillers of their host plant. This suggests that *Tetramesa* species on grasses can be sampled/collected year-round. The *Tetramesa* larvae feed on vascular and structural tissues inside the tiller of *E. curvula*. Sustained damage caused by phytophagous insects that feed on vascular, photosynthetic, structural, or underground tissues is more likely to prevent recovery and cause mortality (Ireson et al., 2000; Sheppard & Smyth, 2012). The fact that *Tetramesa* sp. 4 and sp. 5 are present and abundant throughout the year is beneficial in terms of their predicted efficacy in Australia as the target weed will have no temporal escape from herbivory.

There is a demand for biological control practitioners to conduct pre-release efficacy assessments to identify and prioritise potential agents that inflict measurable damage on the target weed (van Klinken and Raghu, 2006; Milbrath et al., 2018). Because there are no benefits to the release of ineffective agents and given the costs of developing agents, it would be a waste of resources to release ineffective agents (McClay & Balciunas, 2005). The high numbers of seed produced by each inflorescence that grows on an *E. curvula* individual provides significant propagule pressure and facilitates the spread of dense *E. curvula* monocultures in Australia (Roberts et al., 2021). Both *Tetramesa* species feed on the vascular and structural tissue inside the tiller, which is the structure from which the inflorescence grows. Other *Tetramesa* species, including those associated with *S. pyramidalis* and *S. natalensis,* feeding on these tissues inside the tillers of their host plants result in sterilisation, whereby the tiller is killed before flowering or the inflorescence is malformed and seedless (Sutton et al., 2021). Their presence would therefore hinder the host plant’s ability to reproduce and spread, making them ideal candidate agents.

The potential efficacy of both *Tetramesa* species was assessed by sampling five sites in the Eastern Cape Province of South Africa every three months between 2021 and 2022. *Tetramesa* sp. 4 had no significant effect on either the tiller reproduction or survival, while *Tetramesa* sp. 5 caused a significant increase in tiller survival but did not have a significant effect on the probability of tiller reproduction. However, the overlapping confidence intervals provide little support for this result. The positive correlation between the presence of *Tetramesa* sp. 5 and probability of tiller survival may be attributed to the ability of *E. curvula* to produce tillers rapidly in response to stress (Lazarides, 1997; Roberts et al., 2021 and references therein). Grasses have also been shown to compensate for stresses imposed on them (e.g., grazing pressures) by growing at an increased rate or producing more leaves and/or tillers per plant (Oesterheld & McNaughton, 1991). The

Tetramesa species associated with *S. pyramidalis* and *S. natalensis* had a cumulative effect on their host plants, whereby the presence of both species significantly reduced the host plant survival and reproduction probability (Sutton et al., 2021). There was also no inter-specific interaction found between *Tetramesa* sp. 4 and sp. 5 (Sutton et al., 2021). *Tetramesa* species can often be highly damaging to their host plants, but the two *Tetramesa* species identified in this study don't appear to possess the damaging potential that would make them suitable to be developed as agents. However, the overlapping confidence intervals (particularly for the effects of *Tetramesa* sp. 4 on tiller survival/reproduction) suggest an inaccurate ability to predict the effects of *Tetramesa* spp. presence/absence on tiller survival/reproduction. A possible explanation for this inaccuracy may be the relatively low number of tillers occupied by *Tetramesa* during the predicted efficacy assessments (i.e., a sample size issue). As such, it is recommended that damage surveys continue before final conclusions are drawn. Potential efficacy assessments of *Tetramesa* sp. 4 and sp. 5 can be re-evaluated following the conclusion of host-specificity studies, although the results from this study indicate that they may not be sufficiently damaging even if they are shown to be host specific. However, the high incidence and coverage of *Pediobius* sp. 30, a parasitoid of *Tetramesa*, suggests that *Tetramesa* sp. 4 and sp. 5 are heavily parasitised in their native range. Parasitised *Tetramesa* larvae are likely to cause substantially less damage to their host plant prior to their death, relative to healthy *Tetramesa* larvae. High levels of parasitism experienced by both *Tetramesa* species in their native range could mask the levels of damage they cause to *E. curvula,* resulting in an under-estimation of their damage potential. Furthermore, *Tetramesa* sp. 4 and sp. 5 population densities might be heavily regulated by their parasitoids in their native range. Therefore, if *Tetramesa* sp. 4 and sp. 5 are introduced to Australia and, as such, released from *Pediobius* sp. 30, they could reach higher population densities. The *Tetramesa* species associated with *S. pyramidalis* and *S. natalensis* had density-dependant damaging effects on their host plants (Sutton et al., 2021). This suggests that higher abundances of *Tetramesa* sp. 4 and/or sp. 5 may result in more significant damage inflicted on their host plant.

4.1.4. Ecological niche modelling

Agents found in climatically similar regions in the target weed's native range are expected to be more likely to become established in the invaded range and thus, become successful agents (Robertson et al., 2008). Climatically matched regions are identified by using climate matching software to identify geographic regions in the weed's native range that are climatically similar to the climate of the invaded range (e.g., Mukhurjee et al., 2011; Scott et al., 2016; Sutton, 2019). Although climate matching has faced criticism as some climatically similar regions may not contain host-plant populations (Robertson et al., 2008), the native range of *E. curvula* in South Africa is extensive, covering the majority of the country, and falls within most of the regions deemed climatically suitable.

