Application to release the gall-inducing thrips (*Acaciothrips ebneri*) for biological control of the weed prickly acacia (*Vachellia nilotica* subsp. *indica*)

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Executive summary

Prickly acacia (*Vachellia nilotica* subsp. *indica* (Benth.) Kyal. & Boatwr.) was introduced into Australia for shade, fodder and ornamental purposes in the 1890s. It has spread into central and western Queensland and into semi-arid and arid areas elsewhere in Australia. It is a declared weed in all Australian states and the Northern Territory. Infestations of prickly acacia increase mustering times and costs, impede stock access to water, increase soil water loss through transpiration, and reduce pasture production. Nine subspecies of *Vachellia nilotica* (L.) P.J.H.Hurter & Mabb. are recognised in its native range and they occur across much of Africa and the Indian subcontinent. Prickly acacia populations in Australia have been identified as *V. nilotica* subsp. *indica*.

Biological control is the most economically viable management option for prickly acacia in Australia. The biological control program started in the early 1980s with native-range surveys in India, Kenya, Pakistan and South Africa. A total of six insects have been released as biological agents for the control of prickly acacia. Two agents have become established, a seed feeding bruchid (*Bruchidius sahlbergi* Schilsky) from Pakistan and a leaf feeding looper (*Chiasmia assimilis* Warren) from Kenya and South Africa. The impact of *B. sahlbergi* on prickly acacia is relatively small while *C. assimilis* has established only at coastal sites, not in the inland regions where major prickly acacia infestations occur. The need for effective biological control for prickly acacia remains a high priority.

Surveys were redirected into Ethiopia based on herbarium records and bioclimatic modelling. The gall-inducing thrips (*Acaciothrips ebneri* Karny) was prioritised as a prospective biological control agent based on field host range and damage intensity. In its native range, the gall-inducing thrips occurs widely on both juvenile and mature prickly acacia plants in northern and eastern regions of Ethiopia. Additionally, gall-inducing thrips were only found on *V. nilotica* subsp. *tomentosa* and *V. nilotica* subsp. *indica* (both with moniliform fruits), but not on *V. nilotica* subsp. *leiocarpa* (with non-moniliform fruits) and other co-occurring *Vachellia* species (e.g., *Vachellia etbaica* (Schweinf.) Kayl. & Boatwr. and *Vachellia seyal* (Delile) P.J.H. Hurter).

Adult thrips feed and induce rosette galls on the axillary and terminal buds and continued feeding by adults and the developing larvae inside the galls results in shoot-tip dieback. Early signs of gall induction can be seen within a week of adult feeding under quarantine conditions. The entire life cycle from egg to adult takes about 25 days at a glasshouse temperature of 28 ± 2°C. A bioclimatic model based on its native range distribution predicts that central and western Queensland, central Northern Territory and coastal areas of Western Australia are climatically suitable for *A. ebneri* establishment.

The assessment of host specificity is shown in Figure 1. Preliminary no choice tests in a South African quarantine laboratory recorded the thrips inducing galls only on Australian prickly acacia (*V. nilotica* subsp. *indica*) and not the South African subspecies (*V. nilotica* subsp. *kraussiana*). The gall-inducing thrips was imported into a high security quarantine facility at the Ecosciences Precinct, Brisbane, Australia for colony establishment and host specificity tests in December 2015. A total of 59 test species from the family Fabaceae with a minimum of five replications were screened for host acceptability (adult feeding, gall induction, egg laying and larval development) (Figure 1). All test species were used in no-choice trials. The thrips induced galls and produced progeny only on Australian prickly acacia (*V. nilotica* subsp. *indica*) indicating it is highly host specific at the subspecies level and suitable for release in Australia.

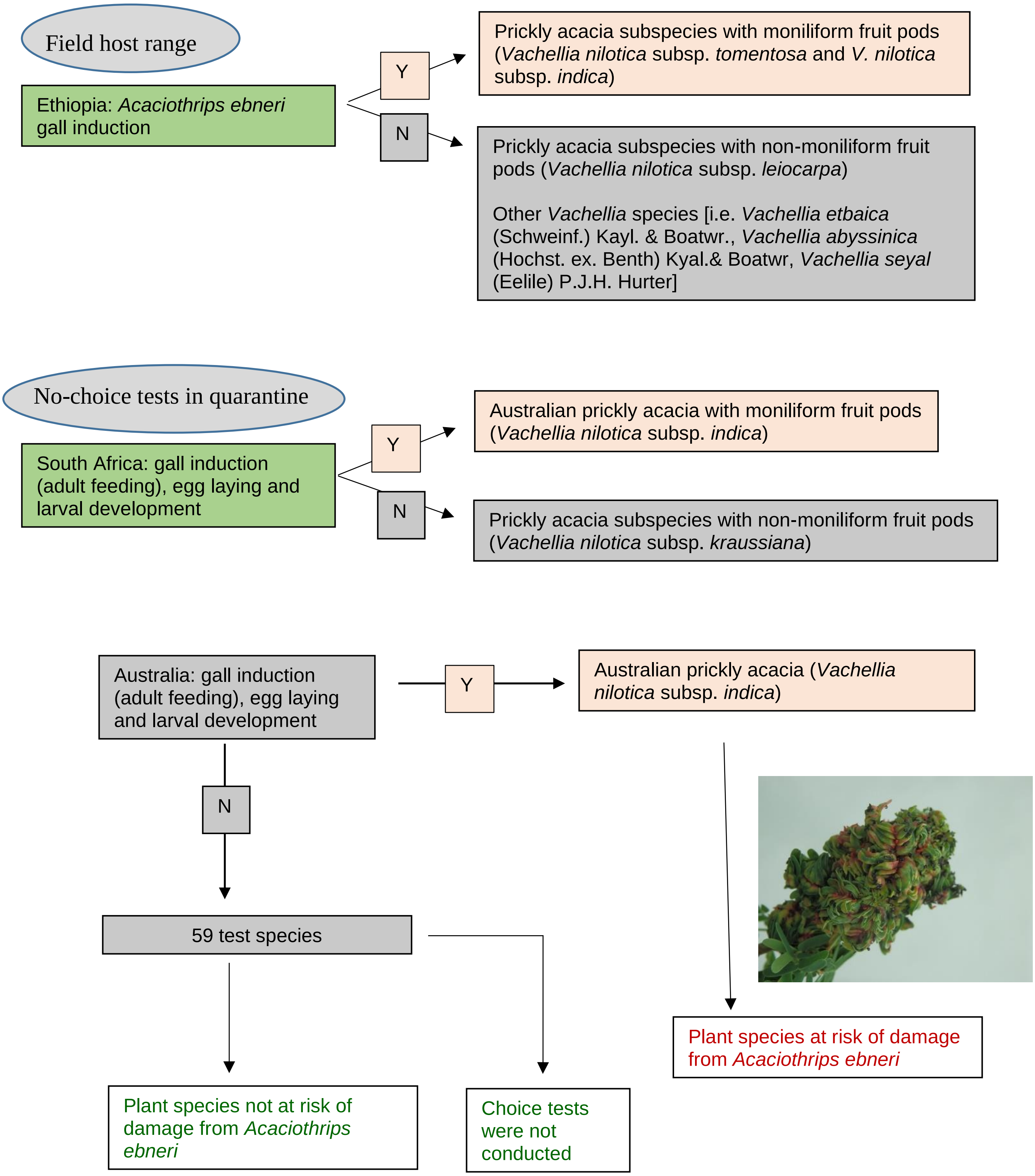

Figure 1. Flow chart used for host specificity tests undertaken for *Acaciothrips ebneri* for the target species, *Vachellia nilotica* subsp. *indica*. The results of host specificity testing are also shown.

