Prioritising biological control agents for release against *Sporobolus pyramidalis* and *Sporobolus natalensis* (Poaceae) in Australia

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Abstract

Sporobolus pyramidalis Beauv. and *S. natalensis* (Steud.) Th. Dur. & Schinz. (giant rat's tail grass) (Poaceae), invade rangelands and pastures in eastern Australia, costing the livestock industry approximately AUS$ 60 million per annum in grazing losses. Mechanical and chemical control options are costly and largely ineffective. Biological control is viewed as the most promising control option, however this management strategy has largely been avoided for grasses, due to their perceived lack of suitably host-specific and damaging natural enemies. In this thesis, the prospects for using biological control against *S. pyramidalis* and *S. natalensis* in Australia was assessed, in light of these potential challenges.

Climate matching models were used to identify high-priority geographic regions within the plants' native distributions to survey for potential biological control agents. High-priority regions to perform surveys were identified by modelling the climatic suitability for *S. pyramidalis* and *S. natalensis* in sub-Saharan Africa (i.e. their potential native ranges'), and climatic compatibility with regions where biological control is intended in Australia. High-priority regions for *S. pyramidalis* included: (1) coastal East Africa, ranging from north-eastern South Africa to Uganda, including south-eastern DRC, (2) some parts of West Africa, including inland regions of the Ivory Coast and western Nigeria, (3) northern Angola and (4) eastern Madagascar, and for *S. natalensis* included: (1) eastern South Africa, (2) eastern Zimbabwe, (3) Burundi, (4) central Ethiopia and (5) central Madagascar. Prospective control agents collected from these regions have the highest probability of establishing and proliferating in Australia, if released.

In surveys of the insect assemblages on *S. pyramidalis* and *S. natalensis* in the climatically-matched region of eastern South Africa fifteen insect herbivores associated with the grasses were identified. Insect feeding guild, geographic distributions, and seasonal abundances suggest that three stem-boring phytophagous wasps, *Tetramesa* sp. 1, *Tetramesa* sp. 2 and *Bruchophagus* sp. 1 (Hymenoptera: Eurytomidae), have potential as control agents. Species accumulation curves indicated that additional surveys in South Africa are unlikely to yield additional potential control agents.

Field host-range surveys of 47 non-target grass species in South Africa showed that *Tetramesa* sp. 1, *Tetramesa* sp. 2, and *Bruchophagus* sp. 1, were only recorded from *S. pyramidalis* and *S. natalensis*. Integrating field host-range with phylogenetic relationships between plant species indicated that no native Australian *Sporobolus* species or economic crops and pastures are expected to be attacked by these wasps. All three wasp species are predicted to be suitably host-specific for release in Australia. Three other endophagous herbivores attacked non-target native African *Sporobolus* species that share a close phylogenetic relationship to native Australian *Sporobolus* species, and therefore, demonstrate considerable risk of non-target damage. These species should not be considered as potential control agents.

Under native-range, open-field conditions, *Tetramesa* sp. 1 caused an approximately 5-fold greater reduction in plant survival and reproductive output than *Tetramesa* sp. 2 and *Bruchophagus* sp. 1. *Tetramesa* sp. 1 in combination with *Tetramesa* sp. 2 did not significantly increase the level of damage, while *Bruchophagus* sp. 1 may decrease the efficiency of *Tetramesa* sp. 1, if released in combination. *Tetramesa* 1 is therefore the most promising candidate agent. Prioritising potential agents using predicted efficacy allowed otherwise equally suitable prospective agents to be prioritised in a strategic manner.

Prioritising which natural enemies to target as biological control agents is a complex task. Field host range and damage assessments in the native range may provide more realistic data than typical studies performed under artificial conditions in a laboratory or quarantine. Moreover, it could assist practitioners in prioritising the most suitable agent(s) at the earliest stage in the programme as possible. This study demonstrated that grasses are suitable targets for biological control as they can harbour host-specific and damaging natural enemies.

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Publications Arising from this Thesis

Sutton, G.F. 2019. Searching for a needle in a haystack: Where to survey for climatically-matched biological control agents for two invasive grasses (*Sporobolus* spp.) invading Australia. *Biological Control* 129: 37-44.

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Chapter 1: General introduction

Invasive alien plant species constitute a major threat to native biodiversity, agricultural productivity and ecosystem functioning (Mooney, 2005). They are considered to be some of the most ecologically and environmentally harmful invasive taxa worldwide (Vilà et al., 2011). The global economic losses associated with invasive alien plants and pastures alone is estimated at US$ 95 billion per annum (2001 value) (Pimentel et al., 2001). In Australia, invasive alien plants cost the economy approximately US$ 5.4 billion in the 2001/2002 financial year, rising to US$ 13.8 billion in 2011/2012 (Hoffmann and Broadhurst, 2016).

Sporobolus pyramidalis Beauv. and *Sporobolus natalensis* (Steud.) Th. Dur. & Schinz. (Poaceae), collectively known as giant rat's tail grass, are two problematic African grasses that have invaded Australia, notably along the eastern seaboard (Palmer, 2012). They invade rangelands and natural pastures, where they reduce carrying capacity by up to 80% (Vogler and Bahnisch, 2006; Bray and Officer, 2007), and cost the livestock industry approximately AUS$ 60 million per annum (2001 value) (Bray and Officer, 2007). As with most invasive grasses, mechanical and chemical control options are costly, labour-intensive and are largely ineffective against these species (Witt and McConnachie, 2004). Biological control is considered the most promising management tool for the control of these two grasses in Australia, however, no biological control agents are currently available (Palmer, 2012).

The aim of this thesis was to identify and prioritise potential biological control agents from the native ranges' of *S. pyramidalis* and *S. natalensis*. The following chapter introduces invasive alien plants and their management using biological control, with particular emphasis on the biological control of invasive grasses. A brief introduction to the two target weeds, *S. pyramidalis* and *S. natalensis,* is provided.

1.1. Invasive alien plants and biological control

Invasive alien plant species (hereafter ‘weeds’) are being transported around the globe at an unprecedented rate due to globalisation (Mack et al., 2000). Weeds can spread and proliferate into non-native habitats after being transported outside of their indigenous distribution, where they cause significant environmental and economic damage (Richardson et al., 2000; Pyšek et al., 2012). The serious environmental and economic threats posed associated with weed invasions results in governments having to spend substantial amounts of money to manage them (Simberloff, 2013). Control interventions to limit the impact and spread of weeds in South Africa amount to approximately US$ 30.5 million per annum (2012 value) (van Wilgen et al., 2012), while approximately US$ 87.5 million was spent on controlling weeds in Australia in a single year (2004 value) (Sinden et al., 2004).

Traditionally, weed control has primarily used mechanical (i.e. physical removal) and chemical control methods (i.e. the use of herbicides) (Williams et al., 2010). While these control methods can be effective, they inevitably require follow-up applications, can be expensive, and their effectiveness is usually limited over large spatial scales (Patten et al., 2017; Lake and Minteer, 2018; Quirion et al., 2018). Moreover, concerns over the adverse environmental effects and non-target impacts of mechanical and chemical control on native species limit their use (Crone et al., 2009; Ray et al., 2018).

Classical weed biological control (hereafter ‘biological control’) is a cost-effective, sustainable and environmentally-friendly management option for the control of weeds (McFadyen, 1998; Zachariades et al., 2017). Biological control is defined as “the actions of parasites, predators, and pathogens in maintaining another organism's density at a lower average than would occur in their absence" (DeBach, 1964; McFayden, 1998). This approach is predicated on the enemy-release hypothesis (ERH), which states that alien plants establish and proliferate in non-native habitats due to a release from specialist, co-evolved natural enemies that regulate it in its native distribution (Keane and Crawley, 2002). Biological control practitioners take advantage of this "enemy-release" by reuniting the invader with its specialist natural enemies from its native distribution (Keane and Crawley, 2002). The majority of biological control agents are phytophagous insects, although mites, nematodes and fungal pathogens are also frequently employed (Schwarzländer et al., 2018).

The aim of biological control is not to eradicate the weed, but rather to reduce the density of the weed to below a threshold where environmental and/or economic benefits are accrued (Hoffmann et al., 2019). Typically, biological control agents are required to be strictly monophagous (i.e. use only the target weed for feeding and reproduction), although narrowly oligophagous species (i.e. use a few closely-related plant species for feeding and reproduction) can be used, depending on the presence of potential non-target host plants in the intended region of introduction (Moran, 1980). To date, 468 biological control agent species have been intentionally released against a diverse suite of invasive plants and across an array of environmental conditions, consisting of 175 weed entities from 48 different families, the majority of which belong to the Asteraceae (44 species), Cactaceae (25 species) and the Fabaceae (23 species) (Winston et al., 2014; Schwarzländer et al., 2018). Biological control is widely viewed as an efficacious weed management strategy, with 115 target weed species (65.7% of all weeds targeted) being considered under some level of control due to the action of biological control agents worldwide (Schwarzländer et al., 2018). Moreover, biological control has an exemplary safety record, with more than 99% of 512 control agent introductions being associated with no known non-target effects (Hinz et al., 2019).

Approximately 3,207 non-native plant species have been recorded in Australia, with 398 having been declared as noxious weeds, defined as "any plant that requires some form of action to reduce its effect on the economy, the environment, human health and amenity" (Invasive Plants and Animals Committee, 2016). These weeds have been categorised into seven primary groups, based on their life-form, including: trees (57 species), shrubs (122 species), vines (32 species), herbs (182 species), aquatic macrophytes (32 species), succulents (2 species) and grasses (51 species). The capacity of weeds to negatively impact the Australian economy and preservation of natural resources was recognised by the national government by producing a national weeds strategy (Invasive Plants and Animals Committee, 2016). The national weeds strategy identified the serious negative impacts of weeds and proposed that weed management forms an essential component of the protection of natural resources and economic endeavours. Moreover, between 1999 and 2013, the Australian government identified 32 of these species as Weeds of National Significance (WoNS), which were selected due to their current and projected negative environmental and economic impacts. WoNS are considered high-priority species for active and strategic management interventions (Invasive Plants and Animals Committee, 2016).

To date, 202 biological control agents (i.e. species or genotypes) have been released for the management of 56 weeds in Australia (Schwarzländer et al., 2018). As such, Australia is amongst the foremost proponents of biological control, along with Canada, New Zealand, the United States of America (USA) and South Africa (Schwarzländer et al., 2018). Thirty-nine of the 56 (69.6%) weeds targeted for biological control in Australia are considered to be under some degree of control due to biological control (Schwarzländer et al., 2018). Despite invasive grasses constituting a significant proportion of the weeds (~ 14 %), and being some of the most environmentally and economically damaging invasive plants in Australia, biological control has not yet been used as a management strategy to control invasive grasses in Australia.

1.2. Grass invasions

Grasses (Poaceae) are the most successful angiosperm family worldwide consisting of ~ 11 000 species (Linder et al., 2017). They occupy a greater land area than any other vegetation type, cover one-third of the globe and contribute approximately 33% of global primary productivity (Tscharntke and Greiler, 1995). Grasses are believed to have been present on earth for at least the last *c*. 96 – 75 million years (Bremer, 2002; Bouchenak-Khelladi et al., 2010). Beginning in the early Miocene (23 – 5.3 Mya), when C_3 photosynthetic grasses started replacing temperate forests, grasses have dramatically altered the composition and ecology of the biosphere (Owen-Smith, 2013).

Grasses are arguably the most valuable plant family worldwide due to their importance as food crops, biofuel crops, medicinal plants, building materials, musical instruments, lawns, and pastures for grazing (Tscharntke and Greiler, 1995; Bouchenak-Khelladi et al., 2010). Approximately 50% of global caloric intake is derived from three species of grass (rice, wheat and maize) (Bruinsma, 2017). Grasses have been cultivated by humans for at least the last *c*. 9 000 - 10 000 years (Purugganan and Fuller, 2009), and extensively planted for grazing/pasture (Cook and Dias, 2006). As a result, grasses have been deliberately introduced into many non-native regions across the globe (Lonsdale et al., 1994; Williams and Baruch, 2000; Cook and Dias, 2006; Overholt and Franck, 2017), making them amongst the most widespread and abundant weeds of natural and agricultural habitats worldwide (Daehler, 1998; Pyšek et al., 2012). As such, the Poaceae (4807 weedy species) are second to only the Asteraceae (5094 weedy species) in terms of the total number of weedy species per plant family worldwide (Randall, 2017), including 3 of the world's top 100 invasive species, namely: *Arundo donax* L. (giant reed), *Imperata cylindrica* (P.) Beauv. (cogongrass) and *Spartina anglica* C.E. Hubbard (smooth cordgrass) (Lowe et al., 2000).

There is a strong asymmetry with regards to which geographic regions that are invaded by grasses, and which regions are the source of invasive grasses (Visser et al., 2016). Africa is a common source for invasive grasses (Visser et al., 2016), owing to the tolerance of many grass species to heavy grazing, having co-evolved with large mammalian herbivores (Cook and Glem, 2000). Tolerance of heavy grazing has been viewed as a highly desirable trait by agronomists, whereby many African grasses are considered to be more valuable pasture species than the majority of native pasture species in other geographic regions (Williams and Baruch, 2000; Cook and Dias, 2006; Overholt and Franck, 2017; van Klinken and Friedel, 2017; but see Firn, 2009 and references therein). For example, native Australian grasses have evolved under relatively light grazing pressure by native marsupials, and thus, are unable to tolerate the heavy grazing pressure imposed by commercial livestock grazing (Cook and Glem, 2000). As such, many grasses, particularly of African origin, were intentionally introduced into Australia in the 1900's as part of the Australian Commonwealth Plant Introduction Scheme to improve the quality and quantity of pastures and forage for grazing livestock (Lonsdale, 1994; Cook and Dias, 2006; van Klinken and Friedel, 2017). Approximately 2 250 non-native grass species (~ 22% of the world's grass species) have been introduced into Australia (Lonsdale, 1994; Cook and Dias, 2006; van Klinken and Friedel, 2017). Several grasses were widely planted (providing ample propagule pressure), and in many instances, multiple agronomic lines were introduced during this period (increasing intra-specific genetic diversity) (e.g. Firn, 2009), which are both strong predictors of invasion success (Hui and Richardson, 2017). Invasive grasses are now found in almost every terrestrial and freshwater aquatic habitat across Australia (van Klinken and Friedel, 2017).

Invasive grasses are a pervasive problem in Australia (Godfree et al., 2017). Fifty-one of the 398 (12.8%) declared noxious weeds, and four of 32 (12.5%) Weeds of National Significance (WoNS), in Australia, are invasive grasses. Grass invasions are associated with serious negative environmental and economic consequences, both globally and in Australia, including but not limited to: reducing native biodiversity, threatening native plant and wildlife populations, reducing grazing and agricultural productivity, national security, altering fire regimes and disrupting nutrient cycling and other ecological processes (Fig. 1.1).

Grass invasions are often relatively insidious, which exacerbates the problem by allowing many species to establish, proliferate and transform native habitats without being detected (Godfree et al., 2017). In 2009, the Australian government listed the *Invasion of northern Australia by Gamba Grass and other introduced grasses* as a primary threat to Australian biodiversity, under the *Australian Environment Protection and Biodiversity Conservation Act of 1999* (EPBC Act). This, along with listing of 4 invasive grasses as WoNS, highlights the current and future threat posed by invasive grasses to native ecosystems across Australia and necessitates their active and strategic management, through systematic eradication and control interventions.

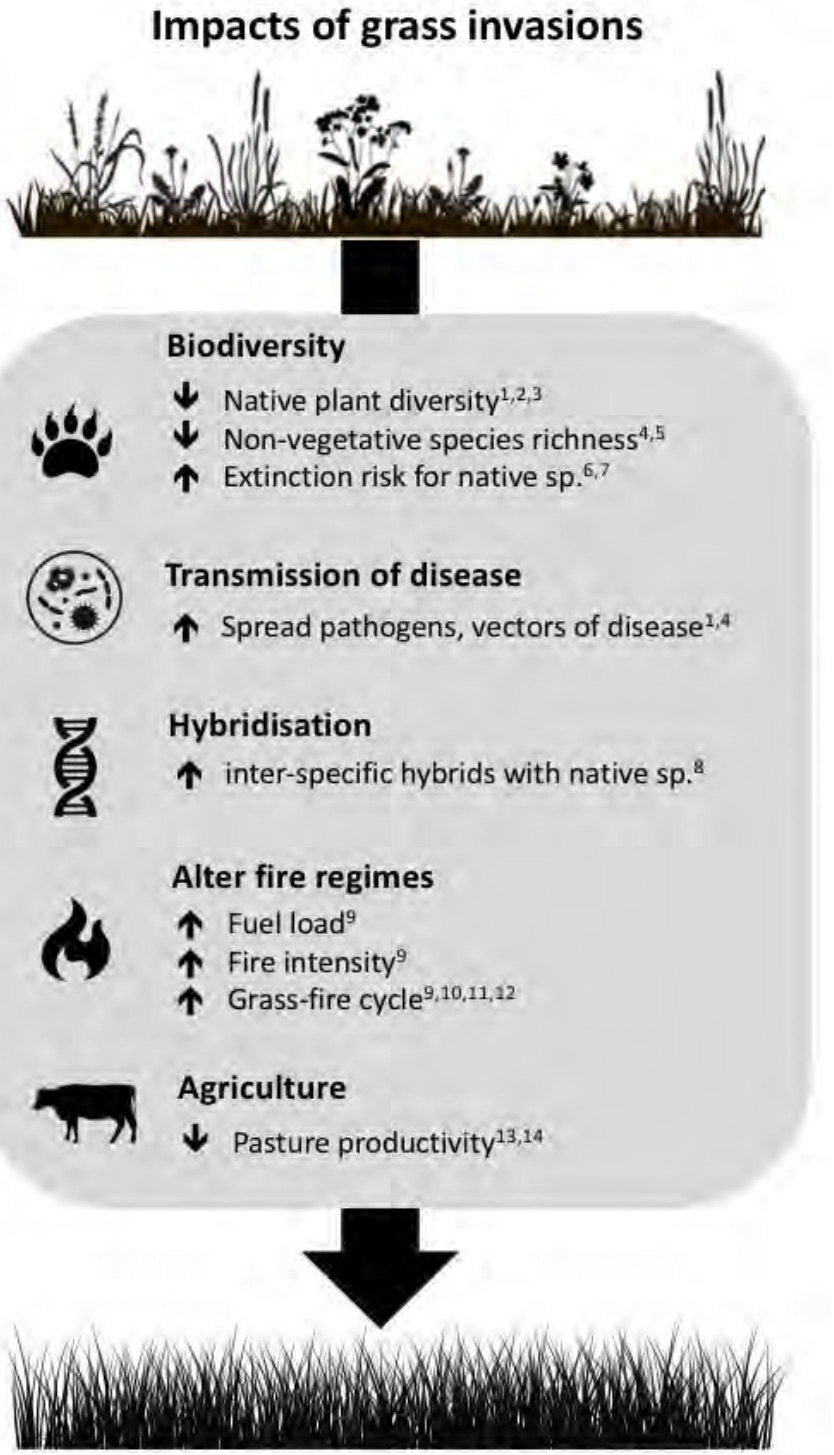


Figure 1.1. Impacts associated with alien grass invasions, including examples from: [1] *Pennisetum setaceum* (D'Urso et al., 2017); [2] *Brachiaria mutica* (Ferdinands et al., 2005); [3] *Agropyron cristatum* (Heidinga and Wilson, 2002); [4] *Arundo donax* (Racelis et al., 2012; Moran et al., 2017); [5] *Phragmites australis* (Benoit and Askins, 1999); [6] *Bromus inermis* (Williams and Crone, 2007); [7] *Cenchrus ciliaris* (Edwards et al., 2019); [8] *Spartina alterniflora* (Ayres et al., 2004); [9] *Andropogon gayanus* (Setterfield et al., 2010); [10] *Melinis minutiflora* (D'Antonio and Vitousek, 1992); [11] *Schizachyrium condensatu* (D'Antonio and Vitousek, 1992); [12] *Bromus tectorum* (D'Antonio and Vitousek, 1992); [13] *Eragrostis curvula* (Firn, 2009); [14] *Sporobolus* spp. (Witt and McConnachie, 2004).

1.3. Biological control of grasses

High-impact weeds, such as many invasive grass species (e.g. *A. donax*; *S. anglica*), are traditionally the focus of management programmes, being targeted for active control interventions, usually in the form of mechanical and/or chemical control, along with prescribed burning (Lake and Minteer, 2018). However, the control of invasive grasses is problematic due to the ineffectiveness, high costs and logistical constraints of implementing control strategies over appropriate spatial scales (Grice et al., 2012; Quirion et al., 2018). Novel methodological approaches and control interventions are required to achieve the desired degree of control over many invasive grasses and biological control arguably offers the greatest potential (Palmer, 2012; van Klinken and Friedel, 2017).

Historically, very few invasive grasses have been targeted for biological control (Pemberton, 1996; Casagrande et al., 2018; Schwarzländer et al., 2018), as grasses have been considered poor biological control targets (Wapshere, 1990; Evans, 1991; Pemberton, 1996, 2002). This stems from the perception that grasses support a small, unspecialised, and insufficiently damaging natural enemy community to exploit for potential biological control agents (Gill and Blacklow, 1984; Wapshere, 1990; Pemberton, 2002). Grasses are expected to harbour relatively unspecialised herbivore assemblages as they lack the diversity and quantity of secondary chemical compounds (i.e. feeding deterrents, toxins, stimulants) typically found in dicotyledons, which are considered the primary drivers of herbivore specialisation (Ehrlich and Raven 1964; McNaughton et al. 1985; Moore and Johnson 2017; but see Kellogg 2015). Grasses are believed to be relatively tolerant of herbivory since they have evolved in the presence of grazing by large mammals and harvesting (Tscharntke and Greiler, 1995). This tolerance is ascribed to the rapid regrowth potential of grasses from basal meristems and underground storage organs, and their extensive tillering ability (Coughenour, 1985). Herbivores targeting above-ground biomass, may therefore, not be able to cause enough damage to regulate grass populations. Grasses also typically have a low essential nutrient content, and the presence and abundance of such nutrients are usually positively correlated with insect performance and host choice (Scheirs et al., 2003). Moreover, grasses produce an array of structural defences that may deter herbivores and/or reduce palatability and digestibility, with silica being a particularly important grass anti-herbivore defence (McNaughton et al., 1985).

The risk posed to economically valuable crops and/or native biodiversity, by introducing grass biological control agents, has been considered too great to warrant implementing biological control (Wapshere, 1990). The concerns over non-target damage to valued grasses stems from the close phylogenetic relationships between grasses targeted for biological control, and non-target crops, pastures and native grass species (Soreng et al., 2017). For example, Wapshere (1990) took a particularly pessimistic stance regarding the potential for the biological control of invasive grasses in Australia (specifically *Nassella trichotoma* (Nees) Arech. (Serrated Tussock), due to the presence of closely-related valued pasture species (i.e. several native *Stipa* species). Phytophagous surveys conducted on *I. cylindrica* and *Sorghum halepense* (Pers.) L. (Johnsongrass) in the 1970's and 1980's, which did not yield any suitable agents, reinforced the perception of grasses as poor targets for biological control (see Witt and McConnachie, 2004).

Only two invasive grasses, namely: *S. alterniflora* and *A. donax*, have had any biological control agents released against them, to date. *Spartina alterniflora* is native to the Atlantic and Gulf Coasts of the USA, but is an alien invader on the Pacific Coast (Daehler and Strong, 1995). In 2000, the planthopper *Prokelisia marginata* (Van Duzee) (Hemiptera: Delphacidae) was released against *S. alterniflora* in Willapa Bay, Washington (USA) (Grevstad et al., 2003). Three insect herbivores have been released as biological control agents on *A. donax* in the USA, namely: the Arundo wasp *Tetramesa romana* Walker

(Hymenoptera: Eurytomidae), the Arundo scale *Rhizaspidiotus donacis* (Leonardi) (Homoptera: Diaspididae), and the Arundo leaf sheath-miner *Lasioptera donacis* Coutin & Faivre-Amiot (Diptera: Cecidomyiidae) (Goolsby et al., 2009a; Goolsby and Moran, 2009; Goolsby et al., 2017). It is striking that only two grasses have biological control agents released against them, especially given the serious environmental and economic consequences often associated with grass invasions.

Despite the largely negative attitude to grass biological control in the literature, several authors have argued that biological control of invasive grasses is possible (Witt and McConnachie, 2004; Overholt et al., 2016; van Klinken and Friedel, 2017; Casagrande et al., 2018), and in recent years, we have seen the initiation of a handful of grass biological control projects (e.g. *A. donax*, *S. alterniflora*), and several other biological control agents approved for release globally. For example, *Archanara* spp. (Lepidoptera: Noctuidae) were recently approved for release on *Phragmites australis* (Cav.) Trin. ex Steudel (common reed) in the USA (Blossey et al., 2020), and the rust fungus *Uromyces pencanus* Arth. & Holw. (Uredinales) has been approved for release against *Nassella neesiana* (Trin. & Rupr.) Barkworth (Chilean needlegrass) in New Zealand (Anderson et al., 2017). Given the success of biological control for the management of other plant-life forms (McFadyen, 1998; Schwarzländer et al., 2018), and the relative unsuitability of traditional weed control methods for invasive grass management (e.g. mechanical and chemical control), practitioners may be missing an opportunity to control invasive grasses by avoiding biological control.

1.4. Giant rat's tail grasses: *Sporobolus pyramidalis* and *Sporobolus natalensis*

Sporobolus pyramidalis and *S. natalensis* are tufted perennial grasses originating from Africa that have become highly invasive along the eastern seaboard of Australia (Fig. 1.2).

These two species are commonly considered to be a single weed entity in Australia, referred to as giant rat's tail grass. However, recent molecular studies have concluded that *S. pyramidalis* and *S. natalensis* are sister species (Shresha et al., 2003; 2005), and will be treated as such for the purpose of this thesis. They grow to approximately 0.6 – 2.0 m tall, with an inflorescence of up to 45 cm in length. The inflorescence is originally "rat's tail-like" in structure becoming "pyramid-shaped" when fully matured, although this is less pronounced in *S. natalensis* (Fig. 1.3.). Typical forms of each species can be morphologically distinguished from each other by their obtuse (*S. pyramidalis*) or acute to sub-acute upper glume (*S. natalensis*) (Fish et al., 2015). *Sporobolus pyramidalis* and *S. natalensis* pose a serious environmental and economic threat to pasture and grazing lands in eastern and northern Australia.

1.4.1. Taxonomy

Sporobolus R.B.r. (Poaceae) is a large genus of approximately 160 species distributed primarily across the tropical and sub-tropical regions of the world (Clayton and Renvoize, 1986). Species within this genus are defined by a single-flowered spikelet, 1-nerved (but sometimes 3-nerved) lemmas, fruit with cistoid pericarps and ligules with a hairy membrane (Simon and Jacobs, 1999; Peterson et al., 2014). The taxonomy of the *Sporobolus* genus has a long and confusing history.

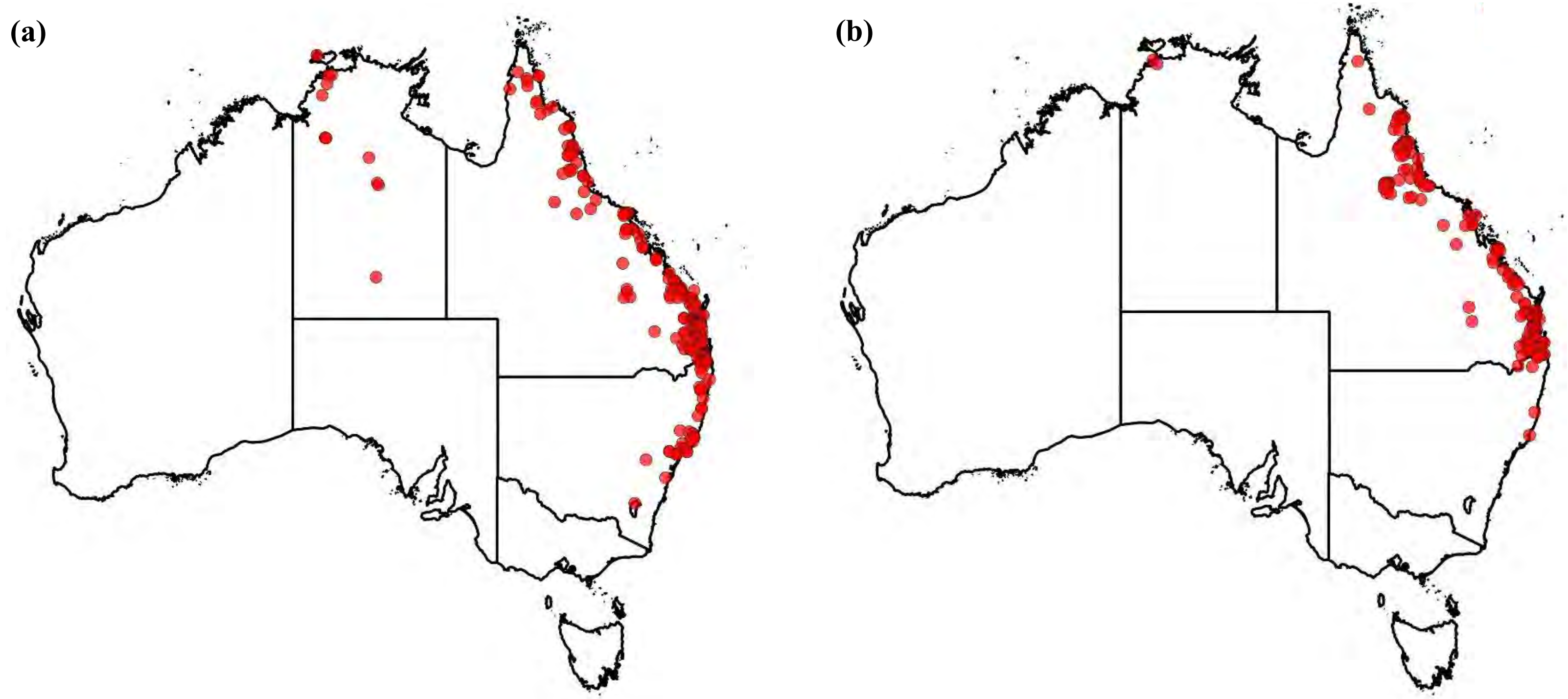


Figure 1.2. Invaded range of (a) *Sporobolus pyramidalis*, and (b) *S. natalensis* in Australia. Maps were downloaded from the Australian Virtual Herbarium Portal (AVH, 2019).

(a) (b)

Figure 1.3. Typical morphology of (a) *Sporobolus pyramidalis*, with inflorescence branches spreading away from the main culm by more than 30°, and (b) *S. natalensis*, with inflorescence branches densely compacted on the main culm (i.e. inflorescence branches spreading away from the main stem by less than 30°) (Photographs: Sheldon Navie; Copyright of the Queensland Government, 2016; Weeds of Australia – Biosecurity Queensland Edition).

The genus was erected by Robert Brown in 1810 for three Australian species that were originally thought to belong to *Agrostis* L. (see Simon and Jacobs, 1999). Over the next 200 years, the genus has undergone numerous revisions and taxonomic treatments.

Several molecular phylogenies have been produced in recent years, allowing for the determination of phylogenetic relationships between *Sporobolus* species (Shrestha et al., 2003, 2005; Peterson et al., 2014; Soreng et al., 2017). The two weedy species, *S. pyramidalis* and *S. natalensis*, belong to the monophyletic and morphologically homogenous *indicus* complex, along with six native Australian *Sporobolus* spp. (Simon and Jacobs, 1999; Table 1.1). An additional 13 native *Sporobolus* species that are not part of this complex are found in Australia (Simon and Jacobs, 1999; Table 1.1). One of these native Australian species, namely *Sporobolus pamelae*, is listed as 'endangered' according to the Queensland Nature Conservation (Wildlife) Regulation 2006. None of the native Australian *Sporobolus* species occur in Africa (Fish et al., 2015). There are 38 *Sporobolus* species that are native to southern Africa (Fish et al., 2015), many of which are more closely-related to native Australian *Sporobolus* species than they are to *S. pyramidalis* and *S. natalensis*.

Members of the *indicus* complex are extremely difficult to delineate and identify due to morphological intergradation (Simon and Jacobs, 1999). Morphological identifications are complicated further by inter-specific hybridization between some species (Simon and Jacobs, 1999). The presence of closely-related native Australian *Sporobolus* species, several of which belong to the *indicus* complex along with the two target weeds, will likely place a significant constraint on the host-specificity of any candidate agent considered for this biological control programme.

Table 1.1. List and status of non-native and native *Sporobolus* species present in Australia.

Species name	Common name	Origin[a]	*Sporobolus indicus* complex[b]
(a) Non-native species in Australia			
Sporobolus natalensis (Steud.) T.Durand & Schinz	Giant rat's tail grass	Africa	Yes
Sporobolus pyramidalis P.Beauv.	Giant rat's tail grass	Africa	Yes
Sporobolus africanus (Poir.) Robyns & Tournay	Parramatta grass	Africa	Yes
Sporobolus fertilis (Steud.) Clayton	Giant Parramatta grass	Asia	Yes
Sporobolus jacquemontii Kunth	American rat's tail grass	America	Yes
Sporobolus coromandelianus (Retz.) Kunth	Small drop seed	Africa and Asia	No
(b) Native species in Australia			
Sporobolus blakei De Nardi ex B.K.Simon		Australia	Yes
Sporobolus creber De Nardi	Slender rat's tail grass	Australia	Yes
Sporobolus elongatus R.Br.	Slender rat's tail grass	Australia	Yes
Sporobolus laxus B.K.Simon	Lax rat's tail grass	Australia	Yes
Sporobolus sessilis B.K.Simon		Australia	Yes
Sporobolus actinocladus (F.Muell.) F.Muell.	Fairy grass	Australia	No
Sporobolus australasicus Domin	Dropseed	Australia	No
Sporobolus caroli Mez	Yakka grass	Australia	No
Sporobolus contiguus S.T.Blake		Australia	No
Sporobolus disjunctus R.Mills ex B.K.Simon		Australia	No
Sporobolus latzii B.K.Simon		Australia	No
Sporobolus lenticularis S.T.Blake		Australia	No
Sporobolus mitchellii (Trin.) C.E.Hubb. ex S.T.Blake	Rat's tail couch	Australia	No
Sporobolus pamelae B.K.Simon		Australia	No
Sporobolus partimpatens R.Mills ex B.K.Simon		Australia	No
Sporobolus pulchellus R.Br.		Australia	No
Sporobolus scabridus S.T.Blake		Australia	No
Sporobolus virginicus (L.) Kunth	Salt couch grass	Australia	No

[a] - Species origins were taken from Simon and Jacobs (1999) and Fish et al. (2015)
[b] - Species were defined as belonging to the *indicus* complex following Simon and Jacobs (1999), Shresha et al. (2003, 2005) and Peterson et al. (2014)

1.4.2. Biology and distribution

Sporobolus pyramidalis and *S. natalensis* are tropical, perennial grasses originating from Africa, that were introduced into Australia in contaminated seed lots during the 1960's (Anon, 1997; cited in Bray et al., 1998). They inhabit a wide variety of soil types, ranging from dry or stony soils to alkaline and sandy soils, and habitats, ranging from savannah to roadsides (Palmer, 2012). They can produce inflorescences throughout the year in Australia and in the native range, if conditions are favourable, although peak growth usually occurs in the wet and warm summers (Vogler and Bahnisch, 2006). In South Africa, the plants maintain above-ground shoots year-round, including during both winter and drought (G.F. Sutton, pers. obs.). Individual inflorescences can produce up to 1000 seeds each (Vogler and Bahnisch, 2006), with seed densities in dense infestations reaching 85 000 seeds/m^2 and seed viability ranging between 90-100% (Bray and Officer, 2007). A seed bank can form and may remain viable for up to 10 years (Bray and Officer, 2007). The mucilaginous seed coat (when wet) adheres to passing livestock and machinery and will fall off once it dries sufficiently. This mode of dispersal is expected to be one of the main contributing factors to the spread of *S. pyramidalis* and *S. natalensis* in Australia (Bray et al., 1998). Bray et al. (1998) demonstrated that cattle may also contribute to the spread of *S. pyramidalis* and *S. natalensis* as approximately 28% of seed they were fed was excreted as viable seed 2-3 days after ingestion.

In its native range, *S. pyramidalis* occurs from southern Africa extending north to Yemen, including the islands of Madagascar and Mauritius (Van Oudtshoorn, 1999). In its invaded range of Australia, *S. pyramidalis* is distributed from Central Coast (New South Wales) to Cooktown (Queensland) (Fig. 1.2a). The native distribution of *S. pyramidalis* is usually limited to areas that receive at least 500 mm of precipitation per annum, although weed infestations in Australia are typically found in areas that receive more than 600 mm (Vogler and Bahnisch, 2006). Gallagher et al. (2010) found that *S. pyramidalis* occupies a significantly different climatic niche in Australia than it does in its native range, where Australian populations occur in two novel ecoregions, namely: temperate broadleaf and mixed forests, and desert and xeric shrublands.

Sporobolus natalensis has a native range that stretches from southern Africa to Ethiopia in the north (Van Oudtshoorn, 1999). In Australia, it is distributed from Port Macquarie (New South Wales) to Rockhampton (Queensland) (Fig. 1.2b). The most problematic *S. natalensis* infestations occur between the New South Wales border and Rockhampton, notably: Moura, Mackay, Townsville, Ingham and Mareeba (Queensland) (Bray and Officer, 2007). In Queensland, the majority of infestations occur in productive black spear grass pastures (*Heteropogon contortus* (L.) P. Beauv. ex. Roem. & Schult.) (Tothill, 1983).

An eco-climatic modelling study indicated that climatic conditions across approximately 30% (223 million hectares) of Australia were potentially suitable for *S. pyramidalis* and *S. natalensis*, including about 60 % of Queensland (108 million hectares) (Vogler et al., 1998). Current indications are that *S. pyramidalis* and *S. natalensis* are rapidly filling this climatically suitable habitat across eastern Australia, having increased their invaded ranges' approximately 5-fold over the last 10-15 years (90 000 to 450 000 hectares) (J. Wright, pers. comm. cited in: Bray et al., 1998; Bray and Officer, 2007). Without appropriate control interventions, *S. pyramidalis* and *S. natalensis* will almost certainly continue to spread across eastern and northern Australia.

1.4.3. Impacts

The primary impact of *S. pyramidalis* and *S. natalensis* is the significantly reduced economic viability of invaded rangelands and pastures. The unpalatable and fibrous leaves of *S. pyramidalis* and *S. natalensis* in comparison to sympatric pasture species results in selective grazing of the more palatable species (Bray and Officer, 2007). Selective grazing reduces inter-specific competition between valuable pasture species and *S. pyramidalis* and *S. natalensis*, allowing the latter to out-compete and replace the more palatable species (e.g. Firn, 2009). A negative feedback loop then occurs between the two weed species and overgrazing, whereby as weed densities increase, the selective overgrazing of nutritious pasture becomes more acute. This results in more severe overgrazing, if no concomitant reduction in stocking rates is undertaken, producing more favourable conditions for weed proliferation and spread.

Once paddocks are heavily infested by *S. pyramidalis* and *S. natalensis* and dense monocultures are formed, the carrying capacity of a pasture diminishes due to a substantial reduction of high-quality forage (Bray and Officer, 2007). Livestock stocking rates must then be reduced proportional to the quantity of quality forage available, which leads to infested pastures reducing stock production by up to 50% (Innes, 1997; cited in Bray et al., 1998). Cattle grazing on heavily infested pastures can also take up to 12 months longer to gain similar weight as on uninfested pasture (Bray and Officer, 2007). This results in carrying capacities of infested pastures being up to 80% lower than uninfested pastures (Vogler and Bahnisch, 2006; Bray and Officer, 2007). The potential economic costs of infestations are estimated at approximately AUS $ 60 million per annum (2001 value) due to reduced grazing potential and control costs (Bray and Officer, 2007).

The adverse environmental and economic effects of *S. pyramidalis* and *S. natalensis* infestations have resulted in their declaration as category 2 weeds in Queensland, and category 3 weeds in New South Wales, necessitating that, by law, landholders are required to take appropriate steps to control and clear their properties (Bray and Officer, 2007).

1.4.4. Control methods

Bray and Officer (2007) recommend that land-managers maintain a highly competitive pasture to limit the amount of suitable habitat for *S. pyramidalis* and *S. natalensis* germination and seedling emergence. This is achieved through minimising disturbance and creation of bare patches, by resting of the pasture periodically and altering stocking rates to protect against overgrazing. Several additional preventative measures are recommended to limit seed dissemination, namely: land-holders are advised to check for lodged seeds and wash vehicles and machinery in a dedicated wash-down area, minimise movement of livestock between infested and uninfested pastures, and maintain buffer zones alongside fence-lines and roads (Bray and Officer, 2007).

Once *S. pyramidalis* and *S. natalensis* establish in a paddock, a variety of control options have been developed depending on the land-use and severity of the infestation (Bray and Officer, 2007). These control interventions consist of manual extraction or slashing, spraying herbicidal applications (e.g. fluropropanate and glyphosate) and prescribed burning. Preliminary trials indicated that prescribed burns of 125°C may kill up to 50% of the soil seed-bank (Bray et al., 1998). However, follow-up trials demonstrated that prescribed fires may encourage the development of dense infestations by reducing background inter-specific competition and promoting compensatory seed production (Bray and Officer, 2007). While these techniques can be effective over small spatial-scales and/or relatively sparse infestations, their efficacy is limited otherwise (Bray and Officer, 2007).