Many of the sites included in this study fell within areas that were deemed climatically suited to the areas where the agents would be released in Australia. The southern coast of South Africa (George – Cape St. Francis), and inland Eastern Cape (Alice and Fort Beaufort) were identified as climatically suited areas. It may be beneficial to collect any agent populations for release in these regions as they do host healthy populations of *E. curvula*. There are however many areas outside of the region that was sampled that are also climatically suited to the invaded range of *E. curvula.* An effort was made to sample *E. curvula* at sites throughout its native range, covering as much of its distribution within South Africa as possible across wide climatic regions. While it is important to prioritise potential agents that can survive the climate of the invaded range, monophagous herbivores often occupy smaller biogeographic distributions than their oligophagous and polyphagous counterparts and may only occur in portions of the weed's native range (Strong et al., 1984). Therefore, it is advisable to perform surveys across as much of the weed's native range as possible while prospecting for new potential agents. MaxEnt identified south-west Kenya and south-west Uganda as regions with high climatic similarity to the invaded range of *E. curvula.* Uganda, however, is not included within the native range of *E. curvula* and as such, it is unlikely that large populations will be found here (Roberts et al., 2021). Given the magnitude of the climatic similarity scores for south-west Kenya, it is highly recommended to conduct surveys in this region as this may identify additional candidate agents. Furthermore, other techniques, such as genetic matching, could also be useful to identify regions of the target weed's native range that should be sampled for prospective biocontrol agents, if further surveys are required (Paterson et al., 2009; Gaskin et al., 2011).

Several authors have criticised the use of species distribution models for invasive species distribution modelling due to model extrapolation (Elith & Leathwick, 2009; Elith et al., 2010). MaxEnt can begin to extrapolate when the models are projected to climatic space outside of the range of the environmental data used to calibrate the model (Elith et al., 2010; Webber et al., 2011). Most of the climatically suitable regions identified by the MaxEnt models were in extrapolation space (Fig. 15a, 16a). Typically, this would call for some caution in drawing inferences for practical implications from these data (Elith and Leathwick, 2009; Elith et al., 2010). However, it is well known that many IAP's, including *E. curvula*, occupy climatic niches in their invaded range that are outside the climatic niches of their native range (Gallagher et al., 2010; Atwater et al., 2018). As such, despite model extrapolation in these areas, we believe that these predictions are valid and relatively robust.

4.2. Implications for biological control

This thesis identified two species of *Tetramesa* previously unknown to science that are associated with *E. curvula*. Sutton et al. (2021) also identified two previously unknown *Tetramesa* species associated with *S. pyramidalis* and *S. natalensis*. Surveys performed on several other native African grass species during this research has resulted in the discovery of several *Tetramesa* species native to Africa (Sutton et al., 2021). At least four *Tetramesa* species are currently being formally described by the relevant taxonomic expert, Prof. Hossein Lotfalizadeh (Plant Protection Research Department, East-Azarbaijan Agricultural and Natural Resources Research and Education Centre, *pers. comm.*). Prior to the *Tetramesa* species identified in this research and those identified by Sutton et al. (2021), only four *Tetramesa* species were known from the African continent (van Noort, 2022). *Tetramesa* is a genus that is well known for host-specific species, although in some instances, this may not be the case (Phillips, 1936; Dawah, 1987; Lotfalizadeh et al., 2020). *Tetramesa* appear to have co-evolved and radiated with grasses (Dawah, 1987). The diversity of Poaceae and the discovery of several new species of *Tetramesa* in African grasses suggests that there are potentially many more species to be discovered, as insect communities associated with grasses are poorly studied (Sutton et al., 2019). The success of some species of *Tetramesa* as grass agents (e.g., *A. donax*), and the discovery of several new *Tetramesa* species on African grass species, is promising for new biological control programs against African grass species.

The natural enemies associated with *E. curvula* in its native range have been surveyed and assessed for their suitability as candidate agents. Agent prioritisation encompasses several aspects to consider for assistance in the decision-making process, as selecting agents is not an insignificant task (Blossey, 1995). These aspects include host-specificity, predicted efficacy, and geographic distribution (Harris, 1973; Goeden, 1983; Wapshere, 1985; van Klinken and Raghu, 2006). Endophagous natural enemies are more likely to demonstrate higher degrees of host specificity as they have had to co-evolve with their host plants to overcome its structural and/or chemical defences (Cornell, 1989; Hardy and Cook, 2010; Cronin et al., 2015). Endophagous feeders, such as the two *Tetramesa* spp. identified in this study, would therefore usually be considered as good candidate agents. Furthermore, endophagous feeders typically consume tissues that result in damage to their host plant (e.g., consuming vascular tissue disrupts nutrient transport within the plant), which links to their predicted efficacy (Paterson et al., 2014). Herbivores with wide geographic ranges are capable of surviving an array of climatic and biogeographic elements (Bell et al., 2013), and are therefore more likely to become established in the introduced range, especially if collected from regions in the native range that are deemed climatically suited. Thus, herbivores that are host specific, damaging to their host, and geographically widespread should be prioritised as potential agents.

There are several Australian native species of *Eragrostis* that are closely related to *E. curvula,* however, three were found to be at risk of potential attack by both *Tetramesa* spp. (*E. trachycarpa, E. leptocarpa,* and *E. parviflora*). The phylogenetic proxies used as hosts in South Africa indicate a host-range for both *Tetramesa* species that is likely to include Australian *Eragrostis* species, and it is recommended that both be given low priority as potential agents for the biological control of *E. curvula*. The native range distributions of the three at-risk Australian native *Eragrsotis* species have substantial overlap with the invaded range of *E. curvula* (Simon & Alfonso, 2011). Moreover, *E. trachycarpa* is structurally similar to *E. plana* and *E. lehmanniana, E. parviflora* is structurally similar to *E. curvula,* and *E. leptocarpa* is structurally similar to *E. curvula* and *E. trichophora.* As such, it is expected that these Australian native *Eragrostis* species will be included in the host range of *Tetramesa* sp. 4 and sp. 5. It is therefore recommended that *E. trachycarpa, E. leptocarpa,* and *E. parviflora* be given priority while testing the fundamental host-range of the *Tetramesa* species in quarantine. Focussing the laboratory-based no-choice tests on the three at-risk Australian native *Eragrostis* species could substantially reduce the time, effort, and money used to determine the suitability of the two *Tetramesa* species as agents. However, to avoid a ‘false-positive’ result, choice testing should also be included.