1 Information on the target species

1.1 Classification

Order:	Fabales
Family:	Fabaceae
Clade:	Mimosoideae
Tribe:	Acacieae
Genus:	*Vachellia* Wight & Arn.
Species:	*nilotica* (L.) P.J.H.Hurter & Mabb.
Subspecies:	*indica* (Benth.) Kyal. & Boatwr.
Common names:	prickly acacia, gum Arabic tree, babul, thorn mimosa, Egyptian acacia, thorny acacia

1.2 Description

Vachellia nilotica is a polytypic species exhibiting significant morphological (Brenan, 1983; Dwivedi, 1993), phenological (Marohasy, 1992) and genetic diversity (Nagarajan, 2001; Nagarajan et al., 2001; Nongonierma, 1976; Wardill et al., 2005). Nine recognised subspecies of *V. nilotica* exist in its native range (Table 1), with each subspecies having a distinct geographic range (Brenan, 1983). Subspecies are differentiated mainly by the shape, size and degree of pubescence of the pods (Brenan, 1983). The degree of pubescence of young branchlets, the habit of the tree, and the shape of the crown are also important characteristics (Taylor and Dhileepan, 2019). The prickly acacia populations in Australia were originally identified as *V. nilotica* subsp. *indica* based on morphological characters (Brenan, 1983) and subsequently confirmed by molecular studies (Comben et al., 2021; Wardill et al., 2005).

Prickly acacia (*V. nilotica* subsp. *indica*) is a multipurpose tree native to the Indian subcontinent and east Africa (Comben et al., 2021). It is a Weed of National Significance and is widespread throughout the grazing areas of northern Australia. Prickly acacia is a thorny shrub or small tree growing to about 4-5 m high but occasionally to 10 m (Figure 2a). It is usually single stemmed but may be multiple stemmed at the base, especially after damage by fire or frost. Prickly acacia forms dense thorny thickets (up to 900 plants/ha) and mature plants live for *ca*. 40 years (Dhileepan, 2009). Mature trees have brown to black rough bark and tend to lose most of their thorns. The tree has a deep taproot and several branching lateral roots close to the soil surface. Leaves are green, bipinnate and about 3-4 cm long with pairs of stout and sharp thorns growing at their base (Figure 2b). Each leaf is made up of 10-25 pairs of leaflets along its length (Figure 2b). The inflorescence is ball shaped and golden yellow, occurring on the stem in groups of 2-6 at the leaf base (Figure 2c). The fruit pods are covered with fine hairs and are grey-green (Figure 2b). The plants have distinct flat necklace shaped (moniliform) fruit pods (Figure 2b), each 10-15 cm in long, bearing 8-15 seeds (Spies and March, 2004). A mature tree can produce up to 300,000 seeds per year. When buried in soil, seeds can remain viable for up to 7 years (Dhileepan, 2009) (Figure 3). The plant is dispersed largely by livestock (particularly cattle) ingesting mature seed.

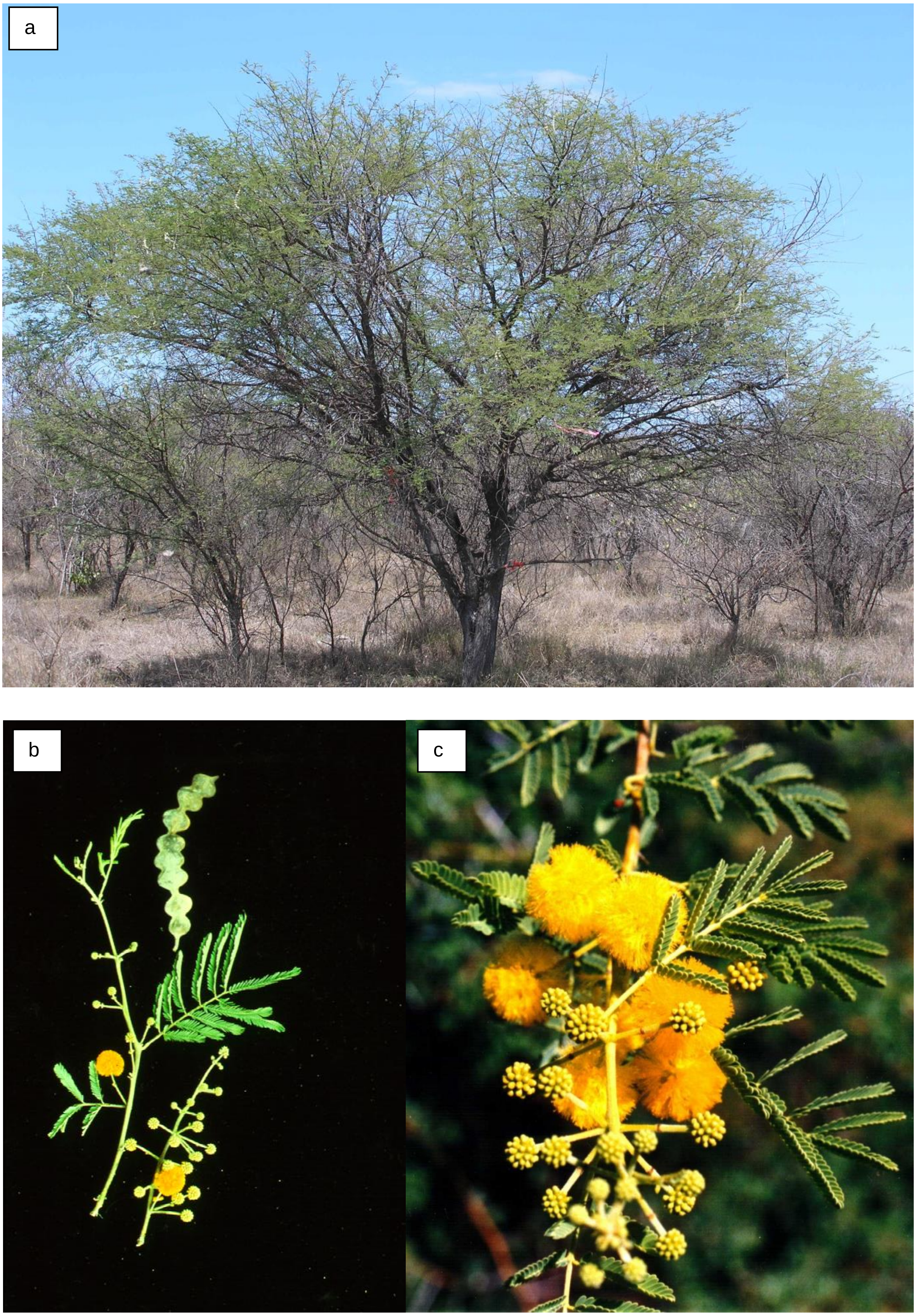

Figure 2. *Vachellia nilotica* subsp. *indica* (a) mature plant, (b) a shoot tip showing leaves, flowers and a moniliform fruit pod, (c) flowering clusters.

Table 1. *Vachellia nilotica* subspecies and their native range.

Sub-species	Fruit pods	Native range
Vachellia nilotica subsp*. indica* (Benth.) Kyal. & Boatwr.	Moniliform	Asia (India, Pakistan, Yemen, Oman, and Myanmar) Africa (Ethiopia)
V. nilotica subsp. *cupressiformis* (J.L.Stewart) Ali & Faruqi	Moniliform	Indian subcontinent (India and Pakistan)
V. nilotica subsp. *nilotica* (L.) P.J.H. Hurter & Mabb.	Moniliform	Africa (Cameroon, Chad, Egypt, Ethiopia, Sudan, Mali, Nigeria, Niger, Senegal and Sudan) Asia (Iran, Iraq, Oman, Saudi Arabia and Yemen)
V. nilotica subsp. *tomentosa* (Benth.) Brenan	Moniliform	Africa (Senegal, Mali, Ivory Coast, Ghana, Niger, Nigeria, Sudan and Ethiopia)
V. nilotica subsp. *adstringens* (Schumach. & Thonn.) Kyal. & Boatwr.	Non-moniliform	Africa (Algeria, Cameroon, Chad, Egypt, Gambia, Libya, Mali, Nigeria, Senegal, Sudan, and Somalia)
V. nilotica subsp. *hemispherica* Ali & Faruqi	Non-moniliform	Indian subcontinent (India and Pakistan)
V. nilotica subsp. *kraussiana* (Benth.) Kyal. & Boatwr.	Non-moniliform	Africa (Angola, Botswana, Malawi, Mozambique, Namibia, South Africa – Natal & Transvaal, Zambia, Zimbabwe, Swaziland and Tanzania)
V. nilotica subsp. *leiocarpa* (Benth.) Kyal. & Boatwr.	Non-moniliform	East Africa (Kenya, Somalia, Ethiopia and Tanzania)
V. nilotica subsp. *subalata* (Vatke) Kyal. & Boatwr.	Non-moniliform	East Africa (Sudan, Ethiopia, Uganda, Kenya and Tanzania)