1.4.5. Biological control

The increasing concerns about the rapid proliferation and impacts of *S. pyramidalis* and *S. natalensis* during the 1990's and inadequate control interventions resulted in both species being approved as targets for biological control in Australia by the National Resource Management Standing Committee in 2007 (Palmer, 2012). Surveys were performed at approximately 90 sites across parts of the native range in southern Africa between 2001 and 2003 for insect herbivores and fungal pathogens (i.e. potential biological control agents) associated with *S. pyramidalis*, *S. natalensis* and a closely-related native African species, *Sporobolus africanus* (Poir.) Robyns & Tournay (Parramatta grass), that is also invasive in Australia (Witt and McConnachie, 2004). These surveys constitute the only known inventory of herbivores and fungal pathogens associated with these three grass species.

Approximately 70 insect species were recorded on the three grasses. The majority of these insects were incidental visitors, phytophagous pests, predators or parasitoids, and thus were of little interest in a biological control context (Palmer, 2003 unpublished report; Witt and McConnachie, 2004). At least 23 pathogens were recorded, five of which were primary pathogens (Vánky, 2003; Witt and McConnachie, 2004). Witt and McConnachie (2004) proposed that only the stem-galling wasp, *Tetramesa* sp. (Hymenoptera: Eurytomidae), and a smut-fungus, *Ustilago sporoboli-indici* (Ustilaginales), showed promise as candidate biological control agents. A brief overview of the biology, host-specificity, predicted efficacy and biological control prospects offered by each species is provided below.

1.4.5.1. *Tetramesa* sp. (Hymenoptera: Eurytomidae)

Tetramesa sp. is a phytophagous stem-boring wasp associated with *S. pyramidalis* and *S. natalensis* in southern Africa. Larvae feed within the culm, resulting in malformation of the inflorescence. It was not recorded from at least four sympatric grasses (e.g. *Sorghum* sp., *Hyparrhenia* sp., *Pennisetum* sp. and *Cymbopogon* sp.) in southern Africa during preliminary field host-range surveys (Palmer, 2003 unpublished report). *Tetramesa* sp. was found at many sites, often in high abundance, and was associated with a 29% reduction in culm heights (Witt and McConnachie, 2003 unpublished report). The abundance, damage caused and apparently narrow host-range of *Tetramesa* sp. were used to prioritise this species as a candidate agent. A laboratory colony of the wasp could not be successfully reared, so traditional laboratory host-range testing and efficacy assessments were never performed (Palmer, 2012). The failure to establish a culture was ascribed to the wasp undergoing winter diapause which could not be replicated under artificial conditions (Palmer, 2008 unpublished report), and/or heavy parasitism (Witt and McConnachie, 2003 unpublished report). However, failure to rear *Tetramesa* sp. may be due to inappropriate cage materials, as has been found for *Tetramesa romana* Walker (Hymenoptera: Eurytomidae) being reared on *A. donax*, which couldn't be reared effectively until cages were made with black silk organza instead of standard white mesh cages (Goolsby and Moran, 2009).

1.4.5.2. *Ustilago sporoboli-indici* (Ustilaginales)

Ustilago sporoboli-indici is a leaf-smut fungus associated with *S. pyramidalis*, *S. natalensis* and *S. africanus* in southern Africa. The host-range and pathogenicity of the smut was assessed under laboratory conditions against *S. pyramidalis*, *S. natalensis* and 10 native Australian *Sporobolus* species. Fundamental host-range testing indicated that *U. sporoboli-*

indici produced major disease symptoms on four native Australian *Sporobolus* species, precluding its use as a biological control agent in Australia (Yobo et al., 2009). Nevertheless, recent field surveys indicated that *U. sporoboli-indici* is already present on *S. natalensis* in south-east Queensland, representing the first record for this species in Australia (Vitelli et al., 2017). It is unknown whether *U. sporoboli-indici* was co-introduced with *S. pyramidalis* and/or *S. natalensis* to Australia, or whether Australia is part of the fungus's native range. Vánky (2003) reported that the assumed native range of *U. sporoboli-indici* included: Africa, Asia (China) and the Philippines, where it has been recorded from an array of *Sporobolus* species. To determine the native/non-native status of *U. sporoboli-indici* in Australia, additional surveys are required, with particular emphasis on whether it is recorded on parapatric native Australian *Sporobolus* species.

1.4.5.3. Current status

The broad host-range of *U. sporoboli-indici* and the inability to rear and perform host-range assessments on *Tetramesa* sp. resulted in the biological control project being terminated in 2007 (Palmer, 2012). However, increasing concerns over the spread and negative environmental and economic impacts of *S. pyramidalis* and *S. natalensis* infestations across Australia led the Queensland Department of Fisheries and Forestry, in conjunction with Rural Industries, to re-initiate the *Sporobolus* biological control project in 2016. Biological control is viewed as the most promising control option for the management of *S. pyramidalis* and *S. natalensis* in Australia (Palmer, 2012; van Klinken and Friedel, 2017).

1.5. Thesis aims and structure

The primary aim of this thesis was to source and develop potential biological control agents for the management of *S. pyramidalis* and *S. natalensis* in Australia. An ecologically-motivated and quantitative approach to native-range phytophagous surveys was adopted during this study to identify and prioritise candidate biological control agents. It was imperative that the native-range surveys were performed systematically to ensure that all potential candidate agents were located in the areas that were surveyed, and by doing so, demonstrate that performing additional surveys were unlikely to yield any additional candidate agents.

One of the first steps in a new biological control programme is to identify appropriate geographic areas to search for biological control agents in the target weed's native distribution (Goolsby et al., 2006). In Chapter 2, climate-matching models were developed to identify geographic regions that were most likely to yield climatically compatible control agents to weed infestations in Australia. This is particularly relevant for *S. pyramidalis* which occupies a significantly different climatic niche in Australia than in its native range (Gallagher et al., 2010). Candidate agents sourced from climatically-matched regions in a target weeds native range are expected to stand the best chance of establishing and proliferating in their introduced range.

Native-range surveys are required to gain a detailed account of the potential pool of biological control agents and can provide valuable ecological data to assist in agent selection and prioritisation (Goolsby et al., 2006; van Klinken and Raghu, 2006). In Chapter 3, the structure of the herbivore communities associated with *S. pyramidalis* and *S. natalensis* in South Africa was described. Prospective biological control agents were identified using a combination of traits, including geographic distribution, seasonal abundance and feeding biology of the herbivores recorded. Survey completeness was assessed by quantifying whether sampling effort was sufficient to document the full suite of candidate biological control agents associated with *S. pyramidalis* and *S. natalensis* in South Africa.

Field host-range surveys in a target weeds' native range are used to evaluate the suitability of candidate agents with respect to their predicted host-range in the region where biological control is intended. However, the utility of such an approach is often limited because key non-target species that are most at risk in the region where biological control is intended, do not occur in the weeds' native range (Clement and Cristofaro, 1995; Schaffner et al., 2018). The host-range of insects, fungal pathogens and herbivorous mites demonstrates a strong phylogenetic signal, whereby the probability that two plant species will be attacked by a shared enemy is negatively correlated with the phylogenetic distance between the two species (Weiblen et al., 2006; Gilbert and Webb, 2007; Gilbert et al., 2012; Wheeler and Madeira, 2017; but see Becerra, 1997; Rapo et al., 2019). In Chapter 4, a methodological approach was developed to investigate the potential realised host-range of multiple candidate biological control agents for *S. pyramidalis* and *S. natalensis* (identified in Chapter 3), by taking advantage of the phylogenetic constraints on herbivore host-range. This study prioritised candidate agents based on their host-specificity by integrating field host-range data with published plant phylogenies, to provide an ecologically-motivated estimate of risk posed to key non-target grass species in Australia.

Traditionally, the screening, prioritisation and petition to release biological control agents has been based almost exclusively on the perceived risk posed to economic crops and native biodiversity in the region of intended control (McFadyen, 1998; Hinz et al., 2019). Several biological control practitioners have advocated that pre-release efficacy assessments be conducted to demonstrate that the candidate agent has an impact on key target weed productivity parameters (McEvoy and Coombs, 1999; van Klinken and Raghu, 2006).

Moreover, many programmes release multiple control agents to increase the likelihood of weed suppression (Denoth et al., 2002), despite negative competitive interactions between control agents frequently being cited as a constraint to successful biological control (e.g. Paynter and Hennecke, 2001; Jones and Lake, 2018). As such, using predicted efficacy and complementarity between candidate agents is advocated to maximise the probability of weed suppression (van Klinken and Raghu, 2006; Milbraith and Nechols, 2014). In Chapter 5, field surveys in the native ranges' of *S. pyramidalis* and *S. natalensis* were performed to evaluate the predicted efficacy and inter-specific competitive interactions between multiple candidate agents, which was used to prioritise only the most damaging candidate agents for biological control.

In Chapter 6, the data presented in Chapters 2 - 5 were discussed in the context of the prospects for biological control of *S. pyramidalis* and *S. natalensis* in Australia, based on the climatic-compatibility, geographic distribution, seasonal incidence, feeding biology, field host-range, and predicted efficacy and complementarity of a suite of candidate agents identified from the two grasses native ranges'. Recommendations are made for the development of the *Sporobolus* biological control programme in Australia. Moreover, the value of native-range field surveys for identifying and prioritising candidate biological control in a parsimonious manner was explored, and the suitability and challenges of using biological control as a management technique for the suppression of invasive grasses was discussed.

Chapter 2: Searching for a needle in a haystack: where to survey for climatically-matched biological control agents for two grasses (*Sporobolus* spp.) invading Australia

2.1. Introduction

The success of classical weed biological control is largely based on the ability of a candidate agent to establish and proliferate in the regions where it is released, which may often depend on climatic factors (Wapshere, 1983; Byrne et al., 2004). Climatic factors, such as temperature, precipitation and humidity, have a direct influence on insect development, survival and reproduction, as well as fungal pathogen germination and infection (Lovell et al., 2004). These factors are among the foremost determinants of the establishment, distribution and abundance of weed biological control agents in their introduced range (Byrne et al., 2002; Cowie et al., 2016).

Climatic incompatibility between source and release regions has long been recognised as one of the primary reasons why biological control agents have failed to establish significant populations upon introduction into a novel region (McClay and Hughes, 1995; Rafter et al., 2008; Robertson et al., 2008). It is estimated that 44% of weed biological control agents have failed to establish due to climatic incompatibility (McEvoy and Coombs, 2001). Candidate control agents sourced from climatically-matched regions in their native range to their intended release sites are more likely to establish and proliferate upon release and provide control over the target weed than agents sourced from climatic regions that are incompatible (Wapshere, 1983). Moreover, sourcing, testing and releasing climatically-incompatible biological control agents is a significant waste of time and resources (Byrne et al., 2004). For example, the cost of finding and developing a candidate biological control agent was estimated at $ 475 334 (in 2014 NZ$) (Paynter et al., 2015a), so it is important that the most promising potential agents are selected to avoid this significant use of resources on an agent that is not climatically suited, and may, therefore not be effective. Climate matching techniques have been used to prioritise regions in the native range of a target weed to conduct surveys for climatically-matched candidate biological control agents (Robertson et al., 2008; Rafter et al., 2008). This reduces the expenses of field work in the native range and increases the chances of the agent being effective at controlling the weed.

One limitation of climate matching studies is that they have focused on the degree of climatic matching between regions where control agents are to be released and their native range (Robertson et al., 2008). This neglects the likelihood of regions in the native range containing populations of the host plant. Invasive plants frequently occupy climatic niches in their invaded range which are significantly different from the niche occupied in their native range (Gallagher et al., 2010), or due to the weed being released from its natural enemies and/or genetic drift (Mooney and Cleland, 2001). Moreover, the native ranges of many weeds targeted for biological control are often poorly described due to a lack of surveys and the logistical difficulties of accessing certain geographic regions (McFadyen, 2003). As such, the native range of a target weed may possess gaps which are in fact part of the plants native range (i.e. the plant has been falsely assumed as absent from such regions), and which should be considered when prioritising regions to survey for biological control agents (Scott et al., 2016). Regions that are unlikely to contain populations of the target weed could be excluded prior to initiating phytophagous surveys, while regions that are not formally included in the target weeds described native range can be identified and included in the potential regions to be surveyed for biological control agents. Knowledge of the complete potential native range of a target weed provides greater coverage of its fundamental niche, and may allow for a more complete representation of its phytophagous fauna (Bell et al., 2013)

A number of climate-matching models have been developed, which can be broadly categorised into (1) correlative models, which use statistical functions between species presence (and assumed absence) and its response to environmental covariates (e.g. MaxEnt and Boosted Regression Trees) (Elith and Leathwick, 2009), and (2) mechanistic or process-based models, which link eco-physiological parameters of the species to environmental covariates, either derived experimentally or inferred from known distributions (e.g. CLIMEX; Sutherst and Maywald, 1985), to predict environmental suitability for a species (Dormann et al., 2012). Both correlative (e.g. Mukhurjee et al., 2012; Russel et al., 2017) and mechanistic climate matching models (e.g. Robertson et al., 2008; Rafter et al., 2008) have been used by weed biological control practitioners to prioritise regions in a weeds native distribution to perform surveys. In recent years, much debate has ensued over the validity, and respective advantages and disadvantages of the different modelling techniques (e.g. Peterson et al., 2007; Kriticos et al., 2013). This thesis does not intend to be a proponent of one model class over the other, particularly for application in weed biological control. Rather, it follows Dormann et al. (2012) and Kriticos et al. (2013), in recognising that both correlative and mechanistic models have their own strengths and weaknesses, which need to be considered when interpreting model outputs.

Sporobolus pyramidalis and *S. natalensis* have a widespread native range in Africa and a small region in the Middle East, with *S. pyramidalis* distributed from South Africa to Yemen in the north, as well as the islands of Mauritius and Madagascar (Fig. 2.1a), while *S. natalensis* is distributed from South Africa to Ethiopia in the north (Fig. 2.1b). The broad native ranges of both species make surveying their entire distributions unfeasible, highlighting the need to prioritise climatically matched regions to survey for candidate control agents, while maximising the geographic sampling coverage of the target weeds phytophagous assemblage. Moreover, the disjunct known native distributions of *S.*

pyramidalis (e.g., Nigeria) and *S. natalensis* (Fig. 2.1) call into question whether the full native distribution of these species has been recorded.

In this study, climate matching models were used to identify regions within the native distributions of *S. pyramidalis* and *S. natalensis* where phytophagous surveys should be conducted to limit the risk of climatic incompatibility with intended biological control agent release sites in Australia. Thereafter, regions which were not likely to contain target weed populations were identified and excluded from consideration, and regions that were climatically suitable for *S. pyramidalis* and *S. natalensis* but were not part of the known native range were identified. Candidate control agents collected from these climatically-matched regions were expected to have the greatest probability of establishing and forming vigorous populations at their intended release sites in Australia (Robertson et al., 2008). This approach allows for a scientifically-motivated allocation of time and resources when conducting surveys for candidate control agents for *S. pyramidalis* and *S. natalensis*, and may ultimately improve the likelihood of achieving successful control of these two highly-damaging grass invaders in Australia.

2.2. Methods and materials

2.2.1. Species occurrence records

Species distribution data were compiled from a variety of sources. Global occurrence records were primarily sourced from the Global Biodiversity Information Facility (GBIF) portal (accessed at: http://data.gbif.org) (see Appendix 1 for details). These records were supplemented with data obtained from numerous Australian state and territory herbaria, which were accessed through the Australian Virtual Herbarium (AVH) online database (https://avh.chah.org.au/), the South African National Biodiversity Institute (SANBI), scientific literature, collaborators and from field surveys conducted by the author. The compiled species occurrences were cleaned in the following manner: (1) duplicates were removed, (2) co-ordinate errors were corrected where possible (occurrences were removed if not), (3) co-ordinates lacking sufficiently fine-scale precision (e.g. to the nearest 0.5°) were excluded, (4) occurrences growing in managed areas were excluded (e.g. botanical gardens), and (5) co-ordinates with spatial errors exceeding 10km (as recorded in the GBIF database) were removed. Species occurrence datasets compiled from museum specimens and/or online herbaria (e.g., GBIF and AVH), are typically derived from numerous surveys lacking a systematic sampling protocol. As such, they are likely to exhibit geographic and environmental biases (e.g. spatial autocorrelation), which can significantly reduce model quality (Beck et al., 2014). Filtering of species occurrence data may limit the inherent biases in the data and improve model quality (Veloz, 2009). Filtering was implemented using two approaches. First, species occurrences were thinned to retain a single sample point per 5' pixel to avoid pseudo-replication (cell filtering). Second, species occurrences were then thinned at distances which minimised the influence of spatial autocorrelation (spatial filtering) (Boria et al., 2014) (see Appendix 2 – Spatial autocorrelation curves). Species occurrence datasets were thinned using the '*spThin*' package (Aiello-Lammens et al., 2015), and spatial autocorrelation analyses were performed using the '*ecospat*' package (Di Cola et al., 2017).

2.2.2. Environmental predictors

Climate data were obtained by downloading the standard set of 19 bioclimatic variables from the WorldClim ver. 1.4 database (Hijmans et al., 2005) (data available at: www.worldclim.org/download.htm). This dataset was representative of annual and seasonal means and variation of temperature and precipitation metrics averaged over the 1950 – 2000 time period (current climate) at a 5 arc minute resolution. This spatial resolution reflected the accuracy of species distribution records obtained from the AVH and GBIF databases (~ 10km) (Gallagher et al., 2013). These variables are known to potentially influence species distributions, and have been used to model the abiotically suitable areas for multiple weed invaders, including multiple grass species in both study regions of the current study (Gallagher et al., 2010; Gallagher et al., 2013; Bocksberger et al., 2016).

Little biological information is available to guide the selection of environmental covariates to calibrate models for *S. pyramidalis* and *S. natalensis*. As such, eight predictors were selected that have previously been used to model grass distributions in Africa and Australia (e.g., Gallagher et al., 2010; Bocksburger et al., 2016). These eight variables were reduced to a set of uncorrelated variables for each species x region model. This was performed by computing Pearson's correlation coefficients between all pairs of predictors, whereby predictors which were highly correlated ($|r| > 0.70$) were excluded from the final predictor set (Dormann et al., 2013). Producing a set of uncorrelated predictors decreases the risk of calibrating overfit models. Thereafter, preliminary MaxEnt models were calibrated for each species x region, and the fitted response curves investigated for uncorrelated predictors which had little influence on predicted species presence (i.e. response curves with a single, flat horizontal line), which were removed from the final environmental predictor set. The final reduced set of environmental predictors for *S. pyramidalis* consisted of: (a) native-range model – bio2 (mean diurnal temperature range [mean of monthly max temp - min temp]), bio6 (minimum temperature of the coldest month) and bio12 (mean annual precipitation), and (b) invaded-range model - bio5 (Max Temperature of Warmest Month), bio6, bio12 and bio17 (Precipitation of Driest Quarter), and for *S. natalensis* consisted of: (a) native-range model – bio1, bio6 and bio12, and (b) invaded-range model – bio1, bio12 and bio17 (see Hijmans et al., 2005 for further details) (see Appendix 3).

2.2.3. Climate matching models

The Maximum Entropy Species Distribution Model ver. 3.4.1 (hereafter 'MaxEnt') (Phillips et al., 2006; Phillips and Dudík, 2008), implemented in the '*dismo*' package in R (Hijmans et al., 2017), was used to prioritise regions in the native range of *S. pyramidalis* and *S. natalensis* to survey for potential biological control agents. MaxEnt was chosen as it outperforms other correlative ecological niche modelling algorithms (Wisz et al., 2008; but see Peterson et al., 2007), and has been shown to perform as well as mechanistic climate models, for both insects (e.g. Kumar et al., 2014) and plant pathogens (e.g. Narouei-Khandan et al., 2020). MaxEnt uses species occurrence points (latitudes and longitudes) and environmental predictor layers to predict a species' geographic distribution (Philips et al., 2006). It has been used to identify regions in a target weeds' native range to search for climatically-matched biological control agents in a number of studies (e.g. Mukherjee et al., 2011; Russell et al., 2017).

Climatic matches between intended biological control agent release sites in Australia and the weeds native ranges' were determined by generating individual MaxEnt models for *S. pyramidalis* and *S. natalensis* calibrated with invaded range occurrences (i.e., species occurrences from Australia only). These models were then projected into geographic space over Africa (native-range). These models identify regions in the native range which are most likely to contain climatically-matched natural enemies to their intended release sites in Australia (e.g. Mukherjee et al., 2011; Russel et al., 2017).

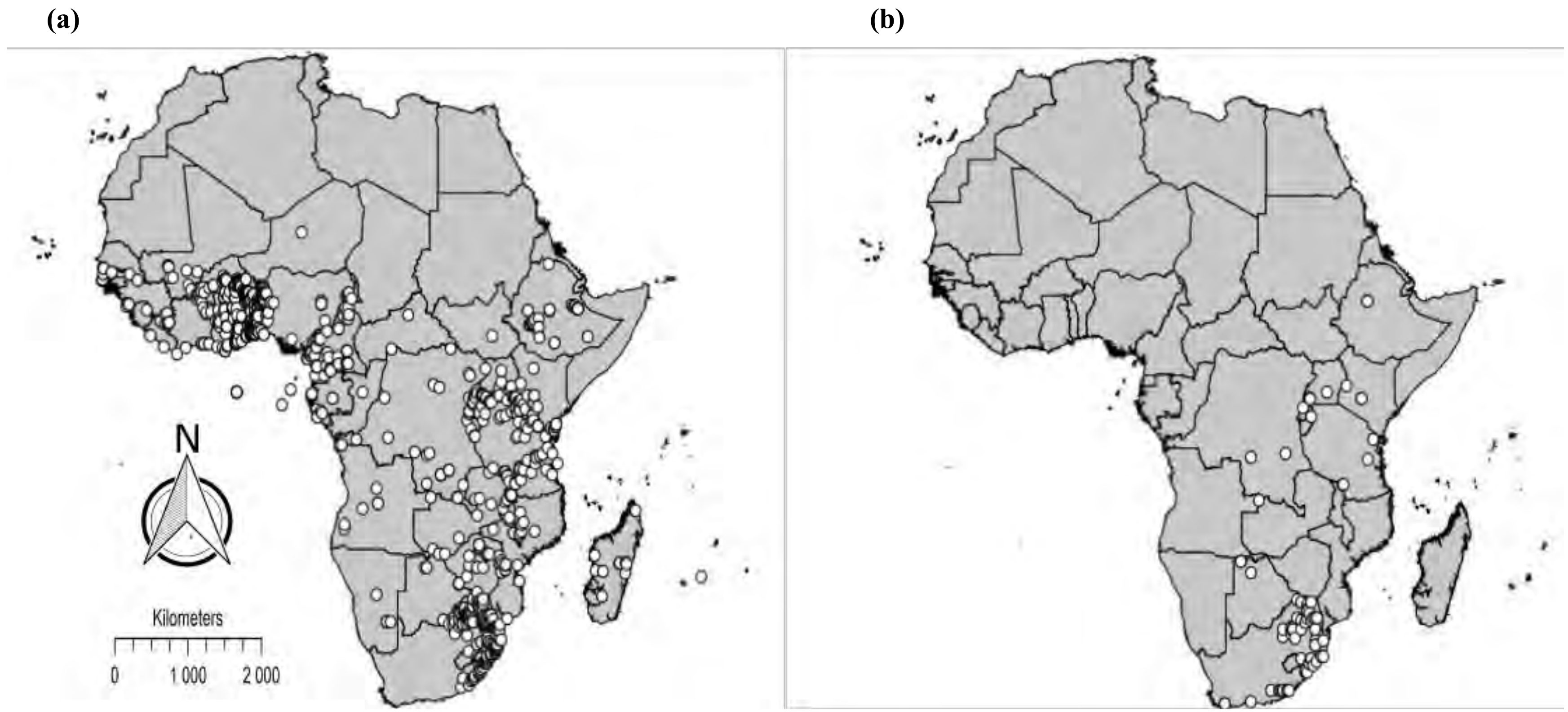


Figure 2.1. Native ranges' of (a) *Sporobolus pyramidalis* and (b) *S. natalensis*. Each filled, white circle represents a unique species locality. Species occurrences were downloaded from the Global Biodiversity Information Facility (Appendix 1).

However, climate matching studies have been advised to consider both climatic suitability for plant growth and survival, as well as the degree of climatic matching with the invaded range of the target weed (Robertson et al., 2008; Scott et al., 2016). Accordingly, an additional set of MaxEnt models were calibrated for *S. pyramidalis* and *S. natalensis* using species occurrence data from their native ranges' (i.e., species occurrences from Africa only). Models calibrated with native-range occurrences were indicative of favourable climatic conditions for plant growth, survival and reproduction. Higher suitability values were interpreted as regions that were more likely to contain populations of the target weeds.

Default MaxEnt settings have been shown to occasionally produce models that are overfit (Shcheglovitova and Anderson, 2013). Overfitting occurs when the model fits the calibration data too well, and thus, performs poorly when tested against evaluation data (Boria et al., 2017). Overfit models are a major concern when transferring models in space or time (Yates et al., 2018), such as the invaded-range models calibrated in this chapter, because they may lack generality, and thus, may provide erroneous projections in novel climatic space (see Radosavljevic and Anderson, 2014 and references therein). As such, MaxEnt users are recommended to perform model tuning experiments to estimate optimal MaxEnt complexity and settings configurations (Shcheglovitova and Anderson, 2013; Radosavljevic and Anderson, 2014; Galante et al., 2018). Model tuning has been shown to produce simpler and more realistic MaxEnt models and geographic predictions than models calibrated with default settings (see Galante et al., 2018 and references therein). Model tuning experiments were performed by estimating optimal model complexity (i.e. models that best approximated reality while minimising the degree of model overfitting), by varying the feature classes and regularization multiplier used to build MaxEnt models (Shcheglovitova and Anderson, 2013). MaxEnt allows users to specify a range of feature classes (FC), which control the function and shape of the environmental response curves used to calibrate models (Philips and Dudik, 2008). Using complex FC's can allow MaxEnt to fit complex species responses to environmental variables, albeit, this can also lead to overfitting of MaxEnt models (Shcheglovitova and Anderson, 2013). Thereafter, MaxEnt also allows users to specify a species-specific regularization multiplier (RM) to penalise models for including unnecessary environmental variables and features into the model, and thus, protects against overfitting (Shcheglovitova and Anderson, 2013).

Optimal FC and RM settings configurations were determined using two approaches. First, threshold-dependent omission rates were compared with theoretical expectations of omission rates to estimate the degree of overfitting associated with each FC and RM combination. The omission rate is the proportion of evaluation (test) occurrence localities that are not predicted to fall within the projected model surface once the model is converted into a binary prediction output (Boria et al., 2014). Overfit models have omission rates higher than the theoretical expectation for the threshold applied (Shcheglovitova and Anderson, 2013). The 10th percentile calibration omission rate (hereafter 'OR10') was applied in this study to estimate model overfitting. OR10 sets the binary prediction threshold at a value that excludes the 10% of the calibration localities from the model with the lowest prediction values, and therefore has an expected omission rate of 0.10 (Boria et al., 2014). As such, this first criterion selected models calibrated with MaxEnt settings which best approximated the expected 0.10 omission rate. Models with omission rates increasingly higher than the expected value were considered as more overfit (Boria et al., 2017). Second, optimal model settings were determined by selecting model configurations which produced the lowest value for the Akaike Information Criterion corrected for small sample sizes (AICc) (i.e. ΔAICc = 0; Muscarella et al., 2014). The AICc criterion simultaneously scores models according to their complexity and goodness-of-fit, whereby models with the lowest AICc are selected as the best models (Galante et al., 2018). AICc was used as the primary evaluation metric as it is calculated using MaxEnt models built using the entire species occurrence dataset, unlike OR10 (and numerous other metrics frequently used for model evaluation) which may be spatially biased due to the partitioning of the species occurrence dataset into training and evaluation sets (Sanín and Anderson, 2018).

Numerous authors have raised concerns over the use of correlative climate models and projecting these models into new regions or time periods due to issues with extrapolation into novel/non-analogous climate (e.g. Elith and Leathwick, 2009; Sutherst and Bourne, 2009; Elith et al., 2011). As such, critical evaluation of the performance of correlative climate models are required (Elith et al., 2010; Webber et al., 2011). Here, multivariate environmental similarity surfaces (hereafter 'MESS'; *sensu* Elith et al., 2010) were computed to assess whether MaxEnt models were extrapolating or interpolating. MESS maps measure the similarity of any given point to a set of reference points [reference points were defined as only the species presence points used to calibrate models as per Kriticos et al. (2014)], allowing the delineation of geographic regions that were within the range of covariates used to calibrate the model. Negative MESS values indicate geographic regions outside the range of climate variables used to calibrate the model (i.e. extrapolation space or MESS-), while MESS values between 0 and 100 indicate geographic regions inside the range of climatic variables used to calibrate the model (i.e. interpolation space or MESS+). Typically, MESS maps are used as a measure of prediction uncertainty or caution against inferences in extrapolation space (Elith et al., 2010). In this chapter, a more conservative approach was taken, whereby any geographic region that was in extrapolation space (MESS-) was masked from the native-range and invaded-range model projections for both focal species. As such, any inferences made from the resulting MaxEnt projections are limited to geographic regions that were interpolating (MESS+) (Elith and Burgman, 2014). MESS masks were made by thresholding MESS maps at zero, and then filtering out all regions that were in extrapolation space (MESS-) (Webber et al., 2011). The MESS mask was then overlaid over MaxEnt model projections (both native-range and invaded-range models). MESS analyses were implemented in the '*dismo*' package in R (Hijmans et al., 2015).

Given that MaxEnt is a presence-only modelling algorithm, model calibration requires a user-defined geographic background to sample the climate of representative grid cells where the focal species is absent (i.e. background points), whereby the definition of the background extent can have a significant effect on model output (VanDerWal et al., 2009). The background should ideally represent the geographic areas available to the focal species, omitting areas where species absence is due to historical factors, dispersal constraints and/or biotic interactions (Sanín and Anderson, 2018). Often, the background is defined by a minimum convex polygon drawn around the species occurrence points (e.g. Darwell and Althoff, 2017). A more ecologically relevant approach may be to define the background using a biophysical classification. Here, the Koppen–Geiger classification was used because it classes the Earth's climate into zones based on vegetation, precipitation and temperature with class boundaries specifically chosen to match large-scale vegetation changes (see Webber et al., 2011). Species occurrences for both *S. pyramidalis* and *S. natalensis* in their native and invaded ranges' were intersected with Koppen-Geiger climate zones. Only Koppen-Geiger zones containing at least 1 species occurrence were retained in the background definition.

2.2.4. High-priority survey regions

To identify high-priority regions to search for candidate biological control agents of *S. pyramidalis* and *S. natalensis*., native-range and invaded-range model projections were overlaid. Regions receiving high model scores for both model sets were assigned the highest priority for phytophagous surveys, as they were deemed most likely to contain target weed populations, and to yield climatically-matched agents for intended release sites for biological control in Australia. All models were projected and visualised using MaxEnt's logistic output, with values ranging from 0 (low climatic match) to 1 (high climatic match) (Mukherjee et al., 2011). Increasing warmer colours indicated higher model prediction scores (i.e., red pixels indicate high-priority survey regions), while increasing cooler colours indicate successively lower prediction scores (i.e., blue pixels indicate inappropriate survey regions). For ease of visualisation, a binary map of high-priority survey regions was created for both *S. pyramidalis* and *S. natalensis* by masking out any regions from the native-range and invaded-range models with MaxEnt projection scores < 0.70. The 0.70 value was used as it constrained the high-priority search area to a greater extent than other thresholding rules typically applied (e.g. minimum training presence [MTP] and OR10; Liu et al., 2005; data not shown), which was advantageous for the current study which sought to identify and prioritise only the most climatically matched regions to survey for biological control agents. However, all inferences made on model outputs were performed on the continuous logistic MaxEnt output. All modelling and statistical analyses were conducted in R. ver. 3.4.2 (R Core Team, 2018).

2.3. Results

2.3.1. Model tuning and evaluation

Spatial filtering substantially reduced the number of species occurrences used for model calibration for both *S. pyramidalis* and *S. natalensis* (Table 2.1). For *S. pyramidalis*, the final spatially filtered data set consisted of 320 (native-range model) and 43 (invaded-range model) unique occurrences to be used for model calibration. For *S. natalensis*, the final spatially

Table 2.1. Summary of filtering on species occurrence datasets used to calibrate MaxEnt models for *Sporobolus pyramidalis* and *S. natalensis*. Total = unfiltered datasets, cell filtered = removing duplicate records from individual raster cells, and spatially filtered = spatial filtering of occurrence records to remove spatially autocorrelated species occurrences identified from computing mantel correlograms in the '*ecospat*' package in R (Di Cola et al., 2017) (see Appendix 2).

	No. of records		
	Total	Cell filtered	Spatially filtered[a]
Sporobolus pyramidalis			
Native-range model	1,204	610	320
Invaded-range model	281	133	43
Sporobolus natalensis			
Native-range model	75	64	40
Invaded-range model	136	91	27

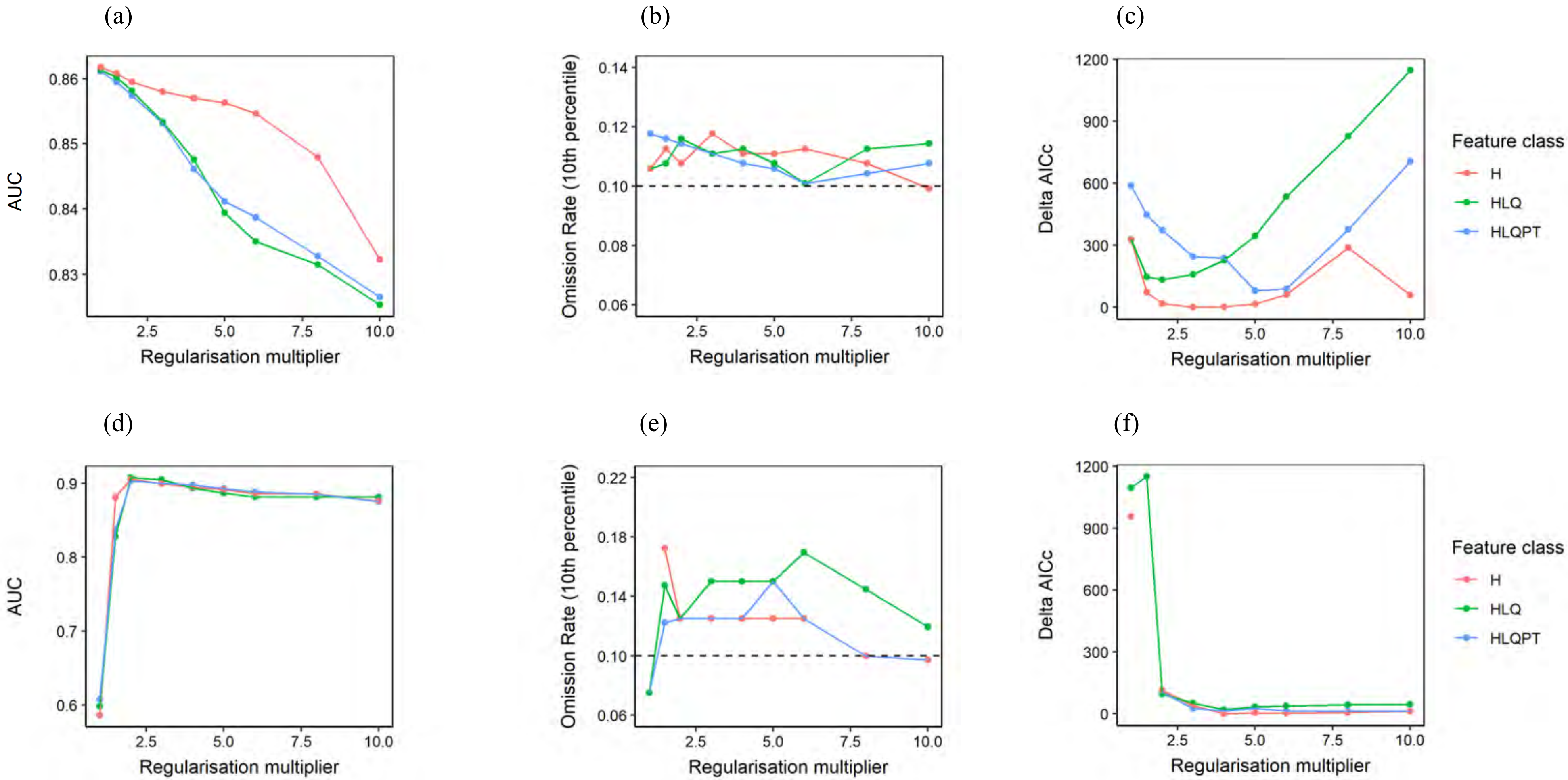
[a] The spatially filtered dataset was used as the species occurrence input for model calibration

filtered data set consisted of 40 (native-range model) and 27 (invaded-range model) unique occurrences to be used for model calibration.

The optimal and co-optimal model settings configurations differed amongst the species x region candidate models (Fig. 2.2, 2.3). Default MaxEnt model settings were not identified as being an optimal model solution for any set of candidate models. The optimal settings configuration for the *S. pyramidalis* native-range and invaded-range models, and the *S. natalensis* invaded-range model, consisted of using 'hinge' features only, and a regularisation multiplier of 4.0 (Fig. 2.2, 2.3). The *S. natalensis* native-range model was run using 'hinge' features only, and a regularisation multiplier of 3.0 (Fig. 2.3). Predictive performance across all models was high, with all models demonstrating high predictive accuracy and low omission rates. AUC scores were all > 0.89, and 10^{th} percentile omission rates (OR10) approximated the expected omission rate of 0.10, indicating effective discriminatory ability between suitable and unsuitable climatic regions and no significant overfitting, across all models.

Figure. 2.2. (next page) Model tuning experiments for *Sporobolus pyramidalis*, (a-c) native-range models and (d-f) invaded-range models. Metrics used to assess model fit included, (a, d) evaluation AUC indicates the discriminatory ability of the model with values closer to 1 provides better discriminatory power, (b, e) indicate the omission rates in comparison to the expected 10% omission rate following thresholding using the 10th percentile training omission rate (the horizontal black line represents the expected omission rate of 0.10), and (c, f) represent the difference in AICc scores between candidate models. Optimal model settings were determined with respect to varying feature class combinations (H = hinge; HLQ = hinge + linear + quadratic; HLQPT = hinge + linear + quadratic + product + threshold) and regularization multipliers (RM) ranging from 0.5 to 10.0.

Figure. 2.3. (following page) Model tuning experiments for *Sporobolus natalensis*, (a-c) native-range models and (d-f) invaded-range models. See Fig. 2.2. for details.



(a)
(b)
(c)
(d)
(e)
(f)
AUC
Omission Rate (10th percentile)
Delta AICc
Regularisation multiplier
Feature class
H
HLQ
HLQPT
0.86
0.85
0.84
0.83
0.14
0.12
0.10
0.08
0.06
1200
900
600
300
0
0.9
0.8
0.7
0.6
0.22
0.18
0.14
2.5
5.0
7.5
10.0

Figure. 2.2.

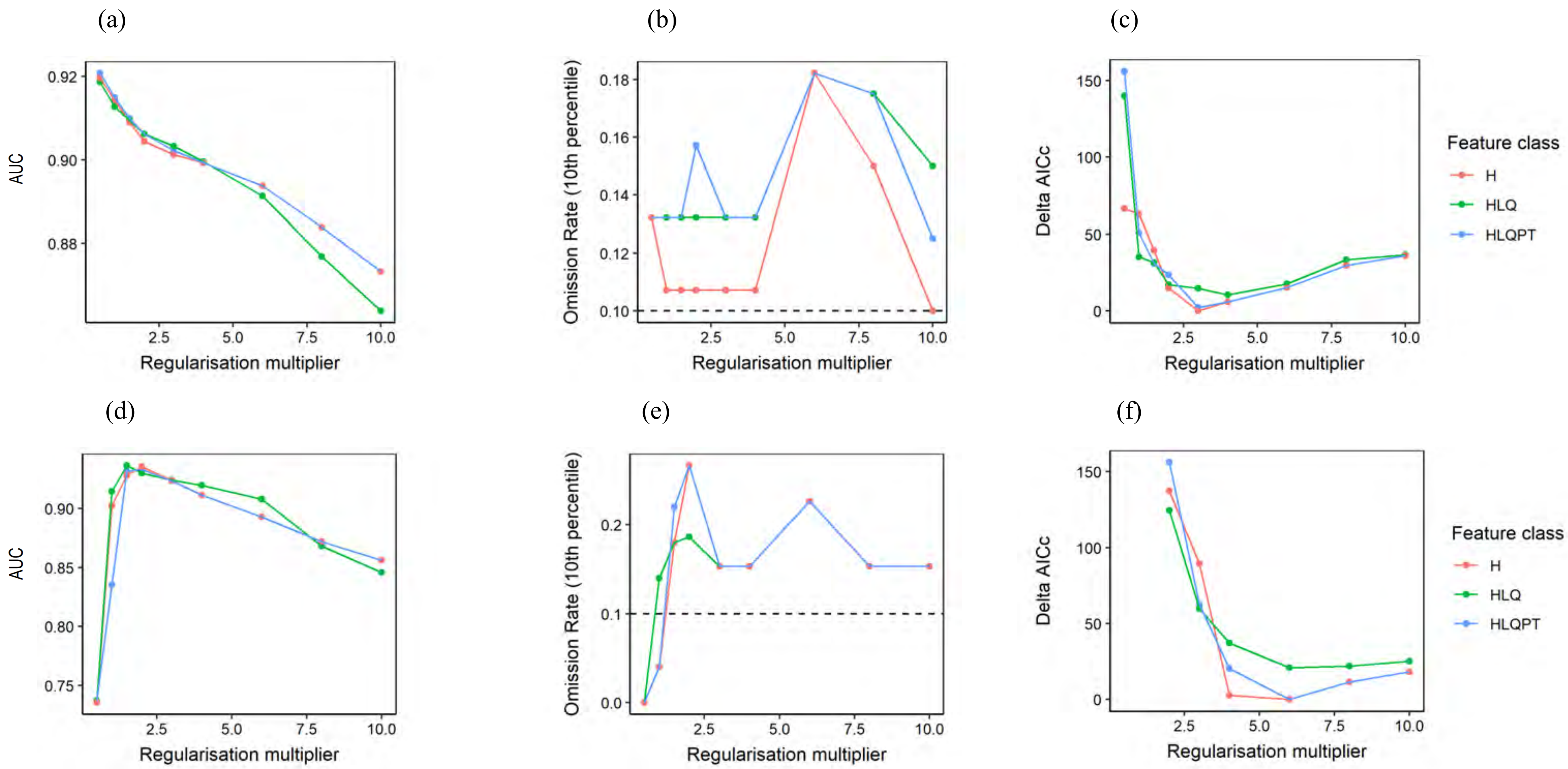

(a)
(b)
(c)
(d)
(e)
(f)
AUC
Omission Rate (10th percentile)
Delta AICc
Regularisation multiplier
Feature class
H
HLQ
HLQPT
0.92
0.90
0.88
0.18
0.16
0.14
0.12
0.10
150
100
50
0
2.5
5.0
7.5
10.0
0.85
0.80
0.75
0.2
0.1
0.0

Figure. 2.3.