If neither *Tetramesa* species is suitably host-specific, the biological control program should focus on finding new candidate agents outside of the regions that were sampled in this study. If subsequent surveys do not identify new candidate agents, other control methods are the only option. Mites or fungal pathogens should also be investigated as potential agents, and because they weren’t included in this study, surveys to identify them can be performed in South Africa.

The wide geographic range and the number of sites *Tetramesa* sp. 4 and sp. 5 were recorded in indicates that they can endure a broad range of abiotic climatic factors, which coupled with their presence in climatically similar regions to the target weed’s invaded range, infers that they have a high potential to become established in Australia. While both species are stem-boring, endophagous herbivores that consume both vascular and structural tissue, predicted efficacy assessments revealed that the presence of either *Tetramesa* species caused no significant damage to *E. curvula,* while *Tetramesa* sp. 5 abundance, in fact, increased the probability of tiller survival. Although these data were not very well supported, they do suggest that even if the two *Tetramesa* species are suitably specific, they may not be suitably damaging.

Candidate agent prioritisation efforts have included scoring systems that require detailed information about the life cycles, distributions, percentage parasitism, and host-specificity of the candidate insects (Goeden, 1983; Harris, 1973). More empirical approaches have been developed as well, wherein the herbivory type, and frequency required to effectively control target weeds is assessed (van Klinken & Raghu, 2006). These approaches require data that can only be collected from laboratory-based tests and are therefore inappropriate for agent prioritisation using field-based assessments (Sutton et al., 2021). The high costs of quarantine can be mitigated by only using it for assessments on potential agents that have some quantifiable inferred suitability, i.e., as a function of host-specificity and predicted efficacy (Sutton et al., 2021). A standardised approach to ranking potential agents based on the inferred suitability would be highly valuable to those who practice weed biological control (Sutton et al., 2021). This thesis shows that field-based methods can be used effectively to reduce the time required in quarantine by prioritising novel candidate agents using field-host range data, field-based damage surveys and ecological niche modelling.

4.3. Conclusion

This study used field-based methods to identify and prioritise potential biological control agents for *E. curvula* in Australia. The methods used in this study could assist in other biological control programmes against novel targets, by reducing costs usually incurred at the start of a biological control programme from importing, culturing, and performing fundamental host range and efficacy tests of potential agents that do not have the required level of host specificity. This study highlighted that the field-based methods are effective at identifying insects that may not be suitable for use as biological control agents. Although fundamental host-range tests are still required to ultimately determine if the potential agents identified in this study are suitable agents, few insects and relatively few plants will be required for this process. Thus, the methods used in this study are useful to reduce the costs associated with, and time required for quarantine-based host range testing.

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Appendix 1 – Model diagnostics