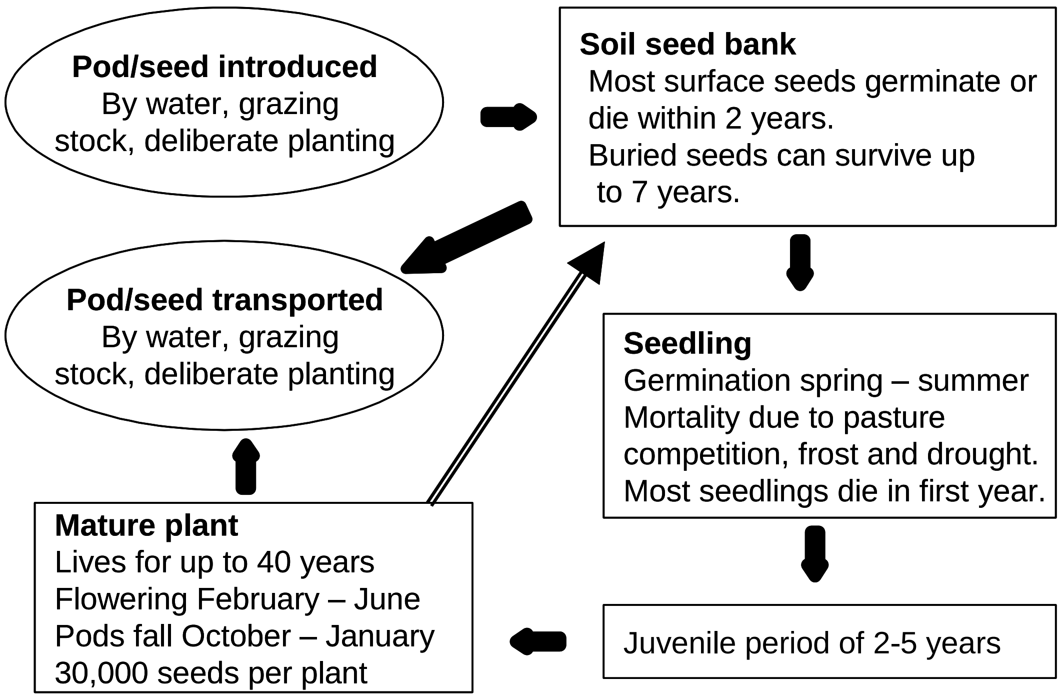

Figure 3. Life cycle of prickly acacia in Australia.

1.3 Native range and centre of origin

Vachellia nilotica is widespread in the drier areas of Africa and the Indian subcontinent (Brenan, 1983). Prickly acacia (*V. nilotica* subsp. *indica*) is native to the Indian subcontinent (India, Bangladesh and Pakistan), the Middle East (Yemen and Oman) and East Africa (Ethiopia) (Comben et al., 2021; Dhileepan et al., 2019; Wardill et al., 2005).

1.4 Australian and overseas distribution

Prickly acacia was introduced into Queensland in the late 1890s as an ornamental tree (Bolton, 1989). From the mid-1920s, the tree was widely used as a shade tree and fodder for sheep in western Queensland. A change from sheep to cattle as the predominant grazing species and a series of wet years resulted in its spread. Prickly acacia has been regarded as a problematic weed since 1926 (Pollock, 1926), and was declared as a noxious weed in Queensland in 1957. Prickly acacia is now a Weed of National Significance in Australia (Australian Weeds Committee, 2013).

In central and western Queensland, particularly the Mitchell grass downs, over 6.6 million ha of arid and semi-arid natural grasslands and 2,000 km of bore drains (Mackey, 1997; Spies and March, 2004) are infested by prickly acacia (Figure 4 and 5). Infestations are mainly around Winton, Richmond, Cloncurry, Hughenden and Longreach. Scattered infestations are found along the Queensland coast between Bowen and Maryborough, the Barkly Tablelands and Arnhem Land in the Northern Territory, Cordillo Downs Station in northeast of South Australia and on the Durack River and nearby areas, west of Wyndham in Western Australia (Figure 5). In the Northern Territory, there are fewer than 10 infestations recorded, covering an estimated 600 ha in total, most of which occur on properties along the Barkly Highway. A minor infestation has also been recorded in the Katherine district (Gracie, 1998). Prickly acacia has the potential to infest vast areas of Australia's native grassland ecosystems in northern Australia (Figure 4) (Kriticos et al., 2003).

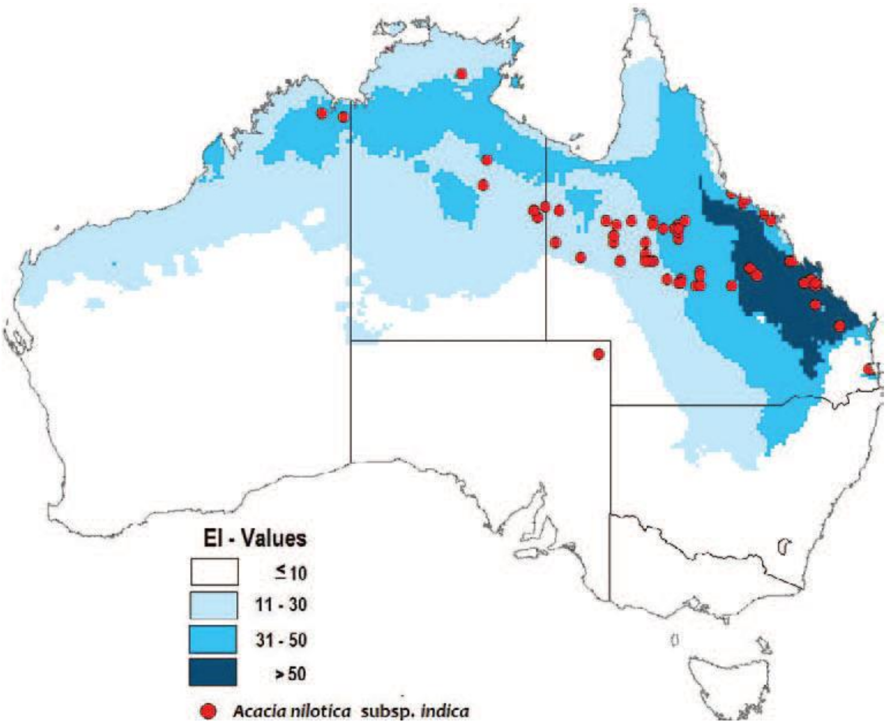

Figure 4. Current and potential distribution of prickly acacia under current climate conditions using CLIMEX after Kriticos et al. (2003).

Figure 5. Prickly acacia infestation near Bowen, Queensland.

Prickly acacia has also been introduced into various other countries including Indonesia, Iran, Iraq, Nepal, New Caledonia, Vietnam and the West Indies as a multipurpose tree (Dhileepan, 2009). A different subspecies of *V. nilotica* (with non-moniliform fruits) is a major environmental weed in the Baluran National Park in Java, Indonesia (Zahra et al., 2020).

1.5 Close relatives in Australia

Prickly acacia was formally known as *Acacia nilotica* subsp. *indica*. *Acacia* (*sensu lato*) has been split into seven genera (*Acacia* Martius, *Vachellia* Wight & Arn., *Senegalia* Raf., *Parasenegalia* Seigler & Ebinger, *Pseudosenegalia* Seigler & Ebinger, *Acaciella* Britton & Rose and *Mariosousa* Seigler & Ebinger) (Miller et al., 2017). Species within *Acacia* subg. *Acacia* (including prickly acacia) have been transferred to the genus *Vachellia* Wight & Arn. Species within subgenus *Aculeiferum* have been transferred to *Senegalia*, *Acaciella*, *Parasenegalia*, *Pseudosenegalia* and *Mariosousa* (Miller et al., 2017). Species within *Acacia* subg. *Phyllodineae* (the majority of Australian *Acacia* species) remain in the *Acacia* (*sensu stricto*) genus.