2.3.2. *Sporobolus pyramidalis*

Much of sub-Saharan Africa was climatically suitable for *S. pyramidalis*, with the notable exceptions of western South Africa, Namibia and Botswana, and northern Kenya, eastern Ethiopia and Somalia (Fig. 2.4a). The east coast of South Africa and central Ethiopia received the highest climatic suitability scores, while much of the east coast of Africa, ranging from Zimbabwe and Mozambique in the south, to southern Kenya in the north, and parts of Madagascar, received slightly lower suitability scores. Western Angola and south-eastern Democratic Republic of Congo (DRC) also received high climatic suitability scores, despite very few known *S. pyramidalis* occurrences in these regions. Much of coastal West Africa received relatively high suitability scores, particularly regions along the coast between Ghana and western Nigeria. MESS maps indicated that the climatic suitability projections for *S. pyramidalis* were in interpolation space, and thus, extrapolation is unlikely to be a significant influence on the model output (Fig. 2.4b).

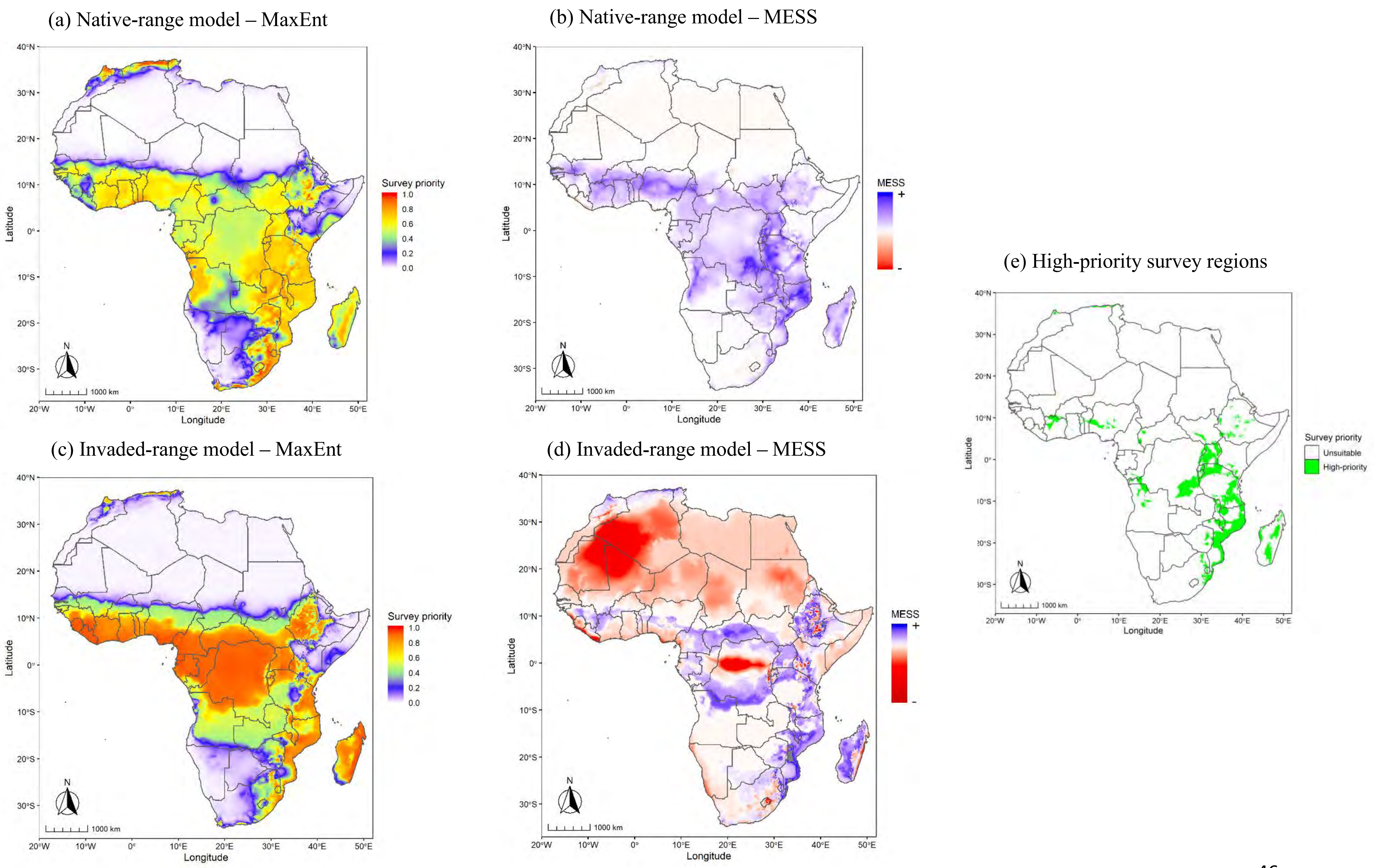
Large parts of Central and West Africa, ranging from Guinea in the west to Ethiopia in the east, including most of DRC, were projected to be climatically compatible with *S. pyramidalis* infestations in Australia (Fig. 2.4c). However, the MESS surface indicated that much of this area, particularly central DRC and coastal West Africa, were regions into which the MaxEnt models were extrapolating (Fig. 2.4d), and thus, these regions were excluded from consideration, due to model uncertainty. Given the open-ended response curves (i.e. fitted environmental responses allowed *S. pyramidalis* to maintain high suitability beyond the limits of the data used to calibrate the model) of multiple environmental variables (see Appendix 4), the high climatic suitability scores in extrapolation space are not surprising. Large parts of coastal East Africa, ranging from Kenya to north-eastern South Africa, as well as eastern Madagascar, were projected to be compatible with *S. pyramidalis* infestations in Australia, and were regions where the model was interpolating (Fig. 2.4d).

High-priority geographic regions to survey for potential biological control agents of *S. pyramidalis* were: (1) coastal East Africa, ranging from north-eastern South Africa to Uganda, including south-eastern DRC, (2) some parts of West Africa, including inland regions of the Ivory Coast and western Nigeria, (3) northern Angola and (4) eastern Madagascar (Fig. 2.4e).

2.3.3. *Sporobolus natalensis*

The climatically suitable area for *S. natalensis* occurs over a restricted and localised distribution across sub-Saharan Africa (Fig. 2.5a). Most of the climatically suitable area for *S. natalensis* occurs within South Africa, particularly along the south and east coast, with smaller suitable regions scattered throughout east Africa, from the eastern highlands of Zimbabwe to central Ethiopia (Fig. 2.5a). The west coast of South Africa and east coast of Madagascar are predicted to be climatically suitable for *S. natalensis* (Fig. 2.5a), however, the MESS maps indicate that these regions are where the model is extrapolating, and thus, were excluded from consideration, due to model uncertainty (Fig. 2.5b).

Figure 2.4. (next page) Climate matching maps for *Sporobolus pyramidalis* indicating: (a) climatic suitability in the native range (models calibrated with native-range occurrences), (b) MESS surface indicating where MaxEnt the *S. pyramidalis* native-range model in (a) was in extrapolation (MESS-) and interpolation space (MESS+), (c) climatic similarity to weed infestations in Australia (models calibrated with invaded-range occurrences), (d) MESS surface indicating where MaxEnt the *S. pyramidalis* invaded-range model in (c) was in extrapolation (MESS-) and interpolation space (MESS+), and (e) a binary projection of high-priority regions to conduct surveys for potential biological control agents, which was created by overlaying (a) and (c), and masking model outputs to only allow inferences to be made in regions where the MaxEnt models were in interpolation space (b, d).


(a) Native-range model – MaxEnt
(b) Native-range model – MESS
(c) Invaded-range model – MaxEnt
(d) Invaded-range model – MESS
(e) High-priority survey regions
Survey priority
1.0
0.8
0.6
0.4
0.2
0.0
MESS
+
-
Survey priority
Unsuitable
High-priority
Latitude
Longitude
40°N
30°N
20°N
10°N
0°
10°S
20°S
30°S
20°W
10°W
0°
10°E
20°E
30°E
40°E
50°E
N
1000 km

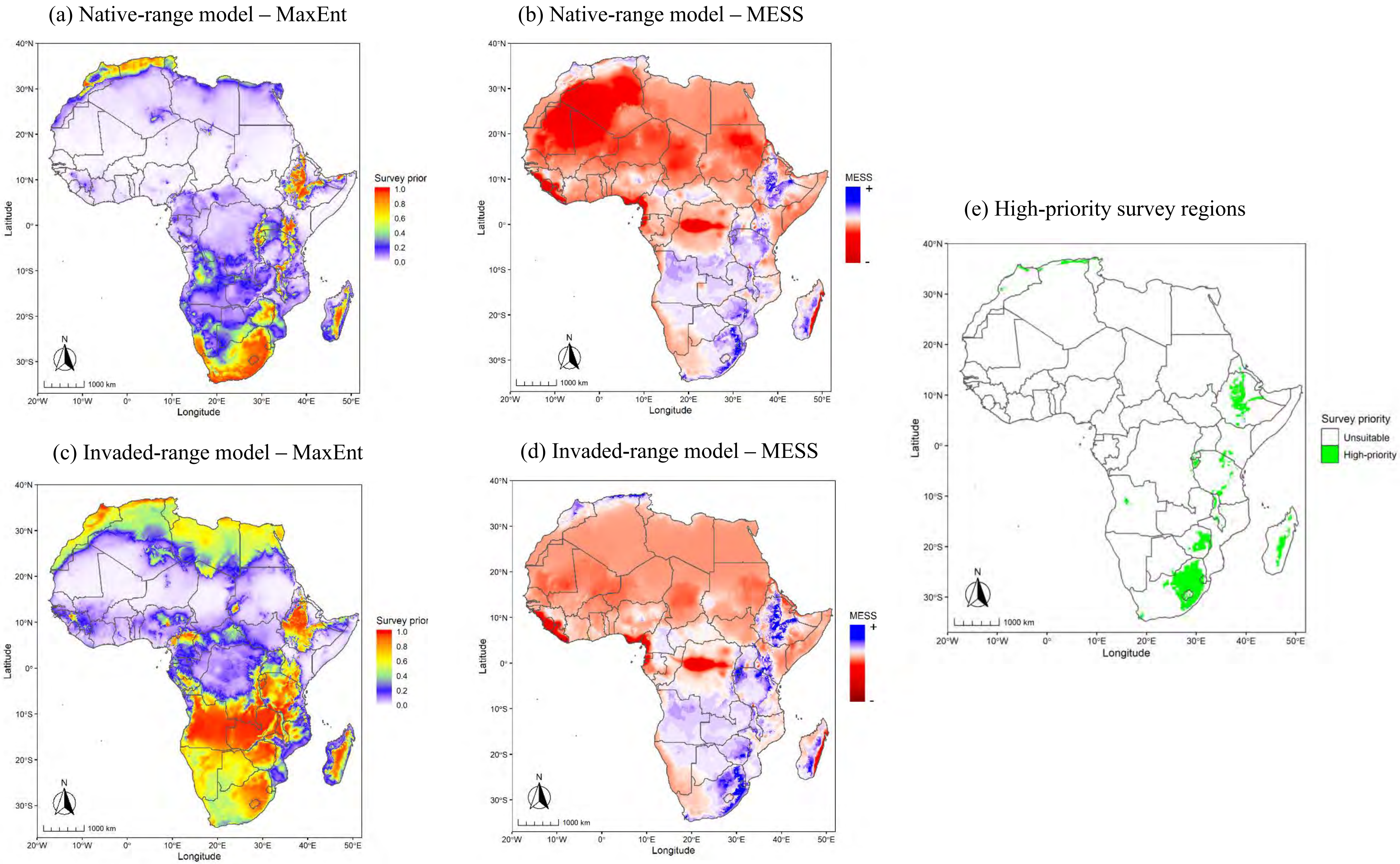
Much of Angola, Zambia and Zimbabwe received high climatically compatibility scores with *S. natalensis* infestations in Australia (Fig. 2.4c). Other climatically compatible regions were eastern South Africa, western Ethiopia, central Madagascar and parts of Tanzania (Fig. 2.4c). MESS maps indicated that the climatic compatibility projections for *S. natalensis* were in interpolation space, and thus, extrapolation is unlikely to be a significant influence on the model output (Fig. 2.4d).

High-priority geographic regions to survey for potential biological control agents of *S. pyramidalis* were: (1) eastern South Africa, (2) eastern Zimbabwe, (3) Burundi, (4) central Ethiopia and (5) central Madagascar (Fig. 2.5e).

2.4. Discussion

Biological control agents that have been collected from climatically-matched regions in a weeds' native range are expected to have the greatest probability of establishing at their intended release sites, and therefore becoming successful control agents (Robertson et al., 2008). These regions are typically identified using climate-matching techniques which identify regions within the weeds' native distribution that are climatically similar to weed infestations (i.e. the invaded range) (e.g., Rafter et al., 2008; Mukhurjee et al., 2011; Scott et al., 2016).

Figure 2.5. (next page) Climate matching maps for *Sporobolus natalensis* indicating: (a) climatic suitability in the native range (models calibrated with native-range occurrences), (b) MESS surface indicating where MaxEnt the *S. natalensis* native-range model in (a) was in extrapolation (MESS-) and interpolation space (MESS+), (c) climatic similarity to weed infestations in Australia (models calibrated with invaded-range occurrences), (d) MESS surface indicating where MaxEnt the *S. natalensis* invaded-range model in (c) was in extrapolation (MESS-) and interpolation space (MESS+), and (e) a binary projection of high-priority regions to conduct surveys for potential biological control agents, which was created by overlaying (a) and (c), and masking model outputs to only allow inferences to be made in regions where the MaxEnt models were in interpolation space (b, d).


(a) Native-range model – MaxEnt
(b) Native-range model – MESS
(c) Invaded-range model – MaxEnt
(d) Invaded-range model – MESS
(e) High-priority survey regions
Survey prior
1.0
0.8
0.6
0.4
0.2
0.0
MESS
+
-
Survey priority
Unsuitable
High-priority
Latitude
Longitude
N
1000 km

However, climate-matching has been criticised because regions in the native range that are predicted to be climatically compatible to weed infestations in the adventive range may not be suitable for plant growth, reproduction and survival, and may therefore not contain host-plant populations (Robertson et al., 2008). Moreover, many weeds targeted for biological control have undescribed or relatively unknown native distributions, and therefore large parts of their native distributions may go unsampled when conducting surveys for biological control agents. This study identified geographic regions in the native ranges of *S. pyramidalis* and *S. natalensis* to search for climatically-compatible biological control agents, while simultaneously excluding regions from consideration that were unlikely to contain populations of the host plants. This was achieved by overlaying climate models indicative of climatic compatibility with weed infestations in Australia (i.e. models calibrated using invade-range occurrences only) and climatic suitability for plant growth and survival in the native range (i.e. models calibrated using native-range occurrences only).

The traditional climate matching approach projected large parts of sub-Saharan Africa to be climatically compatible with *S. pyramidalis* and *S. natalensis* weed populations in Australia. However, for a variety of reasons discussed below, sending practitioners to conduct surveys for potential biological control agents in some of these regions would be erroneous. Firstly, correlative niche models, such as the MaxEnt software used in this chapter, may perform poorly when projected into novel climatic conditions (either in space or time), due to issues with extrapolation (Elith et al., 2010; Yates et al., 2018). Given that MaxEnt predicts suitable habitat for a focal species by fitting response curves to environmental covariates, when the models are projected into climatic space outside of the range of the environmental data used to calibrate the model, this can lead to extrapolation and potentially misleading projections of climatic suitability (Elith et al., 2010; Webber et al., 2011). Indeed, if we consider the *S. pyramidalis* models presented in this chapter, much of central and northern Congo and DRC was projected to be climatically compatible with weed infestations in Australia. However, these projections were based on extrapolation outside of the range of the environmental data used to calibrate the climate-matching models, and therefore, may not actually be climatically matched to Australian climate occupied by *S. pyramidalis*. Drawing any inferences or practical implications from these data would be 'inherently risky' (Elith and Leathwhick, 2009; Elith et al., 2010).

Second, regions in the native range that are deemed climatically matched with weed infestations may not be suitable for plant growth, reproduction and survival, and therefore may not contain populations of the target plant (Robertson et al., 2008). This pattern may emerge because invasive plants frequently occupy climatic niches in their invaded range which are significantly different from the niche occupied in their native range (Atwater et al., 2018), including *S. pyramidalis* in Australia (Gallagher et al., 2010), or due to the weed being released from its natural enemies and/or genetic drift (Mooney and Cleland, 2001). For example, the invaded-range *S. natalensis* models indicated the much of Angola was a climatic match with weed infestations in Australia, and that the model was not extrapolating in this region. However, there are no known records of *S. natalensis* in Angola, and the native-range models indicate that Angola was climatically unsuitable for *S. natalensis,* and as such, would likely represent a waste of resources sending researchers to survey this region for potential biological control agents, as it is unlikely the plant will be found.

Several high-priority surveys regions would not have been identified without modelling the potential native range of *S. pyramidalis* and *S. natalensis.* The native-range models for both *S. pyramidalis* and *S. natalensis* indicates that their native ranges' may be broader than currently described. For example, north-western Angola was climatically suitable for *S. pyramidalis*, despite no confirmed records from this region. Moreover, much of inland Nigeria was climatically suitable for *S. pyramidalis*. The distinct lack of records east of the geopolitical border between Benin and Nigeria, despite hundreds of records west of this border, is likely a consequence of under-sampling in Nigeria, rather than the true absence of *S. pyramidalis* in Nigeria (Chikoye et al., 2004). This is an important result for weed biological control because a small section of northern Angola, far south-western DRC and western Nigeria, were also modelled as being climatically matched with *S. pyramidalis* infestations in Australia, and thus, represent potentially important regions to survey for candidate biological control agents. A similar situation exists for *S. natalensis* and eastern Zimbabwe and central Madagascar. If practitioners travelled to and surveyed at known localities of the target weed, these regions would not have been identified as high-priority regions to perform surveys. By modelling the potential native ranges' of *S. pyramidalis* and *S. natalensis*, several regions that may form part of the native range of *S. pyramidalis* (e.g. northern Angola and much of Nigeria) and *S. natalensis* (e.g. eastern Zimbabwe, central Madagascar) were identified, all of which were climatically-matched with weed infestations in Australia, and thus, represent high-priority survey regions. This approach may be particularly relevant for weeds with an uncertain/unknown native range, such as weeds from Africa and South America, where logistic constraints may have limited the accessibility to these regions, and thus, may have poorly described distributions (McFadyen, 2003).

Multiple high-priority regions to survey *S. pyramidalis* and *S. natalensis* for potential biological control agents were identified. High-priority regions for *S. pyramidalis* included: (1) coastal East Africa, ranging from north-eastern South Africa to Uganda, including south-eastern DRC, (2) some parts of West Africa, including inland regions of the Ivory Coast and western Nigeria, (3) northern Angola and (4) eastern Madagascar, and for *S. natalensis* included: (1) eastern South Africa, (2) eastern Zimbabwe, (3) Burundi, (4) central Ethiopia and (5) central Madagascar. This does not imply that all of these regions need to be surveyed for biological control agents as there are a number of additional factors other than climate that have to be considered. For example, genetic matching of invasive weed populations to their source population in the native range (e.g. Goolsby et al., 2006; Paterson et al., 2009) and logistic constraints (e.g. funding, time, researcher safety, Nagoya Protocol) (Silvestri et al., 2010) are critical factors that determine where native-range biological control surveys should be performed. The climate modelling approach used in this chapter aimed to demonstrate how high-priority survey regions could be identified (based on climatic factors) in an ecologically justifiable manner.

In conclusion, climate modelling (using the MaxEnt software) was used to identify climatically-matched regions in the native range of *S. pyramidalis* and *S. natalensis* to survey for potential biological control agents. High-priority regions to perform surveys were identified by considering the climatic suitability for *S. pyramidalis* and *S. natalensis*, and climatic compatibility with regions where biological control is intended in Australia, while excluding regions where climate models were extrapolating (as they may produce unreliable projections of climatic suitability in these regions). This approach significantly reduced the total search area identified by the traditional climate matching approach, whereby only climatic compatibility is considered. Validating native-range model projections with focused surveys confirming the presence/absence of *S. pyramidalis* and *S. natalensis* populations across the native range should be prioritised in the near future. The climate modelling approach outlined in this chapter allows evidence-based decisions to be made regarding the prioritisation of regions to perform native-range weed biological control surveys. It may limit wasted expenditure conducting surveys in regions that are unsuitable (i.e. regions that were not actually climatically matched with weed infestations, or that may not contain the host-plant).

Chapter 3: Herbivorous insects associated with *Sporobolus pyramidalis* and *Sporobolus natalensis* (Poaceae) in South Africa: implications for biological control

3.1. Introduction

The suitability of biological control as a management strategy for invasive weeds is dependent on the size and structure of their natural enemy assemblages, and the degree of host-specificity required in the region where biological control is to be implemented (Goolsby et al., 2006). One group of plants that has been considered particularly unsuitable for biological control are invasive grasses. Historically, very few invasive grasses have been targeted for biological control (Pemberton, 1996; Bell et al., 2011; Schwarzländer et al., 2018). This largely stems from the perception that grasses support a small and unspecialised natural enemy community to exploit for potential biological control agents (Gill and Blacklow, 1984; Pemberton, 2002). Biological control programmes require that at least one natural enemy demonstrates a sufficiently narrow host range to not pose any significant risk to economic crops and/or native biodiversity in the region of intended control (Hinz et al., 2019).

Architecturally simple plants, such as grasses, are perceived to lack the diversity of resources and niches that are traditionally considered drivers of natural enemy diversification, the abundance of which is typically positively correlated with insect and fungal pathogen species richness (Strong et al., 1984; Tscharntke and Greiler, 1995; Clay, 1995). Moreover, most grasses lack the diversity and quantity of secondary chemical compounds (i.e. feeding deterrents, toxins, stimulants) typically found in dicotyledons, which are considered the primary drivers of natural enemy specialisation (Ehrlich and Raven, 1964; McNaughton et al., 1985; Moore and Johnson, 2017; but see Kellogg, 2015). Indeed, fewer than 0.2% of grasses produce alkaloids, while many other important secondary chemicals are almost entirely absent from grasses (McNaughton et al., 1985). Hence, grasses are expected to harbour relatively small and unspecialised herbivore assemblages (Gill and Blacklow, 1984; Wapshere, 1990; Pemberton, 2002), which may place a significant constraint on availability of potential biological control agents, making grasses unsuitable targets for biological control.

An array of factors interact to determine the size, structure and specificity of herbivorous natural enemy assemblages, including, but not limited to: plant geographic range, size, taxonomic isolation, structural complexity, density, detectability, predictability, secondary compounds, habitat characteristics and life-cycle (Lawton, 1983; Strong et al., 1984; Bernays and Graham, 1988; Greiler and Tscharntke, 1992). For example, Tscharntke and Greiler (1995) demonstrated that 97% of the variation of insect species richness for 15 grass species was described by plant size and life-cycle (i.e. perennial versus annual). Ecological theory predicts that short-lived and/or annual host plants should promote generalisation of consumers, whereas long-lived and/or perennial host plants should promote specialisation (Strong et al., 1984). As such, we would expect that annual grasses would support a smaller and more generalist pool of herbivores, while perennial grasses would support a larger and more specialised pool of herbivores (Greiler and Tscharntke, 1992; Tscharntke and Greiler, 1995; Magalhães et al., 2007; Hardy and Cook, 2010). The pattern of lower insect herbivore species richness on annual grasses than on perennials has also been observed for fungal pathogens (Clay, 1995). As such, we would expect that grasses will differ in their suitability as targets for biological control due to the relative influence of factors that determine the structure of their phytophagous assemblages, and thus, the availability of candidate biological control agents.

To address the knowledge gaps on herbivore assemblages on grasses and assess the suitability of biological control of invasive grasses, it will be imperative to conduct thorough native-range surveys for target grasses to get a complete picture of the assemblage of potential biological control agents that may be available. At the onset of a new biological control programme, practitioners typically conduct faunistic surveys in the target weeds native range. Native-range surveys have ranged from relatively *ad hoc* short-term efforts (e.g. Olckers, 1999; Paterson et al., 2017) to extensive long-term surveys that produced extensive natural enemy inventories (e.g. Harley et al., 1995; Palmer and Pullen, 1995; Goolsby et al., 2003). Goolsby et al. (2006) propose that "the entire outcome of a [biological control] programme depends on the suite of potential agents that is discovered." However, two common pitfalls were identified by Goolsby et al. (2006) that limit the usefulness of native-range surveys, namely: (1) it is often difficult to evaluate how comprehensive (i.e. how complete) the sampling of the natural enemy assemblage was, which means practitioners cannot determine if additional sampling would likely yield more potential control agents, and (2) in general, native-range surveys have collected little data to aid the prioritisation of potential control agents.

Species accumulation curves can be used to determine whether additional sampling in the native-range would likely yield additional control agents, and aid practitioners in deciding when to stop surveys in a particular biogeographic region and continue searches elsewhere (Cripps et al., 2006; Bell et al., 2014). While biological control programmes only require that a single natural enemy be suitably host-specific, the larger the pool of potential biological control agents is, the more likely practitioners are to have at least one suitable agent. Moreover, many biological control programmes strive to release multiple agents in an attempt to improve the likelihoods of successful control (Denoth et al., 2002). This not only requires that at least two suitable agents are available for the given project, but typically, natural enemies with contrasting feeding strategies are prioritised to increase the cumulative stress on the host-plant (Harris, 1985; Hoffmann and Moran, 1998). As such, the larger the community of natural enemies, the greater the likelihood of finding multiple biological control agents, which is even more important when herbivores with complimentary feeding guilds are sought.

Data and observations gathered during native range-surveys should guide the prioritisation of which natural enemies possess the greatest potential as biological control agents, in terms of host specificity and damage to the target weed (Goolsby et al., 2006). A multitude of factors can be used to prioritise potential agents (e.g. Blossey, 1995; van Klinken and Raghu, 2006; Dhileepan et al., 2013; Paterson et al., 2014). For example, the feeding biology (mode of damage) of a natural enemy can be used as a proxy for whether the species could inflict suitable damage, if released. Natural enemies that damage plant structural tissues are more likely to be effective control agents than leaf-feeders, seed-attackers or flower-feeders (Harris, 1973; Goeden, 1983; but see Impson et al., 2011). A large geographic distribution suggests a tolerance for a wide range of abiotic conditions, which may translate into greater probability of establishment and coverage of weed infestations. Natural enemies that occur on the host-plant throughout the year (seasonal incidence) are more likely to inflict sustained damage, which should maximise plant mortality, and minimise reproductive output and regeneration capacity (Egli and Olckers, 2017). While agent host-specificity and predicted efficacy are the primary determinants of a natural enemy's suitability as a biological control agent, feeding biology, geographic coverage and seasonal incidence can be used as early indicators of natural enemy suitability, and could be considered as coarse-filters for prioritising potential control agents for detailed host-specificity and predicted efficacy assessments.

Native-range surveys were performed across southern Africa on *S. pyramidalis* and *S. natalensis* between 2001-2003 in search of potential biological control agents. Two potential control agents were identified, namely: the stem-galling wasp, *Tetramesa* sp. (Hymenoptera: Eurytomidae), and the smut-fungus *Ustilago sporoboli-indici* (Ustilaginales) (Witt and McConnachie, 2004). The smut-fungus expressed too broad a host-range to be considered as a control agent and has subsequently been recorded in Australia (Yobo et al., 2009; Vitelli et al., 2017), while *Tetramesa* sp. could not be reared under laboratory conditions, thus, the project was terminated (Palmer, 2012). The increasingly severe negative environmental and economic impacts associated with *S. pyramidalis* and *S. natalensis* in Australia, and the ineffectiveness of current control methods, has resulted in this biological control programme being reinitiated. However, there is no indication as to how comprehensive the prior native-range surveys were that were performed in South Africa (i.e. whether all potential biological control agents were recorded), or where additional phytophagous surveys could be performed. A comprehensive inventory of the assemblage of natural enemies associated with *S. pyramidalis* and *S. natalensis*, that simultaneously prioritises natural enemies as potential biological control agents, is required to assess the prospects for and guide the biological control of these two invasive grasses.

In this chapter, the assemblage of insects associated with *S. pyramidalis* and *S. natalensis* in South Africa is described. South Africa was identified as a high-priority survey region because most of the eastern part of South Africa was a high climatic-match to weed infestations in Australia for both species (Chapter 2). Sampling effort is quantified to determine whether sufficient sampling has been performed to document the full complement of potential biological control agents in South Africa. The potential these species offer as biological control agents for weed infestations in Australia is discussed with regards to their feeding biology, geographic distribution and seasonal incidence (potential to inflict sustained damage).

3.2. Methods and materials

The insect assemblages associated with *S. pyramidalis* and *S. natalensis* in South Africa were described using two field survey designs. Firstly, repeat-surveys at a subset of sites in a localised area were performed to determine changes in insect community assemblage patterns over time (long-term repeat surveys; hereafter 'LTS'). Secondly, nationwide surveys (hereafter 'NWS') were performed to investigate changes in insect community assemblage patterns over broad spatial and environmental scales.

3.2.1. Plant surveys

3.2.1.1. Long-term repeat surveys (LTS)

The first component of this study aimed to describe the phytophagous fauna associated with *S. pyramidalis* and *S. natalensis* over a 12-month period between November 2017 and November 2018. Bi-monthly surveys (i.e. surveys were performed every ~ 2 months) were performed at 22 sites across the KwaZulu-Natal Province (South Africa). An effort was made to ensure that varying host-plant community combinations were surveyed, namely (1) *S. pyramidalis* only, (2) *S. natalensis* only, and (3) both *S. pyramidalis* and *S. natalensis*. The majority of LTS sites were located within the climatically-prioritised survey region (Chapter 2) but were also chosen to maximize the geographic extent of survey coverage, and therefore the range of habitats and climatic conditions under which surveys were conducted.

3.2.1.2. Nationwide surveys (NWS)

The second component of this study aimed to complement the LTS by providing additional information regarding the composition of the phytophagous fauna associated with *S. pyramidalis* and *S. natalensis* over a larger spatial extent than the LTS's. Nationwide surveys were prioritised as insect assemblages on plants can vary between biogeographic regions (e.g. Bell et al., 2014), and may therefore provide a more representative account of their phytophagous assemblages.

Nationwide surveys were performed between March 2017 until February 2019 across the entire native range of *S. pyramidalis* and *S. natalensis* in South Africa. An effort was made to visit each site on at least two occasions, although this was not always possible due to logistic constraints. Sites were selected to cover the widest possible area of the known distributions of *S. pyramidalis* and *S. natalensis* in South Africa, both within and outside of the climatically-prioritised survey regions (less than 20% of NWS sites were located outside of the climatically-prioritised survey regions) (Chapter 2).

3.2.2. Insect assemblages

At each site, plants were visually inspected for ~ 10 minutes for any signs of damage to the host-plants (e.g. emergence holes, stem deformation, gall formation) and ectophagous feeders, after which ten plants were bagged for dissection. An effort was made to stratify sampling by collecting five damaged and five undamaged plants during each site visit. However, this way not always possible (i.e. some sites did not yield any damaged plants, while other sites were so heavily infested that no undamaged plants could be collected). Each insect survey represents ten tillers being dissected, and ten minutes of visual searching for damaged tillers and ectophagous insects per plant species. Each tiller (including the roots, base of the tiller, culm and inflorescence) was dissected under a microscope in search of endophagous feeders. The presence and abundance of each endophagous species was recorded per tiller. All ectophagous and endophagous insects that were recovered were either preserved in 95% ethanol or placed in emergence chambers and reared through to adulthood. Immature insects were reared in pill-vials or small ventilated tubes (Dawah, 1987).

Voucher specimens were identified to the lowest taxonomic level possible by experts at the South African National Collection of Insects (ARC-PHP, Pretoria, South Africa) and then sent to local and international experts in the relevant taxonomic group, if necessary. Voucher specimens were deposited in the South African National Collection of Insects, and can be queried using Rhodes University accession numbers (RU). A thorough literature review was performed for specimens which were identified to the genus/species level to ascertain whether the specimen was phytophagous/parasitic and for any associated host-plant records. Unidentified specimens were grouped into morphospecies.

3.2.3. Sampling effort

Species accumulation curves and total species richness estimates were computed to determine whether adequate sampling had been performed to detect the full phytophagous species assemblages associated with *S. pyramidalis* and *S. natalensis*. Species accumulation curves were computed separately for (1) ectophagous and (2) endophagous species, owing to the difference in sampling intensity (no. of surveys performed to detect ectophagous versus endophagous species). Moreover, species accumulation curves were plotted for the endophagous assemblages associated with (1) *S. pyramidalis* and (2) *S. natalensis* separately to determine whether community composition differed between host plant species.

Species accumulation curves plotted the observed species richness against the number of surveys performed (hereafter 'Observed'). Three non-parametric species richness estimators were used to calculate estimated species richness, namely: Chao 2 ('chao2'), second-order jackknife ('jack2'), and the bootstrap estimator ('boot') (see Bell et al., 2013 and references therein). Sampling effort was considered sufficient if observed species richness was comparable to estimated species richness (i.e. approached the same asymptote) (Longino et al., 2002). Uncertainty associated with species accumulation curves was depicted by computing 95% confidence intervals around the fitted model.

3.3. Results

3.3.1. Plant surveys

A total of 77 sites across the native distributions of *S. pyramidalis* and *S. natalensis* in South Africa were visited over an 18-month sampling period (Fig. 3.1). Many sites were surveyed on more than one occasion. *Sporobolus pyramidalis* was surveyed on 167 occasions at 72 sites. These surveys consisted of 96 surveys at 16 LTS sites (surveyed every ~ two months) and 71 surveys at 56 NWS sites. *Sporobolus natalensis* was surveyed on 51 occasions at 16 sites. These surveys consisted of 36 surveys at six LTS sites and 15 surveys at ten NWS sites.

Most survey sites were located on disturbed roadsides, commonages, overgrazed pastures and vacant plots of land, although in the KwaZulu-Natal Province in particular, several sites were located in undisturbed savanna bushveld. Whenever sites were disturbed, *S. pyramidalis* and *S. natalensis* grew in relatively dense monocultures. At many sites, *S. pyramidalis* and/or *S. natalensis* grew sympatrically with other native African *Sporobolus* species (e.g. *S. africanus*, *S. fimbriatus*, *S. nitens*). The field host-range of insect herbivores recorded on *S. pyramidalis* and *S. natalensis* is investigated in the next chapter (Chapter 4).

3.3.2. Insect assemblages

Twenty-one insects were associated with *S. pyramidalis* and *S. natalensis* in South Africa, after omitting species where less than ten individuals were recorded in total and species that were only recorded on one occasion, or from one site (Table 3.1). Fifteen species were herbivorous, three were predators and three were parasitoids. Of the 15 herbivores, nine were ectophagous and six were endophagous. Although several ectophagous species were recovered, often in high numbers, on *S. pyramidalis* and/or *S. natalensis*, these species were incidentals, polyphagous, crop pests, predators or parasitoids, and thus were of little interest in the context of prospecting for candidate biological control agents (Table 3.1).

All six endophagous herbivores were found on both *S. pyramidalis* and *S. natalensis,* with none of these herbivores being restricted to either species. Phytophagous wasps constituted the majority of the herbivorous endophagous assemblages associated with *S. pyramidalis* and *S. natalensis*. Four of the six (67%) endophagous feeders recorded were phytophagous wasps belonging to the Eurytomidae family (Hymenoptera). The two remaining species consisted of a shoot-galling fly (Chloropidae sp. 1; Diptera: Chloropidae) and a shot-hole borer (Scolytidae sp. 1; Coleoptera: Scolytidae) (Table 3.1). All six species were reared on potted *S. pyramidalis* and/or *S. natalensis* plants under greenhouse conditions. This confirmed that these species were herbivorous, and not parasitic.

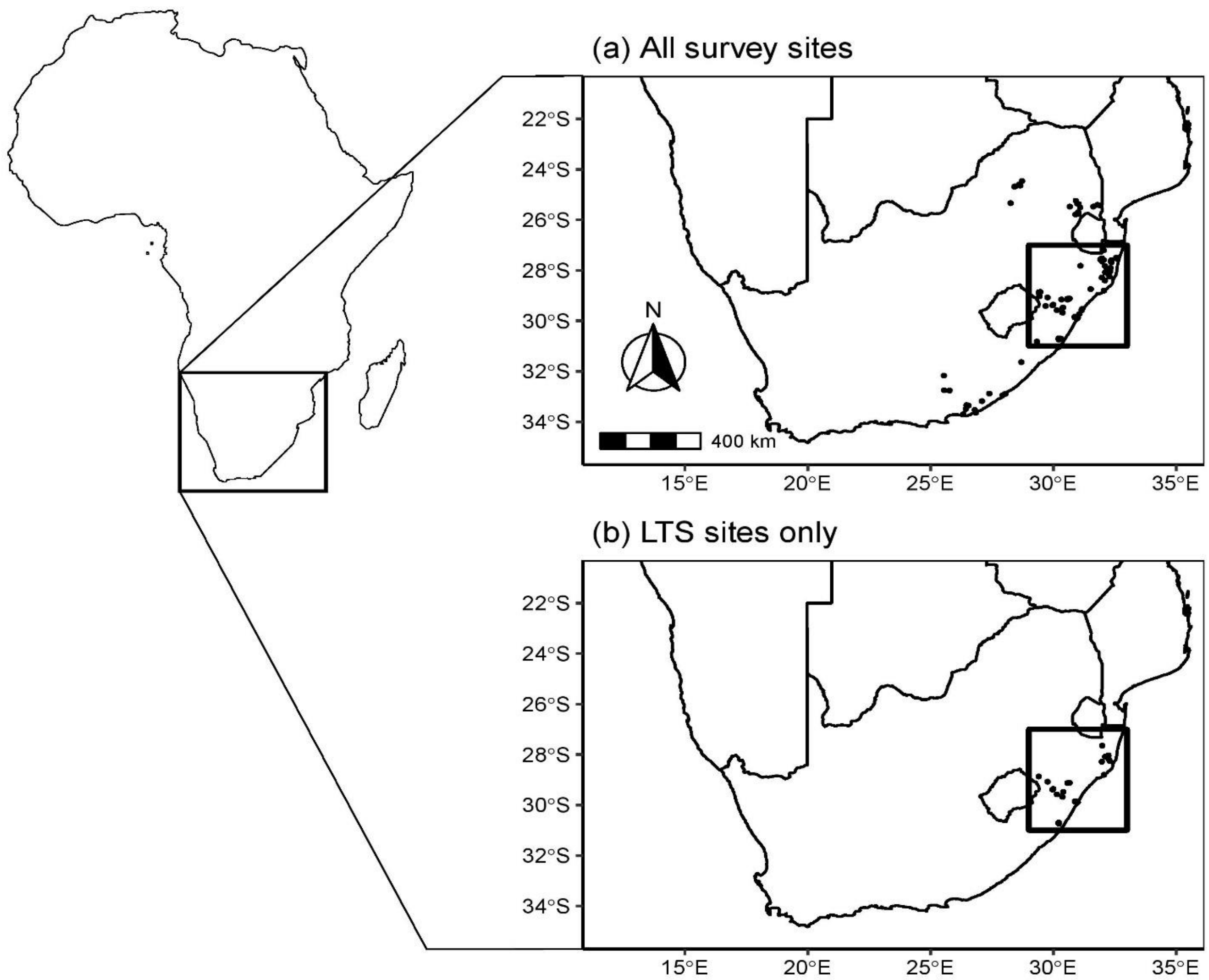


Figure 3.1. Distribution of survey sites across South Africa that were surveyed for herbivorous insects associated with *Sporobolus pyramidalis* and/or *S. natalensis*, representing (a) all survey sites, and (b) the 23 long-term repeat survey sites ('LTS sites') that were visited on a bi-monthly basis. The black bounding box inset in both panel (a) and (b) indicates the geographic region where all LTS sites were distributed.