Insect species richness

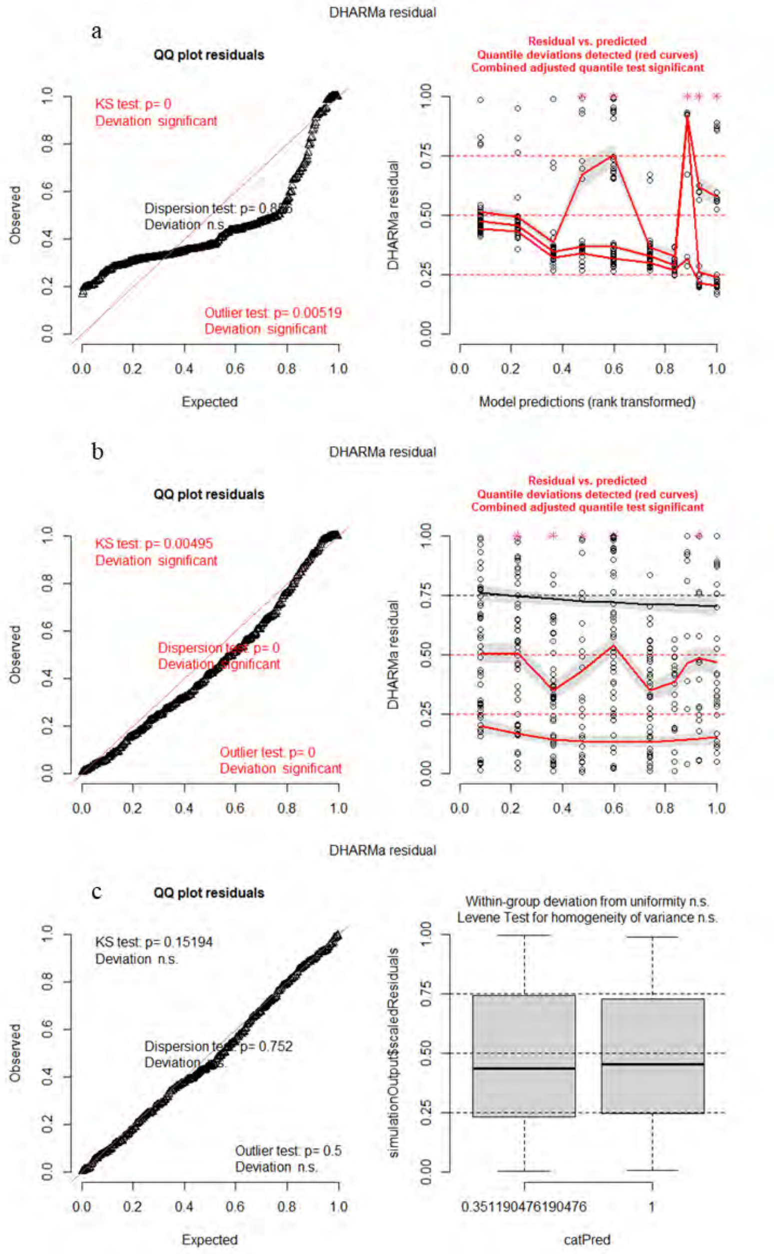


Fig. S1 Model diagnostics results (QQ plot residuals and Residuals vs. Predicted) for the GLMM's used in the analyses. a: insect species richness GLMM fitted with a Gaussian distribution, b: insect species richness GLMM fittted with a Poisson distribution, and c: insect species richness GLMM fitted with a negative-binomial distribution (the model of best fit).

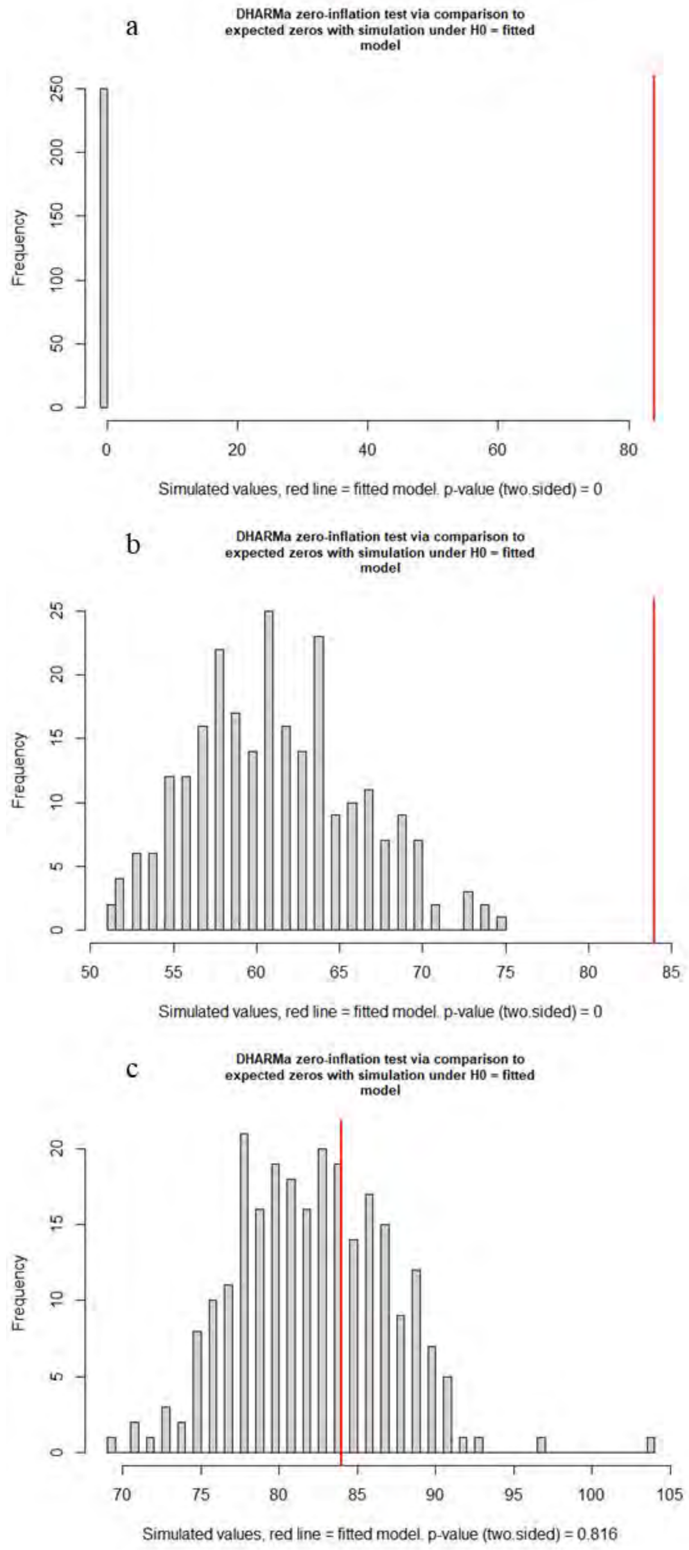


Fig. S2 Further model diagnostics; DHARMa zero-inflation plots. a: Insect species richness GLMM fitted with a Gaussian distribution. b: insect species richness GLMM fitted with a Poisson distribution, and c: insect species richness GLMM with a negative-binomial distribution (the model of best fit).