The *Vachellia* genus is of Afro-Asian origin and contains 164 species (Kodela and Wilson, 2006). Current knowledge suggests that *Vachellia* is more closely related to mimosoid genera than it is to *Acacia s.s*. (Taylor and Dhileepan, 2019). Molecular work suggests that *Vachellia* is nested within the old Mimoseae and is sister to *Neptunia* Lour. and other basal Mimoseae including *Prosopis*, *Desmanthus* and *Leucaena* (Bouchenak-Khelladi et al., 2010; Miller and Burd, 2014; Murphy, 2008) (Figure 6). Eleven *Vachellia* species occur in

Australia of which nine are native to Australia (Pedley, 2002) (Table 2). *Vachellia nilotica* subsp. *indica* and *Vachellia farnesiana* are the two species naturalised in Australia (Kodela and Wilson, 2006). All native *Vachellia* species are restricted to northern Australia (Table 2).

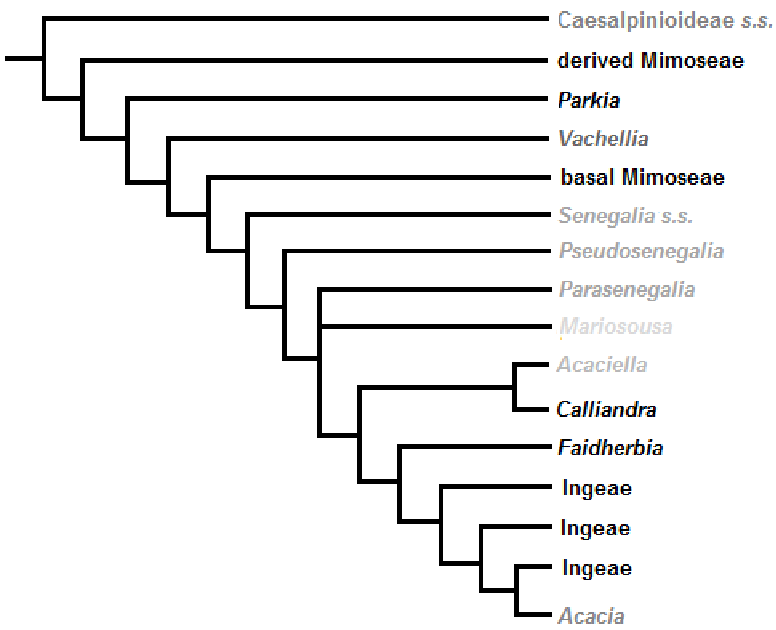

Figure 6. Schematic representation of relationships within the former Mimosoideae (Taylor and Dhileepan, 2019).

Table 2. *Vachellia* species found in Australia and their distribution.

Species	Status	Distribution
Vachellia bidwillii (Benth.) Kodela	Native	QLD and NT
Vachellia clarksoniana (Pedley) Kodela	Native	QLD and WA
Vachellia ditricha (Pedley) Kodela	Native	WA, NT and QLD
Vachellia douglasica (Pedley) Kodela	Native	QLD and NT
Vachellia pachyphloia (W.Fitzg.) Kodela	Native	NT and WA
Vachellia pallidifolia (Tindale) Kodela	Native	Qld and NT
Vachellia suberosa (A. Cunn. ex Benth.) Kodela	Native	WA, NT and QLD
Vachellia sutherlandii (F. Muell.) Kodela	Native	WA, NT and QLD
Vachellia valida (Tindale & Kodela) Kodela)	Native	WA, NT and QLD
Vachellia nilotica subsp. *indica* (Benth.) Kyal. & Boatwr.	Naturalized/invasive	WA, NT and QLD
Vachellia farnesiana (L.) Wight & Arn.	Naturalized/invasive	QLD, NSW, WA, NT and SA

QLD = Queensland; NT = Northern Territory; WA = Western Australia; NSW = New South Wales; SA = South Australia.

1.6 Beneficial aspects

In its native ranges, prickly acacia is widely used in agroforestry, social forestry, reclamation of wastelands and rehabilitation of degraded forests (Bargali and Bargali, 2009). In traditional agroforestry systems, *V. nilotica* provides fuel, fodder, gum, tannin and timber (Dhileepan, 2009). Root nodulations in this species help with in biological nitrogen fixation (Dreyfus and Dommergues, 1981) and enhance soil fertility (Pandey and Sharma, 2003). In addition, the plant is rich in anti-oxidants such as alkaloids, volatile essential oils, phenols and phenolic glycosides, resins, oleosins, steroids, tannins and terpenes (Ali et al., 2012). Different parts of the plant have been shown to have inhibitory activities against the Hepatitis C virus and the human immunodeficiency virus, as well as having antioxidant, anti-bacterial, anti-hypertensive and anti-spasmodic activities (Ali et al., 2012).

Prickly acacia has been introduced into various countries including Australia, Indonesia, Iran, Iraq, Nepal, New Caledonia, Vietnam and the West Indies as a multipurpose tree. In Australia, prickly acacia (*V. nilotica* subsp. *indica*) was originally introduced as a beneficial plant for the western Queensland areas to increase lambing percentages and wool production by providing shade trees on natural grassland (Palmer et al., 2012). At low densities, prickly acacia provides shade, and leaves and fruit pods are used as fodder for sheep during dry periods, thus increasing stock productivity (Carter and Cowan, 1988). Prickly acacia also provides a nitrogen supplement to Mitchell grasslands. There has also been interest in using prickly acacia in Australia for biomass energy production.

1.7 Pest status

Prickly acacia is a major weed in the grazing areas of western Queensland and costs primary producers AUD 9 million/year by decreasing pasture production, forming impenetrable thickets that hinder the mustering of livestock and restricts their access to watercourses. Infestations alter pasture composition by outcompeting native and perennial pasture species and favouring less desirable annual species. In the Mitchell grass downs, prickly acacia poses a threat to nearly 25 rare and threatened animal species, including the endangered carnivorous marsupial Julia Creek dunnart (*Sminthopsis douglasi* Archer), and two endangered plant communities by modifying grasslands (Spies and March, 2004).

Prickly acacia is a serious weed of northern Australia and has been declared a Weed of National Significance (http://weeds.ala.org.aPu/WoNS/pricklyacacia/). Prickly acacia infests over seven million hectares of the Mitchell grass downs in western Queensland, as well as scattered infestations in coastal Queensland, the Northern Territory, South Australia and Western Australia (Bolton, 1989; Mackey, 1997; Spies and March, 2004). It is a declared species in all Australian states and the Northern Territory. In Queensland, prickly acacia is a category 3 restricted invasive plant under the *Biosecurity Act 2014* (www.daf.qld.gov.au/data/assets/pdf_file/_0007/73753/prickly-acacia.pdf). In the Northern Territory, prickly acacia is declared Class-A weed, and a target for eradication (https://nt.gov.au/environment/weeds/weeds-in-the-nt/A-Z-list-of-weeds-in-the-NT/prickly-acacia). In Western Australia, all non-native acacias are declared pests and prickly acacia is a target for eradication (https://www.agric.wa.gov.au/declared-plants/acacia-declared-pest). In New South Wales, prickly acacia is a prohibited weed under the *Biosecurity Act 2015* (https://weeds.dpi.nsw.gov.au/Weeds/PricklyAcacia). In South Australia, prickly acacia is a declared plant under the Natural Resources Management Act 2004 (https://www.pir.sa.gov.au/biosecurity/weeds_and_pest_animals/weeds_in_sa/plant_policies/pest_weed_policies/declared_plants_2/prickly_acacia_policy.pdf). In Victoria, prickly acacia is a declared noxious weed (http://vro.agriculture.vic.gov.au/dpi/vro/vrosite.nsf/pages/weeds_prickly_acacia). In Tasmania, prickly acacia is a declared weed under the Tasmanian Weed Management Act 1999 (https://dpipwe.tas.gov.au/invasive-species/weeds/weeds-index/declared-weeds-index/prickly-acacia).

1.8 Other control options

Chemical control

There are a variety of control options for prickly acacia with the most important being chemical control for low and medium density infestations (DAF, 2018; Spies and March, 2004). For dense infestations, chemical treatment is very expensive (DAF, 2018). Current chemical control methods are basal barking, cut stump application, stem injection and overall foliar spray. Residual chemicals are used for the treatment of infestations in bore drains (DAF, 2018). Basal bark spraying is the most effective way of treating low and medium density infestations across a paddock (DAF, 2018). Chemical control can achieve a kill of greater than 90%, but follow up treatments are needed to treat missed plants and to control seedling recruitment (Spies and March, 2004). The cost of 10-20¢ per tree becomes prohibitive in high-density infestations and other control measures are necessary (Spies and March, 2004). For high-density infestations, aerial application appears promising but is equally expensive.