Table 3.1. Summary of arthropods recorded on *Sporobolus pyramidalis* and *S. natalensis* in South Africa.

Insect taxa		Feeding biology	Coverage[1]	Incidence[2]	Host range[3]	Potential biocontrol agent
Family	Species					
Thysanoptera	Phlaeothripidae *Haplothrips stofbergi* Faure	Herbivore (ectophagous)	0.72	0.73	Polyphagous[a]	No
Hemiptera	Aphididae *Hysteroneura setariae* (Thomas)	Herbivore (ectophagous)	0.55	0.57	Polyphagous[b]	No
	Lygaeidae *Horridipamera nietneri* (Dohrn)	Predator	0.21	0.20	-	No
	Ricaniidae *Mulvia albizona* Germar	Herbivore (ectophagous)	0.03	0.03	Polyphagous[c]	No
	Cicadellidae *Balclutha rosea* (Scott)	Herbivore (ectophagous)	0.31	0.35	Polyphagous[d]	No
	Cicadellidae *Exitianus taeniaticeps* Kirchbaum	Herbivore (ectophagous)	0.18	0.17	Polyphagous[d]	No
Diptera	Chloropidae Chloropidae sp. 1 -	Herbivore (endophagous)	0.18	0.14	?	Yes
	Chloropidae *Thaumatomyia natalensis* (Becker)	Herbivore (incidental)	0.26	0.23	Polyphagous[e]	No

Coleoptera	Chrysomelidae *Asbecesta cyanipennis* Harold	Herbivore (ectophagous)	0.01	0.03	Polyphagous[f]	No
	Chrysomelidae *Monolepta* spp. -	Predator	0.09	0.05	Polyphagous[f]	No
	Meloidae *Decapotoma lunata* Pallas	Herbivore (ectophagous)	0.12	0.06	Polyphagous[g]	No
	Coccinellidae *Cheilomenes lunata* (Fabricius)	Predator	0.15	0.08	-	No
	Scolytidae Scolytidae sp. 1 -	Herbivore (endophagous)	0.13	0.13	?	Yes
	Curculionidae *Umzila capeneri* Marshall	Herbivore (ectophagous)	0.27	0.15	Polyphagous[e]	No
Hymenoptera	Eurytomidae *Tetramesa* sp. 1 -	Herbivore (endophagous)	0.35	0.32	?	Yes
	Eurytomidae *Tetramesa* sp. 2 -	Herbivore (endophagous)	0.21	0.22	?	Yes
	Eurytomidae prob. *Bruchophagus* sp. 1 -	Herbivore (endophagous)	0.23	0.21	?	Yes
	Eurytomidae	Herbivore	0.11	0.09	?	Yes

Eurytomidae sp. 4 -	(endophagous)				
Eurytomidae *Eurytoma* spp. -	Parasitoid	0.15	0.12	-	No
Ormyridae *Ormyrus* sp. -	Parasitoid	0.13	0.10	-	No
Eupelmidae *Eupelmus* sp. -	Parasitoid	0.06	0.04	-	No

[1] Coverage = proportion of sites each insect was recorded in
[2] Incidence = proportion of sampling events each insect was recorded in
[3] Host range was only reported for herbivorous insects; ? = unknown host range
[a] Way (2008); [b] Blackman and Eastop (2000); [c] Picker et al. (2004); [d] van Antwerpen et al. (2011); [e] G.F. Sutton (unpublished data); [f] Igbinosa et al. (2007); [g] Habou et al. (2014)

Below, a brief introduction to each herbivorous endophagous feeder is provided, with emphasis on its geographic distribution, abundance and feeding biology. These six endophagous natural enemies were given high-priority as potential biological control agents during this study. As species-level identifications could not be obtained for the six endophagous feeders, it was not possible to determine their host-range based on literature searches, as was done for the ectophagous feeders recorded on *S. pyramidalis* and *S. natalensis* (Table 3.1). Endophagous herbivores are typically more specialised and damaging than ectophagous feeders, and thus, are more likely to be suitably specific to investigate as biological control agents (Wapshere, 1990; Cronin et al., 2015).

3.3.2.1. *Tetramesa* sp. 1 (Hymenoptera: Eurytomidae)

Tetramesa sp. 1 (Hymenoptera: Eurytomidae) was the most widespread endophagous species associated with *S. pyramidalis* and *S. natalensis* in South Africa. This species is easily recognised by the “golden shoulder” patch (Fig. 3.2; Witt and McConnachie, 2004). It was collected at the most sites (35% of all sites), and is the most commonly encountered species being recorded during 32% of all insect surveys performed (Table 3.1). It was recorded from the warm and tropical north-eastern regions of KwaZulu-Natal and Limpopo, however, it was entirely absent from field sites in the Mpumalanga Province (Table 3.2). *Tetramesa* sp. 1 was recorded equally as often during summer and winter (Table 3.2).

Figure 3.2. (next page) Photographs of pinned specimens, including: (a) *Tetramesa* sp. 1 (female) (Hymenoptera: Eurytomidae; Rhodes University [RU] accession number RU1180), (b) *Tetramesa* sp. 1 (male; note the plumose antennae) (RU1190), (c) *Bruchophagus* sp. 1 (female) (Hymenoptera: Eurytomidae) (RU1202), and (d) *Bruchophagus* sp. 1 (male; note the plumose antennae) (RU1195).

(a) *Tetramesa* sp. 1 (female)

(c) *Bruchophagus* sp. 1 (female)

(b) *Tetramesa* sp. 1 (male)

(d) *Bruchophagus* sp. 1 (male)

Table 3.2. Provincial variation in geographic coverage and incidence of endophagous herbivorous insects associated with *Sporobolus pyramidalis* and *S. natalensis* in South Africa. The provinces surveyed include: KZN = Kwa-Zulu Natal, LMP = Limpopo, MPA = Mpumalanga and ECP = Eastern Cape.

Insect species[1]	Seasonal coverage[2]		Seasonal incidence[3]		Province							
					KZN		LMP		MPA		ECP	
	Summer	Winter	Summer	Winter	*C*	*I*	*C*	*I*	*C*	*I*	*C*	*I*
Tetramesa sp. 1 Hymenoptera: Eurytomidae RU 1180, 1190	0.31	0.36	0.31	0.29	0.24	0.33	0.57	0.57	0	0	0	0
Tetramesa sp. 2 Hymenoptera: Eurytomidae RU 1204-1207	0.22	0.18	0.23	0.17	0.37	0.23	0.29	0.29	0.41	0.41	0	0
Bruchophagus sp. 1 Hymenoptera: Eurytomidae RU 1171-1176, 1195, 1202	0.21	0.18	0.22	0.17	0.19	0.20	0.71	0.71	0.25	0.25	0	0
Eurytomidae sp. 4 Hymenoptera: Eurytomidae RU 1208-1210	0.14	0.18	0.12	0.16	0.14	0.09	0	0	0	0	0.17	0.17
Scolytidae sp. 1 Coleoptera: Scolytidae RU 1185, 1189	0.16	0.30	0.17	0.25	0.19	0.15	0	0	0	0	0	0
Chloropidae sp. 1 Diptera: Chloropidae RU 1211-1219	0.25	0.36	0.19	0.29	0.22	0.15	0.14	0.14	0	0	0.17	0.17

[1] RU = Rhodes University accession numbers

[2] Coverage = proportion of sites each insect was recorded from; hereafter '*C*'

[3] Incidence = proportion of surveys each insect was recorded from; hereafter '*I*'

This species is an endophagous stem-borer. Larvae cause extensive damage to the tiller by tunnelling up and down the stem (Fig. 3.3a). Most larvae are encountered just above the first and second node of the tiller. No obvious gall symptoms can be observed, with endophagous feeding being indicated by a slight swelling and discolouration to the tiller (although these external signs of damage are not always observed). Emergence holes located above the 1st to 4th node of the culm are characteristic signs of infestation by *Tetramesa* sp. 1 (Fig. 3.3b). Larval feeding appears to result in tillers being sterilised (i.e. not producing an inflorescence), and in many instances, tillers will die within the season they were attacked (see Chapter 5). Once larvae are about to complete development within the tiller, the entire tiller above the site of attack will become brown and appear desiccated, while any portion of the tiller below the site of attack still appears green and healthy. This results in tillers breaking at the site where larvae were found in the stem, especially where multiple individuals were located in close proximity to one another in the stem. The species is apparently multi-voltine, at least in the KwaZulu-Natal Province, where all life-stages were recorded year round. Larvae and pre-adults typically overwinter inside the culm.

3.3.2.2. *Tetramesa* sp. 2 (Hymenoptera: Eurytomidae)

Tetramesa sp. 2 (Hymenoptera: Eurytomidae) was recorded at 21% of all field sites and during 22% of all insect surveys (Table 3.1; Fig. 3.3b). It was recorded from the warm and tropical north-eastern provinces of KwaZulu-Natal, Limpopo and Mpumalanga (Table 3.2). *Tetramesa* sp. 2 was recorded equally as often during summer and winter (Table 3.2).

This species is an endophagous stem-borer. Larvae cause extensive damage to the inflorescence. Most larvae and pre-adults are encountered in the apical 30 cm of the inflorescence. No obvious gall symptoms can be observed, although a slight swelling and

(a) (b) (c)

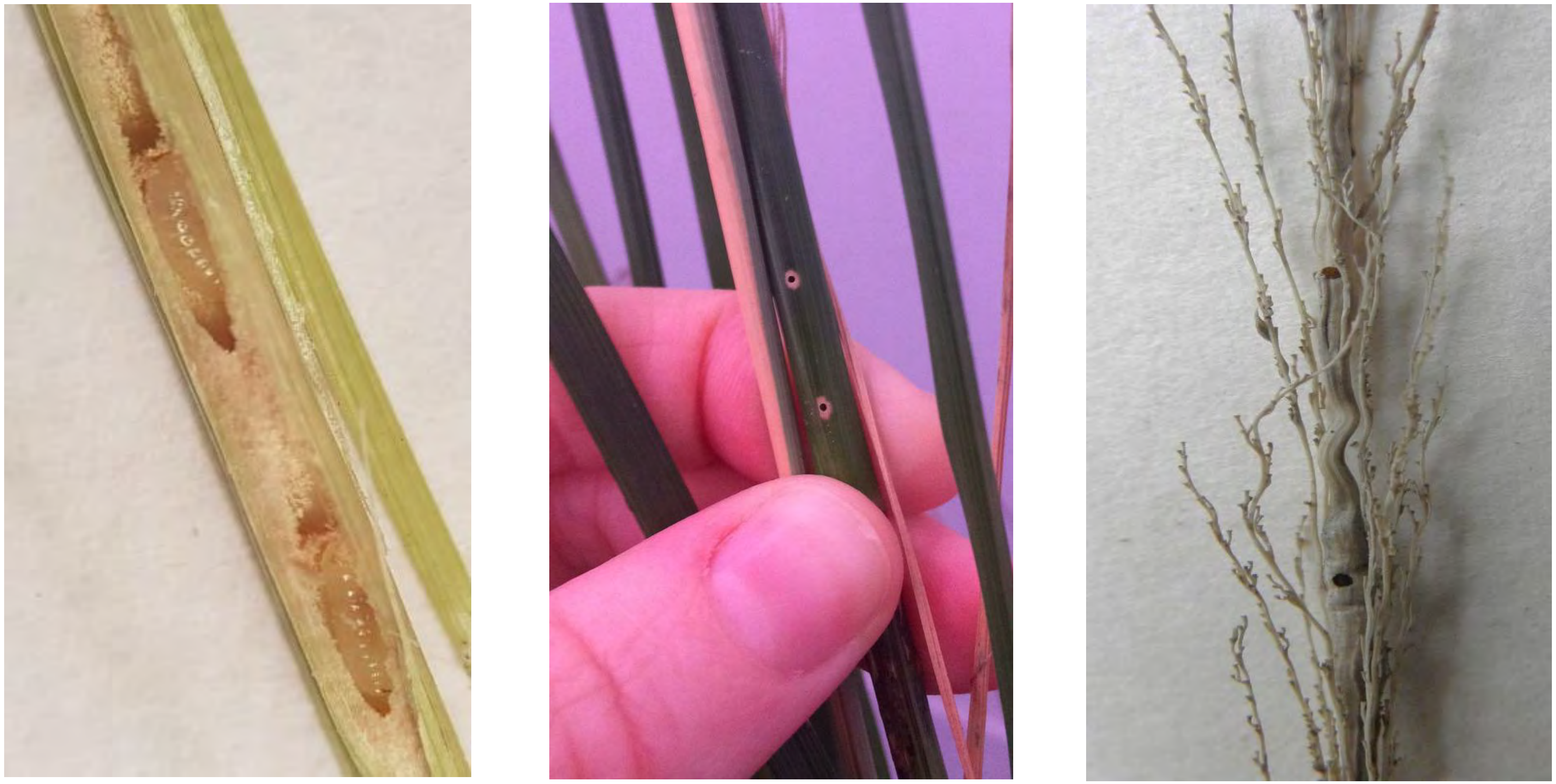


Figure 3.3. Symptomology of *Tetramesa* spp. associated with *Sporobolus pyramidalis*, (a) larvae of *Tetramesa* sp. 1 inside the tiller, (b) typical emergence holes for *Tetramesa* sp. 1, and (c) characteristic 'zigzagged' damage associated with larvae of *Tetramesa* sp. 2.

discolouration to the tiller can occasionally be observed. Late-season damage is more apparent with damaged tissue becoming “zigzagged” or “wavy” in appearance (Fig. 3.3c). Larval feeding appears to result in tillers being sterilised, although this does not appear to translate into reduced tiller survival (see Chapter 5). Larvae and pre-adults typically overwinter inside the culm.

3.3.2.3. *Bruchophagus* sp. 1 (Hymenoptera: Eurytomidae)

Bruchophagus sp. 1 (Hymenoptera: Eurytomidae) was recorded at 23% of all field sites and during 21% of all insect surveys (Table 3.1). This species was easily identifiable owing to its enlarged, bulbous abdomen (Fig. 3.2). It was recorded from the warm and tropical north-eastern provinces of KwaZulu-Natal, Limpopo and Mpumalanga. *Bruchophagus* sp. 1 was particularly widespread and common in the drier Limpopo Province, being recorded from 71% of sites in the province (Table 3.2). It was recorded attacking *S. pyramidalis* and *S. natalensis* throughout the year (Table 3.2).

Bruchophagus sp. 1 is an endophagous stem-borer. Larvae damage the tiller by tunnelling up and down the stem. Most larvae are encountered just above the second to fourth node of the tiller. Larval feeding does not appear to translate into reduced tiller survival or reproduction (see Chapter 5). Larvae and pre-adults typically overwinter inside the culm.

3.3.2.4. Eurytomidae sp. 4 (Hymenoptera: Eurytomidae)

Eurytomidae sp. 4 (Hymenoptera: Eurytomidae) was only recorded from 11% of all field sites and during 9% of all insect surveys (Table 3.1), and was recorded as often during summer as it was during winter (Table 3.2). It was the only phytophagous wasp recorded from either of the two target weeds from the Eastern Cape Province (Table 3.2). This species is an endophagous stem-borer, however there appears to be no appreciable impact on the host plants. The few larvae and adults that were recovered were found in the apical 30 cm of the inflorescence.

3.3.2.5. Scolytidae sp. 1 (Coleoptera: Scolytidae)

An undescribed Scolytid beetle, Scolytidae sp. 1 (Coleoptera: Scolytidae), was recorded at 13% of all field sites and during 13% of all insect surveys (Table 3.1). Scolytidae sp. 1 was recorded ~ 2 fold more frequently during winter than it was during summer (Table 3.2). It was only recorded in the KwaZulu-Natal Province (Table 3.2). This species is a shot-hole borer that causes extensive damage to the host plants. Larval and adult feeding within the culm often results in tiller sterilisation and tiller death.

3.3.2.6. Chloropidae sp. 1 (Diptera: Chloropidae)

An undescribed chloropid fly, Chloropidae sp. 1 (Diptera: Chloropidae), was a widespread and relatively common herbivore associated with *S. pyramidalis* and *S. natalensis* in South Africa. Overall, it was recorded at 18% of all field sites and during 14% of all insect surveys (Table 3.1), and was recorded more often in winter than in summer (Table 3.2). It is a stem-borer, whose larvae tunnel up and down the culm, although this does not appear to cause an appreciable reduction in tiller reproduction or survival.

3.3.3. Sampling effort

Species accumulation curves indicated that the entire ectophagous (Fig. 3.4a) and endophagous arthropod assemblages (Fig. 3.4b) associated with *S. pyramidalis* and *S. natalensis* were recorded. Approximately four times the number of surveys were required before the full ectophagous assemblage (~ 80 surveys required) was recorded than the entire endophagous assemblage (~ 20 surveys required) (Fig. 3.4). Nevertheless, the confidence intervals around expected species richness for all three richness estimators (i.e. 'boot', 'chao2' and 'jack2') converged on observed species richness for both the ectophagous and endophagous assemblages (Fig. 3.4), providing strong support that no additional arthropod species are likely to be found in South Africa, if additional sampling was performed.

For both *S. pyramidalis* and *S. natalensis*, observed endophagous species richness reached a similar asymptote as estimated species richness, irrespective of which richness estimator was used (Fig. 3.5). The rate at which new endophagous species were accumulated was comparable for *S. pyramidalis* and *S. natalensis*, with the entire endophagous assemblage associated with each host plant being recorded within the first ~ 30 - 40 surveys (Fig. 3.5).

3.4. Discussion

To date, very few grasses have been targeted for biological control, largely owing to the perception that grasses lack a suite of suitably host-specific and damaging natural enemies to develop as biological control agents (Wapshere, 1990; Evans, 1991; Pemberton, 1996). This chapter reports on faunistic surveys that found 15 herbivorous insects associated with *S. pyramidalis* and *S. natalensis* in South Africa. The number of herbivores associated with *S. pyramidalis and S. natalensis* is significantly lower than many weeds targeted for

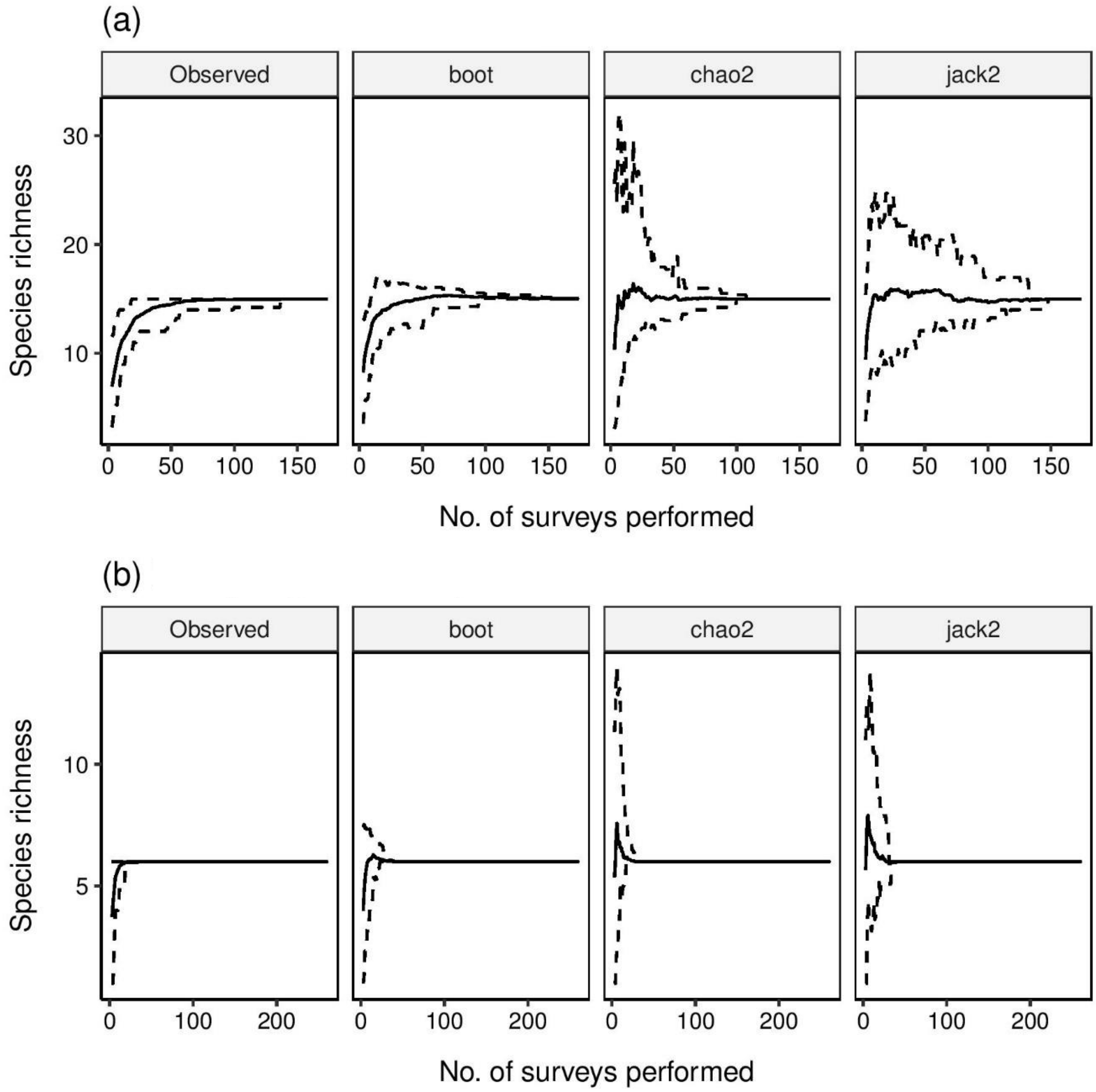


Figure 3.4. Species accumulation curves indicating observed versus expected species richness for (a) ectophagous and (b) endophagous insects on *Sporobolus pyramidalis* and *S. natalensis* in South Africa, according to three species richness estimators. The solid black line indicates observed ('Observed') or predicted ('boot', 'chao2', 'jack2') insect species richness. Dashed lines indicate 95% confidence intervals around the fitted species richness estimators. Sampling effort was deemed sufficient where observed/predicted species richness curves reached an asymptote, and the confidence intervals converged on the species richness curves.

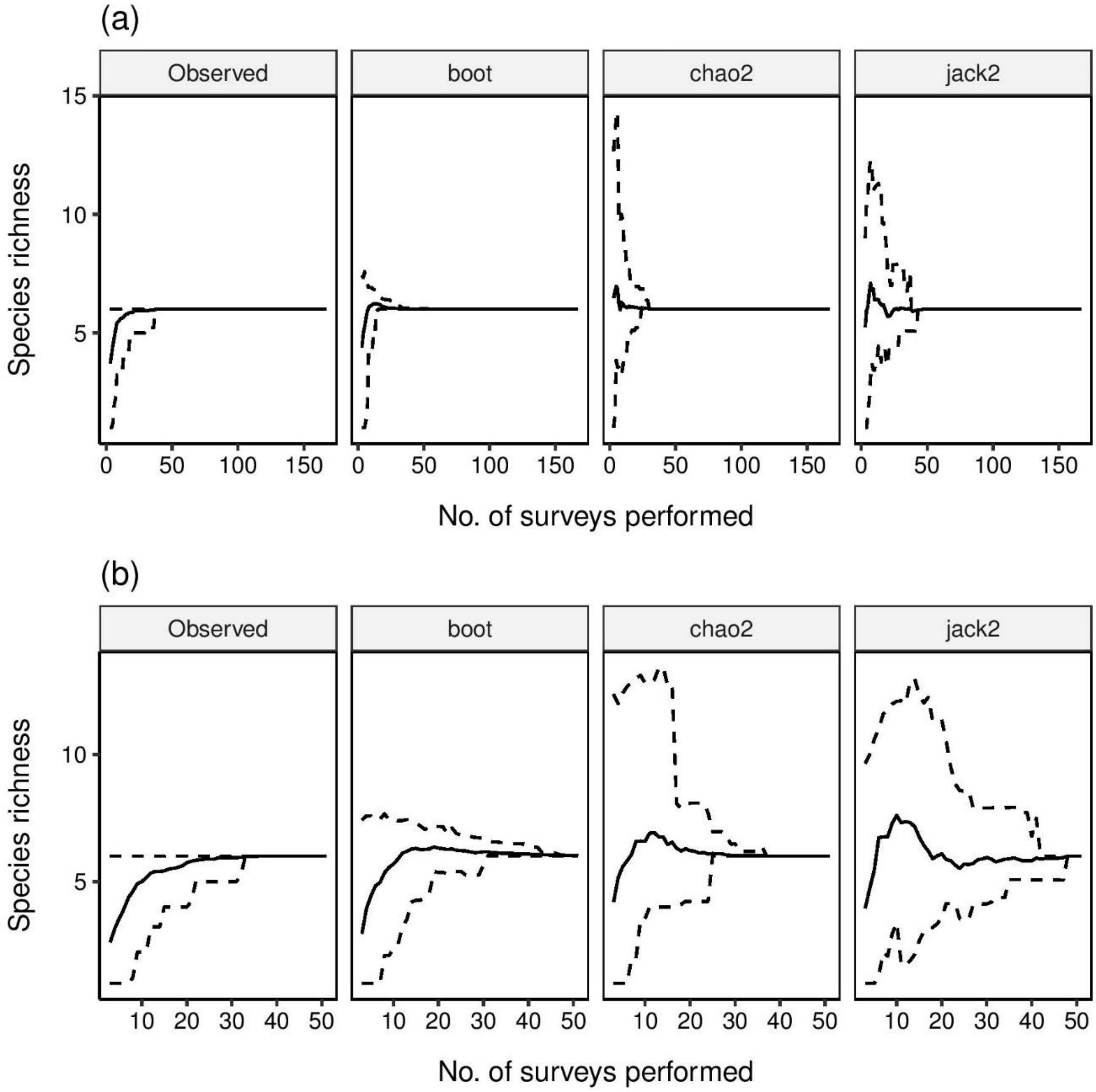


Figure 3.5. Species accumulation curves indicating observed versus expected endophagous insect species richness for (a) *Sporobolus pyramidalis* and (b) *S. natalensis* in South Africa, according to three species richness estimators. The solid black line indicates observed ('Observed') or predicted ('boot', 'chao2', 'jack2') insect species richness. Dashed lines indicate 95% confidence intervals around the fitted species richness estimators. Sampling effort was deemed sufficient where observed/predicted species richness curves reached an asymptote, and the confidence intervals converged on the species richness curves.

biological control. For example, in their native distributions, *Lantana camara* L. (Verbenaceae) supports 550 phytophagous species (Palmer and Pullen, 1995) and *Mimosa pigra* L. (Mimoseae) harbours 441 phytophagous species (Harley et al., 1995). However, the fifteen herbivores associated with *S. pyramidalis* and *S. natalensis* is in line with several weeds targeted for biological control, that have successfully released control agents, such as: *Pereskia aculeata* Miller (Cactaceae), which supports 15 herbivores in its native distribution and has two biological control agents released against it in South Africa (Paterson et al., 2014), and *Lygodium microphyllum* (Cav.) R.Br. (Schizaeaceae), which supports 22 herbivores in its native distribution (Goolsby et al., 2003), and has three biological control agents released against it in the USA (Winston et al., 2014). As such, the size of the insect assemblage found for *S. pyramidalis* and *S. natalensis* in the native range may not be a limiting factor in their potential for biological control.

While 15 herbivores associated with *S. pyramidalis* and *S. natalensis* is significantly fewer than the 171 herbivores associated with *P. australis* in its native distribution (Tewksbury et al., 2002), it is consistent with other grass species that have been surveyed for natural enemies in their native distribution, such as: *A. donax* (Tracy and DeLoach 1998; Goolsby and Moran 2009; Goolsby et al. 2009a; Goolsby et al. 2017), *Calamagrostis epigeios* (L.) Roth (wood small-reed) (Dubbert et al. 1998), and *Leymus* (= *Elymus*) *cinereus* (Scribn. & Merr.) A. Löve (Great Basin wildrye) (Youtie et al., 1987). The significantly larger herbivore assemblage associated with *P. australis*, is likely explained by the plant architecture hypothesis, which states that the physical structure of the above-ground portion of the host plant will be a primary determinant of insect community structure (Lawton, 1983). Numerous studies have demonstrated a correlation between insect species richness and plant architecture, with insect species richness is typically positively correlated with increasing plant size and greater structural heterogeneity (e.g. Strong et al., 1984; Forbes et al., 2017).

For example, Tscharntke and Greiler (1995) showed that for 10 grass species, larger plant size was correlated with increased herbivore species richness. As such, the large herbivore assemblage recorded on species such as *P. australis* (~ 10 times more herbivores), in comparison to those assemblages recorded on *S. pyramidalis* and *S. natalensis*, is not surprising given its larger size and more complex structural architecture (Strong et al., 1984). However, *A. donax* is comparatively as large and structurally heterogenous as *P. australis*, yet *A. donax* supports 8-fold fewer herbivores (~ 20 herbivores) than *P. australis* (~ 161 herbivores), in their respective native distributions' (Tracy and Deloach, 1998; Tewksbury et al., 2002). Clearly, plant size and structural heterogeneity are just two of many factors that potentially interact to determine insect community structure and richness on grasses.

The herbivore communities recorded on grass species are often dominated by ectophagous species (Tscharntke and Greiler, 1995), or have relatively equal numbers of ectophagous to endophagous feeders (Tewksbury et al., 2002; Witt and McConnachie, 2004). This is consistent with the herbivorous insect community recorded on *S. pyramidalis* and *S. natalensis* in South Africa, which is only slightly dominated by ectophagous (60%) over endophagous feeders (40%). Cornell (1989) proposed the defence theory, which states that as plant defence levels increase, the ratio of endophages to ectophagous should increase. As such, heavily-defended plants or plant tissues are expected to have higher endophagous-to-ectophagous species, because endophagous species are believed to be more efficient at avoiding plant defences (e.g. selective feeding, tissue manipulation) (Cornell, 1989). Grasses are typically not heavily defended, at least chemically (McNaughton et al., 1985; Vicari and Bazley, 1993), thus, it is not unexpected that *S. pyramidalis*, *S. natalensis*, and many other grasses, are dominated by ectophagous feeders, or equal ratios of the endophagous-to-ectophagous species. This might be some cause for concern as endophagous herbivores are typically more specialised than ectophagous feeders, and thus, are more likely to be suitably specific to investigate as biological control agents (Wapshere, 1990). However, a small endophagous herbivore assemblage is only a limiting factor if the pool of herbivores is not found to contain suitably host-specific species to serve as control agents. There are many biological control programmes that have found and released biological control agents, despite supporting small herbivore assemblages, due to the availability of at least one monophagous herbivore (e.g. McConnachie et al., 2004; Paterson et al., 2011). The finding of multiple potential biological control agents on *S. pyramidalis* and *S. natalensis* suggests that invasive grasses may be better targets for biological control than originally predicted.

Although subtle differences between host plant species (e.g. morphology, nutrition or chemical defences) often results in contrasting herbivore community composition (e.g. Adair, 2004; Madeira et al., 2016), this does not appear to be the case for *S. pyramidalis* and *S. natalensis*. All six endophagous feeders found on *S. pyramidalis* were also found on *S. natalensis*, and no herbivore species was found on only one of the two species. Morphological studies have struggled to distinguish *S. pyramidalis* from *S. natalensis*, owing to the extreme structural homology, and inter-specific hybridization of the two species (Simon and Jacobs, 1999). However, recent genetic evidence suggests that *S. pyramidalis* and *S. natalensis* may be distinct species (Shresha et al., 2005). The implications of *S. pyramidalis* and *S. natalensis* being a single versus distinct species is unlikely to have a significant impact on this biological control programme.

The relatively small number of herbivores recorded on *S. pyramidalis* and *S. natalensis* in South Africa was not due to inadequate sampling. Sampling completeness for species inventories can be defined as: reasonable (> 50% of total estimated richness), comprehensive (70-80%) and exhaustive (90-100%) (Cardoso et al., 2009). Sampling completeness for ectophagous and endophagous herbivores on *S. pyramidalis* and *S. natalensis* in South Africa was exhaustive (~100%). Irrespective of which of the three non-parametric richness estimator was used, the entire ectophagous and endophagous assemblages associated with these two grasses appears to have been sampled. As such, all the potential biological control agents for *S. pyramidalis* and *S. natalensis* have likely been sampled in South Africa. The exhaustive sampling coverage is in contrast to most insect inventories, including those looking for potential biological control agents in a weeds' native-range, which often yield much lower survey completeness values (30-70%) (e.g. Heard and Pettitt, 2005; Bell et al., 2013). These differences are likely driven by the fact that the smaller size of the herbivore community associated with *S. pyramidalis* and *S. natalensis* than typically occurs on weeds targeted for biological control, and the fact that the current study only surveyed the two target weeds over a subset of their native ranges'.

Additional surveys for potential biological control agents could be performed in other biogeographic regions across the native distributions of *S. pyramidalis* and *S. natalensis*. Vast geographic survey coverage is advised when prospecting for potential control agents as monophagous herbivores are expected to occupy a narrower biogeographic distribution than oligophagous and polyphagous species, and therefore may only be encountered in a sub-set of a weed's native range (Strong et al., 1984). For example, many herbivores associated with *Parkinsonia aculeata* L. (Fabaceae) demonstrated a restricted distribution, being limited to one or a few biogeographical units (Bell et al., 2013), while Paterson et al. (2014) only recorded the biological control agent *Phenrica guerini* Bechyné (Coleoptera: Chrysomelidae) at 31 % of *Pereskia aculeata* sites, limited to Rio de Janeiro Province in Brazil, despite extensive surveys throughout the weed's native range, spanning four countries and six sampling regions. Therefore, it is recommended that in addition to surveys in South Africa, phytophagous surveys on *S. pyramidalis* and *S. natalensis* be conducted in other high-priority survey regions identified in Chapter 2, such as: coastal West Africa, East Africa and eastern Madagascar. These surveys would be expected to increase the size of the herbivore pool recorded on the two target weeds, and yield additional potential biological control agents. This approach would provide a more complete representation of the phytophagous assemblage associated with the target weeds, and maximise the probability of locating narrowly-distributed, host-specific and suitably damaging herbivores (Strong et al., 1984).

While some practitioners have warned against under-sampling during native-range surveys (e.g. Goolsby et al., 2006), other projects have undoubtedly used unnecessary resources by over-sampling the native-range. For example, Heard and Pettitt (2005) demonstrated that 11 of the 12 biological control agents released against *M. pigra* in Australia were located in the native-range within 23 surveys performed at only three sites. If survey effort had been analysed iteratively during the *M. pigra* biological control project, surveys could have been terminated at an appropriate stage and valuable resources would not have been spent on performing an additional 723 collections and visiting 274 additional sites (Heard and Pettitt, 2005). In the current study, species accumulation curves were computed iteratively during the native-range component of the *Sporobolus* biological control programme. This resulted in surveys for natural enemies ceasing after 2 years. The resources freed up by not continuing native-range surveys across South Africa were spent on pre-release efficacy and agent complementarity studies in an attempt to improve the agent prioritisation process (Chapter 5).

Now that the natural enemy assemblages associated with *S. pyramidalis* and *S. natalensis* in South Africa have been comprehensively surveyed and described, the next step is to prioritise which natural enemies are best suited for development as biological control agents. Agent selection and prioritisation is not a trivial undertaking (Blossey, 1995). An array of criteria have been developed to aid the decision-making process, incorporating aspects such as: host-specificity, predicted efficacy, geographic distribution, abundance, phenological synchrony, rate of increase (i.e. reproductive potential) and mortality factors (e.g. Harris, 1973; Goeden, 1983; Wapshere, 1985; van Klinken and Raghu, 2006). Natural enemies that have a strong physiological dependency on their host-plant, such as endophagous feeders, mites and fungal pathogens, are typically more specialised and damaging than their ectophagous counterparts (Cornell 1989; Cronin et al., 2015). This pattern emerges as the more strongly-dependent the consumer is on the host plant, the more likely the consumer is to develop mechanisms to counter host plant defences and/or to be protected from predation and parasitism, thus selecting for greater host specificity (Raman 1993; Hardy and Cook 2010). As such, endophagous feeders, such as the six insects recorded on *S. pyramidalis* and *S. natalensis* are considered good candidates for biological control.

On the basis of their feeding biology, geographic coverage, seasonal incidence and preliminary field observations the three most promising insect herbivores for the biological control of *S. pyramidalis* and *S. natalensis* in Australia are the stem-boring wasps: *Tetramesa* sp. 1, *Tetramesa* sp. 2 and *Bruchophagus* sp. 1. *Tetramesa* spp. appear to have radiated amongst the Poaceae, and are typically host-specific, with most species attacking a single host-plant or a few close relatives (Claridge, 1961; Dawah, 1987; Dubbert et al., 1998). For example, the Arundo wasp, *T. romana* has already been released in the USA for the biological control of *A. donax*, due to its narrow host-range (Goolsby and Moran, 2009). Presently unidentified *Tetramesa* spp. are under evaluation as biological control agents of *Taeniatherum caput-medusae* (L.) Nevski (medusahead) and *Megathyrsus* (=*Panicum*) *maximus* (Jacq.) B. K. Simon & S. W. L. Jacobs (Guineagrass) in the USA (Massimo Cristofaro, pers. comm.). *Bruchophagus* spp. are typically seed-attacking herbivores, otherwise they gall leaf and stem tissue (Bouček, 1988). Due to their often restricted host-range and damaging nature, several *Bruchophagus* spp. have been used considered as weed biological control agents (Neser & Prinsloo, 2004), albeit none have been released to date (Winston et al., 2014). *Bruchophagus* spp. are known to attack plants spanning several families (most notably Fabaceae) (Bouček, 1988). However, we are not aware of any published record of a phytophagous *Bruchophagus* spp. attacking any grasses (Poaceae), which suggests that the current species may be reclassified at a later stage. We are currently in the process of obtaining a formal description for *Bruchophagus* sp. 1, and both *Tetramesa* sp. 1 and *Tetramesa* sp. 2.

The wide geographic distributions across South Africa and the relatively large number of sites where the wasps were recorded suggests a tolerance for a wide range of abiotic conditions, which may translate into greater probability of establishment and coverage of weed infestations in Australia, especially given the high climatic-match between South Africa and Australian field sites (Chapter 2). All three species are stem-borers whose larvae damage plant vascular tissue. Internal stem-feeding is likely to result in significantly reduced host plant fitness (Goeden, 1983; Cronin et al., 2015). Moreover, all three wasp species were recorded during a large number of field surveys, in both summer and winter. The sustained year-round damage caused to the plant suggests that these wasps may be suitably damaging because they will attack the plant throughout its reproductive window (Briese et al., 1994; Egli and Olckers, 2017), and this sustained damage is likely to maximise plant mortality and limit potential for regrowth and regeneration (e.g. Ireson et al., 2000).

These field surveys suggest that *Tetramesa* sp. 1, *Tetramesa* sp. 2 and *Bruchophagus* sp. 1 are suitable candidates for the biological control of *S. pyramidalis* and *S. natalensis* in Australia. Further investigation into their field host-range (Chapter 4), and potential to damage the host plant, as well as the interactions between these three wasp species (Chapter 5), is required.

Chapter 4: Using plant phylogeny and the phylogenetic conservatism of insect herbivore host-range to prioritise weed biological control agents from native-range surveys

4.1. Introduction

The safety, efficacy and sustainable use of weed biological control is largely dependent on the ability of practitioners to select and prioritise only those agents that pose negligible risk to non-target species in the area of intended control. This is achieved by assaying and describing the agents' potential host-range (Wapshere, 1974; Hinz et al., 2019). Traditionally, these host-range assessments have been performed as laboratory-based no-choice test studies (Briese, 2005; Schaffner et al., 2018). These tests describe the "fundamental host-range" of the candidate agent (i.e. all plant species that are suitable for feeding and supporting complete development) (van Klinken, 2000). This is despite numerous agents attacking a smaller subset of plants under natural field conditions than they do under laboratory conditions (i.e. the "realised host-range"; van Klinken, 2000) (see Hinz et al., 2014 and references therein).

The discrepancy between fundamental and realised host-range is typically ascribed to the artificial conditions experienced by candidate agents reared and tested in the laboratory, such as: physical confinement, prior experience, starvation and deprivation, artificial growing conditions, and presence of other plant volatiles (Marohasy, 1998; Sutton et al., 2017; Schaffner et al., 2018). The artificial conditions can drastically influence insect behaviour, including aspects of host-searching and host-acceptance, and thus can confound host-range assessments obtained from laboratory-based trials (Marohasy, 1998; Briese, 2005; Sheppard et al., 2005; Schaffner et al., 2018). There is consensus amongst biological control practitioners that by allowing candidate agents to express their natural host-finding and host-selection behaviours, host-range assessments conducted under natural conditions allow for the most representative and accurate prediction of the risk posed by a candidate agent (Schaffner et al., 2018; Hinz et al., 2019).