Insect species abundance

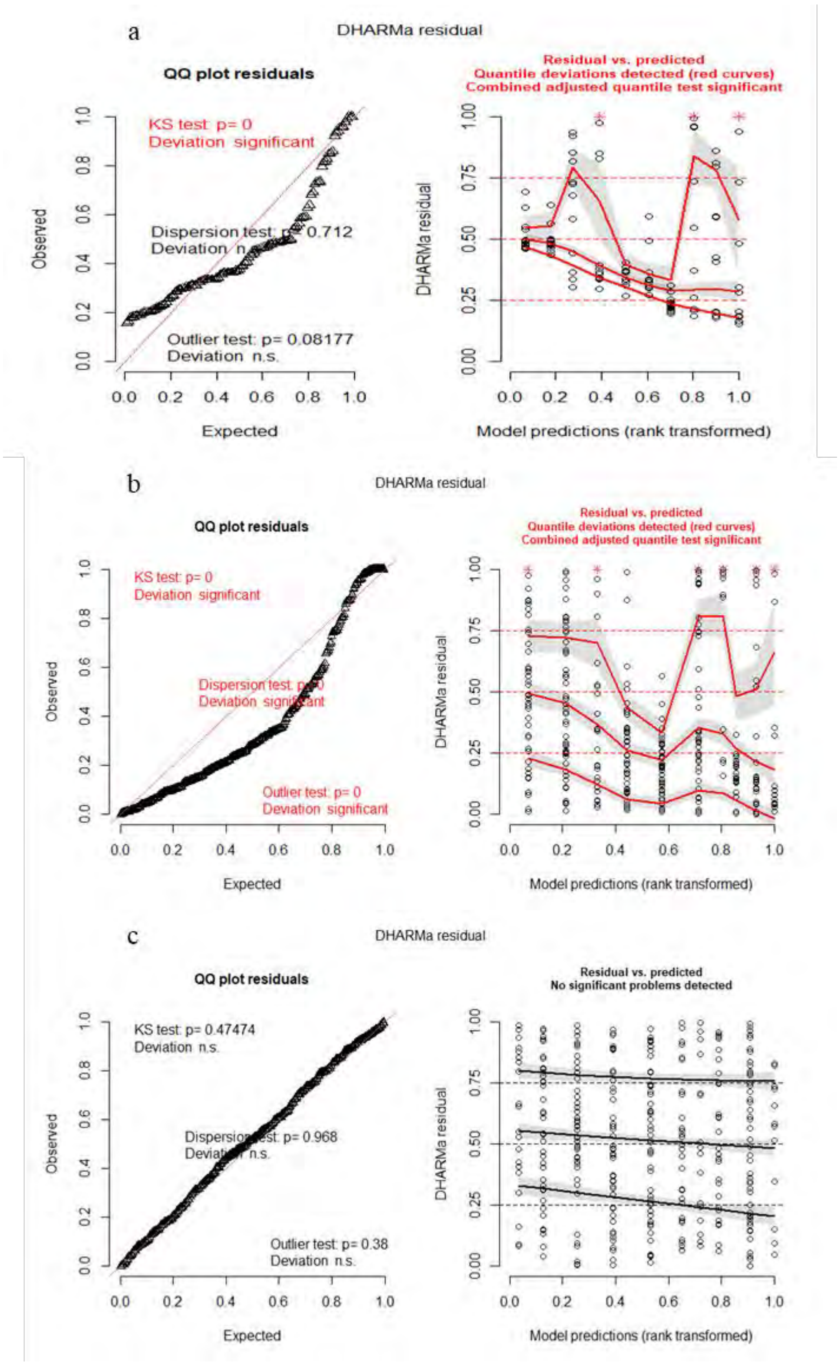


Fig. S3 Model diagnostics results (QQ plot residuals and Residuals vs. Predicted) for the GLMM's used in the analyses. a: insect species abundance GLMM fitted with a Gaussian distribution, b: insect species abundance GLMM fittted with a Poisson distribution, and c: insect species abundance GLMM fitted with a negative-binomial distribution (the model of best fit).