Mechanical control

There are three main mechanical methods used for control of prickly acacia - tractor grubbing, cutter bar, and double chain pulling (DAF, 2018). Tractor grubbing can kill 90% of the trees, and is cost competitive compared with application of herbicides (Spies and March, 2004). Cutter bar and double chain pulling methods are expensive but appear to be the best available methods for medium to heavy infestations (DAF, 2018). Chain pulling achieves a 70% kill of larger plants, but is relatively ineffective against seedlings (Spies and March, 2004).

Fire

Prickly acacia trees are not susceptible to fire, instead fire stimulates prickly acacia seed germination (Parsons and Cuthbertson, 1992). However, fire is effective on prickly acacia seedlings, and can be used to control seedlings when germination is prolific.

Agronomic practices

Pasture competition has little effect on the establishment of prickly acacia seedlings. Grazing by livestock has limited impact on prickly acacia populations (Radford et al., 2002). In some situations, grazing by camels and goats, in conjunction with traditional methods, have been shown to reduce the cost of control (Spies and March, 2004; Tiver et al., 2001). Restriction of stock movement from infested areas during the period of fruit pod drop can minimise the spread of prickly acacia.

Biological control

Biological control offers a sustainable long-term control option for prickly acacia. Biological control of prickly acacia in Australia commenced in the early 1980s, with native range surveys conducted in Pakistan, Kenya, South Africa and India (Dhileepan, 2009; Dhileepan et al., 2014). Six biological control agents have been released in Australia since 1985. The two agents that successfully established are the seed-feeding *Bruchidius sahlbergi* Schilsky (Lepidoptera: Bruchidae) from Pakistan and a leaf-feeding geometrid *Chiasmia assimilis* Warren (Lepidoptera: Geometridae) from Kenya and South Africa. The impact of *B. sahlbergi* on prickly acacia has been insignificant (Radford et al., 2001) while *C. assimilis* has established only at coastal sites, and not widely in the arid inland regions where the major infestations of prickly acacia occur (Palmer et al., 2007). A third agent, *Cuphodes profluens* Meyrick (Lepidoptera: Gracillariidae) from Pakistan may have become established temporarily at a coastal site in Queensland before prickly acacia trees were removed from the location (Palmer et al., 2012). The other three agents, the leaf-feeding beetle, *Homichloda barkeri* Jacoby (Lepidoptera: Chrysomelidae) from Kenya, the leaf-feeding moth, *Chiasmia inconspicua* Warren (Lepidoptera: Geometridae) from Kenya and the leaf-feeding moth, *Cometaster pyrula* Hopffer (Lepidoptera: Noctuidae) from South Africa, did not establish at all (Palmer et al., 2012).

Surveys in India identified five further insects and two rust fungi as prospective biological control agents (Dhileepan et al., 2013). However, when these agents were tested, they were either not sufficiently host specific or difficult to rear in quarantine (Dhileepan et al., 2014; Taylor and Dhileepan, 2018).

A literature search (Dwivedi, 1993) and herbarium records suggested that prickly acacia (subsp. *indica*) and other *V. nilotica* subspecies with moniliform (necklace shaped) fruits occur in Ethiopia. Recent surveys (Dhileepan et al., 2019) and genetic studies (Comben et al., 2021) confirmed the native geographic range extension of prickly acacia (*V. nilotica* subsp. *indica*) populations in Ethiopia. A CLIMEX model has predicted that areas in Ethiopia are climatically similar to the arid inland regions of western Queensland where prickly acacia is a major problem (Dhileepan et al., 2018; Senaratne et al., 2006). Hence, the search for new biological control agents were redirected to Ethiopia. Potential survey sites were identified in Ethiopia based on herbarium records and the CLIMEX model. Surveys in Ethiopia identified a gall-inducing thrips [*Acaciothrips ebneri* (Karny), Thysanoptera: Phlaeothripidae] inducing shoot-tip rosette galls as a prospective biological control agent based on field host range and damage potential (Dhileepan et al., 2018).

1.9 Approved target for biological control

Prickly acacia (*V. nilotica* subsp. *indica*) is an approved target species for biological control in Australia since the 1980s (the biological program pre-dates the approval process that has occurred since 1983; https://weeds.org.au/overview/lists-strategies/).

1.10 Stakeholders

The Australian Government (Department of Agriculture, Water Resources and the Environment), state and territory Governments (Queensland, Northern Territory and Western Australia), local governments and land managers across northern Australia, particularly cattle producers, have considerable interest in the control of prickly acacia (Table 3). Community and Natural Resource Management (NRM) groups are also concerned about the impact of the weed on land used for amenity, biodiversity conservation and agriculture (Table 3).

Meat and Livestock Australia, AgriFutures Australia (formerly Rural Industries Research and Development Corporation) and the Queensland State Government have provided substantial funding towards biological control research.

Table 3. The stakeholders for prickly acacia management in Australia.

Australian Government	
Department of Agriculture, Water and the Environment	
Industry	
Meat and Livestock Australia AgriFutures Australia AgForce	
State Governments	
Queensland Government Northern Territory Government The Government of Western Australia	
Local Governments	**Community and NRM groups**
City of Townsville Mount Isa City Council Barcaldine Regional Council Blackall-Tambo Regional Council Central Highlands Regional Council Charters Towers Regional Council Isaac Regional Council Longreach Regional Council Local Government Association of Queensland Rockhampton Regional Council Whitsunday Regional Council Boulia Shire Council Burdekin Shire Council Burke Shire Council Carpentaria Shire Council Flinders Shire Council McKinlay Shire Council Richmond Shire Council Winton Shire Council	Desert Channels Queensland Fitzroy Basin Association Kimberley NRM Kimberley Rangelands Biosecurity Association Mary-Burnett Regional Group Northern Gulf Resource Management group Northern Queensland Dry Tropics Prickly acacia alliance forum Prickle bush management group Rangelands NRM Southern Gulf NRM

2 Information on the potential agent

Acaciothrips ebneri is native to northern Africa and has been reported only on *Vachellia nilotica*. The gall-inducing thrips is found only on three subspecies of *V. nilotica*, all with moniliform (necklace shaped) fruits – subsp. *indica* (Ethiopia), subsp. *tomentosa* (Ethiopia, Sudan, Nigeria, and Senegal) and subsp. *nilotica* (Egypt). The gall-inducing thrips is not found on other *V. nilotica* subspecies with non-moniliform (not necklace shaped) fruits.

2.1 Taxonomy

Order:	Thysanoptera
Family:	Phlaeothripidae
Subfamily:	Phlaeothripinae
Genus:	*Acaciothrips* Priesner
Species:	*Acaciothrips ebneri* (Karny)

A whole genome sequence of this species has been completed and submitted in the GenBank (BankIt2484584 Acaciothrips_ebneri_mitochondria MZ645927 – is currently pending public release).

2.2 Description

The gall-inducing thrips was identified as *Acaciothrips ebneri* (Karny) by Laurance Mound (CSIRO, Canberra). *Acaciothrips ebneri* lacks forewing duplicated cilia, and has an extra pair of slightly larger setae on the head (Laurance Mound, personal communication, June 2020). Adults are macropterous, each with a body length of 1.7—2.4 mm; body and all legs including tarsi medium to dark brown; antennal segments III—IV mainly yellow, V—VI variably yellow at base; forewing shaded on basal half but pale toward apex. The head is slightly longer than wide, without strong sculpture; with two pairs of prominent postocular setae; mouth-cone short and rounded; maxillary stylets about one-third of head width apart, retracted half-way to postocular setae. Antennae are 8-segmented; segment III with one sense cone, IV with only 2 major sense cones; VIII not constricted at base. Pronotum is transverse, with five pairs of major setae but those on the anterior margin are not elongate. The surface is transversely linear-reticulate, with complete notopleural sutures. Metanotum with linear-reticulate sculpture. Prosternal basantra is absent, with complete mesopresternum transversely; metathoracic sternopleural sutures are present. Fore tarsal tooth are absent in both sexes. Forewings are not constricted medially, without duplicated cilia on posterior margin. Pelta is triangular; tergites II–VII each with two pairs of sigmoid wing-retaining setae; tergite IX setae S1 and S2 bluntly pointed, slightly shorter than tube, S2 setae of male shorter and stouter than S1; male sternite VIII largely occupied by pore plate except near posterior margin (Laurance Mound, personal communication, March 2021).