Faunistic surveys in the target weeds native distribution are particularly suitable for identifying and screening biological control agents, because the field host-range of multiple candidates can usually be assessed across a large number of non-target plants simultaneously (e.g., Cordo et al., 1995; Forno et al., 1995). During these surveys, non-target species that grow sympatrically with the target weed are surveyed to determine the field host-range of potential biological control agents, with much of the survey effort dedicated to surveying congeneric plants for the same insects, fungal pathogens and herbivorous mites that occur on the target weed (e.g. Palmer and Pullen, 1995; Harley et al., 1995; Heard et al., 2004; Dhileepan et al., 2013). Performing common-garden experiments for a similar number of host plants and candidate agents would be logistically impractical.

The host-range of insects, fungal pathogens and herbivorous mites demonstrates a strong phylogenetic signal, whereby the probability that two plant species will be attacked by a shared herbivore is negatively correlated with the phylogenetic distance between the two species (Weiblen et al., 2006; Gilbert and Webb, 2007; Gilbert et al., 2012; Cripps et al., 2016; Wheeler and Madeira, 2017; but see Becerra, 1997). This occurs because more closely-related plant species are more likely to share similar traits that are strong mediators of plant-herbivore interactions and resulting host-range patterns (Futuyuma, 2000; Pearse and Hipp, 2009; Cripps et al., 2016). The phylogenetic signal of host-range underpins fundamental host-range assessments and thus the risk assessment of candidate biological control agents (Wapshere, 1974; Wheeler and Madeira, 2017). Host-range testing of potential biological control agents follows the centrifugal phylogenetic model, which stipulates that host-range testing should focus on congeneric and closely related non-target plant species, with progressively fewer non-target species tested from more distantly related phylogenetic groups (Wapshere, 1974; Briese, 2003). The exemplary track record of the safety of weed biological control provides strong support for the use of phylogenetic relationships between the target weed and non-target species to assess the suitability and risk associated with introducing a candidate control agent (Hinz et al., 2019).

Host range assessments in the native range should survey a large number of non-target species that have been selected *a priori*, according to the centrifugal phylogenetic model (Wapshere, 1974; Briese, 2005). To date, biological control projects that have used faunistic surveys in the area of origin to identify and assess the host-range of candidate agents, have typically only surveyed a limited number of non-target species and/or have been limited to congeners of the target weed (e.g. Balciunas et al., 1994; Palmer and Pullen, 1995; Harley et al., 1995; McClay et al., 1995; Palmer and Pullen, 2001; Olckers et al., 2002; Paterson et al., 2014; Dhileepan et al., 2018; but see Cordo et al., 1995). The greatest benefits from native range surveys would be achieved if the non-target species selected to be surveyed in the native-range resembled the test plant list (based on the centrifugal phylogenetic model) drafted for fundamental no-choice testing under quarantine conditions. While this may not be possible at the species-level, practitioners may be able to identify native species in the area of origin that are as closely-related to the target weed (hereafter ‘phylogenetic proxy’) as those native non-target species considered most at risk in the target weeds invaded range. Surveying phylogenetic proxies could provide practitioners with an ecologically-motivated tool to screen and prioritise potential biological control agents for detailed host-range testing under quarantine conditions. The extensive resources required to source, import and perform host-range testing for a novel biological control agent (Paynter et al., 2015a), the importance of which is ever increasing due to more stringent regulations (Briese et al., 2002a), provides a strong argument for performing native-range host-range assessments that help prioritise agents that have the greatest chance of success.

Host-range of prospective biological control agents can be influenced by a multitude of factors other than plant phylogeny, such as: plant traits (e.g. structural traits and/or chemical compounds) (Cripps et al., 2016), the relative abundance of host plants (Moffatt et al., 2013), the presence of predators and parasitoids (Bernays and Graham, 1988), and the presence of congeneric herbivores (Futuyuma, 2000; Madeira et al., 2008). Native-range surveys may allow practitioners to identify factors, other than plant phylogeny, that influence host-plant selection and resulting insect host-range patterns, and that may be crucial for consideration during laboratory-based host-range assessments (e.g. Heard et al., 2004), and when assessing the risk posed by candidate agents to non-target species (e.g. Sheppard et al., 2006).

In this chapter, a phylogenetically-reasoned approach to native-range surveys was used to identify and prioritise prospective biological control agents for the management of two invasive grasses in Australia, *Sporobolus pyramidalis* and *S. natalensis.* The primary aims of this chapter were to: (1) perform field host-range surveys to identify prospective biological control agents for these two weeds, and (2) integrate these field host-range data with published plant phylogenies to select and prioritise only those candidate agents that should be subjected to detailed host-range testing under quarantine conditions. Agent prioritisation was based on the predicted risk each prospective agent poses to native Australian *Sporobolus* species and other non-target grass species.

4.2. Methods and materials

4.2.1. Plant and insect sampling

The distribution of sites where field host-range surveys were performed and the methods used to sample insect communities were provided in Chapter 3. The same sampling protocols were used to survey the insect assemblages associated with *S. pyramidalis* and *S. natalensis*, and all non-target grass species.

4.2.2. Field host-range and agent prioritisation

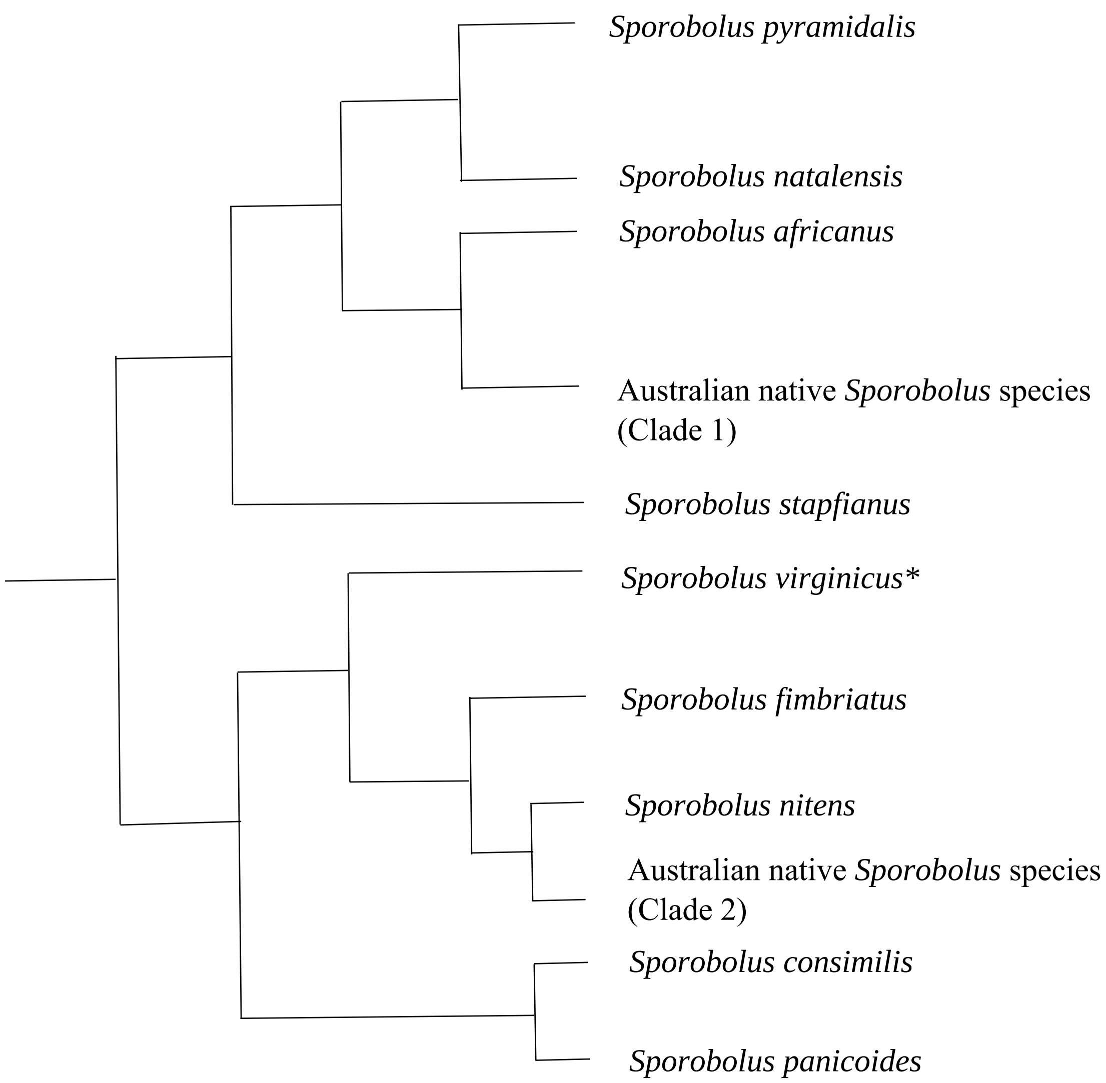
Surveys for phytophagous insects were conducted on 47 grass species that grew sympatrically with the two target weeds (i.e. 49 grass species were surveyed in total including the two target weeds). The plant species surveyed were selected based on availability at each site, and in accordance with the centrifugal phylogenetic model (Wapshere, 1974), and modifications proposed by Briese (2003). The primary prioritisation criteria was the phylogenetic proximity of the non-target species with the target weeds, whereby congeners (i.e. any *Sporobolus* species) were given the highest priority. An effort was made to survey a broad range of non-target species of increasingly distant phylogenetic proximity to the target weeds. Several additional criteria were used to prioritise which non-target species to survey, including: sympatry, relative abundance, and structural similarity to the target weeds. The reason for including each criterion, example non-target grasses surveyed under each criterion and key references are provided in Table 4.1.

To prioritise candidate biological control agents based on their predicted host-range, and thus risk posed to key non-target species, a phylogenetic approach was used to predict which non-target plant species were likely to be at risk in the weeds adventive range, despite many of these species not being present in South Africa. This approach assumes that the phylogenetic distance between two plant species is the primary determinant of herbivore host-range, as traits that mediate plant-herbivore interactions are phylogenetically conserved (Futuyuma, 2000; Pearse and Hipp, 2009).

Several molecular phylogenies have been produced in recent years allowing for the determination of phylogenetic relationships between native African and Australian *Sporobolus* species (Shrestha et al., 2003, 2005; Peterson et al., 2014; Soreng et al., 2017; summarised in Fig. 4.1). The two weedy species, *S. pyramidalis* and *S. natalensis*, belong to the monophyletic and morphologically homogenous *indicus* complex, along with five native Australian *Sporobolus* spp. (Simon and Jacobs, 1999; Fig. 4.1). An additional 13 native *Sporobolus* species that are not part of the *indicus* complex are also found in Australia (Simon and Jacobs, 1999; Fig. 4.1). One of these native Australian species, namely *Sporobolus pamelae*, is listed as 'endangered' according to the Queensland Nature Conservation (Wildlife) Regulation 2006. None of the native Australian *Sporobolus* species occur in Africa (Fish et al., 2015). There are 38 *Sporobolus* species that are native to southern Africa (Fish et al., 2015), all of which are more closely- related to native Australian *Sporobolus* species than they are to *S. pyramidalis* and *S. natalensis*. Any native African *Sporobolus* species that was surveyed for insects was defined as a 'phylogenetic proxy', and was used to infer the potential risk posed by candidate biological control agents to native Australian *Sporobolus* species. Any herbivore associated with a phylogenetic proxy during native-range surveys was assumed to pose a threat to native Australian *Sporobolus* species in the same clade, and clades that were closer in phylogenetic distance to the clade containing *S. pyramidalis* and *S. natalensis*.

Table 4.1. Criteria used to prioritise non-target grass species for field host-range surveys.

Agent prioritisation criterion	Reason for inclusion	Example non-target grasses	Key references
1. Phylogenetic proximity	Herbivore host-range is typically phylogenetically conserved, whereby the more-closely related two species are phylogenetically, the more-likely they are to share a common herbivore.	A diverse suite of grasses of varying phylogenetic proximity to the target weeds were surveyed, although increasing attention was given to more-closely related species (see Table 4.3)	Wapshere, 1974; Futuyuma, 2000
1. (a) Phylogenetic proxy	Phylogenetic proxy is a special case of 1. phylogenetic proximity. Phylogenetic proxies were defined as any congeneric species (i.e. any *Sporobolus* spp.) that was as closely, or more-closely related to native Australian *Sporobolus* spp. than to the two target weeds. Any herbivore associated with a phylogenetic proxy was considered more likely to attack native Australian *Sporobolus* spp. than the two target weeds, due to the phylogenetic conservatism of herbivore host-range	Any African *Sporobolus* spp. (e.g. *Sporobolus africanus*, *S. fimbriatus*, *S. nitens*, ect…), especially those species in the same clade as native Australian *Sporobolus* spp.	Wapshere, 1974; Futuyuma, 2000
2. (a) Sympatry (native range)	Non-target species that grew in close proximity with the target weeds in the native-range are most likely to be attacked	Only non-target species that grew in sympatry with the target weeds in the native-range were surveyed	Briese, 2003
2. (b) Sympatry (invaded range)	Non-target species that grow in close proximity with the target weeds in the invaded-range are most likely to be attacked. Non-target grass species growing in proximity of the target weeds in both South Africa and Australia were given high priority. Non-target grass species growing in sympatry with two target weeds in South Africa that had congeners in Australia were also prioritised.	*Heteropogon contortus*; *Themeda triandra*; *Panicum maximum*	Briese, 2003
3. Relative abundance	Candidate biological control agent host-range can be influenced by the relative frequency of potential host plants, whereby more abundant species are more likely to be attacked	*Eragrostis curvula*; *Panicum maximum*; *Themeda triandra*	Heard et al., 2004; Moffatt et al., 2013
4. Structural similarity	Plants that share similar traits are expected to be at greater risk to non-target effects, as plants traits are strong mediators of plant-consumer interactions and resulting host-range.	*Sporobolus fimbriatus*; *Eragrostis plana*; *Eragrostis curvula*; *Panicum* spp.	Futuyuma, 2000



Australian native *Sporobolus* species (Clade 1):

Sporobolus blakei De Nardi ex B.K.Simon
Sporobolus creber De Nardi
Sporobolus elongatus R.Br.
Sporobolus laxus B.K.Simon
Sporobolus sessilis B.K.Simon

Australian native *Sporobolus* species (Clade 2):

Sporobolus actinocladus (F.Muell.) F.Muell.
Sporobolus australasicus Domin
Sporobolus caroli Mez
Sporobolus contiguus S.T.Blake
Sporobolus disjunctus R.Mills ex B.K.Simon
Sporobolus latzii B.K.Simon
Sporobolus lenticularis S.T.Blake
Sporobolus mitchellii (Trin.) C.E.Hubb. ex S.T.Blak
Sporobolus pamelae B.K.Simon†
Sporobolus partimpatens R.Mills ex B.K.Simon
Sporobolus pulchellus R.Br.
Sporobolus scabridus S.T.Blake
* *Sporobolus virginicus* (L.) Kunth is native to both South Africa and Australia
† Conservation priority in Australia (endangered)

Figure 4.1. Summary of phylogenetic relationships between native Australian and South African *Sporobolus* species (Shrestha et al., 2003, 2005; Peterson et al., 2010; Soreng et al., 2017). Clade-level memberships for native Australian *Sporobolus* species were taken from Table 1.2.

For example, any herbivore associated with only the two target weeds, *S. pyramidalis* and *S. natalensis*, would be given high-priority as a biological control agent, as its predicted realised host-range in Australia does not include any native Australian *Sporobolus* species or any valued crop species (Fig. 4.2; Hypothetical agent #1). However, any herbivore attacking *S. africanus*, a native African species which grows sympatrically with the two target weeds in their native distributions, is as likely to attack native Australian *Sporobolus* spp. from the *indicus* complex as the two target weeds, because *S. africanus* is more closely-related to native Australian *Sporobolus* spp. belonging to the *indicus* complex than it is to the two target weeds (Australian native clade #1; Fig. 4.2; Hypothetical agent #2). As such, the herbivore could be excluded from consideration as a candidate biological control agent. Moreover, any candidate agent found attacking other 'phylogenetic proxies' in this study (e.g. *S. fimbriatus*, *S. nitens*, *S. consimilis*), in addition to *S. pyramidalis* and *S. natalensis*, would be hypothesized to potentially attack native Australian *Sporobolus* spp. that do not belong to the *indicus* complex, and by extension all of the Australian *indicus* complex species too (Australian native clade #1; Fig. 4.2.; Hypothetical agent #3), and thus, could also be rejected as potential biological control agents. Only those candidate agents that are not likely to pose a significant threat to key native Australian *Sporobolus* spp. would then be considered suitable for further study under quarantine conditions.

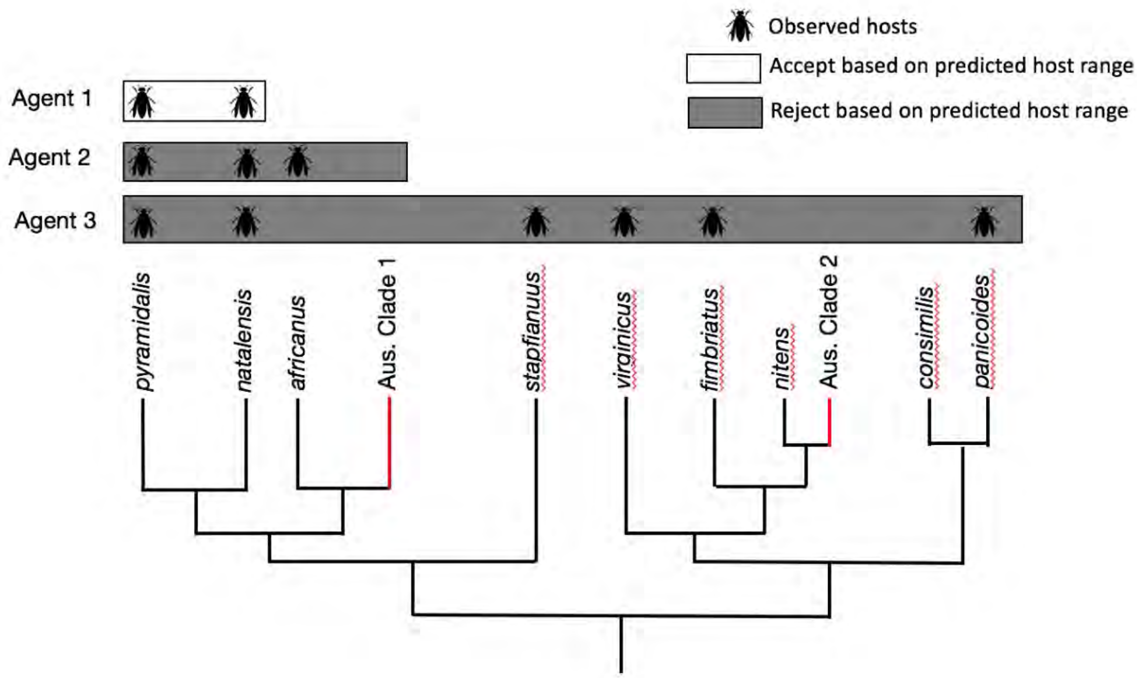


Figure 4.2. Schematic overview of how prospective biological control agents were prioritised from native-range field host-range surveys. Any native African *Sporobolus* species that was surveyed for insects was defined as a 'phylogenetic proxy', and was used to infer the potential risk posed by candidate biological control agents to native Australian *Sporobolus* species (indicated by a red line in the figure).

4.2.3. Field host-range and plant structural similarities

An effort was made to prioritise non-target species for field host-range surveys, that not only represented a broad gradient of phylogenetic distances from the two target weeds, but also prioritise plants based on: their structural similarity to *S. pyramidalis* and *S. natalensis* and the relative abundance of non-target species (Table 4.1). Preliminary field observations suggested that *Tetramesa* sp. 1, *Tetramesa* sp. 2 and *Bruchophagus* sp. 1 avoided *S. pyramidalis* and *S. natalensis* tillers with thin stem diameters.

It was hypothesized that non-target grasses species that had similar stem diameters to *S. pyramidalis* and *S. natalensis* may be at greater risk of attack by herbivorous insects (‘structural analogues’; *sensu* Briese, 2003). To evaluate the hypothesis of tiller diameter-dependent host range, (1) frequency distributions of insect presence/absence per tiller were individually computed for *Tetramesa* sp. 1, *Tetramesa* sp. 2 and *Bruchophagus* sp. 1, versus *S. pyramidalis* and *S. natalensis* tiller diameter, to determine whether the insects did select for tillers with larger stem diameters, and (2) the frequency distributions of non-target grass species tiller diameters were then computed, and the overlap between their tiller diameter distributions with the range of diameters selected for by *Tetramesa* sp. 1, *Tetramesa* sp. 2 and *Bruchophagus* sp. 1 on *S. pyramidalis* and *S. natalensis* were compared.

4.3. Results

4.3.1. Plant and insect sampling

Field host-range surveys were performed at 114 sites (96% of all survey sites), including 92 NWS sites (95% of NWS sites) and 22 LTS sites (100% of LTS sites). Many sites were surveyed on more than one occasion. A total of 47 non-target grasses were surveyed across the native distribution of *S. pyramidalis* and *S. natalensis* (Table 4.2). Across all grass species, 261 site visits and 648 insect surveys (i.e. each insect survey represents 10 stems being dissected per species = 6480 individual plants were dissected, across all species, and 10 minutes of visual searching for damaged stems/ectophagous insects per plant species) were performed. On average, each non-target species was surveyed at 4.88 ± 0.23 unique sites (range: 1 – 72 unique sites per species), and was surveyed on 11 ± 0.36 occasions (range: 1 – 86 surveys per species) (after removing *S. pyramidalis* and *S. natalensis* as they substantially inflated these calculations).

The greatest survey effort was directed to other *Sporobolus* species that grew sympatrically with the two target weeds. *Sporobolus africanus* was the most frequently encountered phylogenetic proxy for the most-closely related native Australian *Sporobolus* spp. to the two target weeds (i.e. species from Australian Clade 1; Fig. 4.2). *Sporobolus fimbriatus* was the next most widespread phylogenetic proxy, for native Australian *Sporobolus* spp. from Clade 2 (Fig. 4.2) being surveyed in the Eastern Cape Province and KwaZulu-Natal Province. The remaining phylogenetic proxies had more localised distributions, and thus were only surveyed in a relatively localised area in South Africa. For example, *S. nitens* and *S. stapfianus* only grew sympatrically with *S. pyramidalis* and *S. natalensis* in the bushveld of the KwaZulu-Natal Province.

Table 4.2. Taxonomic relationships between the two target weeds and sympatric non-target grass species that were included in field host-range surveys, and their reasons for inclusion as a non-target species (Refer to Table 4.1. for details about each reason for inclusion). Species are listed in descending phylogenetic proximity to the two target weeds.

Plant taxonomy		Plant species	Reason for inclusion
Target weeds			
Section	*indicus complex*		
Sporobolus	Yes	*Sporobolus pyramidalis*	Target weed
Sporobolus	Yes	*Sporobolus natalensis*	Target weed
Congeneric species			
Section (sub-section)	**indicus *complex***		
Sporobolus	Yes	*Sporobolus africanus*	Phylogenetic proxy; abundance; structural similarity
Sporobolus	No	*Sporobolus stapfianus*	Phylogenetic proxy
Triachyrum	No	*Sporobolus panicoides*	Phylogenetic proxy
Fimbriatae	No	*Sporobolus fimbriatus*	Phylogenetic proxy; abundance; structural similarity
Virginicae	No	*Sporobolus virginicus*	Phylogenetic proxy
Pyramidati (Actinocladi)	No	*Sporobolus nitens*	Phylogenetic proxy
Crypsis (Crypsis)	No	*Sporobolus consimilis*	Phylogenetic proxy; structural similarity
Species from other tribes within the Chlorioideae			
Tribe	*Sub-tribe*		
Eragrostideae	Eragrostidinae	*Eragrostis capensis*	Sympatry

Eragrostideae	Eragrostidinae	*Eragrostis cilianensis*	Structural similarity; abundance
Eragrostideae	Eragrostidinae	*Eragrostis cylindriflora*	Structural similarity; abundance
Eragrostideae	Eragrostidinae	*Eragrostis curvula*	Structural similarity; abundance
Eragrostideae	Eragrostidinae	*Eragrostis gummiflua*	Sympatry; abundance
Eragrostideae	Eragrostidinae	*Eragrostis capensis*	Sympatry
Eragrostideae	Eragrostidinae	*Eragrostis plana*	Structural similarity
Eragrostideae	Eragrostidinae	*Eragrostis superba*	Sympatry; abundance
Eragrostideae	Eragrostidinae	*Eragrostis* sp. 1	Sympatry; structural similarity
Eragrostideae	Eragrostidinae	*Eragrostis tenuifolia*	Sympatry; abundance
Eragrostideae	Eragrostidinae	*Eragrostis trichophora*	Structural similarity; abundance
Eragrostideae	Eragrostidinae	*Stiburus conrathii*	Sympatry
Cynodonteae	*incertae sedis*	*Dactyloctenium australe*	Sympatry
Cynodonteae	Eleusininae	*Chloris gayana*	Sympatry; abundance; economic crop
Cynodonteae	Eleusininae	*Cynodon dactylon*	Sympatry; abundance
Cynodonteae	Eleusininae	*Eustachys paspaloides*	Sympatry; abundance
Cynodonteae	Eleusininae	*Perotis patens*	Sympatry; abundance
Species from other sub-families within the Poaceae			
Sub-family	*Tribe (Sub-tribe)*		
Arundinoideae	Arundineae (Arundininae)	*Arundo donax*	Sympatry; abundance
Arundinoideae	Molinieae (Moliniinae)	*Phragmites australis*	Sympatry; abundance
Arundinoideae	Molinieae (Moliniinae)	*Phragmites mauritianus*	Sympatry; abundance
Panicoideae	Andropogoneae (Sorghinae)	*Bothriochloa insculpta*	Sympatry; abundance; pasture
Panicoideae	Andropogoneae (Sorghinae)	*Sorghum halepense*	Sympatry; abundance; economic crop
Panicoideae	Andropogoneae (Andropogoninae)	*Andropogon gayanus*	Sympatry; abundance
Panicoideae	Andropogoneae (Andropogoninae)	*Diheteropogon amplectens*	Sympatry; abundance

Panicoideae	Andropogoneae (Andropogoninae)	*Cymbopogon nardus*	Sympatry; abundance
Panicoideae	Andropogoneae (Saccharinae)	*Imperata cylindrica*	Sympatry
Panicoideae	Andropogoneae (Anthistiriinae)	*Hyparrhenia hirta*	Sympatry; abundance
Panicoideae	Andropogoneae (Anthistiriinae)	*Hyperthelia dissolute*	Sympatry; abundance
Panicoideae	Andropogoneae (Anthistiriinae)	*Themeda triandra*	Sympatry; abundance
Panicoideae	Paniceae (Anthephorinae)	*Digitaria eriantha*	Sympatry; abundance
Panicoideae	Paniceae (Melinidinae)	*Urochloa mosambisencis*	Sympatry; abundance
Panicoideae	Paniceae (Melinidinae)	*Melinis repens*	Sympatry; abundance
Panicoideae	Paniceae (Melinidinae)	*Tricholaena monachne*	Sympatry; abundance
Panicoideae	Paniceae (Panicinae)	*Panicum coloratum*	Structural similarity; sympatry
Panicoideae	Paniceae (Panicinae)	*Panicum deustem*	Structural similarity; sympatry; abundance
Panicoideae	Paniceae (Panicinae)	*Panicum maximum*	Structural similarity; sympatry; abundance
Panicoideae	Paniceae (Cenchrinae)	*Setaria megaphylla*	Sympatry; abundance
Panicoideae	Paspaleae (Paspalinae)	*Paspalum notatum*	Sympatry
Aristidoideae	Aristideae	*Aristida canesens*	Sympatry
Pooideae	Poeae (Aveninae)	*Avena fatua*	Sympatry
Pooideae	Bromeae	*Bromus* sp. 1	Sympatry; abundance

4.3.2. Field host-range and agent prioritisation

Six endophagous herbivores are associated with *S. pyramidalis* and *S. natalensis* in South Africa (Chapter 3). These represent the only potential herbivorous biological control agents, as no suitable ectophagous herbivores were recorded (Chapter 3). The majority of non-target grasses possessed their own distinct suite of herbivorous natural enemies, often being relatively species rich. For the purpose of the current study, no herbivores were considered that were not recorded on *S. pyramidalis* and/or *S. natalensis*.

Four of the endophagous herbivores were only recorded from species within the *Sporobolus* genus (Table 4.3). Three of the herbivores, namely: *Tetramesa* sp. 1, *Tetramesa* sp. 2 and *Bruchophagus* sp. 1, were only recorded from *S. pyramidalis* and *S. natalensis* (the two target weeds). Given that none of these three species were recorded on any phylogenetic proxies for native Australian *Sporobolus* species or any non-target species outside of the genus, they are not predicted to attack any non-target grasses in Australia.

Eurytomidae sp. 4 was not limited to the two target weeds, *S. pyramidalis* and *S. natalensis* (Table 4.3). This species was recovered on multiple occasions from a key phylogenetic proxy in *S. africanus*. No other native African *Sporobolus* species, nor other non-target species from other genera were included in its observed field host-range. Eurytomidae sp. 4, thus, poses a potential risk to native Australian *Sporobolus* species in clade #1, due to *S. africanus* being more closely related to the Australian *Sporobolus* species than the two target weeds (Fig. 4.2).

The field host-range of Scolytidae sp. 1 included species spanning multiple grass genera (e.g. *Andropogon gayanus*, *Themeda triandra*), and the key phylogenetic proxy of *S. africanus* (Table 4.3). Scolytidae sp. 1, thus, poses a potential risk to native Australian

Table 4.3. Summary of field host-range surveys. All values are presented as mean ± standard errors of the number of individuals recorded per plant species, per site visit (10 tillers dissected per site visit).

Plant species	No. of unique sites	No. of surveys	Field host-range (mean no. individuals/per 10 tillers)					
			Tetramesa sp. 1	*Tetramesa* sp. 2	*Bruchophagus* sp. 1	Eurytomid sp. 4	Scolytidae sp. 1	Chloropidae sp. 1
Sporobolus pyramidalis	72	167	3.3 ± 0.4	2.5 ± 0.4	1.6 ± 0.3	0.3 ± 0.1	0.3 ± 0.1	0.7 ± 0.1
Sporobolus natalensis	16	51	1.1 ± 0.3	0.6 ± 0.4	0.6 ± 0.3	0.2 ± 0.1	0.1 ± 0.1	0.5 ± 0.2
Sporobolus africanus	34	86	0	0	0	0.5 ± 0.1	0.5 ± 0.1	0.7 ± 0.2
Sporobolus stapfianus	3	3	0	0	0	0	0	2.1 ± 0.7
Sporobolus panicoides	1	1	0	0	0	0	0	0
Sporobolus fimbriatus	13	23	0	0	0	0	0	0.8 ± 0.5
Sporobolus virginicus	2	3	0	0	0	0	0	0
Sporobolus nitens	6	25	0	0	0	0	0	0
Sporobolus consimilis	1	2	0	0	0	0	0	0
Eragrostis capensis	2	2	0	0	0	0	0	0
Eragrostis cilianensis	1	3	0	0	0	0	0	0
Eragrostis curvula	6	16	0	0	0	0	0	1.6 ± 0.8
Eragrostis cylindriflora	1	1	0	0	0	0	0	0
Eragrostis gummiflua	1	1	0	0	0	0	0	0
Eragrostis plana	5	20	0	0	0	0	0	0
Eragrostis sp. 1	1	1	0	0	0	0	0	0
Eragrostis superba	3	18	0	0	0	0	0	0
Eragrostis tenuifolia	4	10	0	0	0	0	0	0
Eragrostis trichophora	4	14	0	0	0	0	0	0
Stiburus conrathii	1	6	0	0	0	0	0	0
Dactyloctenium australe	1	5	0	0	0	0	0	0
Chloris gayana	3	16	0	0	0	0	0	0

Cynodon dactylon	1	2	0	0	0	0	0	0
Eustachys paspaloides	1	1	0	0	0	0	0	0
Perotis patens	3	6	0	0	0	0	0	0
Arundo donax	2	2	0	0	0	0	0	0
Phragmites australis	1	1	0	0	0	0	0	0
Phragmites mauritianus	3	4	0	0	0	0	0	0
Bothriochloa insculpta	2	12	0	0	0	0	0	0
Sorghum halepense	3	11	0	0	0	0	0	0
Andropogon gayanus	4	8	0	0	0	0	2.1 ± 0.4	0
Diheteropogon amplectens	1	2	0	0	0	0	0	0
Cymbopogon nardus	1	6	0	0	0	0	0	0
Imperata cylindrica	1	1	0	0	0	0	0	0
Hyparrhenia hirta	3	13	0	0	0	0	0	0
Hyperthelia dissolute	1	6	0	0	0	0	0	0
Themeda triandra	5	10	0	0	0	3.1 ± 1.2	0	0
Digitaria eriantha	2	3	0	0	0	0	0	0
Urochloa mosambisensis	3	16	0	0	0	0	0	0
Melinis repens	2	7	0	0	0	0	0	0
Tricholaena monachne	1	3	0	0	0	0	0	0
Panicum colouratum	1	1	0	0	0	0	0	0
Panicum deustem	3	8	0	0	0	0	0	0
Megathyrsus maximus	9	40	0	0	0	0	0	0.4 ± 0.2
Setaria megaphylla	1	5	0	0	0	0	0	0
Setaria sphacelata var. *sericea*	1	2	0	0	0	0	0	0
Paspalum notatum	1	5	0	0	0	0	0	0
Aristida canesens	1	3	0	0	0	0	0	0
Avena fatua	1	1	0	0	0	0	0	0
Bromus sp. 1	1	1	0	0	0	0	0	0

Sporobolus species in clade #1 and clade #2, and species from other closely related genera (e.g. *Eragrostis*, *Panicum*, *Themeda*).

Chloropidae sp. 1 was recorded from non-target species spanning multiple grass genera (e.g. *Eragrostis curvula*), and several key phylogenetic proxies, including: *S. africanus, S. fimbriatus* and *S. stapfianus* (Table 4.3). Chloropidae sp. 1, thus, poses a potential risk to native Australian *Sporobolus* species in clade #1 and clade #2, and species from other closely related genera (e.g. *Eragrostis*).

4.3.3. Field host-range and plant structural similarities

Three herbivores (*Tetramesa* sp. 1, *Tetramesa* sp. 2 and *Bruchophagous* sp. 1) were found to have narrow host-ranges despite numerous non-target grass species being structurally analogous to the target weeds (i.e. non-target species had tiller diameter distributions that at least partially overlapped with the two target weeds; Fig. 4.3, 4.4). Frequency distributions of tiller diameter versus insect presence/absence at the individual-tiller level indicated that all three species selected for similar sized tillers. Across all three wasp species, very few tillers with a diameter < 3 mm were attacked (Fig. 4.3). Numerous non-target grasses had tiller diameters (Fig. 4.4) that at least partially overlapped with the distribution of tiller traits for *S. pyramidalis* and *S. natalensis*, yet none of the non-target grasses were attacked by any of the three wasp species (Table 4.3).

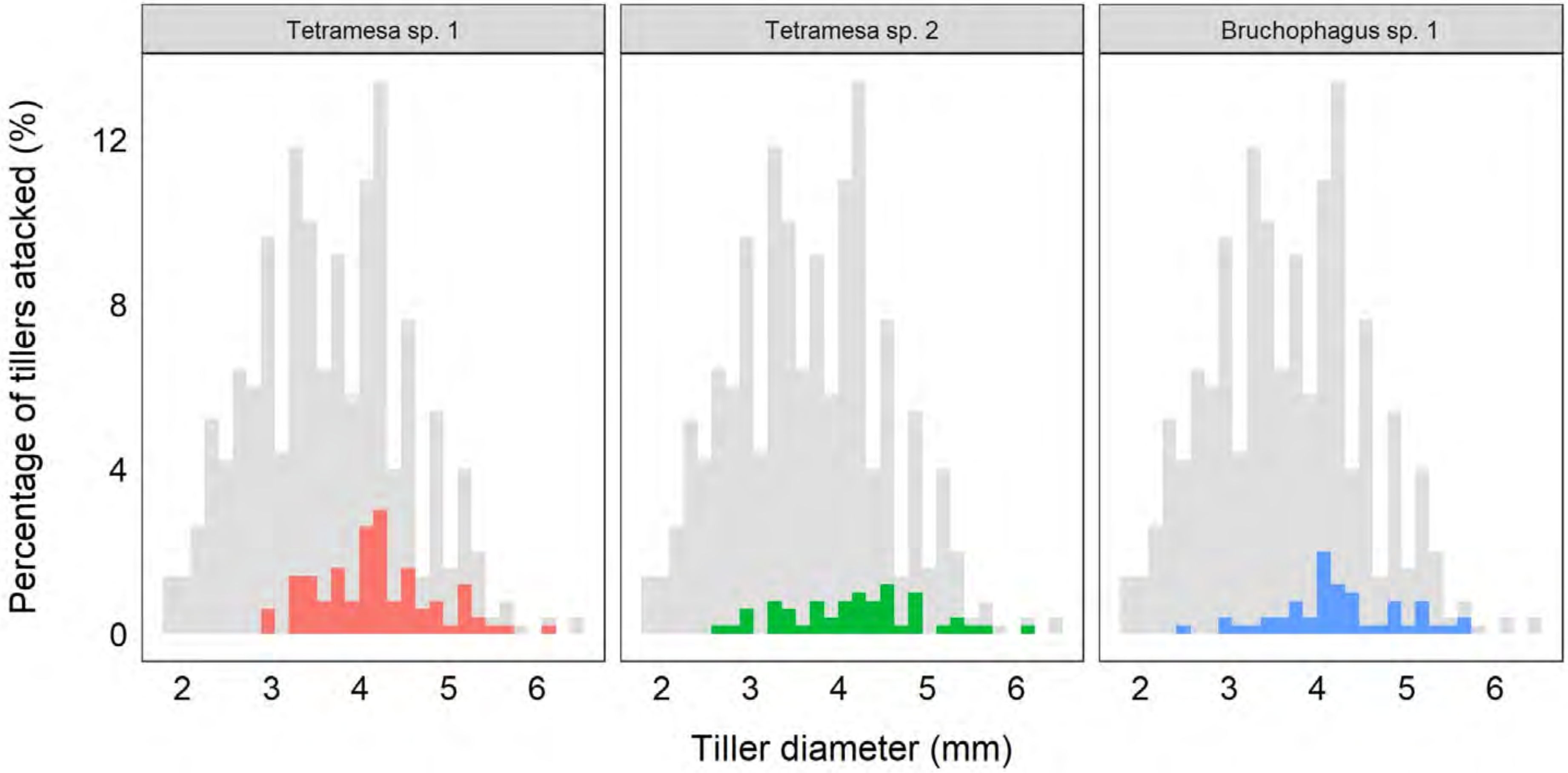


Figure 4.3. Frequency distributions of tiller selection patterns by candidate biological control agents under native-range field conditions with respect to tiller diameter. Colour-shaded bars indicate the tillers in each diameter class that were attacked by the three candidate control agents. Grey-shaded bars indicate tillers in each diameter class that were not attacked by any of the three control agents.

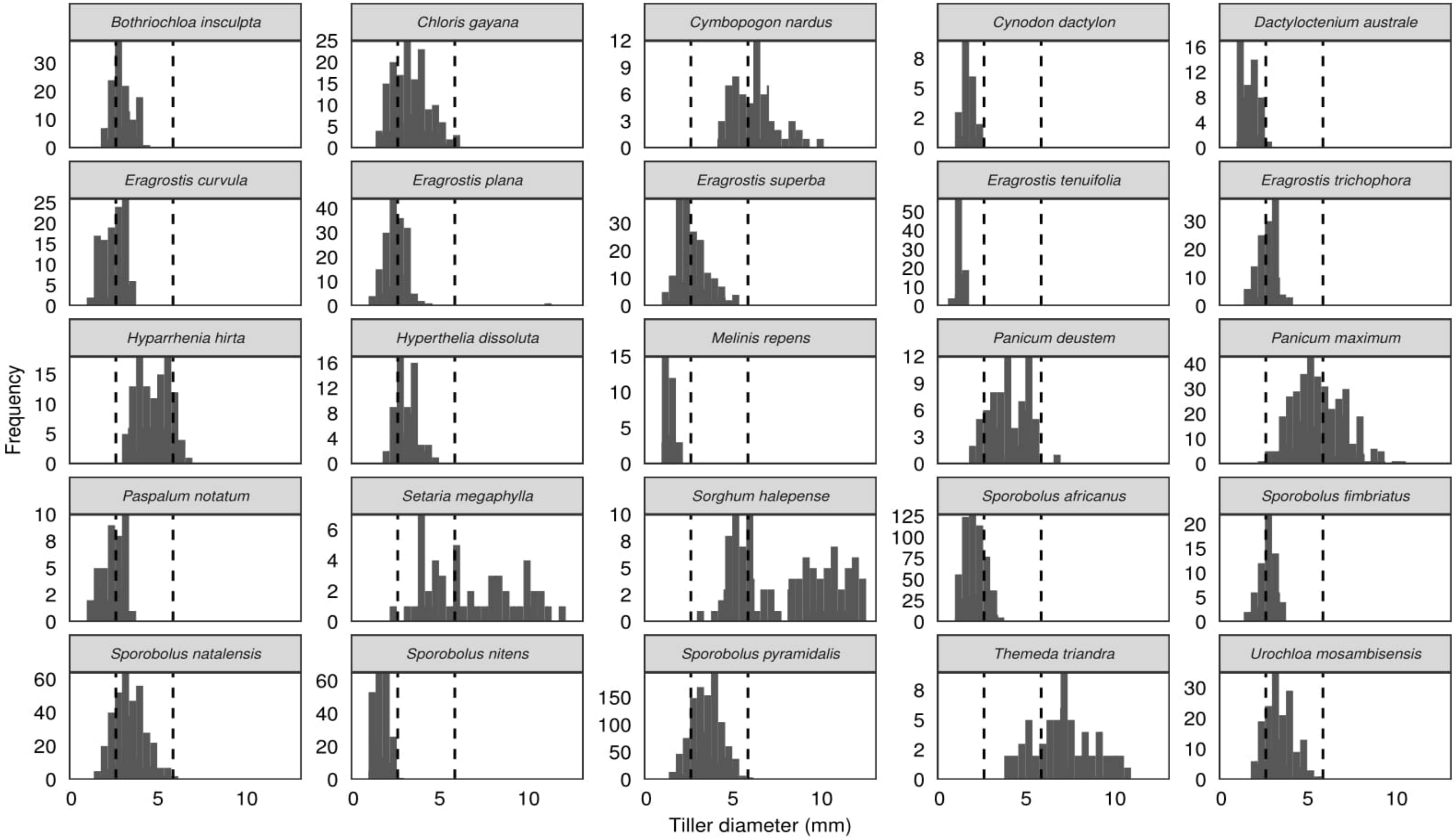


Figure 4.4. Frequency distributions of tiller diameters for *Sporobolus pyramidalis*, *S. natalensis* and non-target species from which structural trait data were collected. Vertical dashed lines indicate the range of tiller diameter values for infested *S. pyramidalis* and *S. natalensis* by the candidate biological control agents (*Tetramesa* sp. 1, *Tetramesa* sp. 2 and *Bruchophagus* sp. 1) under native-range field conditions.