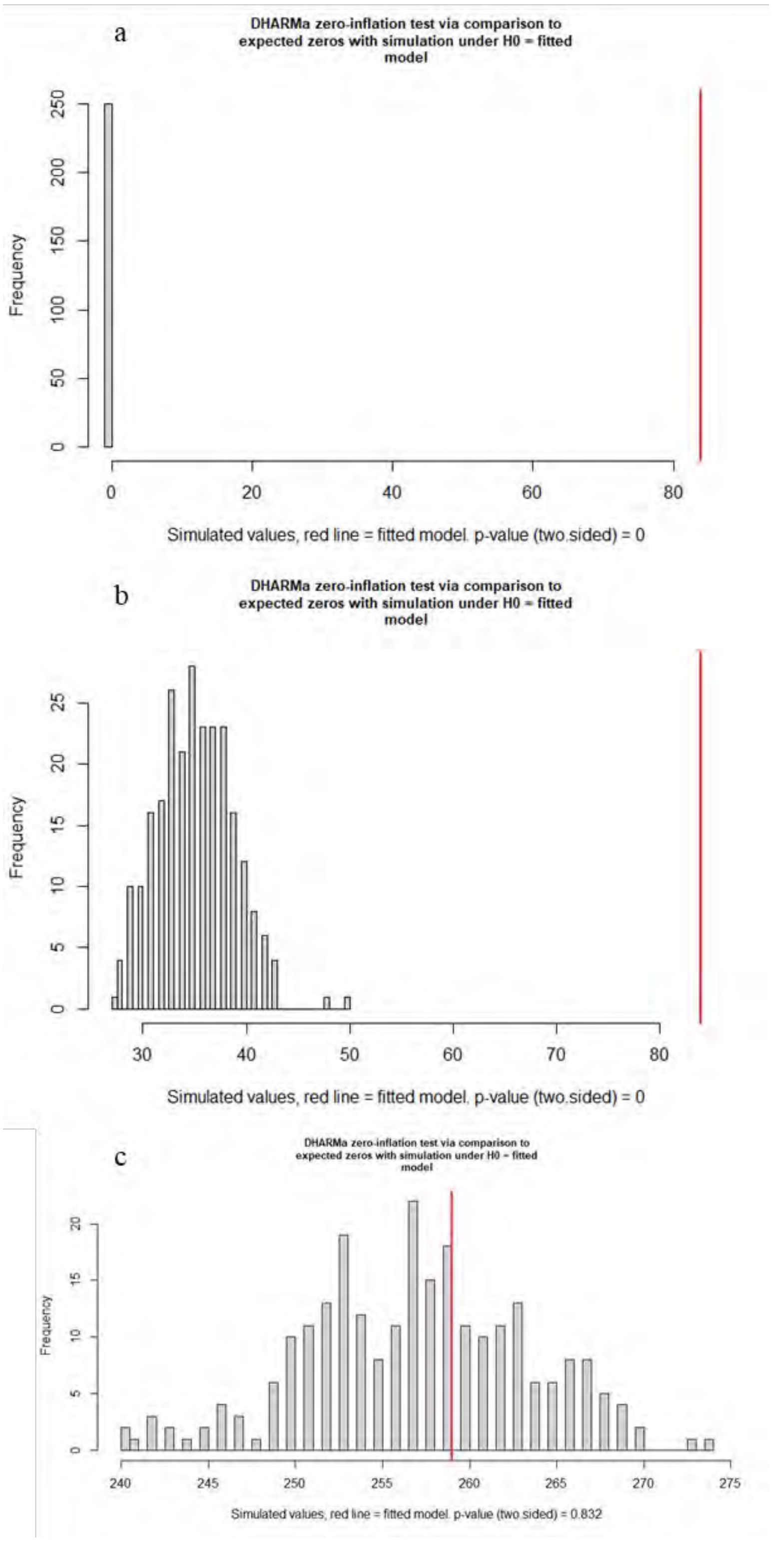


Fig. S4 Further model diagnostics; DHARMa zero-inflation plots. a: Insect species abundance GLMM fitted with a Gaussian distribution. b: insect species abundance GLMM fitted with a Poisson distribution, and c: insect species abundance GLMM with a negative-binomial distribution (the model of best fit).

Tetramesa species abundance

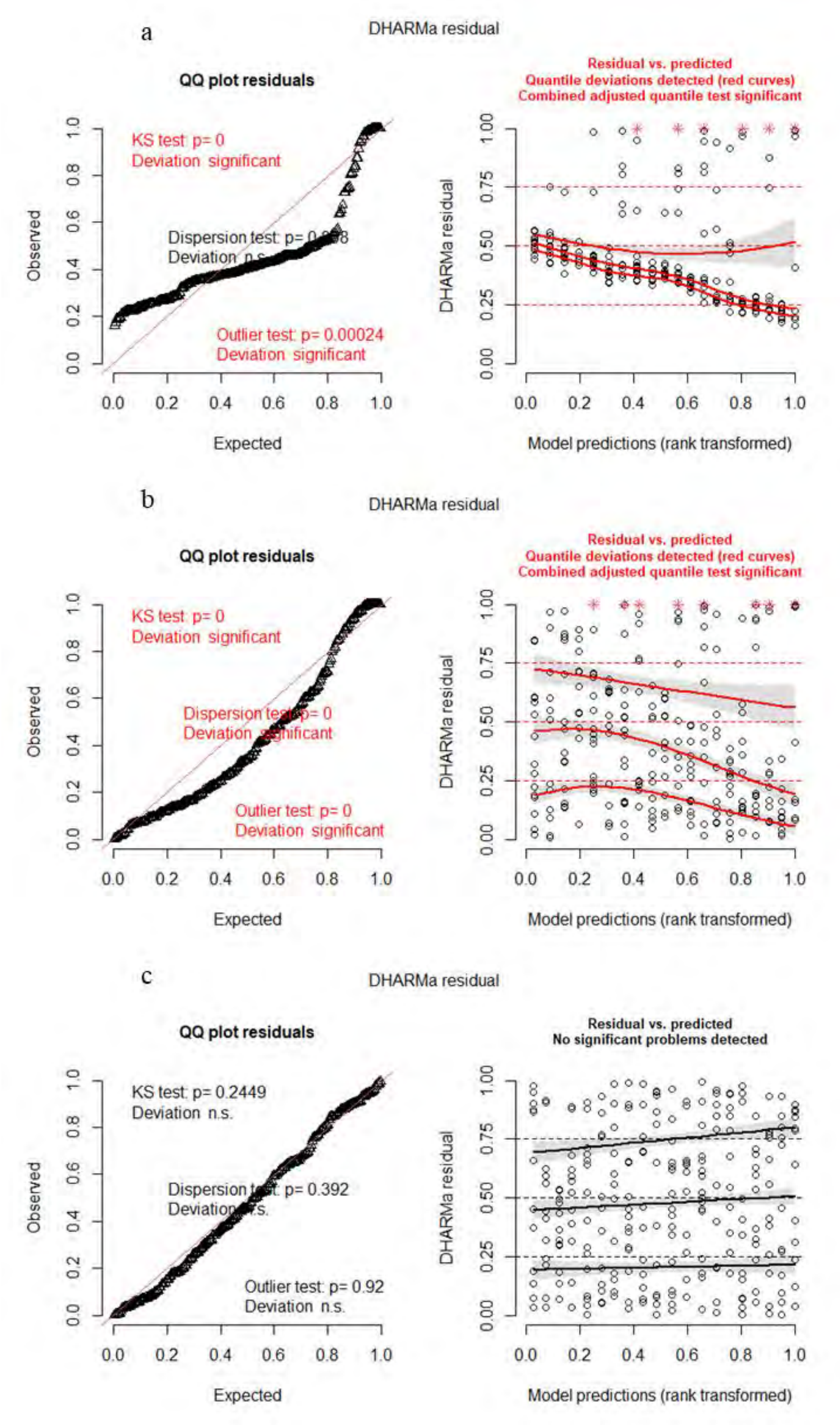


Fig. S5 Model diagnostics results (QQ plot residuals and Residuals vs. Predicted) for the GLMM's used in the analyses. a: *Tetramesa* species abundance GLMM fitted with a Gaussian distribution, b: *Tetramesa* species abundance GLMM fittted with a Poisson distribution, and c: *Tetramesa* species abundance GLMM fitted with a negative-binomial distribution (the model of best fit).