2.3 Biology

The life cycle of *A. ebneri* consists of five stages – egg, larvae, prepupae, pupae and adults (Figure 7). Under quarantine conditions (28 ± 2 °C; 60% RH) the total duration of the life cycle (egg to adult) was about 25 days. Under controlled glasshouse conditions the thrips reproduced sexually, throughout the year.

Adults feed on the axillary and terminal buds externally which induces rosette galls. Once a gall is initiated (in 2-3 days; Figure 8), the adults enter the gall and continue feeding, on the nutritive tissue lining the inner surface of the galls. Adults (1.81 ± 0.09 mm) live for up to 16 weeks, with females living significantly longer (111 ± 26.65 days) than males (41 ± 4.03 days) ($F = 159.32$; $P < 0.001$; Appendix 1). Without any food (only on water), adults can live for 8 ± 0.44 days. Adults are not good fliers, however they are easily carried by the wind from one plant to other plant. Dispersal and mating occur outside the gall. The adults will leave the gall when there is not enough space inside the gall and when the gall turns black (due to necrosis) and shoot tips begin to die back. Adults will move onto new shoot tips to induce new galls or wait for new shoot tips to develop.

All immature stages of the life cycle of the thrips occurs within the galls. Immature stages are unable to feed and develop when removed from the gall or when galls are severed from the plant. Likewise, immature stages removed from a gall and placed on a fresh prickly acacia plant, are unable to feed or induce galls, and so die. It is difficult to study the life cycle within growing galls. Hence, observations of different life stages had to be made by destructive sampling of the galls at regular intervals.

Females lay eggs in clusters, inside the developing galls attached to the nutritive tissues lining the walls inside. Eggs are small (0.36 ± 0.18 mm long), pale green to yellowish in colour, and hatch approximately 6 days after oviposition. Females produce approximately 200 progeny in their lifetime, though it was not possible to accurately record the total number of eggs laid by each female in a gall, as any attempt to open the gall resulted in the death of all immatures.

Destructive sampling of the galls was carried out to study the morphometrics of eggs, larvae, prepupae and pupae. The emerging larvae from eggs feed on the nutritive tissues lining the walls within the galls and develop through two larval instars. Newly emerged larvae (0.54 ± 0.03 mm long) were almost colourless (transparent) and then became yellowish to reddish (Figure 7 and Table 4). The duration of first instar larvae was 3 ± 0.07 days. The second instar larvae (1.14 ± 0.09 mm long) were slightly bigger and bright red with a dark black head, notum, and abdominal tip. The duration of the second instar larvae was 6 ± 0.23 days. The development through the non-feeding and inactive prepupal stage and the two pupal stages were completed within the gall. The dark red pre-pupae (1.38 ± 0.2 mm long) with no wing buds were larger than the second instar larvae with greatly reduced antennae pointing backwards. The developmental duration of first (1.43 ± 0.33 mm long) and second (1.53 ± 0.39 mm long) prepupae were 3 ± 0.32 and 4 ± 0.38 days, respectively (Table 4).

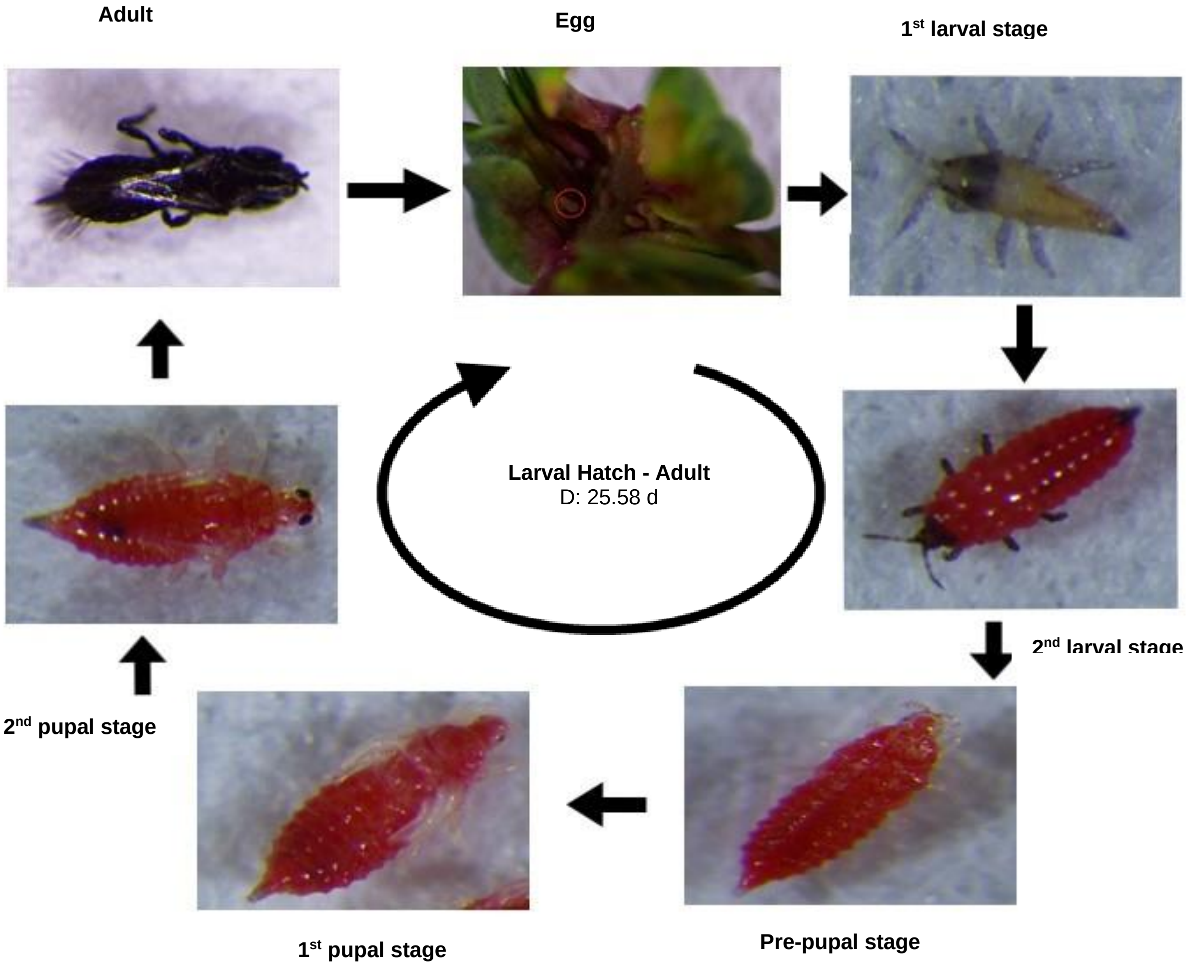

Figure 7. Life cycle of gall-inducing thrips under controlled quarantine conditions at 28 ± 2 ºC.

Table 4. Life cycle and mean development time of *Acaciothrips ebneri* under quarantine conditions.

	Length (mm)	Duration (days)	
Life stage	Mean ± SE	Mean ± SE	Range
Egg	0.36 ± 0.01	6.42 ± 0.16	2 – 10 days
1st instar larvae	0.54 ± 0.03	3.25 ± 0.07	2 – 6 days
2nd instar larvae	1.13 ± 0.09	6.88 ± 0.23	3 – 10 days
Pre-pupae	1.37 ± 0.08	2 ± 0.2	1 – 3 days
1st pupae	1.43 ± 0.06	3.2 ± 0.32	1 – 5 days
2nd pupae	1.53 ± 0.04	3.83 ± 0.38	1 – 7 days
Adult – male	1.62 ± 0.57	41.14 ± 4.03	29 – 60 days
Adult – female	1.81 ± 0.35	111.25 ± 21.38	102 – 126 days
Pre-oviposition period		2.22 ± 0.09	1 – 4 days

Figure 8. Gall induction and development of thrips in a high security quarantine facility at Brisbane, Australia.