4.4. Discussion

Field host-range assessments demonstrated that four phytophagous stem-galling wasps, belonging to the Eurytomidae family; namely: *Tetramesa* sp. 1, *Tetramesa* sp. 2, *Bruchophagus* sp. 1 and Eurytomidae sp. 4; have a narrow host-range, being recorded only from the two target weeds, *S. pyramidalis* and *S. natalensis,* and a closely-related congener in *S. africanus* (Eurytomidae sp. 4 only). This was despite 47 non-target grass species that grew sympatrically with the two target weeds, including seven non-target *Sporobolus* species, being surveyed for herbivorous insects. *Tetramesa* sp. 1 (referred to as *Tetramesa* sp. by Witt and McConnachie, 2004) has previously been recorded as the only insect with potential as a biological control agent for *S. pyramidalis* and *S. natalensis* in Australia (Witt and McConnachie, 2004). As such, the three other stem-boring wasps recorded, namely: *Tetramesa* sp. 2, *Bruchophagus* sp. 1 and Eurytomidae sp. 4., are novel potential biological control agents for this biological control project.

Traditionally, the narrow field host-range observed for these wasps would lead to them being imported into quarantine, where they would be subjected to detailed fundamental host-range testing. However, importation, culturing and host-range testing potential biological control agents is extremely costly (Paynter et al., 2015a). As such, field host-range data were integrated with molecular plant phylogenies to rank candidate agents, prior to importation into quarantine, to select only those candidate agents that have a quantitative and ecologically-plausible chance of satisfying the host-specificity requirements imposed on the programme.

Tetramesa sp. 1, *Tetramesa* sp. 2 and *Bruchophagus* sp. 1 were only recorded on the two target weeds and not from any of the phylogenetic proxy species for native Australian *Sporobolus* species, nor any non-target grasses outside of the *Sporobolus* genus. As such, the predicted realised host-range of these three wasps includes only the two target weeds, and thus, they are neither predicted to attack and develop on any native Australian *Sporobolus* species, nor any economic crops and pastures. All three species should be given high priority as candidate biological control agents, as these data suggest they may be suitable for release in Australia. It is recommended they be imported into quarantine for detailed fundamental host-range assessments.

The results also helped determine which potential biological control agents could be rejected as possible biological control agents, and thus potentially save time and effort in unnecessary quarantine testing. For example, Eurytomid sp. 4 was only recorded from *S. pyramidalis*, *S. natalensis* and *S. africanus*, which indicates a narrow field host-range. However, by using *S. africanus* as a phylogenetic proxy, Eurytomid sp. 4 would be expected to as readily attack native Australian *Sporobolus* species belonging to the *indicus* complex, as the two target weeds, and thus, would be rejected as a candidate agent. More distantly related native Australian *Sporobolus* species outside of the *indicus* complex are unlikely to serve as hosts of Eurytomid sp. 4, as it was not recorded on any of the phylogenetic proxies for these native Australian *Sporobolus* species (e.g. not recorded on *S. fimbriatus*, *S. nitens*). The two remaining herbivores, Chloropidae sp. 1 and Scolytidae sp. 1, were recorded on a number of non-target grasses from multiple grass genera. A similar pattern was observed for *Cryptonevra* sp. (Diptera: Chloropidae) tested as a potential biological control agent of *A. donax* in the USA, which demonstrated a disjunct host-range across multiple grass genera (John Goolsby, pers. comm.). The host-range patterns of Chloropidae sp. 1 and Scolytidae sp. 1 may be explained by a frequency-based host-range (e.g. Moffatt et al., 2013), as the non-target species attacked in the field (e.g., *E. curvula*, *M. maximus*, *T. triandra*) were typically the most abundant grasses at the field sites. However, a number of other potentially interacting factors should not be discounted, such as: structural limitations and/or chemical defences (Rapo et al., 2019).

It must be stressed that the approach adopted in this study is not intended to replace traditional quarantine-based host-range testing. Rather, by combining a phylogenetically-reasoned approach to native-range phytophagous surveys to prioritise candidate biological control agents with traditional no-choice host-range testing under quarantine conditions, biological control practitioners may simultaneously: (1) increase the efficiency and resource expenditure of biological control programmes by selecting only those candidate agents that have a quantitative and ecologically-plausible chance of satisfying the host-specificity requirements imposed on the programme, (2) obtain the most realistic and detailed estimation of a candidate agents fundamental and realised host-range, and (3) increase the transparency of weed biological control by explicitly documenting the decision-making process when selecting and prioritising candidate agents. This transparency is particularly relevant for the biological control of grasses because of the concerns over non-target impacts occurring on economically important cereal crops and pasture species by any introduced control agent (Wapshere, 1990).

The inferences and predictions made here regarding the host-range of candidate agents for the biological control of *S. pyramidalis* and *S. natalensis* are predicated on the well-founded notion that plant phylogeny is the major determinant of herbivore host-range (Wapshere, 1974; Gilbert and Webb, 2007; Gilbert et al., 2012; Wheeler and Madeira, 2017; Hinz et al., 2019). However, herbivore host-range can also be determined by extrinsic and intrinsic factors other than plant phylogeny, such as: plant structural traits (Cripps et al., 2016), secondary chemical defences (Becerra et al., 1997), the presence of predators and parasitoids (Bernays and Graham, 1988), relative host-plant abundance (Moffatt et al., 2013), and the presence of congeneric herbivores (Futuyuma, 2000; Madeira et al., 2008). This is further complicated by plant traits (e.g., chemical defences, structural traits) often demonstrating a phylogenentic signal (e.g. Rasmann and Agrawal, 2011; Cripps et al., 2016).

While the phylogenetic approach used in this thesis was possible for *S. pyramidalis* and *S. natalensis,* because of the phylogenetic signal in the host-range of the candidate agents under study and the availability of appropriate phylogenetic proxies (e.g. *S. africanus*), this may not be the case for other study systems (e.g. where plant chemistry is a stronger determinant of herbivore host-range than phylogeny *per se*; see Rapo et al., 2019). Nevertheless, this chapter demonstrated that surveys of non-target plants, particularly congeners, can greatly contribute to the prediction of candidate agent host-ranges.

Plant structural similarity to non-target species did not appear to influence the host-range of *Tetramesa* sp. 1, *Tetramesa* sp. 2 and *Bruchophagus* sp. 1. Frequency distributions of tiller diameter and tiller heights versus insect presence indicated that all three species selected for similar sized tillers. Across all three species, very few tillers with a diameter < 3 cm were selected for, while no tillers smaller than 2.8 cm were attacked. This finding is consistent with studies on other stem-attacking insects on grasses, including other *Tetramesa* species (e.g. *T. romana*), which have shown that females use stem diameter as an oviposition cue (e.g. Dubbert et al., 1998; Dudley et al., 2006; Blossey et al., 2018). Numerous non-target grasses had tiller diameter distributions that at least partially overlapped with the distribution of tiller diameters selected for by the wasps on *S. pyramidalis* and *S. natalensis*, yet none of the non-target grasses were attacked by any of the three wasp species. If structural traits had an influence on the host-range of these candidate agents, we would have expected structurally analogous non-targets, especially those species that are close-relatives of the two target weeds (e.g. *S. fimbriatus* and *S. africanus*), to be attacked. Clearly, *Tetramesa* sp. 1, *Tetramesa* sp. 2 and *Bruchophagus* sp. 1 use factors other than, or combination with, plant structural traits to select host plants (e.g. chemical cues) (e.g. Macias, 2017).

There are limitations to using a phylogenetic approach to native-range field surveys when identifying and prioritising candidate biological agents. First, molecular phylogenies of the target weed and its close-relatives are not always available. However, these phylogenies can be developed by biological control practitioners (e.g. Shresha et al., 2003; Madeira et al., 2008). Second, performing host-range assessments under native-range field conditions may produce false negatives, whereby a plant is not included in the observed host-range, despite being a true host (Withers et al., 2013), and thus, underestimate the field host-range (Heard et al., 2004). For example, field tests in Mexico likely produced false positives for *Apotoforma rotundipennis* (Walsingham) (Lepidoptera: Tortricidae) and *Aristotelia* sp. (Lepidoptera: Gelechiidae), two candidate biological control agents of *M. pigra* (Heard et al., 2004). False negatives are likely the product of low insect densities, resulting in low oviposition pressure, and unresponsiveness to inferior hosts (Marohasy, 1998; Heard et al., 2004). Given that field host-range surveys are intended to prioritise candidate agents for detailed host-range testing rather than replace traditional no-choice testing, any false-negative results will still be identified prior to the petition to release. Simple sleeve-trials or small-cage experiments could be performed under native field conditions that manipulate the density of any prospective agent to reduce the chances of obtaining a false-negative from native-range host-range assessments. Nevertheless, the potential of obtaining false-negatives during field host-range assessments should be considered when interpreting these data and resulting risk assessments.

There are situations where fundamental host-range testing may not be possible, and where a phylogenetically-reasoned approach to native-range field surveys could potentially be used to demonstrate that a candidate biological control agent is suitably host-specific to be petitioned for release. For example, extensive field host-range surveys across Australia led to the release of *Dasinuera pilifera* Kolesik (Diptera: Cecidomyiidae) on *Acacia baileyana* F. Muell. and *A. decurrens* Willd. (Fabaceae) in South Africa because the control agent could not be reared under quarantine conditions (Adair, 2004). *Dasineura pilifera* was approved for release in South Africa because it demonstrates a host-range limited to *Acacia* s.s. clade ‘E’, based on field host-range data collected in the native range (Australia), which is distantly phylogenetically related to *Vachellia* and/or *Senegalia* species native to South Africa and there are no native *Acacia* s.s. clade 'E' present in South Africa (Kleinjan and Hoffman, 2013). While this approach would not always be feasible (i.e. no appropriate phylogenetic proxies in the native range of the target weed), it does offer practitioners with an option to pursue biological control where quarantine-based host-range testing cannot be performed.

Biological control practitioners have spent many years developing the design, performance and interpretation of fundamental host-range testing (e.g. Marohasy, 1998; van Klinken, 2000; Withers et al., 2013; Paynter et al., 2015b), but less research has focused on improving native-range surveys, despite this being a critical component of a biological control programme (Goolsby et al., 2006). This chapter demonstrated one approach whereby native-range field host-range surveys could be integrated with plant phylogenies to assist practitioners with agent selection and prioritisation decisions at an early stage of a biological control programme. According to their field host-range, *Tetramesa* sp. 1, *Tetramesa* sp. 2 and *Bruchophagus* sp. 1 are specific to *S. pyramidalis* and *S. natalensis*. These wasps have a relatively widespread geographic distribution, can often be found in abundance (Chapter 3), and occur in climatically-similar regions in the two weeds native ranges' to weed infestations in Australia (Chapter 2). The most damaging of these species, or the most damaging combination, should be prioritised for release in Australia, pending the results of no-choice host specificity testing. In the next chapter, the damage caused by each of the three host-specific agents, individually and in combination with one another, was assessed and used as an additional agent prioritisation filter (Chapter 5).

Chapter 5: Prioritisation of potential biological control agents for weedy *Sporobolus* spp. in Australia (Poaceae), based on predicted efficacy and agent complementarity surveys in the native-range

5.1. Introduction

Traditionally, the screening, prioritisation and petition to release biological control agents has been based almost exclusively on the perceived risk posed to economic crops and native biodiversity in the region of intended control (McFadyen, 1998; Hinz et al., 2019). However, the successful biological control of a target weed requires that control agents reduce weed productivity and resulting population densities (McClay and Balciunas, 2005; van Klinken and Raghu, 2006), or some other appropriate metric of success, to below an acceptable economic or ecological threshold (Davis et al., 2006; Paterson et al., 2011a; Hoffmann et al., 2019). Thus, a discrepancy exists between the criteria employed to screen candidate agents for release and the ultimate goal of biological control, namely weed suppression.

In many instances, ineffective biological control agents have been released. This represents a significant waste of time and resources (McFadyen, 1998; Paynter et al., 2015a), and increases the risk of indirect non-target effects (e.g. Pearson and Callaway, 2003). To improve the efficiency and safety of weed biological control, only those agents with quantitative indicators of potential efficacy should be petitioned for release. To do so, practitioners have advocated that pre-release efficacy assessments be conducted to demonstrate that the candidate agent has an impact on key target weed productivity parameters (McClay and Balciunas, 2005; van Klinken and Raghu, 2006). Efficacy assessments typically quantify the candidate agents per-capita impact on plant growth, survival and/or reproduction (van Klinken and Raghu, 2006). Post-release evaluations have indicated that pre-release efficacy assessments can provide a good indication of agent efficacy post-release (Swirepik and Smith, 2002; Goolsby et al., 2016).

Many biological control programmes strive to release multiple control agents to increase the likelihood of weed suppression; however, direct and/or indirect interactions may occur between these agents that can substantially alter their relative impacts on the target weed (Denoth et al., 2002; Milbraith and Nechols, 2014). The "lottery model" and "cumulative stress model" have been proposed to explain why releasing multiple control agents is more successful than releasing a single agent. The "lottery model" states that the release of more agents increases the likelihood of releasing the "correct" biological control agent, and thus assumes that one agent is responsible for providing control over the target weed (Myers, 1985; Denoth et al., 2002). In contrast, the "cumulative stress model" stipulates that the combined impact of multiple agents, typically attacking a range of plants organs, is usually required to exert sufficient damage to control the target weed (Harris, 1985). While there are several examples of positive interactions between agents improving the level of biological control achieved (e.g. Rayamajhi et al., 2010; Aigbedion-Atalor et al., 2019), this is not always the case (e.g. Paynter and Hennecke, 2001; Mnqeta and Paterson 2019). Pre-release investigations of inter-specific competitive interactions between control agents may aid the agent prioritisation process, and maximise agent establishment rates and the degree of weed suppression (Milbrath and Nechols, 2014; Jones and Lake, 2018), while minimising the release of ineffective agents (McEvoy and Coombs, 1999).

Hatcher (1995), with modifications by Turner et al., (2010), proposed four outcomes of inter-specific interactions between multiple biological control agents, which could be used to evaluate how agent combinations are expected to affect weed fitness, and prioritise candidate agents based on predicted efficacy. The interactions are defined as, (1) synergistic, whereby the combined impact is greater than the sum of both agents impacts alone, (2) additive, whereby the two agents are more damaging than the most damaging agent, but less damaging that the sum of both agents impacts alone, (3) equivalent, whereby the two agents are equally as damaging as the more damaging of the two agents alone, and (4) inhibitory, whereby their combined impact is less than the more damaging of the two agents alone. Evaluating the impacts of control agents, singularly and in combination with one another, is not straightforward (Buccellato et al., 2012, 2019). However, it provides valuable data to indicate whether multiple agents or a single agent should be released, and which agent(s) should be prioritised to maximise damage (Milbrath and Nechols, 2014).

The invasiveness of *S. pyramidalis* and *S. natalensis* in Australia is believed to be largely dependent on their ability to produce a large and persistent seed load (Vogler and Bahnisch, 2006). Each inflorescence can produce between 400-1000 seeds, with estimates of 85 000 seeds/m^2 in dense infestations (Vogler and Bahnisch, 2006). For any natural enemy to be considered a suitable candidate for the biological control of *S. pyramidalis* and *S. natalensis*, it should have a demonstratable negative impact on either tiller survival or reproduction, or preferably both.

In this chapter, native-range field surveys were performed to select which of the three apparently monophagous candidate biological control agents of *S. pyramidalis* and *S. natalensis* (Chapter 4), namely: *Tetramesa* sp. 1, *Tetramesa* sp. 2 and *Bruchophagus* sp. 1, alone or in combination, should be imported into Australia for detailed host-range assessments. Specifically, the aims were to determine, (1) were any of the candidate agents considered damaging to the target weeds?, (2) if so, which agent(s) were most damaging?, and (3) are any combinations (i.e. pairs, or all three agents combined) of agents expected to provide a greater degree of control over the target weeds than any agent individually? These data were discussed in the context of prioritising candidate biological control agents based on predicted efficacy and complementarity.

5.2. Methods and materials

5.2.1. Damage surveys

The distribution of survey sites across the native range of *S. pyramidalis* and *S. natalensis* has been described previously (Chapter 3). Briefly, 22 sites were surveyed across the KwaZulu-Natal Province of South Africa on a bi-monthly basis from November 2017 until November 2018. Additionally, NWS surveys were conducted across the majority of the native ranges' of *S. pyramidalis* and *S. natalensis* in South Africa between March 2017 and January 2019. In total, 261 efficacy assessments, totaling 2610 individual stems (i.e. each assessment consists of 10 stems dissected per grass species per site), were performed at 83 sites. The majority of sites were surveyed between 1 and 6 times per plant species (2.13 ± 0.23; mean ± s.e.). Sites were chosen to maximize the geographic extent of survey coverage, and therefore the range of habitats and climatic conditions under which efficacy surveys were conducted.

The field sampling protocol has been provided in Chapter 3. Some additional parameters were collected to investigate the damage caused by endophagous herbivores associated with *S. pyramidalis* and *S. natalensis*. For each tiller: diameter (mm), height (cm), survival (alive/dead), reproduction (producing seed/no seed) and number of emergence holes were recorded. Tiller diameter was measured using a fine-scale caliper approximately 2cm above the first node to ensure consistency. Each tiller was then dissected and all insects recorded according to the protocol described in Chapter 3. The presence and abundance of each endophagous species was recorded per tiller.

5.2.2. Predicting agent efficacy

Generalised linear mixed models (GLMM's) were used to determine the capacity of each of the three potentially host-specific agents to reduce key weed productivity parameters (Bolker et al., 2009). The dependent variables: (1) the probability that an individual tiller was alive/dead (hereafter 'tiller survival'), and (2) the probability that an individual tiller was seeding/not seeding (hereafter 'tiller reproduction'), were modelled with respect to the abundance of *Tetramesa* sp. 1, *Tetramesa* sp. 2 and *Bruchophagus* sp. 1, which were fitted as continuous fixed effects. GLMM's were specified with a binomial distribution and a logit link function. Specifying GLMM's with plant species (*S. pyramidalis* / *S. natalensis*) as a categorical fixed effect did not significantly improve model performance during exploratory analyses (data not shown). As such, field survey data for *S. pyramidalis* and *S. natalensis* were combined for all analyses, and any inferences made from these data were assumed to apply equally to both weed species. To account for potential spatial and temporal non-independence of data, site and sampling event were modelled as random effects (Bolker et al., 2009). Multicollinearity between fixed effects was assessed by calculating generalised variance inflation factors (GVIF's) (Fox and Monette, 1992). GVIF values > 2 are typically taken as evidence for collinearity and any such variable with GVIF > 2 would not be included as a predictor variable in subsequent analyses.

To determine whether interactions between insect species had an influence on *S. pyramidalis* and *S. natalensis* tiller survival and reproductive output, two-way interactions between insect species were included in the models. A three-way interaction between insect species was not included in the model specification due to issues with 'complete separation' (Heinze and Schemper, 2002). Complete separation occurred as the three-way interaction between wasp species fully explained the binary response variable (i.e. all tillers which contained all three wasp species were dead and did not produce any seed). In total, 14 candidate models were fitted for tiller survival and tiller reproduction independently, including: all possible combinations of fixed effects, their two-way interactions, a null + random effects model (i.e. random intercepts for site and sampling date), and a null model consisting of a global random-intercept term only.

Model selection was performed by ranking models according to their AICc scores, whereby any model within 6 AICc points of the top-performing model (i.e. ΔAICc = 0) was retained to form a set of plausible models (Richards et al., 2011). Models that contained uninformative parameters were omitted from the plausible set (Leroux, 2019). This approach omitted models that were more complex versions of equally high-ranked models, but which were specified with fewer parameters (Arnold, 2010). Akaike weights (w_i) were calculated to determine the degree of support for each model being the top-performing model. As there were multiple GLMM's retained in the plausible model set for both tiller survival and tiller reproduction models and no individual model $w_i > 0.9$, model averaging was used to produce a composite model for both model sets (Burnham and Anderson, 2002; Grueber et al., 2011). The significance and magnitude of impact of each wasp species on tiller dynamics was assessed by comparing parameter estimates (± 95 % CI) derived from composite models.

Model diagnostics tests for all plausible GLMM's were performed to assess model fits. Evaluating model fits for GLMM's is difficult, as standard residual plots used for linear models can indicate poor model fits, even if the GLMM is correctly specified (Eveson et al., 2018). Model diagnostic tests were performed using the R packages: '*DHARMa*' (Hartig, 2018) and '*arm*' (Gelman et al., 2018), as the package overcomes this issue by simulating quantile residuals from a fitted GLMM, which are then standardized to values between 0 and 1. Model diagnostics indicated that all plausible GLMM's included in model averaging were a good fit.

To rank the three wasps according to their predicted efficacy, the relative contribution of each wasp species to variation in tiller survival and reproduction was quantified by computing marginal R^2_{GLMM} values (Nakagawa and Schielzeth, 2013). Marginal R^2_{GLMM} values represent the percentage variance explained by fixed effects alone (i.e. no random effects) (analogous to an R^2 value derived from a linear regression) (Nakagawa and Schielzeth, 2013). The most damaging species or species combination was identified as that which, when removed from the global GLMM, resulted in the greatest decrease in percentage variation explained (i.e. marginal R^2_{GLMM}). The decrease in explanatory power was calculated, when each species or species combination was individually removed from a GLMM including all three insect species included as fixed effects, as a percentage of the total marginal R^2_{GLMM} (De Palma et al., 2015). These data are presented as the overall percentage of GLMM model variation explained for ease of interpretation (i.e. 100 - % drop in variation explained). Importantly, these values do not equate to a percentage reduction in tiller survival or reproduction *per se*, but rather how much variation each insect species accounts for in each GLMM model (i.e. an increase in percentage variation accounted for by the model may be the result of increased tiller survival or reproduction with increasing insect abundance). The model variation accounted for by each insect species may overestimate or underestimate the magnitude of their impact depending on covariation amongst the fixed effects, and thus should be interpreted as the relative variation accounted for by each species or species combination (De Palma et al., 2018). Marginal effects plots were computed to visualize the relationships between the abundance of all three phytophagous wasps on tiller dynamics, including the two-way interactions between species pairs using the '*ggeffects*' package (Lüdecke, 2018).

All statistical analyses were performed using *R* ver. 3.5.1. (R Core Team, 2018). GLMM's were fit using the '*lme4*' package (Bates et al., 2015). Model-averaging and model selection were performed using the '*AICcmodavg*' package (Mazerolle, 2017). GVIF's were calculated using the '*car*' package (Fox and Weisberg, 2011).

5.2.3. Agent complementarity

To investigate whether inter-specific competitive interactions may occur between candidate biological control agents, the distribution of tillers containing no insects, a single species, and more than one species was analysed using chi-squared tests. For the three phytophagous wasps being considered as potential biological control agents in this study, there were 8 possible species combinations possible. All pairwise interactions between insect species and their three-way interaction were evaluated. Standardised residuals were calculated to determine which species and species combinations were more or less likely to co-occur in the same stems, whereby negative residuals indicate that a species or species combination occurred less often, while positive residuals indicate that a species or species combination occurred more frequently than expected by chance (Agresti and Kateri, 2011). Species pairs that occurred in the same stem less frequently than expected by chance were deemed potentially antagonistic, species pairs that occurred in the same stem as often or more frequently than expected by chance were deemed potentially complimentary.

5.3. Results

5.3.1. Damage surveys

Although six endophagous feeders are associated with *S. pyramidalis* and *S. natalensis* in South Africa, only the three phytophagous wasps that were identified as being suitably host-specific to warrant consideration as candidate control agents in Chapter 4 are discussed here. The proportion of tillers attacked by candidate biological control agents in the native-range of *S. pyramidalis* and *S. natalensis* was low (Table 5.1). *Tetramesa* sp. 1 attacked more tillers (5.0 – 15.2 % of all tillers) than *Tetramesa* sp. 2 (1.8 – 7.9 %) and *Bruchophagus* sp. 1 (2.2 – 8.5 %). Irrespective of which candidate agent was considered, the proportion of tillers damaged was 3-4 fold lower on *S. natalensis* than *S. pyramidalis*. Very few tillers were attacked by more than a single insect herbivore (Table 5.1).

5.3.2. Predicting agent efficacy

The inclusion of random effects in tiller survival and reproduction GLMM's provided a better fit than the null model (random-intercept term only), justifying the use of GLMM's to account for the hierarchical structure of this field survey dataset. GVIF values for all fixed effects were below the threshold value of 2, indicating that collinearity between the fixed effects was not likely to influence model performance. In the following sections, tiller survival and tiller reproduction dynamics models are discussed separately, with particular emphasis on highlighting the individual contributions made by the three phytophagous wasps in explaining tiller dynamics. The wasp species or species combination that explains the largest amount of variation in tiller dynamics should be given the highest priority as potential biological control agents.

Table 5.1. Proportion of tillers attacked (%) per insect survey.

Plant species	Insect species (% of tillers attacked)[1]						
	Tet1	Tet2	Bru1	Tet1 x Tet2	Tet1 x Bru1	Tet2 x Bru1	Tet1 x Tet2 x Bru1
Sporobolus pyramidalis (n = 1670)	15.2	7.9	8.5	3.7	3.4	2.2	0.9
Sporobolus natalensis (n = 510)	5.0	1.8	2.2	0.6	0.6	0.4	0.0
Total (n = 2180)	11.8	5.9	6.5	2.8	2.5	1.6	0.6

[1] Tet1 = *Tetramesa* sp. 1; Tet2 = *Tetramesa* sp. 2; Bru1 = *Bruchophagus* sp. 1

Three GLMM's were considered plausible in explaining tiller survival dynamics, after omitting candidate models that were more than 6 AICc points from the top-performing model and models with uninformative parameters (Table 5.2). The plausible model set consisted of models that contained various combinations of all three wasp species as predictor variables, including one model that contained an interaction between *Tetramesa* sp. 1 and *Bruchophagus* sp. 1. None of the three plausible models received equivocal support as a top-performing model (Table 5.2), thus model-averaging was used to compute a final composite model for tiller survival dynamics.

Approximately 10.6 – 13.1% of the variation in tiller survival dynamics could be attributed to the impact of the three phytophagous wasps associated with *S. pyramidalis* and *S. natalensis* (Marginal R^2_{GLMM} : 0.106 – 0.131). Inspection of marginal effects plots and negative parameter estimates indicated that individually; *Tetramesa* sp. 1 (β = -0.75 ± 0.14), *Tetramesa* sp. 2 (β = -0.24 ± 0.10) and *Bruchophagus* sp. 1 (β = -0.29 ± 0.13) all had deleterious impacts on tiller survival (Table 5.3; Fig. 5.1a). The impact of all three species was density-dependent, whereby increasing insect abundance was associated with a reduced probability of tiller survival (Fig. 5.1). Partitioning marginal R^2_{GLMM} scores by species indicated that approximately 84% of the variation in tiller survival was attributed to *Tetramesa* sp. 1, while *Tetramesa* sp. 2 and *Bruchophagus* sp. 1 individually accounted for less than 10% of the overall model variation (Fig. 5.2). Marginal effects plots confirmed that attack by *Tetramesa* sp. 1 results in a greater decline in tiller survival than *Tetramesa* sp. 2 and *Bruchophagus* sp. 1 (Fig. 5.1).

Table 5.2. Summary of model selection statistics for generalized linear mixed models (GLMM's) of *Sporobolus pyramidalis* and *S. natalensis* tiller survival, whereby the abundance of *Tetramesa* sp. 1, *Tetramesa* sp. 2 and *Bruchophagus* sp. 1 were specified as fixed effects.. Model selection was performed using Akaike's information criterion corrected for small samples sizes (AICc). K = no. of parameters specified in the model. Plausible models are highlighted in bold.

Model structure	K	AICc	ΔAICc	w_i	LogLik
tet1 x bru1 + tet2	**7**	**542.64**	**0.00**	**0.47**	**-264.29**
tet1 + tet2 + bru1	**6**	**543.98**	**1.34**	**0.24**	**-265.94**
tet1 x tet2 + bru1 †	7	545.72	3.08	0.10	-265.80
tet1 + tet2 x bru1 †	7	545.88	3.24	0.09	-265.88
tet1 + tet2	**5**	**546.52**	**3.88**	**0.06**	**-268.23**
tet1 x tet2 †	6	548.41	5.77	0.02	-268.16
tet1 + bru1	6	551.13	8.49	0.00	-269.52
tet1 x bru1	5	552.37	9.73	0.00	-271.15
tet1	4	554.78	12.14	0.00	-273.37
tet2 + bru1	5	582.03	39.39	0.00	-285.98
tet2 x bru1	6	583.92	41.28	0.00	-285.91
tet2	4	586.41	43.77	0.00	-289.18
bru1	4	592.16	49.52	0.00	-292.06
Null + random effects	3	597.27	54.63	0.00	-295.62
Null	1	719.83	177.19	0.00	-358.92

† Models with uninformative parameters that were omitted from the plausible model set

Table 5.3. Parameter estimates (± SE) for fixed effects of generalized linear mixed models (GLMM's) of *Sporobolus pyramidalis* and *S. natalensis* tiller survival, following model-averaging of the plausible model set (see Table 5.2). Lower and upper 95% confidence intervals (CI) are reported.

Fixed effect	Parameter estimate (± SE)	Lower CI	Upper CI
tet1	-0.75 ± 0.14	-1.01	-0.48
tet2	-0.24 ± 0.10	-0.43	-0.05
bru1	-0.29 ± 0.13	-0.54	-0.03
tet1 x bru1	0.17 ± 0.08	0.01	0.33

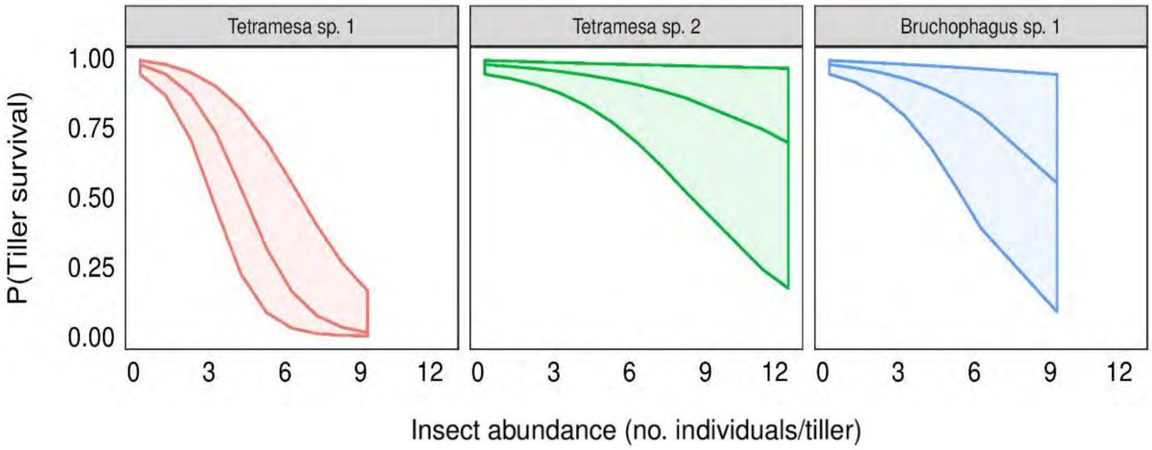


Figure 5.1. Probability of *Sporobolus pyramidalis* and *S. natalensis* tiller survival, with respect to the abundance of abundance of *Tetramesa* sp. 1, *Tetramesa* sp. 2 and *Bruchophagus* sp. 1. Shaded areas indicate 95% confidence intervals of GLMM model predictions, with all other fixed and random effects set to 0. Note the varying x-axis values for the different insect herbivores representing their maximum abundance recorded under field conditions.

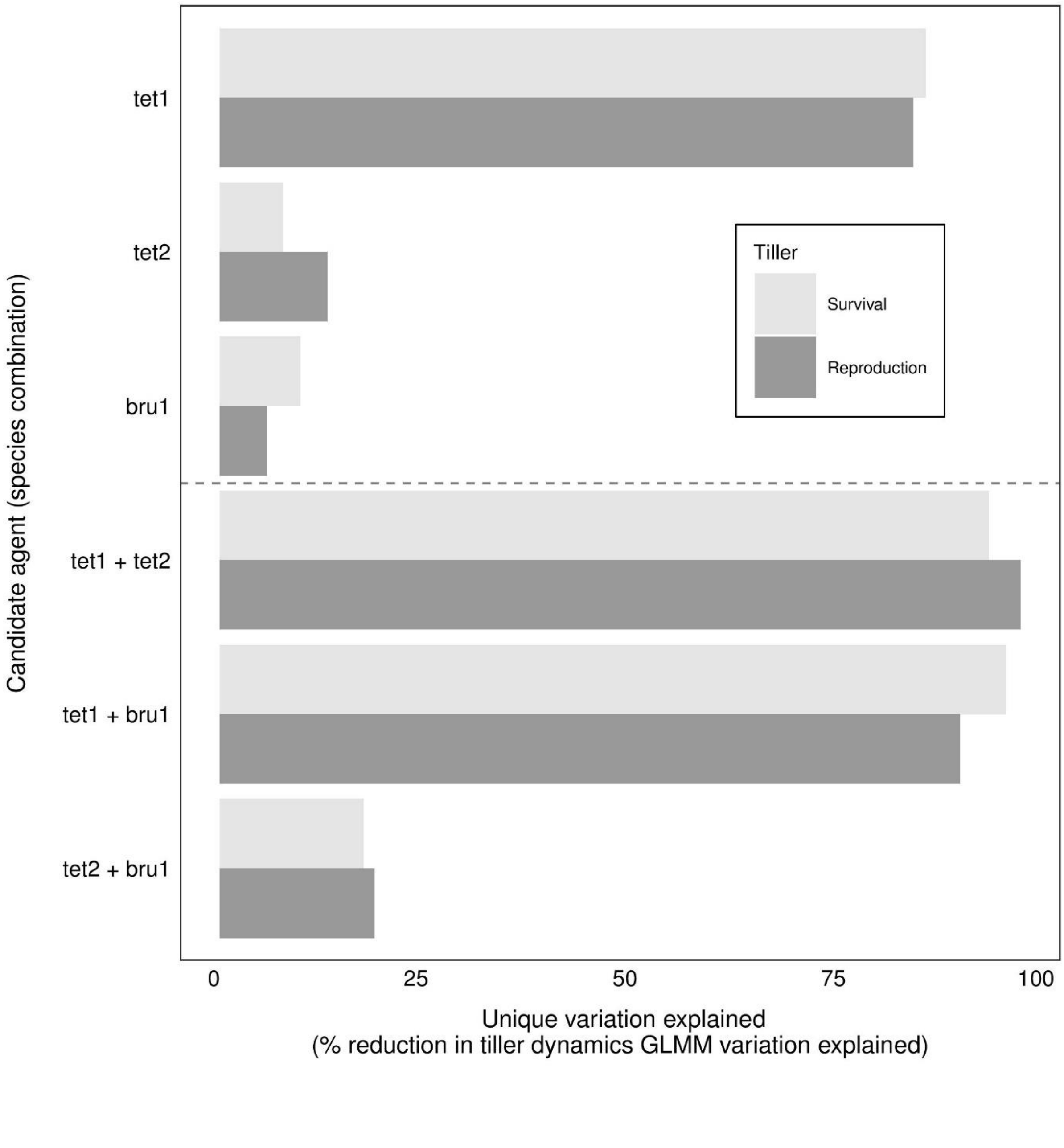


Figure 5.2. The unique contribution of individual insect species, and their two-way interactions, to the percentage of variation accounted for in the generalised linear mixed models (GLMM's) of *Sporobolus pyramidalis* and *S. natalensis* tiller survival (light grey shaded bars) and tiller reproduction (dark grey shaded bars). See text for details regarding how percentage variation scores were calculated.

Three GLMM's were considered plausible in explaining tiller reproduction dynamics after omitting candidate models that were more than 6 AICc points from the top-performing model and models with uninformative parameters (Table 5.4). The plausible model set consisted of models that contained various combinations of all three wasp species as predictor variables, including one model that contained an interaction between *Tetramesa* sp. 1 x *Bruchophagus* sp. 1. None of the three plausible models received unequivocal support as a top-performing model (Table 5.4), thus model-averaging was used to compute a final composite model for tiller reproduction dynamics.

Approximately 13.6 – 17.3% of the variation in tiller reproduction dynamics could be attributed to the impact of the three phytophagous wasps associated with *S. pyramidalis* and *S. natalensis* (Marginal R^2_{GLMM} : 0.136 – 0.173). Inspection of marginal effects plots and negative parameter estimates indicated that *Tetramesa* sp. 1 (β = -0.81 ± 0.15), *Tetramesa* sp. 2 (β = -0.34 ± 0.11) and *Bruchophagus* sp. 1 (β = -0.20 ± 0.13) all had deleterious impacts on tiller reproduction (Table 5.5; Fig. 5.3). The impact of all three species was density-dependent, whereby increasing insect abundance was associated with a reduced probability of the tiller producing seed (Fig. 5.3). Partitioning marginal R^2_{GLMM} scores by species indicated that approximately 83% of the variation in tiller reproduction was attributed to *Tetramesa* sp. 1, while *Tetramesa* sp. 2 and *Bruchophagus* sp. 1 individually accounted for less than 12% of the overall model variation (Fig. 5.2). Marginal effects plots confirmed that attack by *Tetramesa* sp. 1 results in the greatest decline in tiller reproduction (Fig. 5.3).

Table 5.4. Summary of model selection statistics for generalized linear mixed models (GLMM's) of *Sporobolus pyramidalis* and *S. natalensis* tiller reproduction, whereby the abundance of Tetramesa sp. 1, Tetramesa sp. 2 and Bruchophagus sp. 1 were specified as fixed effects. Model selection was performed using Akaike's information criterion corrected for small samples sizes (AICc). K = no. of parameters specified in the model. Plausible models are highlighted in bold.

Model structure	K	AICc	ΔAICc	w_i	LogLik
tet1 x bru1 + tet2	**7**	**727.97**	**0**	**0.76**	**-356.92**
tet1 + tet2 + bru1	**6**	**732.88**	**4.90**	**0.06**	**-360.39**
tet1 + tet2	**5**	**733.38**	**5.50**	**0.05**	**-361.65**
tet1 x tet2 + bru1 †	7	733.74	5.76	0.04	-359.80
tet1 x tet2	6	734.06	6.09	0.03	-360.98
tet1 + tet2 x bru1	7	734.09	6.11	0.03	-359.98
tet1 x bru1	6	739.57	11.59	0.00	-363.73
tet1 + bru1	5	744.73	16.75	0.00	-367.33
tet1	4	745.47	17.39	0.00	-368.66
tet2 + bru1	5	771.06	43.08	0.00	-380.49
tet2 x bru1	6	772.03	44.05	0.00	-379.96
tet2	4	773.07	45.09	0.00	-382.51
bru1	4	784.40	56.42	0.00	-388.17
Null + random effects	3	787.79	59.81	0.00	-390.88
Null	1	960.31	232.34	0.00	-479.15

† Models with uninformative parameters that were omitted from the confidence model set

Table 5.5. Parameter estimates (± SE) for fixed effects of generalized linear mixed models (GLMM's) of *Sporobolus pyramidalis* and *S. natalensis* tiller reproduction, following model-averaging of the plausible model set (see Table 5.4). Lower and upper 95% confidence intervals (CI) are reported.