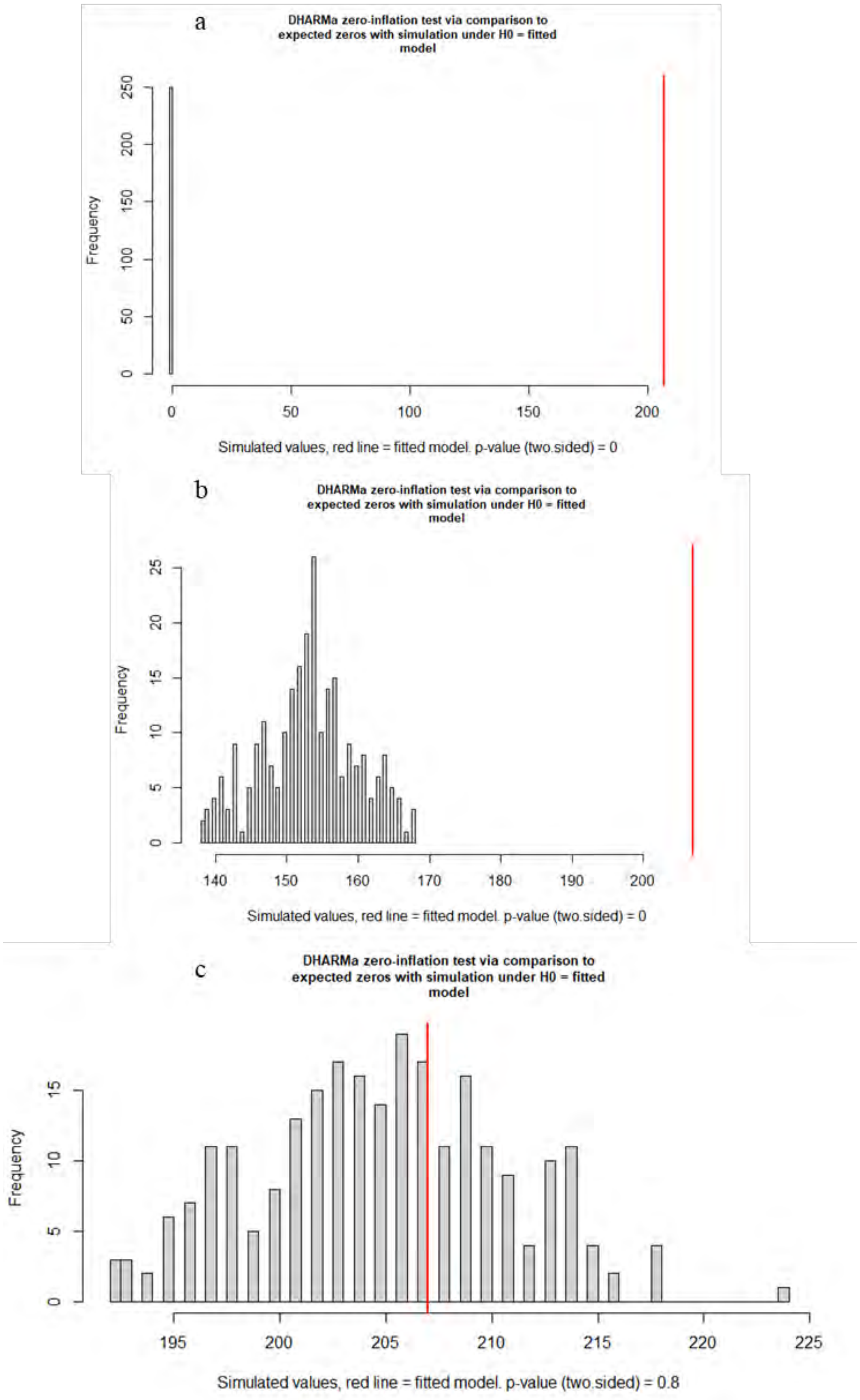
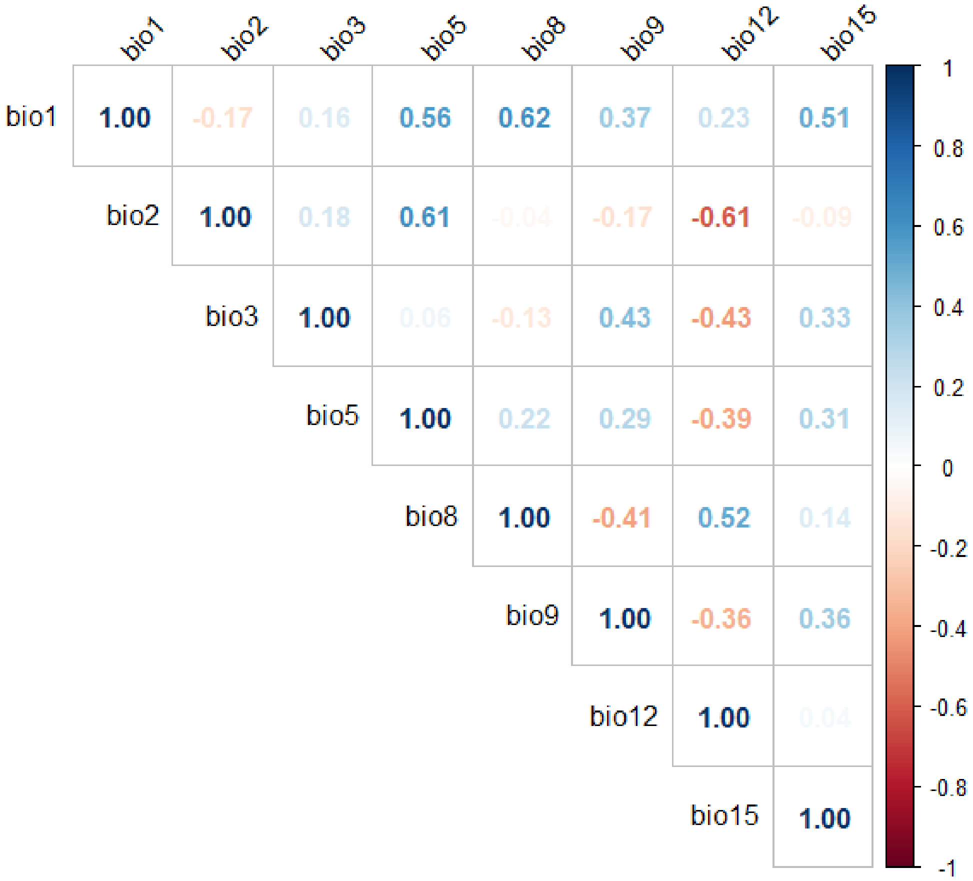