2.4 Native range

Acaciothrips ebneri occurs naturally in Egypt, Ethiopia, Nigeria, Senegal and Sudan (Bournier, 1994; Dhileepan et al., 2018; ThripsWiki, 2021) (Laurance Mound, personal communication).

Prospective survey sites for potential biological control agents in Ethiopia were identified based on herbarium records (Kew Botanic Gardens, , Ethiopian National Herbarium) and a CLIMEX model (Senaratne et al., 2006). Surveys were conducted at 22 sites in July 2014, at 41 sites in December 2015, at 26 sites in November 2016, and at 14 sites in November 2017 in the Adama, Arba Minch, Awash, Dire Dawa, Harar, Melka Werer, Mille, Woldia, Mekele and Shewa Robit areas in Ethiopia (Dhileepan et al., 2018).

Acaciothrips ebneri was widespread on both young and mature prickly acacia plants in northern and eastern regions of Ethiopia (Figure 9). *Acaciothrips ebneri* was only found on *V. nilotica* subsp. *tomentosa* and *V. nilotica* subsp. *indica* at most sites where the subspecies were present, but not on *V. nilotica* subsp. *leiocarpa* (Dhileepan et al., 2018). The gall-inducing thrips did not occur on other *Vachellia* species, including *Vachellia etbaica* (Schweinf.) Kayl. & Boatwr. and *Vachellia seyal* (Delile) P.J.H. Hurter which co-occurred at some sampling sites in Ethiopia (Dhileepan et al., 2018).

Figure 9. Several *A. ebneri* galls on a *Vachellia nilotica* subsp. *indica* plant in Ethiopia.

2.5 Related species to the agent and a summary of their host range

There are 3500 known specie in the family Thysanoptera. The family is classified into two subfamilies, the Idolothripinae containing 80 genera with *ca.* 700 species and the Phlaeothripidae containing 370 genera with *ca.* 2800 species. *Acaciothrips* is a genus in Phlaeothripidae (Mound, 2020). It is a monotypic taxon reported only on *V. nilotica* in Africa.

2.6 Proposed source of the insect

Mature galls containing adults of *A. ebneri* were imported (IP15015295) twice from Ethiopia in 2015 and 2016, respectively (Table 5). The adults were sourced by Dr K. Dhileepan and identified by Dr Laurance Mound (CSIRO, Canberra). Voucher specimens have been deposited in the Australian National Insect Collection. A colony was established in quarantine from both importations and all biological and host specificity testing refer to individuals descended from these two importations (Table 5).

Colonies of thrips will be maintained in this quarantine facility until a decision on this release application is made by the regulators.

Table 5. Permits and importations made for *Acaciothrips ebneri*.

PERMIT	DATE	SPECIES	LOCATION	IMPORTATIONS
IP15015295	20/12/2015	*Acaciothrips ebneri*	Ethiopia	1000 adults were collected from Ethiopia and used for colony establishment and host specificity testing.
IP15015295	26/11/2016	*Acaciothrips ebneri*	Ethiopia	600 adults were collected from Ethiopia and used for colony and host specificity testing.

2.7 Possible interactions with existing biological control programs (of same or related targets, and other targets)

Two agents, a seed feeding *Bruchidius sahlbergi* Schilsky (Lepidoptera: Bruchidae) from Pakistan and a leaf-feeding moth *Chiasmia assimilis* Warren (Lepidoptera: Geometridae) from Kenya and South Africa are established on prickly acacia in Australia. There has been no agent released targeting prickly acacia shoot tips and meristem. *Acaciothrips ebneri* will feed on the axillary and terminal buds externally and will complement the two other agents (*Bruchidius sahlbergi* and *Chiasmia assimilis*) that currently occur in the field.

2.8 Details on the quarantine facility and methods of containment

Mature galls on *V. nilotica* subsp. *indica*, harbouring *A. ebneri* were imported into the high security quarantine facility at Ecosciences Precinct, Brisbane, Australia. This is a Department of Agriculture, Water Resources and the Environment (DAWE) approved facility (Q2274, AA classes 5.3, 6.1 and 7.3). Insects were transferred into the quarantine facility in double sealed containers and then kept in insect-proof cages in the quarantine glasshouse or controlled environment rooms. A colony of *A. ebneri* was established and is being maintained on *V. nilotica* subsp. *indica* within insect-proof cages in the high security quarantine facility.

2.9 The agent's potential for control of the target

Thrips can induce rosette galls on seedlings, juvenile and mature plants and were widespread in the northern and eastern regions of Ethiopia (Dhileepan et al., 2018). Galls can be observed on shoot tips and sprouting axillary buds with the rudiments of leaves near the feeding sites converted into bunches of rosette-like structures. Adult thrips feed on axillary and terminal buds and early signs of gall induction can be seen within a week under optimal quarantine conditions (Figure 8). The thickened and poorly differentiated leaves therefore become crowded at shoot terminals which do not spread out, but curl inwards. With maturation, basal portions of the petioles, rachis, and leaf blades thicken, due to hypertrophied cells. As the gall development progresses, the entire apical regions of the galled shoots become completely deformed (Figure 8 and 10) resulting in shoot-tip dieback. Severe infestation by the gall-inducing thrips in the native range resulted in stunted plant growth in juvenile plants and no flowering and fruiting in infested shoots of mature plants.

A model was developed using the Composite Match Index in Climex Simulator Application 4.0.2.0 to predict regions in Australia that most closely match climate in Ethiopia where *A. ebneri* is abundant (Figure 11). The model suggested that central and western Queensland, central Northern Territory and coastal areas in Western Australia which have major infestations of prickly acacia, should be highly suitable for *A. ebneri* establishment.

Figure 10. Galls induced by *Acaciothrips ebneri* in quarantine in Brisbane, Australia.

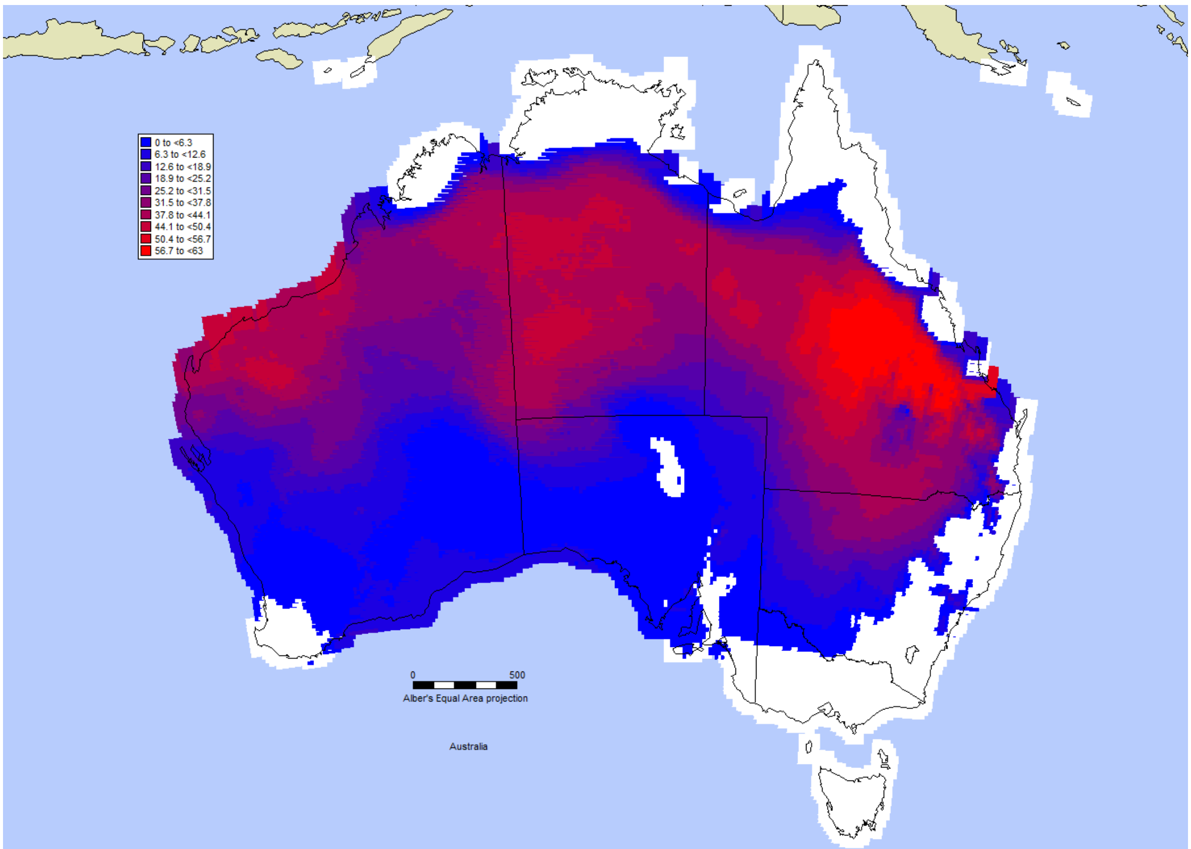

Figure 11. Climate suitability prediction for *Acaciothrips ebneri* using the Composite Match Index in CLIMEX.