Fixed effect	Parameter estimate (± SE)	Lower CI	Upper CI
tet1	-0.81 ± 0.15	-1.11	-0.51
tet2	-0.34 ± 0.11	-0.55	-0.12
bru1	-0.20 ± 0.13	-0.44	0.05
tet1 x bru1	0.27 ± 0.09	0.09	0.45

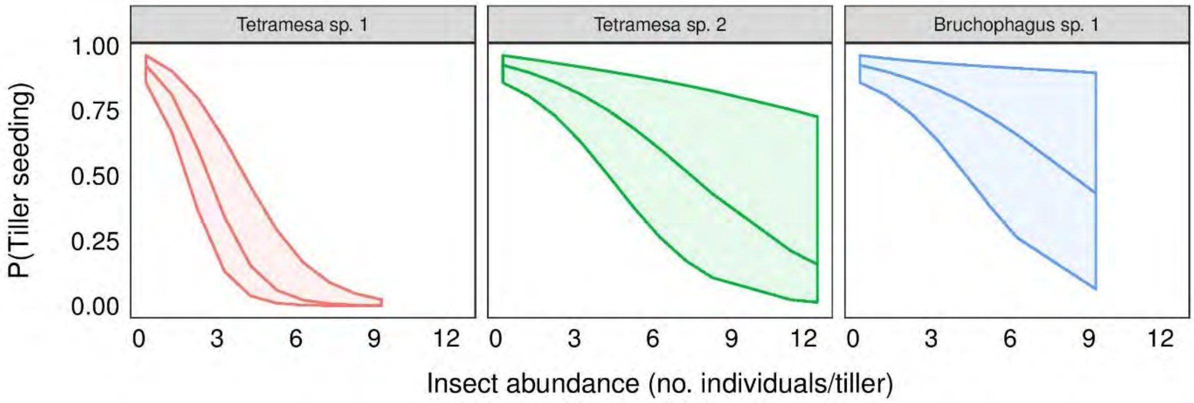


Figure 5.3. Probability of *Sporobolus pyramidalis* and *S. natalensis* tillers producing seed (tiller reproduction), with respect to the abundance of abundance of *Tetramesa* sp. 1, *Tetramesa* sp. 2 and *Bruchophagus* sp. 1. Shaded areas indicate 95% confidence intervals of GLMM model predictions, with all other fixed and random effects set to 0. Note the varying x-axis values for the different insect herbivores representing their maximum abundance recorded under field conditions.

5.3.3. *Agent complementarity*

5.3.3.1. Species co-occurrence

A greater number of tillers were attacked by at least one species than expected (χ^2 = 77.87; d.f. = 2; $P < 0.001$), which was largely explained by *Tetramesa* sp. 1 attacking more tillers than expected by chance (Fig. 5.4). *Tetramesa* sp. 2 attacked fewer tillers than expected, while *Bruchophagus* sp. 1 attacked approximately as many tillers as expected (Fig. 5.3). Pairwise co-occurrence patterns indicated that all combinations of *Tetramesa* sp. 1, *Tetramesa* sp. 2 and *Bruchophagus* sp. 1, including all three species together, were less likely to co-occur in the same tiller than expected (χ^2 = 564.03; d.f. = 7; $P < 0.001$; Fig. 5.4). The largest deviation from expectation occurred between *Tetramesa* sp. 1 and *Bruchophagus* sp. 1, which occupied fewer tillers together than any other insect combination.

5.3.3.2. Multi-species impacts

The combined impact of *Tetramesa* sp. 1 and *Tetramesa* sp. 2 resulted in a greater reduction in tiller survival than either agent in isolation (Fig. 5.5a). However, their combined impact only increased the GLMM percentage variation accounted for by 7.5% (Fig. 5.2). Inspecting the marginal effects plots indicated that a potential antagonism exists between *Tetramesa* sp. 1 and *Bruchophagus* sp. 1, as the impact of *Tetramesa* sp. 1 is reduced when it occurs in the same tiller with *Bruchophagus* sp. 1 (Fig. 5.6a). *Tetramesa* sp. 2 and *Bruchophagus* sp. 1 occurring in the same stem appears to have no appreciable influence on the outcome of herbivory on tiller survival, alone or in combination (Fig. 5.7a).

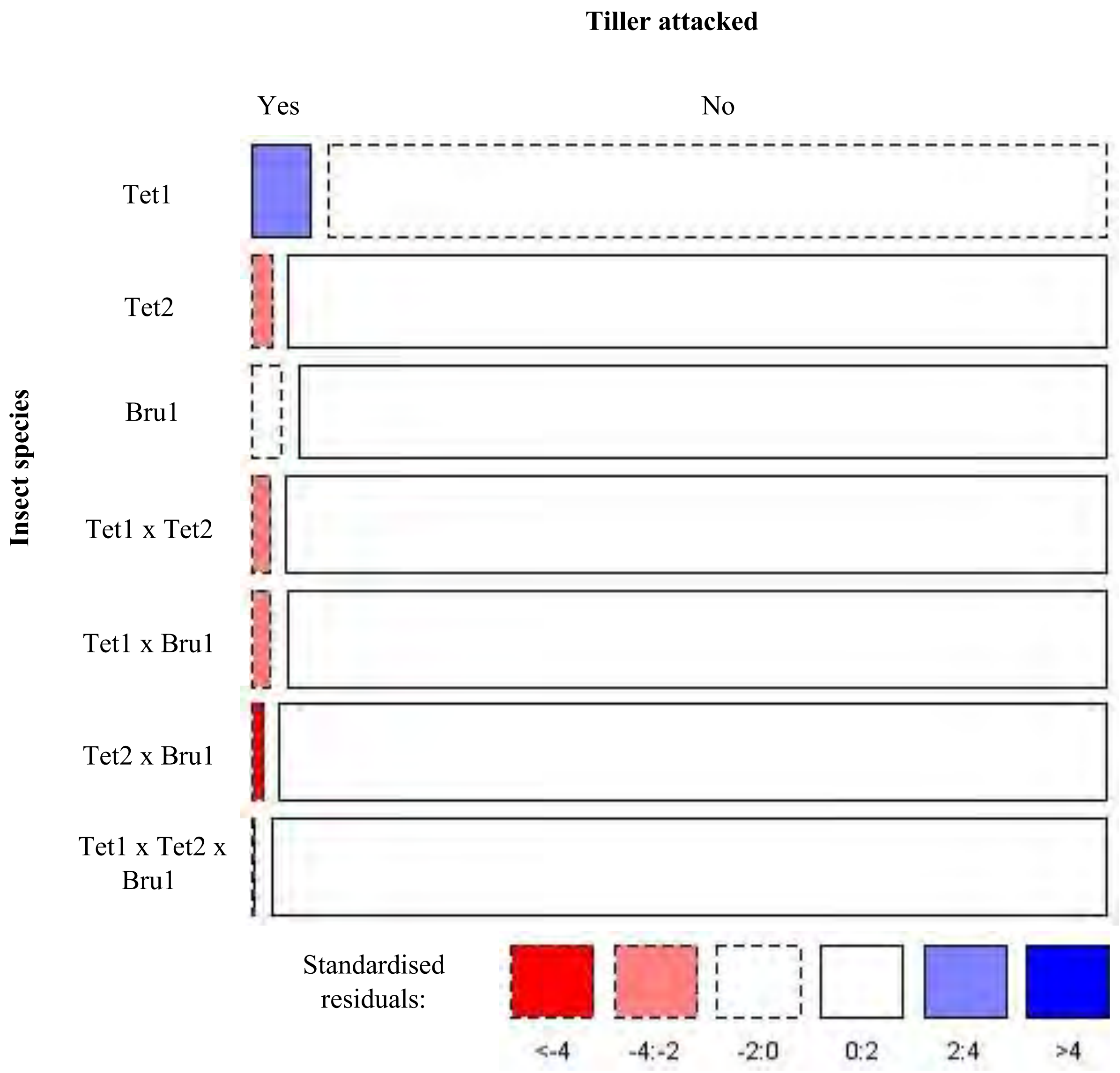


Figure 5.4. Observed versus expected proportion of attacked versus unattacked *Sporobolus pyramidalis* and *S. natalensis* tillers, per insect species and combinations thereof, recorded under native-range field conditions. Deviations from expected tiller attack rates were analysed using a Chi-Squared Frequency Test ($P < 0.05$). Insect species/combinations were recorded in more (blue shading), less (red shading) or approximately as many tillers as expected (no shading), as indicated by standardised residuals of the fitted model. The darker the shading, the greater the magnitude of deviation from expectation.

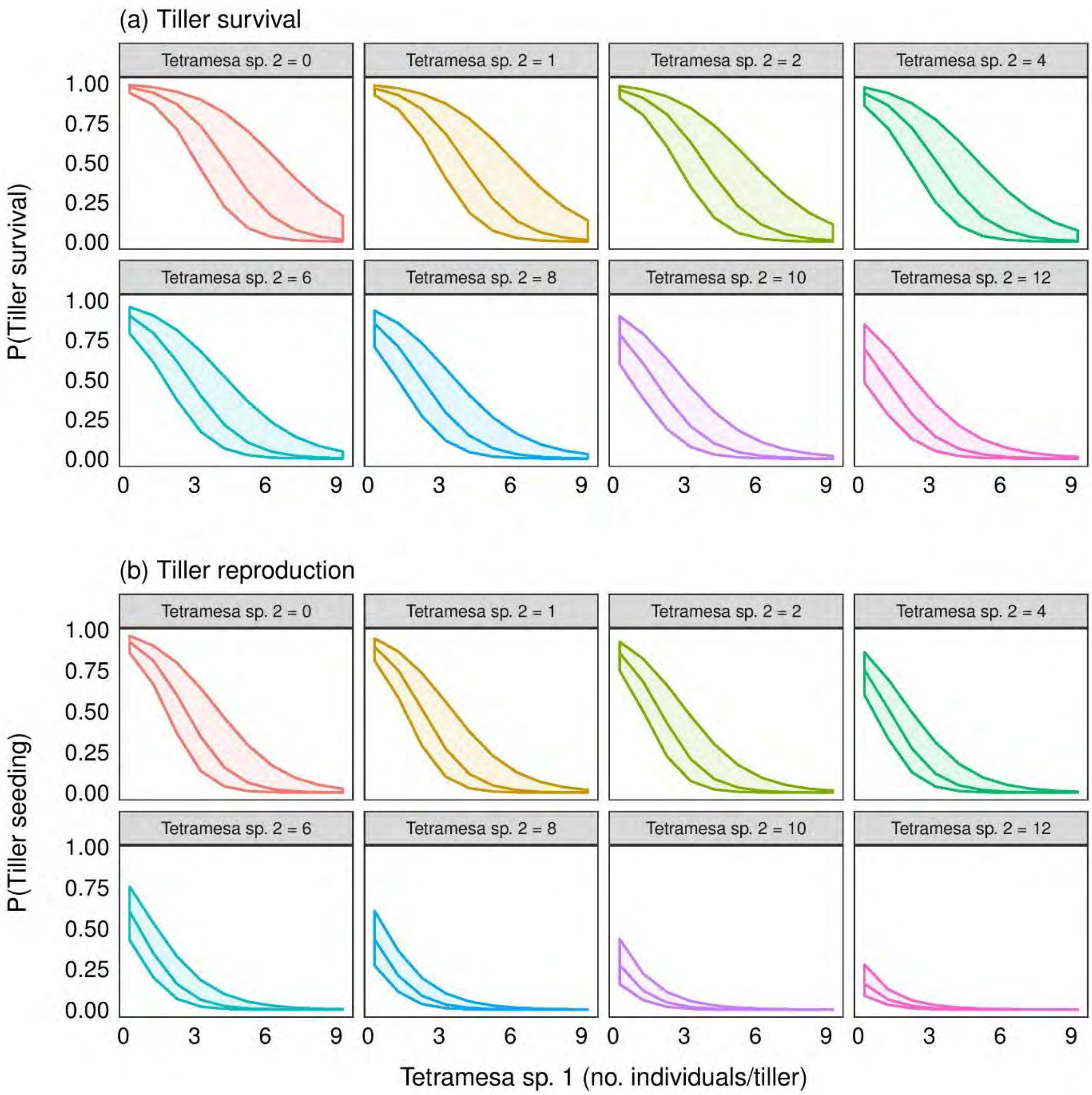


Figure 5.5. Marginal effects plots demonstrating the combined per-capita impact of *Tetramesa* sp. 1 and *Tetramesa* sp. 2 on the probability of *Sporobolus pyramidalis* and *S. natalensis* (a) tiller survival and (b) tiller reproduction. Marginal effects plots assume that all random effects are set to zero (i.e. have no influence on model outputs).

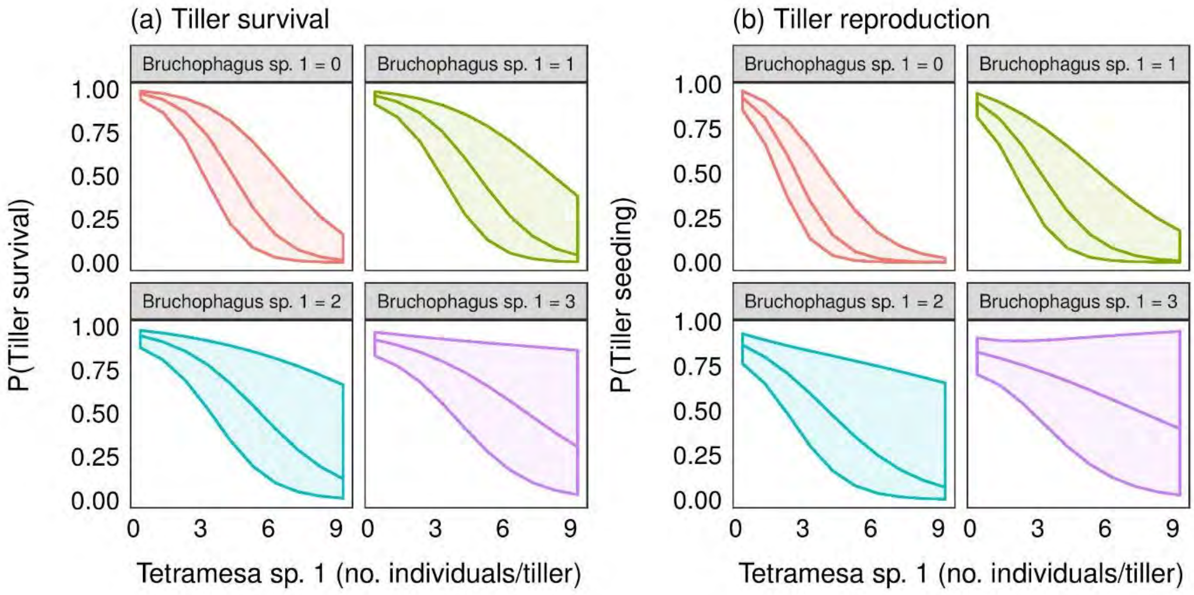


Figure 5.6. Marginal effects plots demonstrating the combined per-capita impact of *Tetramesa* sp. 1 and *Bruchophagus* sp. 1 on the probability of *Sporobolus pyramidalis* and *S. natalensis* (a) tiller survival and (b) tiller reproduction. Marginal effects plots assume that all random effects are set to zero (i.e. have no influence on model outputs).

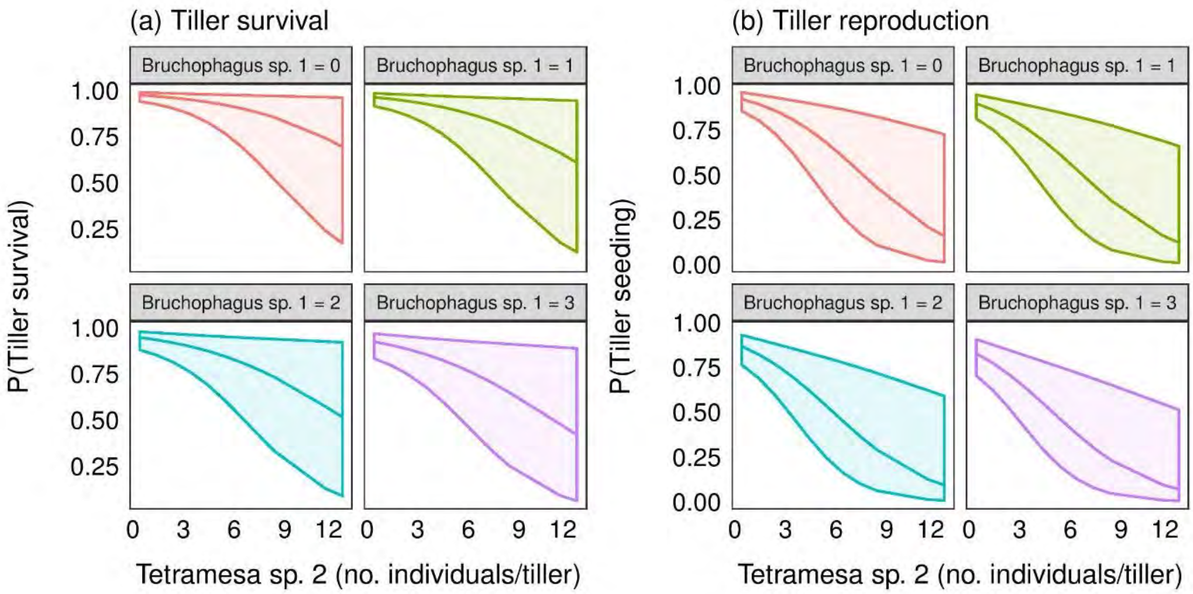


Figure 5.7. Marginal effects plots demonstrating the combined per-capita impact of *Tetramesa* sp. 2 and *Bruchophagus* sp. 1 on the probability of *Sporobolus pyramidalis* and *S. natalensis* (a) tiller survival and (b) tiller reproduction. Marginal effects plots assume that all random effects are set to zero (i.e. have no influence on model outputs).

The combined impact of *Tetramesa* sp. 1 and *Tetramesa* sp. 2 markedly reduced the probability of a tiller producing any seed in comparison to each species alone (Fig. 5.2, 5.5b). Their combined impact increased the GLMM percentage variation accounted for by 12.6% (Fig. 5.2), albeit there was no significant interaction between *Tetramesa* sp. 1 and *Tetramesa* sp. 2 on tiller reproduction (Table 5.5, 5.6). Inspecting the marginal effects plots indicated that a potential antagonism exists between *Tetramesa* sp. 1 and *Bruchophagus* sp. 1, as the impact of *Tetramesa* sp. 1 is reduced when it co-occurs in the same tiller with *Bruchophagus* sp. 1 (Fig. 5.6b). *Tetramesa* sp. 2 and *Bruchophagus* sp. 1 occurring in the same stem appears to have no appreciable influence on the outcome of herbivory on tiller reproduction, alone or in combination (Fig. 5.7b).

5.4. Discussion

Biological control practitioners are under increasing pressure to conduct pre-release efficacy assessments to identify and prioritise only those candidate agents with a demonstrable impact on the target weed (van Klinken and Raghu, 2006; Milbrath et al., 2018). Under native-range field conditions, the three monophagous wasps identified in chapter 4 as high-priority candidate agents based on their predicted host-range caused density-dependent reductions in *S. pyramidalis* and *S. natalensis* tiller survival and reproductive output (ability to produce seed). Although no demographic models, such as stage-structured (e.g. Dauer et al., 2012) or process-based demographic models (e.g. Watt et al., 2008) have been developed to test the potential impacts of the candidate agents identified during this chapter, individual plant level pre-release efficacy assessments can provide a good indication of agent efficacy post-release (Swirepik and Smith, 2002; Goolsby et al., 2016). High seed production is likely a significant contributor to the spread and formation of large and dense *S. pyramidalis* and *S. natalensis* monocultures in Australia (Vogler and Bahnisch, 2006; Bray and Officer, 2007). As such, the ability of the three wasps to reduce the probability of reproduction (i.e. producing seed or not) by up to 90% highlights their promise as biological control agents.

Tetramesa sp. 1 accounted for approximately 84% of the recorded variation in tiller survival and reproduction, while the two remaining agents accounted for < 15 % of such variation. Although *Bruchophagus* sp. 1 was retained as a significant factor in at least one plausible tiller survival and reproduction model, inspection of marginal effects plots suggested that the impact of *Bruchophagus* sp. 1 was negligible when compared to either *Tetramesa* spp. *Tetramesa* sp. 1 and *Tetramesa* sp. 2 should be prioritised over *Bruchophagus* sp. 1, when prioritising candidate agents based on predicted efficacy. *Tetramesa* sp. 1 is the top-priority candidate agent as it is the most damaging herbivore associated with *S. pyramidalis* and *S. natalensis* in South Africa.

Less than 20% of the total variation in *S. pyramidalis* and *S. natalensis* tiller survival and reproduction was accounted for by *Tetramesa* sp. 1, *Tetramesa* sp. 2 and *Bruchophagus* sp. 1. These damage assessments were performed under native-range field conditions where insect herbivores are likely to be less abundant than when introduced into a non-native region, due to predation and parasitism from their own suite of natural enemies (e.g. Prior and Hellman, 2013; Allen et al., 2015). This includes biological control agents; such as *T. romana*, which can reach densities 39 times greater on *A. donax* in the USA (where it is an introduced biological control agent) than in Europe (native range), albeit these population increases may be better explained by favourable climatic conditions in the USA than release from parasitism *per se* (Marshall et al., 2018).

Tetramesa spp. and *Bruchophagus* sp. 1 are heavily parasitized by a diverse suite of parasitoids in South Africa, most notably by undescribed *Ormyrus* sp. and *Eupelmus* sp. (G.F. Sutton, unpublished data). Paynter et al. (2010) proposed that biological control agents with native congeners found on the target weed or its close relatives (i.e. an 'ecological analogue'), in its invaded range, are likely to accumulate novel predators and/or parasitoids. This may limit or prevent the establishment, proliferation and/or spread of the introduced agent (Paynter et al., 2010). Preliminary surveys for phytophagous herbivores across south-eastern Queensland in May 2018 did not recover any eurytomid wasps associated with native Australian *Sporobolus* spp. (G.F. Sutton and M.D. Day, unpublished data). Given the lack of ecological analogues of *Tetramesa* spp. and *Bruchophagus* sp. 1 in Australia, which would be the primary source of potential parasitoids that would attack introduced biological control agents (Paynter et al., 2010), it is not unreasonable to expect that prospective control agents could be more abundant in Australia than in their native ranges'. The density-dependent impact of *Tetramesa* sp. 1 (as well as *Tetramesa* sp. 2 and *Bruchophagus* sp. 1, to a lesser extent) on *S. pyramidalis* and *S. natalensis* survival and reproduction, suggests that if these insects are able to escape their own suite of natural enemies, the magnitude of damage could be higher in Australia than South Africa.

Pre-release efficacy assessments cannot predict the actual impact of prospective biological control agents in their introduced range (Balciunas and Smith, 2006). This occurs because individual plants vary in size, age and life-stage, contribute unequally to overall plant population dynamics (Miller et al., 2009), thus, pre-release studies that quantify candidate agent impacts on individual-level plant parameters (i.e. tiller survival and reproduction), cannot reliably be translated to the plant population-level to predict weed suppression (Garren and Strauss, 2009; Milbrath et al., 2018). For example, substantial reductions in seed production (i.e. reductions of 90 – 98%) attributed to the impact of introduced biological control agents have been recorded with negligible effects on weed population dynamics (e.g. Hoffmann and Moran, 1998). This discrepancy is frequently explained by weed populations not being seed-limited (Maron and Gardner, 2000). If released in Australia, the degree of weed suppression achieved by *Tetramesa* spp. and *Bruchophagus* sp. 1 will be determined not only by the magnitude of their per-capita impacts, but by the degree of seed-limitation of *S. pyramidalis* and *S. natalensis* populations. Limiting seed production and depleting the soil seed-bank will be required for weed populations to become seed-limited, which may take many years (Vogler and Bahnisch, 2006). It will be vital to temper expectations of land-holders and stake-holders to give the biological control agents enough time to impact weed populations.

The size structure of the weed population (i.e. plant ontogeny) is also likely to influence the efficacy of biological control agents with specific resource requirements such as the three wasps investigated in this study. Plant size-dependent oviposition and herbivore attack is a relatively common phenomenon amongst herbivorous insects (Briese, 2000). The pattern of attack by candidate agents of *S. pyramidalis* and *S. natalensis* appeared to be host-plant size-dependent, whereby only plants with a tiller diameter greater than 2.8 - 3.0 mm were selected by females for oviposition (Chapter 4). However, the target weeds are able to produce seed when their diameters are smaller than 2.8 – 3.0 mm in eastern Queensland (invaded range) and South Africa (native range) (G.F. Sutton, unpublished data). If size-dependent oviposition observed in the native-range is realised in Australia, smaller but reproductively active plants within *S. pyramidalis* and *S. natalensis* populations might escape herbivory by introduced control agents, which may buffer weed populations to damage inflicted on larger plants. Ultimately, additional biological control agents may be required that attack these smaller plants to prevent them from producing seed, thereby increasing cumulative stress on weed populations. No prospective agents were recorded attacking smaller plants (with diameters < 2.8 mm) in South Africa, but it is possible that these agents exist elsewhere in the native distributions of *S. pyramidalis* and *S. natalensis*, if required (Chapter 2).

While pre-release efficacy assessments are now becoming more common in weed biological control, the majority of these assessments are performed under controlled experimental conditions in quarantine (e.g. Goolsby et al., 2009b; Balciunas and Smith, 2006; Bitume et al., 2019). Only a few examples exist where efficacy assessments were performed in the target weeds native-range. For example, the impact of three weevils, *Lixus cardui* Olivier, *Trichosirocalus briesei* and *Larinus latum* (Coleoptera: Curculionidae) on their respective *Onopordum* spp. hosts were assessed using native-range field surveys and cage trials (Briese, 1996; Briese et al., 2000, 2002b). Similarly, Balciunas and Burrows (1993) used insecticide exclusion to demonstrate that native herbivores suppress *Melaleuca quinquenervia* (Cav.) Blake in Australia. Balciunas and Burrows (1993) inferred that two herbivores, *Apion* sp. B (Coleoptera: Curculionidae) and *Pomponatius typicus* Distant (Hemiptera: Coreidae) were responsible for the majority of the damage observed on *M. quinquenervia*. The current study used GLMM's to quantify the relative impact of multiple co-occurring herbivores associated with *S. pyramidalis* and *S. natalensis*. These data generate testable hypotheses regarding how to select and prioritise candidate biological control agents from native-range survey data, which so far have been performed at the individual-agent level (e.g. Briese, 1996; Briese et al., 2000, 2002b), or have relied on expert opinion to select and prioritise candidate agents (e.g. Balciunas and Burrows, 1993). Careful post-release evaluations will be required to evaluate the merit of using GLMM's to quantify the relative impacts of and prioritise multiple co-occurring potential biological control agents in the target weed's native distribution. It is expected that this modelling framework may allow practitioners to make evidence-based decisions to rank and prioritise large numbers of co-occurring herbivores encountered during native-range field surveys.

Potentially antagonistic inter-specific interactions between candidate agents were observed for all species pairs. Species co-occurrence patterns indicated that all three insect species were less likely to be found in stems containing individuals belonging to another species, and that more stems were attacked by at least one species than expected, indicating that candidate agents avoided stems that were already attacked. On average, insects avoided stems containing inter-specific competitors more than stems containing intra-specific competitors. Avoidance of host-plant material infested by other species is a relatively common phenomenon amongst biological control agents (e.g. Rayamajhi et al., 2010; Weyl et al., 2012). If multiple control agents were released, inter-specific competition that results in avoidance of infested plant stems may contribute to biological control endeavours by minimising within-plant inter-specific competition between agents. This pattern may result in a larger proportion of tillers being attacked and greater levels of plant damage at the plant population-level (e.g. Paynter and Hennecke, 2001; Rayamajhi et al., 2010).

When two insect species did co-occur in the same tiller, there were variable impacts on the efficacy of either or both species. An additive interaction occurred between *Tetramesa* sp. 1 and *Tetramesa* sp. 2. The additive interaction observed between *Tetramesa* sp. 1 and *Tetramesa* sp. 2 is consistent with the cumulative stress model (Harris, 1985), as their complimentary feeding strategies on different plant parts reduced tiller survival and seed production more than either species alone. Additive interactions between pairs of biological control agents is relatively common, particularly for agents that attack different plant parts, such as *Tetramesa* sp. 1 and *Tetramesa* sp. 2. For example, an undescribed leafhopper (Tribe Erythroneurini, formerly referred to as *Zygina* sp.) and the rust fungus *Puccinia myrsiphylli* (Thuem.) Winter. (Pucciniaceae) reduced the number of tubers, rhizome length and tuber dry weight of *Asparagus asparagoides* L. Druce (Asparagaceae) more than when the plants were exposed to the most damaging agent alone (Turner et al., 2010). If multiple agents are required for the biological control programme against *S. pyramidalis* and *S. natalensis* in

Australia, the complementarity and combined impact of *Tetramesa* sp. 1 and *Tetramesa* sp. 2 could be harnessed to potentially maximise the damage inflicted to the host plants.

An inhibitory interaction occurred between *Tetramesa* sp. 1 and *Bruchophagus* sp. 1, whereby the impact of *Tetramesa* sp. 1 on tiller survival and reproduction was lower in the presence of *Bruchophagus* sp. 1 than when it occurred by itself. This antagonism is likely explained by these two insects competing for the same plant tissues (vascular tissue within the stem). Ecological theory predicts that herbivores that attack the same plant parts (i.e. they are not spatially segregated) are likely to be competitors because they interact directly (e.g. compete for the same resources) (Milbrath and Nechols, 2014). Although the mechanism of competition is unknown, if both agents were released, *Bruchophagus* sp. 1 would be expected to outcompete *Tetramesa* sp. 1, the latter which is significantly more damaging to the target weed. There are currently no known examples of competitive exclusion of a successful biological control agent by an unsuccessful agent (Denoth et al., 2002), but there are examples where the overall level of control has been decreased by the release of additional agents (e.g. Paynter and Hennecke, 2001). Nevertheless, avoidance of plant material containing inter-specific competitors likely minimises the magnitude and impact of negative inter-specific competitive interactions amongst released biological control agents in their introduced range. A pre-cautionary approach suggests that *Bruchophagus* sp. 1 and *Tetramesa* sp. 1 should not both be released together against *S. pyramidalis* and *S. natalensis*, unless *Tetramesa* sp. 1 and *Tetramesa* sp. 2 prove to be insufficiently damaging under field conditions in Australia.

Practitioners may not be able to predict the outcomes of inter-specific interactions between control agents under field conditions from pre-release trials. Buccellato et al. (2012) demonstrated that combined attack by a stem-galling fly, *Procecidochares utilis* Stone (Tephritidae) and a leaf spot pathogen, *Passalora ageratinae* Crous and A. R. Wood

(Mycosphaerellaceae), under greenhouse conditions, resulted in greater reductions in *Ageratina adenophora* (Sprengel) R. M. King and H. Robinson (Asteraceae) fitness parameters compared to when each was released alone (i.e. an additive interaction; *sensu* Turner et al., 2010). A follow-up field trial, however, found an equivalent interaction between *P. utilis* and *P. ageratinae* under field conditions in the weeds invaded range (South Africa) (Buccellato et al., 2019). The current study provides a testable hypothesis that investigating inter-specific interactions and their predicted outcomes for biological control endeavours under natural native-range field conditions may provide a more realistic assessment of inter-specific interactions than pre-release studies performed under artificial conditions.

In conclusion, two of the three phytophagous wasps likely to satisfy the strict host-specificity requirements of the *Sporobolus* biological control programme (Chapter 4), were shown to damage *S. pyramidalis* and *S. natalensis* under native-range field conditions. *Tetramesa* sp. 1 was identified as the primary potential biological control agent for this project as it accounted for most of the reduction in *S. pyramidalis* and *S. natalensis* tiller survival and reproduction. The combined and complimentary impact of *Tetramesa* sp. 1 and *Tetramesa* sp. 2 may increase the degree of biological control achieved, although the magnitude of impact associated with *Tetramesa* sp. 2 was low. The third phytophagous wasp, *Bruchophagus* sp. 1, was not particularly damaging and is potentially antagonistic with *Tetramesa* sp. 1, and thus should be given low priority as a candidate agent. This is arguably the first example that multiple co-occurring potential biological control agents were simultaneously ranked and prioritised based on quantitative predicted efficacy assessments, alone and in combination with one another. While there are certainly difficulties in predicting agent abundance and impact upon introduction (see Augustinus et al., 2020 and references therein), including those performed under native-range open-field conditions, this chapter provides testable hypotheses to potentially aid the selection and prioritisation of damaging natural enemies from native-range surveys. The approach used in the current study may increase the likelihood of achieving successful biological control, reduce wasted expenditure on performing host-range testing on insufficiently damaging candidates, and minimise releasing, ineffective and potentially antagonistic biological control agents. Careful post-release evaluations will be required to evaluate whether the predictions of this study are correct.

Chapter 6: General discussion

Selecting which natural enemies to prioritise for importation and laboratory host-specificity testing is not a trivial undertaking. While biological control practitioners have spent many years and resources developing various components of a typical weed biological control programme, comparatively little effort has been spent on developing agent prioritisation methods as part of native-range surveys. This thesis adopted a multi-faceted, quantitative and ecologically-motivated approach to select and prioritise biological control agents for the management of two invasive grasses in Australia, namely: *Sporobolus pyramidalis* and *Sporobolus natalensis*. In turn, the thesis served as a baseline to begin determining the feasibility of initiating biological control on invasive grasses as a whole.

In this chapter, the importance of improving the selection and prioritisation of potential biological control agents prior to their importation into quarantine is discussed. Thereafter, the potential that biological control offers for the management of invasive grasses is assessed. Finally, the implications of this research for the development and deployment of the biological control programme against *S. pyramidalis* and *S. natalensis* in Australia are outlined.

6.1. Prioritisation of prospective biological control agents

The identification, selection and prioritisation of potential biological control agents is an essential but extremely difficult component of a biological control programme (van Klinken and Raghu, 2006). Practitioners often encounter a large number of herbivorous insects, fungal pathogens and herbivorous mites on plants targeted for classical weed biological control (e.g. Palmer and Pullen, 1995; Harley et al., 1995), which makes importing and performing host-range testing for all potential biological control agents practically and economically infeasible.

Agent prioritisation to date has been a relatively subjective process, with decisions on which potential agents to import and subject to host-range testing being based on factors such as ease of rearing and performing host-range testing (van Klinken and Raghu, 2006). While these are valuable criteria for agent prioritisation, they should be used to complement, not replace, quantitative indications of agent specificity and predicted efficacy. Some practitioners have attempted to formulate less subjective approaches to agent selection. A range of 'rules of thumb' have been developed from ecological theory, retrospective analyses of previous biological control projects, and prior experience / expert opinion (see van Klinken and Raghu, 2006 and references therein). While some of these rules can prove effective, especially those applying to particular weed life-histories and/or guilds of natural enemies (e.g. Briese, 2004), most rules of thumb provide little predictive power, remain untested, and in extreme cases, may reject suitable potential control agents (van Klinken and Raghu, 2006).

Some effort has been made to develop a scoring system to prioritise potential agents (Harris, 1973; Goeden, 1983). These systems require detailed information on the life-cycle, distribution, level of parasitism and host-specificity of candidate agents (Harris, 1973; Goeden, 1983). More recently, practitioners have attempted to take an empirical approach to agent prioritisation, focusing primarily on the type, frequency and magnitude of herbivory required to manage weed infestations (see van Klinken and Raghu, 2006). These data, however, are primarily collected during laboratory-based studies, making them unsuitable for prioritising potential biological control agents from native-range faunistic surveys. The wasted expenditure and resources on testing unsuitable candidate agents, under quarantine conditions, suggests that host-specificity testing should only be performed on potential agents that have some quantifiable *a priori* indication of their suitability, with suitability primarily being a function of host-specificity and predicted efficacy.

Some authors have argued that effective *a priori* agent prioritisation and predicting population dynamics of introduced agents may be impossible (e.g. Simmonds, 1976), often being referred to as the 'holy grail' of biological control (McFadyen, 1998), and likened to 'gazing into crystal balls' (Zalucki and van Klinken, 2006). While there are difficulties in transferring host-range and pre-release efficacy assessments performed in the native-range to the invaded-range (e.g. Wapshere, 1985; Manners et al., 2011), a method to rank and prioritise potential biological control agents from native-range surveys would be of considerable value to weed biological control practitioners.

The relative merit of prioritising potential agents based on native-range field host-range assessments and predicted efficacy studies (i.e. the approach adopted in the current study), versus the traditional "grab-and-run" approach to native-range surveys (*sensu* Sheppard et al., 2006), and then prioritising candidate agents based primarily on laboratory-based host-specific and impact assessments, will require retrospective analyses to be performed and careful post-release evaluation. This thesis took a quantitative and evidence-based approach to native-range host-range and pre-release efficacy assessments, in order to prioritise potential biological control agents at the earliest stage possible for biological control project. Agent prioritisation was performed based on climatic-compatibility with weed infestations in Australia (Chapter 2), agent feeding biology, seasonal incidence and geographic distribution (Chapter 3), predicted host-specificity (Chapter 4), and potential efficacy and complementarity with one another (Chapter 5).

The data presented in this thesis provide testable hypothesis regarding the relative merit of prioritising prospective biological control agents during native-range field surveys vs the traditional "grab-and-run" approach to native-range surveys. While such a study would not necessarily be easy, the data presented here may allow us to initiate this discussion (i.e. by comparing the effort and expense expended on the extra native-range surveys performed here versus if we had performed a standard weed biocontrol faunistic survey). It is hypothesised that performing more extensive native-range surveys may maximise returns on investment in biological control, and improve the safety and efficiency of biological control, by reducing the unnecessary cost and time wasted by importing, culturing and performing fundamental host-range assessments on completely unsuitable candidate agents (Briese et al., 2002a)

6.2. Are grasses suitable targets for biological control?

Historically, very few invasive grasses have been targeted for biological control (Pemberton, 1996; Schwarzländer et al., 2018) as grasses have been considered poor targets with a very low chance of success (Wapshere, 1990; Evans, 1991; Pemberton, 1996, 2002). This stems from the perception that grasses support an (1) unspecialised, and (2) insufficiently damaging natural enemy community to exploit for potential biological control agents (Gill and Blacklow, 1984; Wapshere, 1990; Pemberton, 2002) (see Chapter 1 for details). Moreover, (3) because of the close phylogenetic relationships between many invasive grasses and economic crops (i.e. cereals, pasture), and/or native biodiversity, the risk of introducing biological control agents has been considered too great to warrant consideration (Wapshere, 1990). This thesis, using *Sporobolus* spp. as an example, explored each of these elements that has previously held practitioners back from initiating biological control programmes against grasses. In the following section, an investigation into whether grasses are in fact poor targets for biological control was performed by considering each of these arguments, in light of the results of this study, as well as relevant published literature.

6.2.1. Host-specific natural enemies

In this study, *S. pyramidalis* and *S. natalensis* were associated with at least 15 ectophagous herbivores and six endophagous herbivores in South Africa (Chapter 3), of which, three insects were found only on the two aforementioned grasses (Chapter 4). This result is in accordance with several recent studies, which have demonstrated that grasses can support a natural enemy assemblage that contains at least one host-specific entity that shows promise as a biological control agent (Table 6.1). Moreover, several other grasses that have not been considered as biological control targets, but have been surveyed for natural enemies in their native range, possess an assemblage of phytophagous insects, mites and/or fungal pathogens with at least one potentially host-specific natural enemy. These species include: *Aristida longiseta* Steud. (red threeawn), *Calamagrostis epigejos* (L.) Roth (wood small-reed), *Ehrharta calycina* Sm. (perennial veldtgrass), *Leymus* (= *Elymus*) *cinereus* (Scribn. & Merr.) A. Löve (Great Basin wildrye), *Sporobolus cryptandrus* (Torr.) A. Gray (sand dropseed), *Sitanion hystrix* (Nutt.) J.G. (Smith bottlebrush squirreltail) and *Stipa comata* Trin. and Rupr. (needleandthread) (Spears and Baar, 1985; Youtie et al., 1987; Dubbert et al., 1998; Piątek et al., 2015).

The larger than expected assemblage of host-specific natural enemies on grasses may be due to a lack of knowledge on the factors that influence insect diversification and specialisation (Bernays and Graham, 1988). Indeed, grasses are believed to be relatively depauperate of herbivores, and any herbivores they do host are expected to be generalists due to a lack of secondary chemical defences (McNaughton, 1985). However, numerous alternative mechanisms have been proposed to explain insect host-range patterns and diversification, most notably; the acquisition of enemy-free space (Bernays and Graham, 1988), host-plant life histories (Strong et al., 1984), and structural defences (e.g. trichomes and silica deposits) (Vicari and Bazely, 1993). Structural defences are believed to have played a significant role in promoting diversification and specialisation of grass-associated herbivores (McNaughton et al. 1985; Vicari and Bazley 1993; Moore and Johnson 2017).

Host-range testing has not yet been performed for many of the examples above, and as such, it is uncertain how many of these candidate agents will ultimately be sufficiently host-specific to be approved for release as biological control agents. Nevertheless, the data presented in this thesis (Chapter 4) provides further support that grasses can possess specialised natural enemies that could be investigated as potential biological control agents.

6.2.2. Damaging natural enemies

In contrast to theoretical expectations, this study showed that three potential biological control agents for *S. pyramidalis* and *S. natalensis* inflicted substantial damage to their host grass species, reducing tiller survival and reproductive capacity (Chapter 5). It appears that the damaging nature of these insects is consistent with the published literature. For example, there are a number of host-specific pests that cause serious economic damage to grasses. *Eragrostis tef* (Zucc.) Trotter., an introduced crop species in North America, suffered yield losses of over 70% due to the action of the stem-boring wasp *Eurytomocharis eragrostidis* (Howard) (Hymenoptera: Eurytomidae) (McDaniel and Boe, 1990). *Tetramesa* spp. infestations caused significant reductions in seed weight, germination percentage and germination rate for four different grass species in Idaho, USA (Spears and Barr, 1985).