Fig. S6 Further model diagnostics; DHARMa zero-inflation plots. a: *Tetramesa* species abundance GLMM fitted with a Gaussian distribution. b: *Tetramesa* species abundance GLMM fitted with a Poisson distribution, and c: *Tetramesa* species abundance GLMM with a negative-binomial distribution (the model of best fit).

Appendix 2 – Multicollinearity analysis



	bio1	bio2	bio3	bio5	bio8	bio9	bio12	bio15
bio1	1.00	-0.17	0.16	0.56	0.62	0.37	0.23	0.51
bio2		1.00	0.18	0.61	-0.04	-0.17	-0.61	-0.09
bio3			1.00	0.06	-0.13	0.43	-0.43	0.33
bio5				1.00	0.22	0.29	-0.39	0.31
bio8					1.00	-0.41	0.52	0.14
bio9						1.00	-0.36	0.36
bio12							1.00	0.04
bio15								1.00

Fig. S7 Correlations between potential environmental predictor variables used to calibrate *Eragrostis curvula* MaxEnt models.

Appendix 3 – MaxEnt response curves

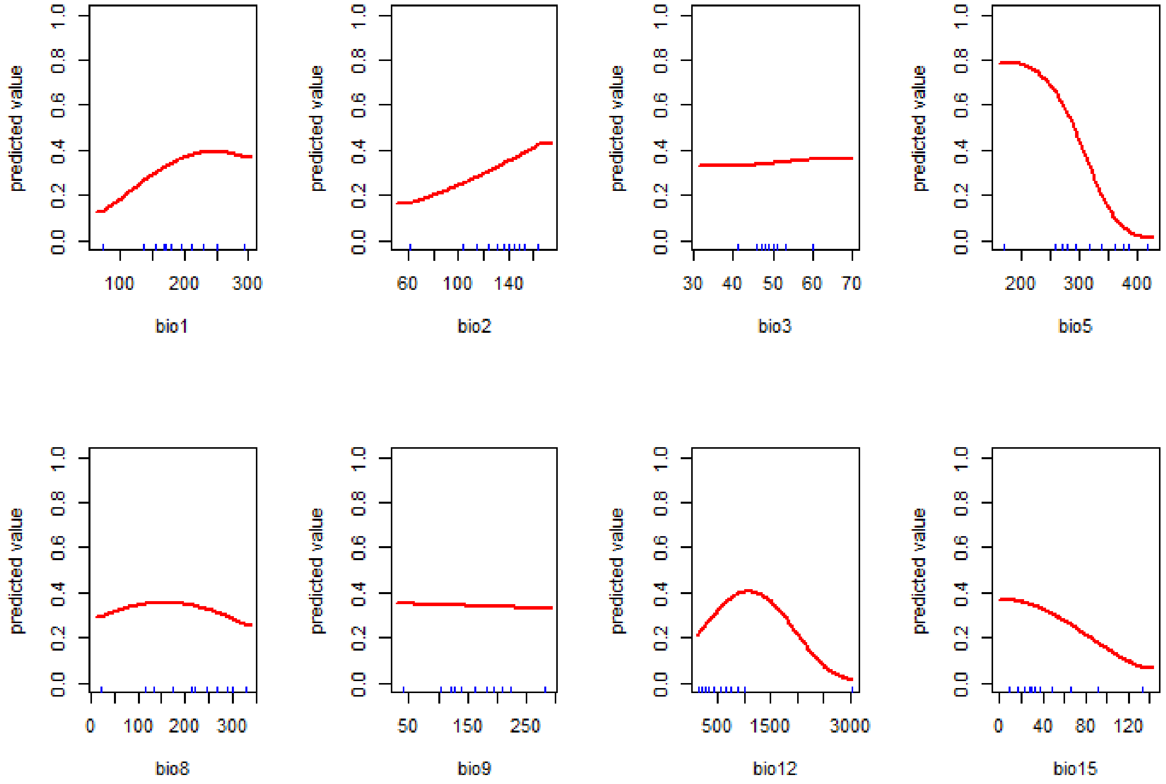


Fig. S8 MaxEnt response curves for the environmental predictor variables used to calibrate the models; bio 1 (mean annual temperature), bio 2 (mean diurnal range; Mean of monthly [max temp - min temp]), bio 3 (isothermality), bio 5 (maximum temperature of the warmest month), bio 8 (mean temperature of the wettest quarter), bio 9 (mean temperature of the driest quarter), bio 12 (mean annual precipitation), and bio 15 (precipitation seasonality).