Table 6.1. Summary and status of current and past biological control projects (listed alphabetically) considered against invasive grasses.

Target weed (common name)	**Country**	**Candidate agent**			**Key references**
		Identity	Status	Control[a]	
Andropogon gayanus Kunth. (gamba grass)	Australia	-	Preliminary surveys underway	-	S. Raghu (pers. comm.)
Arundo donax L. (giant reed)	USA	Multiple potential candidates	-	-	Tracy and DeLoach (1998)
		Tetramesa romana Walker (Hymenoptera: Eurytomidae)	Already present; new genotypes of wasp from origin of invasive plant genotypes were released	Yes	Goolsby and Moran (2009); Goolsby et al. (2016); Marshall et al. (2018)
		Rhizaspidiotus donacis (Leonardi) (Hemiptera: Diaspididae)	Established, 2011	Yes	Goolsby et al. (2009a); Goolsby and Moran (2019)
		Lasioptera donacis Coutin (Diptera: Cecidomyiidae)	Released, 2017	?	Goolsby et al. (2017)
	South Africa	*Tetramesa romana* Walker (Hymenoptera: Eurytomidae)	Already present	?	Angela Bownes (pers. comm.)
		Rhizaspidiotus donacis (Leonardi) (Hemiptera: Diaspididae)	Under evaluation	-	Angela Bownes (pers. comm.)
Avena fatua L. (wild oats)	Australia	*Puccina coronata* f.sp. *avenae* Corda (Uredinales)	Additional testing required	-	Johnston et al. (2000)
Bromus tectorum L. (Cheatgrass)	USA	*Ustilago bullata* Berk. (Ustilaginales)	Under evaluation	-	Meyer et al. (2008)
		Tilletia fusca Ellis & Everhart (Tilletiales)	Under evaluation	-	Meyer et al. (2008)
		Pyrenophora semeniperda (Brittleb. & D.B. Adam) Shoemaker (Pleosporales)	Under evaluation	-	Meyer et al. (2008)

		Stenodiplosis sp. (Diptera: Cecidomyiidae)	Under evaluation	-	M. Cristofaro (pers. comm.)
Cortaderia jubata (Lem.) Stapf (Purple Pampas grass)	New Zealand	*Ustilago quitensis* Lagerh. (Ustilaginales)	Under evaluation	-	Manaaki Whenua Landcare Research Weed Biocontrol Newsletter No. 72 (2015)
		Saccharosydne subandina (Hemiptera: Delphacidae)	Under evaluation	-	Manaaki Whenua Landcare Research Weed Biocontrol Newsletter No. 72 (2015)
Cortaderia selloana (Schult. & Schult.f.) Asch. & Graebn. (Pampas grass)	New Zealand	*Ustilago quitensis* Lagerh. (Ustilaginales)	Under evaluation	-	Manaaki Whenua Landcare Research Weed Biocontrol Newsletter No. 72 (2015)
		Saccharosydne subandina (Hemiptera: Delphacidae)	Under evaluation	-	Manaaki Whenua Landcare Research Weed Biocontrol Newsletter No. 72 (2015)
Digitaria abyssinica (A. Rich.) Stapf. (blue couch grass)	East Africa	Multiple potential candidates	-	-	Sileshi (1995)
Echinochloa crus-galli (L.) Beauv (barnyard grass)	Asia	Multiple potential candidates	-	-	Tosiah et al. (2009)
		Emmalocera leucotaeniella (Ragonot) (Lepidoptera: Pyralidae)	Additional testing required		Tosiah et al. (2009)
Eragrostis curvula (Schrad.) Nees. (African lovegrass)	Australia	-	Preliminary surveys underway		A. McConnachie (pers. comm.)
Hymenachne amplexicaulis (Rudge) Nees (West Indian marsh grass)	Australia	*Ischnodemus variegatus* (Signoret) (Hemiptera: Blissidae)	Pending approval	-	Diaz et al. (2009)
Imperata cylindrica (P.) Beauv. (cogongrass)	USA	Multiple potential candidates	-	-	Van Loan et al. (2002); Overholt et al. (2016)
		Acrapex azumai Sugi (Lepidoptera: Noctuidae)	Additional testing required		Takasu et al. (2014)

		Orseolia javanica Kieffer & van Leeuwen-Reijinvaan (Diptera: Cecidomyiidae)	Additional testing required	-	Overholt et al. (2016)
		No suitable agents found	-	-	Simmonds (1972)
Megathyrsus maximus (= *Panicum maximum*) (Jacq.) B. K. Simon & S. W. L. Jacobs (Guineagrass)	USA	Multiple potential candidates	Additional testing required	-	Mercadier et al. (2009); M. Cristofaro and J.A. Goolsby (pers. comm)
Microstegium vimineum (Trin.) A. Camus (Japanese stiltgrass)	USA	-	Too early to evaluate		Nestory (2016)
Nassella neesiana (Trin. & Rupr.) Barkworth (Chilean needlegrass)	Australia	Multiple potential candidates	-	-	Briese and Evans (1998)
		Uromyces pencanus Arth. & Holw. (Uredinales)	Additional testing required	-	Andersen et al. (2010, 2017)
	New Zealand	*Uromyces pencanus* Arth. & Holw. (Uredinales)	Approved (pending export permits)	-	Andersen et al. (2010, 2017)
	South Africa	No suitable agents found	-	-	Wells (1977)
Nassella trichotoma (Nees) Hack. ex Arechav. (serrated tussock)	Australia	Multiple potential candidates	-	-	Briese and Evans (1998)
		No suitable agents found	-	-	Andersen et al. (2010, 2017)
	South Africa	No suitable agents found	-	-	Wells (1977)
Panicum repens L. (torpedograss)	USA	*Steneotarsonemus* (=*Parasteneotarsonemus*) *panici* (Mohanasundaram) (Acari: Tarsonemidae)	Additional testing required	-	Cuda et al. (2007)
Phragmites australis (Cav.) Trin. ex Steudel (common reed)	USA	Multiple potential candidates	-	-	Tewksbury et al. (2002)

		Archanara geminipuncta (Haworth) (Lepidoptera: Noctuidae)	Approved	-	Blossey et al. (2018); Kiviat et al. (2019); Blossey et al. (2020)
		Archanara neurica (Hübner) (Lepidoptera: Noctuidae)	Approved	-	Blossey et al. (2018); Kiviat et al. (2019); Blossey et al. (2020)
		Platycephala platifrons (Fabricius) (Diptera: Chloropidae)	Shelved - 'second tier priority'	-	Häflinger et al. (2005)
Rottboellia cochinchinensis (Lour.) W.D. Clayton (itchgrass)	New World	*Sporisorium ophiuri* (P. Henn.) Vanky (Ustilaginales)	Additional testing required	-	Ellison and Evans (1995)
Sorghum halepense (L.) Pers. (Johnsongrass)	USA	No suitable agents found	-	-	Domeninchini et al. (1989)
		Multiple potential candidates	-	-	Charudattan and DeLoach (1988)
		Bipolaris spp.	Additional testing required	-	Winder and van Dyke (1990)
		Sporisorium cruentum (J.G. Kuhn) Vanky	Additional testing required	-	Gassó et al. (2017)
Spartina alterniflora Loisel. (smooth cordgrass)	USA	*Prokelisia marginata* (Van Duzee) (Hemiptera: Delphacidae)	Established	Yes	Grevstad et al. (2003)
Spartina anglica C. E. Hubbard (English cordgrass)	USA	*Prokelisia marginata* (Van Duzee) (Hemiptera: Delphacidae)	Additional testing required	-	Wu et al. (1998)
Sporobolus natalensis (Steud.) Dur. & Schinz (giant rat's tail grass)	Australia	*Tetramesa* sp. 1 (Hymenoptera: Eurytomidae)	Under evaluation	-	Witt and McConnachie (2004); Current study
		Tetramesa sp. 2 (Hymenoptera: Eurytomidae)	Under evaluation	-	Current study
		Bruchophagus sp. 1 (Hymenoptera: Eurytomidae)	Under evaluation	-	Current study
		Ustilago sporoboli-indici L. Ling (Ustilaginales)	Rejected; already present	?	Witt and McConnachie (2004); Yobo et al. (2009); Vitelli et al. (2017)

Sporobolus pyramidalis P. Beauv. (giant rat's tail grass)	Australia	*Tetramesa* sp. 1 (Hymenoptera: Eurytomidae)	Under evaluation	-	Witt and McConnachie (2004); Current study
		Tetramesa sp. 2 (Hymenoptera: Eurytomidae)	Under evaluation	-	Current study
		Bruchophagus sp. 1 (Hymenoptera: Eurytomidae)	Under evaluation	-	Current study
		Ustilago sporoboli-indici L. Ling (Ustilaginales)	Rejected; already present	?	Witt and McConnachie (2004); Yobo et al. (2009); Vitelli et al. (2017)
Taeniatherum caput-medusae (L.) Nevski (medusahead)	USA	Multiple potential candidates	Additional testing required	-	Widmer and Sforza (2004)
		Fusarium arthrosporioides Sherb. (Hypocreales)	Rejected	-	Widmer and Sforza (2004)
		Aculodes altamurgiensis de Lillo & Vidović (Acari: Eriophyidae)	Under evaluation	-	De Lillo et al. (2018)
		Tetramesa sp. (Hymenoptera: Eurytomidae)	Under evaluation	-	M. Cristofaro (pers. comm.)
Ventenata dubia (Leers.) Coss (Wiregrass)		Multiple potential candidates	Additional testing required	-	M. Cristofaro (pers. comm.); Alomran et al. (2019)

[a] - Control: Yes - effective biological control; ? - Too early to evaluate or unknown

Yield losses in cereal crops due to the host-specific genetic entities of the herbivorous mite, *Aceria tosichella* Keifer (Wheat curl mite), can reach up to 30% (Harvey et al., 2002). Moreover, the damaging nature of the insects recorded on *S. pyramidalis* and *S. natalensis* in this thesis are consistent with laboratory-based impact assessments (Goolsby et al., 200b), native-range field assessments (Häfliger et al., 2005, 2006; Cortés et al., 2011), and invaded-range post-release evaluations (Grevstad et al., 2003; Goolsby et al., 2016; Moran et al., 2017; Goolsby and Moran, 2019; but see Showler et al., 2018) of biological control agents of grasses.

The few biological control agents that have been released for grasses and scarcity of adequate post-release evaluations limits the conclusions that can be drawn regarding the efficacy of grass biological control agents. However, the damaging nature of the three stem-boring wasps recorded on *S. pyramidalis* and *S. natalensis* during this thesis (Chapter 5) along with the few examples provided above demonstrate that natural enemies of grasses can be highly damaging.

6.2.3. Risk of targeting grasses versus other weed taxa for biological control

An argument has been made that the risk posed to native biodiversity, valued ornamentals, and economic crops, by introducing grass biological control agents, is too great to consider implementation (Wapshere, 1990; Pemberton, 2002). While many programmes have successfully used oligophagous natural enemies to control a target weed, partly due to a lack of economically important close-relatives and native congeners in the weeds' introduced range (e.g. Paterson et al., 2011b), the majority of biological control agents for invasive grasses will likely need to be strictly monophagous, or in extreme cases, demonstrate sub-species level host-specificity (Casagrande et al., 2018). A high level of host-specificity will likely be a requirement that will be imposed on many grass biological control programmes due to the close phylogenetic relationships between invasive grasses and economically important crops (cereals) and pasture/fodder species. For example, Wapshere (1990) regarded the potential for the biological control of invasive grasses in Australia (specifically *N. trichotoma*) to be limited due to the presence of closely-related valued pasture species (i.e. multiple native *Stipa* species).

Biological control programmes have been initiated against weeds that possess native congeners and/or closely-related economic crop species in their adventive range (e.g. *Senecio* spp. in Australia, McFadyen and Morin, 2012), and in the case of the biological control programmes against *Solanum* spp. in South Africa, multiple sufficiently host-specific natural enemies have been released despite a high diversity of native congeners and economic crops (e.g. *Solanum melongena* L. (eggplant) (Cowie et al., 2017). Practitioners conduct host-specificity testing to carefully evaluate the risk posed by candidate biological control agents to native biodiversity and economic crops in the area of intended introduction (Paynter et al. 2015b; Hinz et al., 2019). Over the last 30 years, practitioners have developed the theory and practical applications of host-specificity testing to the point where the host range of a candidate agent is reliably predictable from host-specificity testing (Paynter et al. 2015b; Hinz et al. 2019). Host-range testing, and our ability to assess the safety of a potential biological control agent, should be no different for a candidate being screened against an invasive grass than any other weed taxa, including targeting grasses with native congeners and/or economic crops.

6.3. Implications for the biological control of *Sporobolus pyramidalis*/*S. natalensis*

The current study found that three stem-boring wasps, namely: *Tetramesa* sp. 1, *Tetramesa* sp. 2 and *Bruchophagus* sp. 1, have potential as biological control agents of *S.*

pyramidalis and *S. natalensis*. It is tempting to import, perform host-range testing and ultimately release all three potential agents. However, a single biological control agent is usually responsible for providing control over the target weed (Denoth et al., 2002). While all three wasps were equally host-specific (Chapter 4), quantifying the relative impact of each agent, alone and in combination (Chapter 5), allowed for quantitative, evidence-based prioritisation of the three potential agents, based on predicted efficacy.

Taken together, the findings of this thesis suggest that *Tetramesa* sp. 1, followed by *Tetramesa* sp. 2, should individually be imported and screened for this control project. Assuming each agent is approved for release in Australia, *Tetramesa* sp. 1 should be the first species tested and released, because it caused the greatest reduction in *S. pyramidalis* and *S. natalensis* survival and reproduction (Chapter 5). *Tetramesa* sp. 2 should be the next top priority agent, owing to the fact that it was more damaging than *Bruchophagus* sp. 1, and was not predicted to negatively impact *Tetramesa* sp. 1 populations to the same extent as *Bruchophagus* sp. 1 (Chapter 5). *Tetramesa* sp. 2 should only be imported, tested and petitioned for release once sufficient time has elapsed to allow for *Tetramesa* sp. 1 to establish and impact weed populations (e.g. 10-20 years; McFadyen, 1998), and careful post-release evaluation indicates that *Tetramesa* sp. 1 has not, and is not predicted to provide effective control over *S. pyramidalis* and *S. natalensis* in Australia. The potentially antagonistic interaction between *Tetramesa* sp. 1 and *Bruchophagus* sp. 1 suggests that a precautionary approach should be adopted, whereby *Bruchophagus* sp. 1 should probably not be imported into Australia, at least not until *Tetramesa* sp. 1 and *Tetramesa* sp. 2 have been shown to be insufficiently damaging. This strategy is in accordance with the 'parsimonious' approach to biological control proposed by McEvoy and Coombs (1999), whereby as few agents are released as possible to suppress the target weed. This minimises the risks of direct and indirect non-target impacts occurring and may improve the cost-effectiveness and efficiency of biological control (McEvoy and Coombs, 1999).

Additional biological control agents may eventually be required for the *Sporobolus* biological control programme, either after exhausting the options highlighted in this study (Chapter 4, 5), or because of the potential for small tillers, that can still produce seeds, to escape from herbivory from these biological control agents (Chapter 5), which would require new potential agents with a differing feeding biology to *Tetramesa* sp. 1, *Tetramesa* sp. 2 and *Bruchophagus* sp. 1. It is unlikely that any additional potential control agents would be found if additional surveys were performed in South Africa (Chapter 3). Climate-matching studies identified multiple high-priority regions, outside of South Africa, that could be surveyed for additional control agents, if required.

6.4. Conclusion

This study took a quantitative, and evidence-based approach to native-range, host-range and pre-release efficacy assessments, in order to prioritise potential biological control agents at the earliest stage possible for the biological control programme against *S. pyramidalis* and *S. natalensis*, in Australia. This approach could maximise return on investment in biological control programmes by reducing the unnecessary cost and time wasted by importing, culturing and performing fundamental host-range and efficacy assessments of candidate agents that were never likely to meet the specificity requirements of the project at-hand and/or sufficiently damage the target weed to effect control. Even though grasses have been considered poor targets for biological control in the past, the prospects for control of *S. pyramidalis* and *S. natalensis* are good. This thesis clearly demonstrates that grasses can be suitable targets for biological control.

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Appendix 1 – Species occurrences downloaded from GBIF

The following datasets were download from the Global Biodiversity Information Facility, and were used for MaxEnt modelling in Chapter 2:

Sporobolus pyramidalis occurrences:

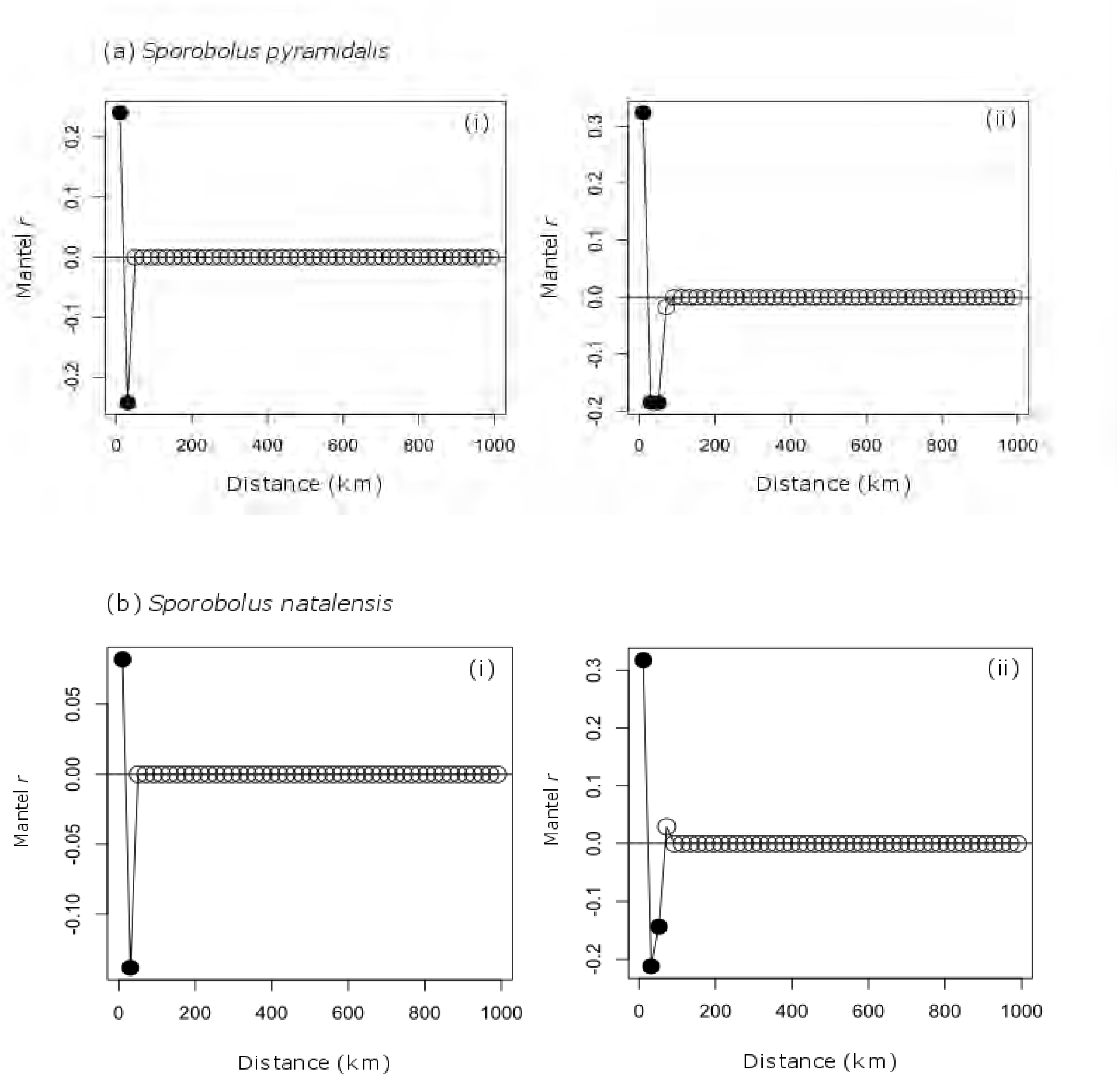
GBIF.org (09 May 2017) GBIF Occurrence Download https://doi.org/10.15468/dl.sdgzul

Sporobolus natalensis occurrences:

GBIF.org (09 May 2017) GBIF Occurrence Download https://doi.org/10.15468/dl.bub3hq

Appendix 2 – Spatial autocorrelation analyses

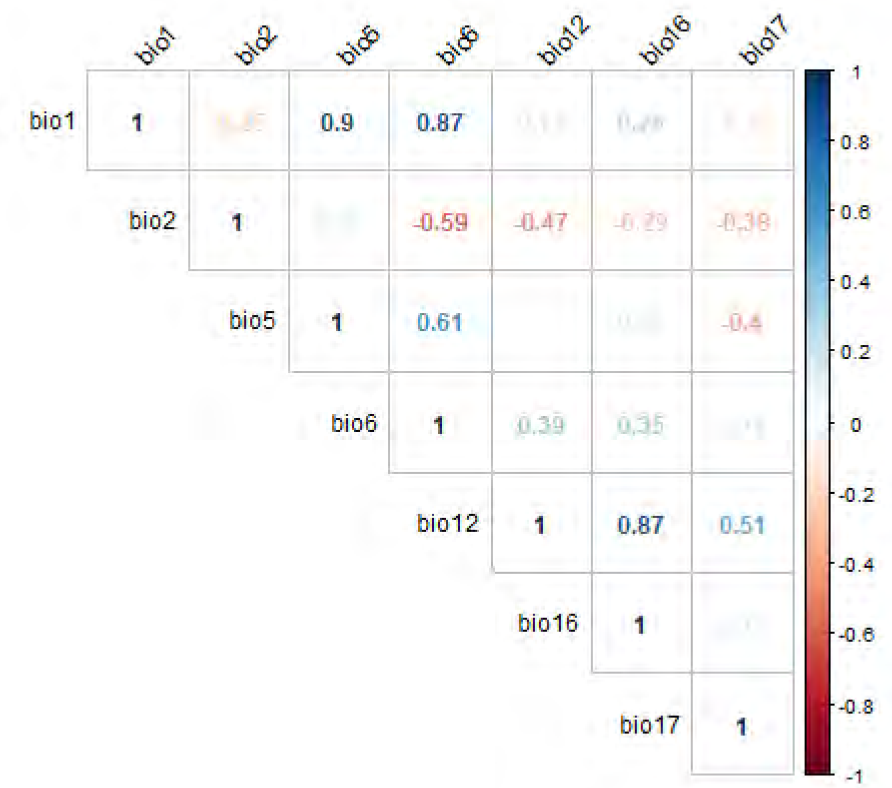
Fig. Mantel correlograms used to spatially-filter (i) invasive and (ii) native species occurrence records for (a) *Sporobolus pyramidalis* and (b) *Sporobolus natalensis*, based on minimising the influence of spatial autocorrelation. MaxEnt models were filtered for each species x region model at distances (km) identified by visually inspecting correlograms for where spatial autocorrelation was not significantly different from zero (white circles).



Appendix 3 – Multicollinearity analyses

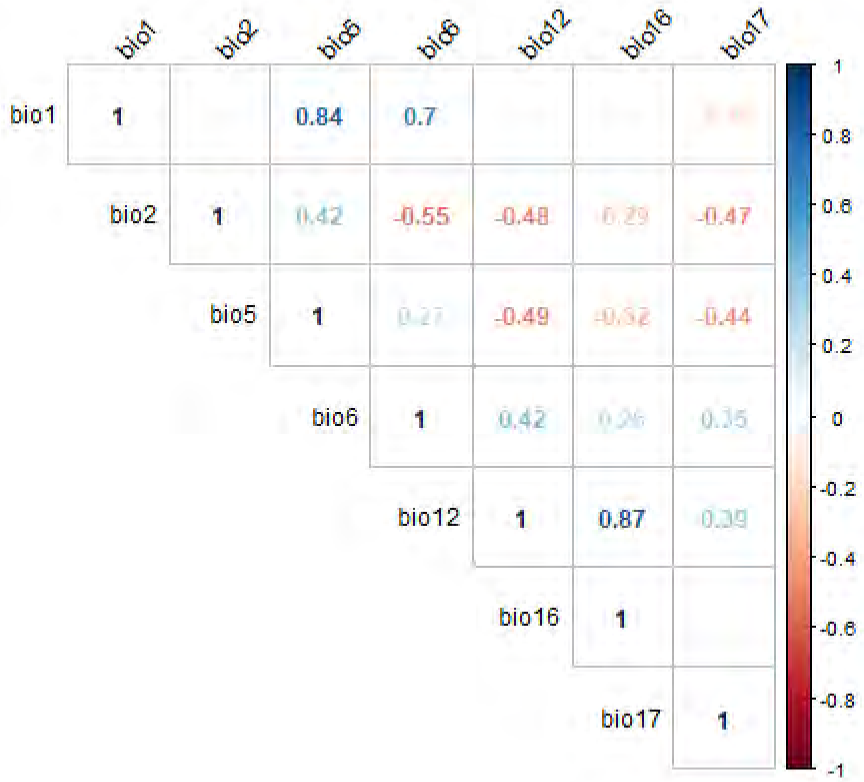
Fig. Correlations between potential environmental predictor variables used to calibrate *Sporobolus pyramidalis* (a, b) and *Sporobolus natalensis* (c, d) MaxEnt models.

(a) *Sporobolus pyramidalis* - Native-range model

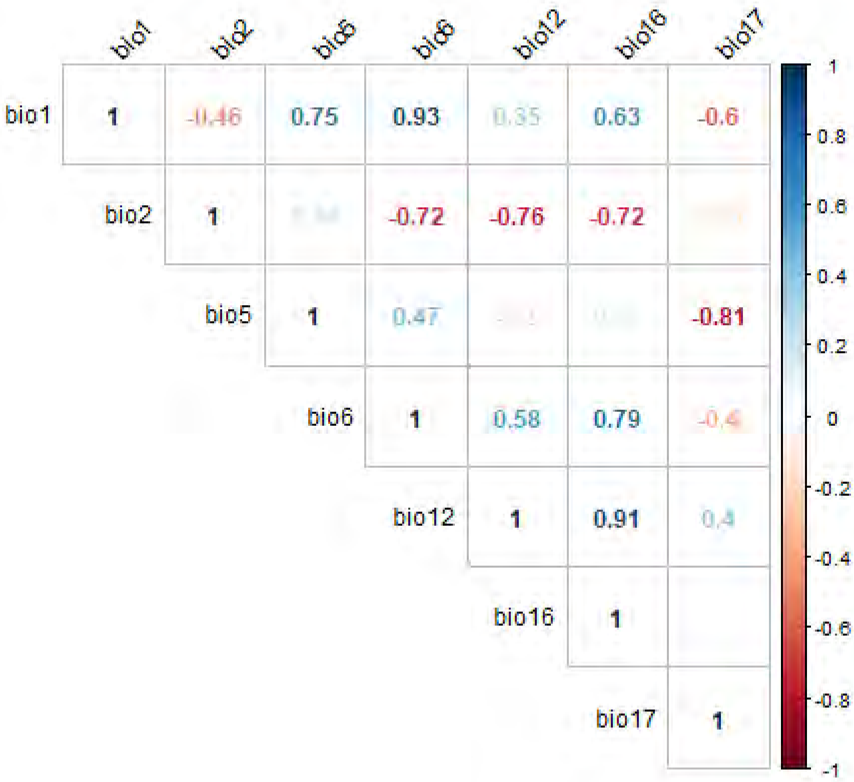


(b) *Sporobolus pyramidalis* – Invaded-range model

(c) *Sporobolus natalensis* - Native-range model



(d) *Sporobolus natalensis* - Invaded-range model



Appendix 4 – MaxEnt response curves

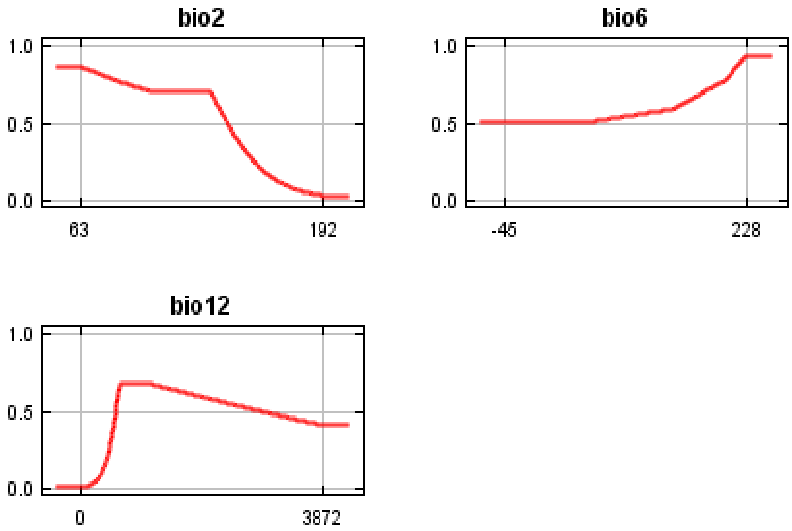
MaxEnt allows users to create two types of response curves, namely (1) marginal and (2) isolated response curves (Philips et al., 2006). Marginal response curves visualise the change in the predicted suitability for the focal species by varying one environmental variable at a time, while holding all other variables used to calibrate the fitted model at their mean value. One drawback of this approach is that strongly correlated environmental variables may confound the interpretation of these plots, as MaxEnt is able to incorporate features that allow environmental variables to co-vary (Clark et al., 2014). The second option is to create isolated response curves, which are derived from models including only the focal environmental variable. Isolated response curves are considered more appropriate when environmental variables are correlated (Philips et al., 2006).

Investigating the shape of response curves is a powerful model diagnostic tool (Webber et al., 2011). For example, environmental variables that demonstrate highly complex curves may provide an indication of model overfitting, while distinct deviations from the curve towards the extremities of environmental variable range (i.e. near the maximum and minimum x-values) may be concerning when extrapolating into novel climatic space (Clark et al., 2014). Moreover, the response curves should be interpreted in relation to the known biology of the focal species to assess the biological realism of the functional response, and thus, the fitted model.

Below, I present the isolated response curves for the optimal species x region MaxEnt model, paying particular attention to the biological plausibility of each fitted response curve, and relating the limits and shapes of the response curves to known biological information and/or field observations.

(a) Sporobolus pyramidalis – native-range model

Three environmental variables were included in the *S. pyramidalis* native-range model: bio2 (mean diurnal range; Mean of monthly [max temp - min temp]), bio6 (minimum temperature of coldest month) and bio12 (mean annual precipitation).

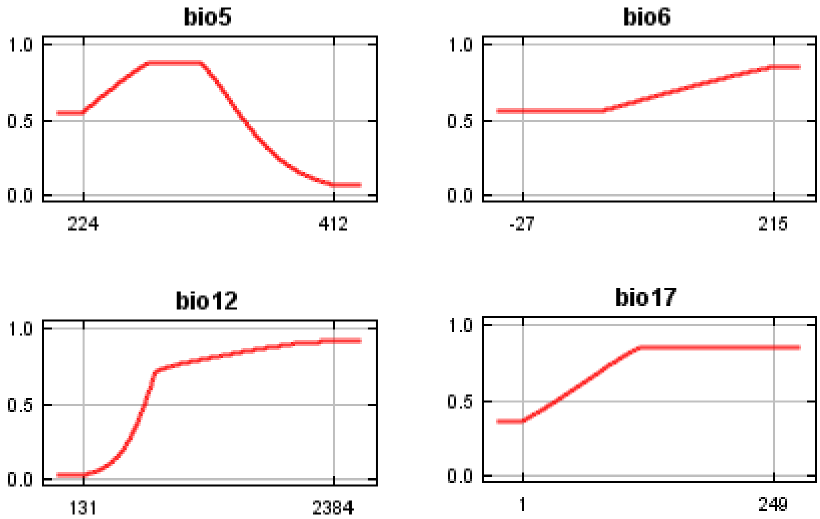


A clear rainfall threshold exists for *S. pyramidalis* in its native range (Africa), whereby predicted suitability increased drastically between 0 and 500-700mm of annual precipitation and remained relatively constant above this threshold (bio12). While little is known of the climatic controls on its distribution in Africa, in Australia, *S. pyramidalis* is only found above the 500-600mm of mean annual precipitation isocline, so the response curve here seems plausible (Vogler and Bahnisch, 2006). Suitability was constant between minimum monthly temperatures between approximately -5°C and 5°C and increased steadily up to 23°C (bio6). Suitability was high for diurnal range (i.e. an indication of temperature fluctuations on a monthly scale) values between 6-12°C but declined steadily to approximately 0 when diurnal range was 12 and 19 °C (bio2). This suggests that large temperature fluctuations negatively impact suitability for *S. pyramidalis*. Indirect support exists to support the influence of diurnal range on the climatic suitability for *S. pyramidalis*. Andrews (1995) and Vogler and Bahnisch (2006) demonstrated that *S. pyramidalis* can germinate over a range of constant temperatures between 15 and 45°C, with the optimal range between 15 and 35°C. These data indicate that *S. pyramidalis* can germinate, at least under experimental conditions, within a thermal range of 20-30°C.

The response curves appear biologically plausible, and there are no immediate causes for concern (i.e. relatively simple and smooth response curves and no drastic increases or declines at the limits of the response curves).

(b) Sporobolus pyramidalis – invaded-range model

Four environmental variables were included in the *S. pyramidalis* invaded-range model: bio5 (max temperature of warmest month), bio6 (minimum temperature of coldest month), bio12 (mean annual precipitation) and bio17 (precipitation of driest quarter).



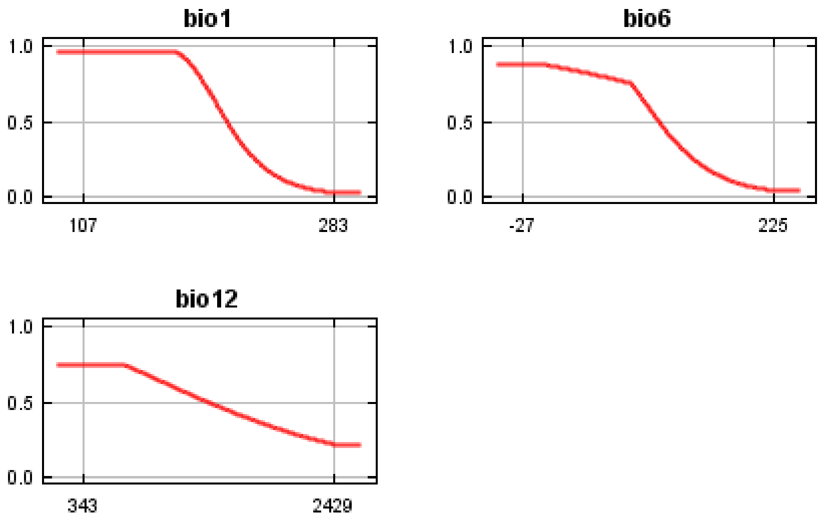
Climatic suitability increases when the maximum monthly temperature ranges between 22°C and 32-33°C, and then gradually decreases to approximately 0 when monthly maximum temperatures reach 41°C (bio5). Suitability is relatively constant for *S. pyramidalis* where minimum monthly temperatures range between -2.7°C and 21.5°C, increasingly slightly between 9/10°C and 21.5°C (bio6). The response curve for bio5 is broadly consistent with the germination biology of *S. pyramidalis* (Andrews, 1995; Vogler and Bahnisch, 2006). *Sporobolus pyramidalis* germinates under laboratory conditions between 15 and 45°C, which is loosely similar to the response curve observed for bio5.

As per the *S. pyramidalis* native-range model (see section (a)), a clear rainfall threshold exists for *S. pyramidalis* in its invaded range (Australia), whereby predicted suitability drastically increased between 0 and 500-700mm of annual precipitation and remained relatively constant above this threshold (bio12). In Australia, *S. pyramidalis* is only found above the 500-600mm of mean annual precipitation isocline (Vogler and Bahnisch, 2006), and a similar functional form of its relationship with mean annual precipitation is found within its native-range, so the response curve here seems plausible. Predicted suitability increases gradually when precipitation in the driest quarter of the year increases from 0 to ~ 125mm, above which suitability remains constant up to 250mm (bio17).

The response curves appear biologically plausible, and there are no immediate causes for concern (i.e. relatively simple and smooth response curves and no drastic increases or declines at the limits of the response curves).

(c) *Sporobolus natalensis* – native-range model

Three environmental variables were included in the *S. natalensis* native-range model: bio1 (mean annual temperature), bio6 (minimum temperature of coldest month) and bio12 (mean annual precipitation).

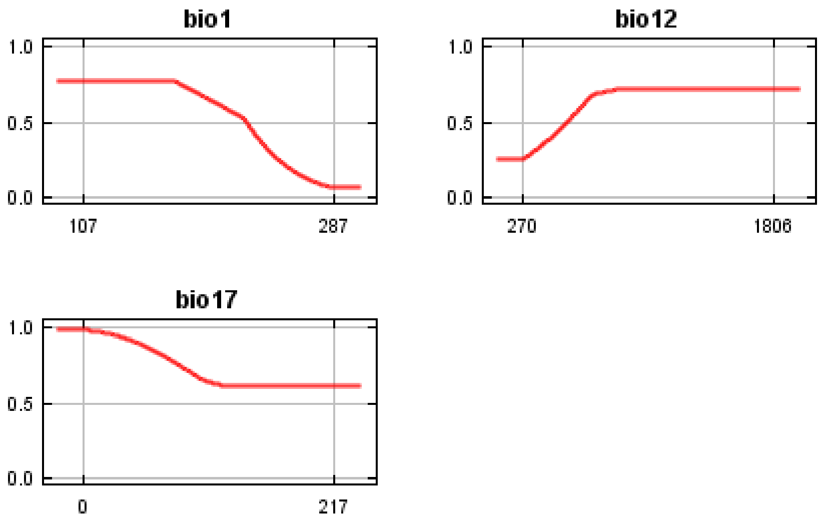


Climatic suitability for *S. natalensis* in its native range is high and relatively constant where mean annual temperatures are between 10.7°C and approximately 18°C, and then gradually decrease to 0 between 18°C and 28°C (bio1). Similarly, suitability is high for minimum monthly temperatures between -2.7°C and approximately 10°C, and then gradually decreases to 0 between 10°C and 22.5°C (bio6). Climatic suitability is relatively high and constant for regions with mean annual precipitation between 343 and approximately 700mm, and then gradually decreases to low suitability between 700mm and 2429mm (bio12). Unfortunately, I am not aware of any published information to corroborate the limits and functional shape of the *S. natalensis* response curves. Field observations by the author in South Africa provide observational support for the validity of the aforementioned response curves, with *S. natalensis* broadly occupying more inland (i.e. colder, and drier) sites in the KwaZulu-Natal and Eastern Cape Provinces of South Africa than *S. pyramidalis*, which primarily occupies hotter and drier sites than *S. natalensis*, albeit there is a range of climatic overlap between the two species, which can and do co-occur at suitable sites in South Africa.

As previously mentioned, without any published information to corroborate the limits and functional shape of the *S. natalensis* response curves, it is difficult to interrogate the response curves presented here. Nevertheless, the response curves appear biologically plausible, and there are no immediate causes for concern (i.e. relatively simple and smooth response curves and no drastic increases or declines at the limits of the response curves).

(d) *Sporobolus natalensis* – invaded-range model

Three environmental variables were included in the *S. natalensis* invaded-range model: bio1 (mean annual temperature), bio12 (mean annual precipitation) and bio17 (precipitation of driest quarter).



Climatic suitability for *S. natalensis* in its invaded range is high and relatively constant where mean annual temperatures are between 10.7°C and approximately 16°C, and then gradually decrease to 0 between 16°C and 28°C (bio1). Climatic suitability is increases gradually between regions with mean annual precipitation between 270mm and approximately 600/700mm. Thereafter, suitability remains constant and high where mean annual precipitation ranges between 600/700mm and 1806mm (bio12). Suitability gradually decreases with increasing precipitation in the driest quarter of the year between precipitation totalling 0 to approximately 100mm, after which suitability for *S. natalensis* remains constant and relatively high (bio17). It is unknown why climatic suitability was higher when the driest quarter of the year received little rainfall than when more than 100mm of precipitation was received. One hypothesis is that moisture stress caused by no/little precipitation during the driest months of the year reduces seed dormancy, and results in a greater proportion of seeds germinating, as has been observed for *S. pyramdalis* (Vogler and Bahnisch, 2006). Irrespective, the implications of the shape of the initial part of the bio17 response curve is unlikely to have a significant influence on model outputs as suitability for *S. natalensis* remained high over the range of bio17 values used to calibrate this model.

As previously mentioned, without any published information to corroborate the limits and functional shape of the *S. natalensis* response curves, it is difficult to interrogate the response curves presented here. Nevertheless, the response curves appear biologically plausible, and there are no immediate causes for concern (i.e. relatively simple and smooth response curves and no drastic increases or declines at the limits of the response curves).

Appendix 5 – Insect oviposition sites

The brackets indicate the niche typically occupied by each of the six endophagous insect herbivores associated with *Sporobolus pyramidalis* and *Sporobolus natalensis* in South Africa. Line drawing kindly provided by Ms. Sariana Faure (Rhodes University, South Africa).