2.10 When and how initial release will be made

A protocol will be prepared for approval from DAWE entomologists, for moving *A. ebneri* out of quarantine. Pending approval adults will be carefully inspected under a microscope to ensure no other associated organisms (parasites or pathogens) are associated with *A. ebneri*. Adults will be transferred into cages with *V. nilotica* subsp. *indica* plants in a non-quarantine glasshouse for mass rearing following the conditions of the DAWE release permit.

Acaciothrips ebneri galls with adults and larvae established on *V. nilotica* subsp. *indica* plants in the non-quarantine glasshouse will be transferred to the Tropical Weeds Research Centre in Charters Towers for mass-rearing and field release. Mature galls from infested plants will be collected and field released in prickly acacia infestations in north and central Queensland in partnership with Prickly Acacia Alliance Forum and other NRM groups. After releases in Queensland, additional releases will be made in the Northern Territory and Western Australia.

3 Host-specificity testing

3.1 Introduction

The field (ecological) host range of *A. ebneri* in Ethiopia was investigated by surveying co-occurring non-target plant species in the field. The fundamental (physiological) host range of *A. ebneri* was studied in quarantine facilities in Pretoria, South Africa and Brisbane, Australia following no-choice host specificity testing.

3.2 Surveys of plant use under natural conditions in the native range

Field surveys were conducted at 22 sites in July 2014, 41 sites in December 2015, 26 sites in November 2016 and 14 sites in November 2017 in the Adama, Arba Minch, Awash, Dire Dawa, Harar, Melka Werer, Mille, Woldia, Mekele and Shewa Robit areas in Ethiopia (Figure 12) (Dhileepan et al., 2018). At each survey site, the occurrence of the *A. ebneri* was recorded, along with details on the *Vachellia nilotica* subspecies status, plant growth stages and co-occurring vegetation (i.e., other *Vachellia* species). Surveys identified natural populations of three *V. nilotica* subspecies (*tomentosa*, *indica* and *leiocarpa*) in Ethiopia. *Acaciothrips ebneri* was only found on *V. nilotica* subsp. *tomentosa* (79% of sites; n = 23) and *V. nilotica* subsp. *indica* (96% of sites; n = 24), both subspecies with moniliform fruits, but not on *V. nilotica* subsp. *leiocarpa* (n = 8), a subspecies with non-moniliform fruits. Furthermore, *A. ebneri* did not occur on other co-occurring *Vachellia* species including *Vachellia etbaica* (Schweinf.) Kayl. & Boatwr., *Vachellia abyssinica* (Hochst. ex. Benth) Kyal.&Boatwr, *Vachellia seyal* (Eelile) P.J.H. Hurter (Dhileepan et al., 2018).

Figure 12. Surveys for *Acaciothrips ebneri* on *Vachellia nilotica* subsp. *indica* in Ethiopia.

3.3 Host specificity testing under laboratory conditions

3.3.1 Test plant list

Once a member of the genus *Acacia*, prickly acacia is now part of the genus *Vachellia*. *Vachellia* is now considered to be more closely related to species within the former Mimoseae tribe than members of *Acacia s.s.* (Taylor and Dhileepan, 2019). A plant list for testing potential biological control agents of *V. nilotica* subsp. *indica* (prickly acacia) was developed based on currently accepted phylogenetic information available in the literature, following the modernisation of the centrifugal-phylogenetic method (Table 6) (Taylor and Dhileepan, 2019). *V. nilotica* belongs to the Fabaceae, Fabales, a basal angiosperm order.

The test list for *Acaciothrips ebneri* is based on the list developed by Taylor and Dhileepan (2019). A proposed host specificity test list was submitted to the Australian Government DAWE for feedback in August 2019 (Shi et al., 2019). No recommendations were received to vary this test list. The host test list contains 59 species and reflects currently accepted phylogenetic relationships while considering the species richness of the various groups in Australia (Table 6). The approximate degree of phylogenetic separation based on the currently known taxonomy of this group is also presented in Table 6. The goal of host-specificity testing is to assess a representative sample of native or economically important species that are close phylogenetically to the target species, with fewer replicate species tested at greater phylogenetic distance from the target species. Eight native *Vachellia* species are included in the list along with the naturalised *V. farnesiana*. We engaged a taxonomist to collect seeds or seedlings of all native *Vachellia* species, including *V. suberosa* from the field. Unfortunately, none of the *V. suberosa* seeds germinated and no seedlings survived in our glasshouse. Given the aforementioned goal of host-specificity testing we believe a test of 8 of the 9 native *Vachellia* species will provide a strong indication of risks of this agent to non-target congeners.

Similar to past test lists, we have included species from each of the four native Mimoseae genera. Three species of *Neptunia* are included because of their close relationship to *Vachellia*. Two species of other basal Mimoseae are included in the list (*Leucaena leucocephala*, which has become widely naturalised in Queensland and is an important pasture forage plant and *Dichrostachys spicata*) as well as two derived Mimoseae (*Adenanthera abrosperma* and *Entada phaseoloides* (L.) Merr.).

Acacia is approximately five degrees apart from *Vachellia*; given this and their high economic and environmental values in Australia, 29 Acacia species were selected in the host test list with all sections represented. Outside of the former Mimosoideae, the test list is greatly reduced compared to past test lists. As Mimosoideae has merged with Caesalpinoideae (s.s.) to form the new Caesalpinoideae (s.l.), a native and an ornamental species from the Caesalpinoideae (s.s.) have been included in the test list: the widespread species *Senna artemisioides* (Gaudich. ex DC) Randell and the common exotic street tree *Delonix regia* (Boj. ex Hook.) Raf. We have also included several species from other legume subfamilies including the large subfamily Faboideae (syn. Papilionoideae). No non-leguminous species are included in the new test list.

3.3.2 Source of prickly acacia (*Vachellia nilotica* subsp. *indica*) and other test plants

Seeds of prickly acacia were field collected in Bowen and Hughenden in Queensland, Australia. Seeds were soaked in hot water for 24-48 hours before planting. Once seeds were swollen, they were then transferred into a germination tray filled with potting mix. All individual seedlings were transferred into single pots filled with potting mix and maintained in a glasshouse set at 27 ºC ± 2 ºC with a 12:12 (light:dark) hour photoperiod for 1 to 3 years before use in host-specificity testing.

Most non-target test plant species were grown from seeds, which were bought from a range of different commercial nurseries in Australia (e.g., Nindethana Seed Service, Fair Dinkum Seeds, Herbalistics Plants Seeds Herbs, AustraHort Seed Merchants and Burringbar Rainforest Nursery) as well as those collected from the field in northern Queensland (Table 6).

3.4 Host specificity testing methods

3.4.1 Background information

There are three basic host specificity assessments: no-choice and choice tests (both conducted in quarantine), and field studies (usually choice trials conducted in the native range). It is widely accepted that no-choice testing provides the best initial screening of plants due to the conservative nature of the test. In no-choice trials, the agent is confined with a test species until death; the agent either attempts to feed (or reproduce) or dies. No choice tests identify the fundamental host range, which is the range of plants species that the agent is potentially capable of utilising. They do not consider behavioural or ecological factors that can affect host selection under natural conditions (realised host range). The probability of an insect rejecting a test plant species in a no-choice trial but accepting it in the field is negligible. In contrast, the probability of an insect accepting a test plant species under the confined conditions of a no-choice trial but rejecting it in the field (i.e., false positive) is widely considered to be high. It is for this reason that test species identified under no-choice conditions to be potential hosts (i.e., supporting complete development of the agent) are then used in choice trials with the target or true host. Choice trials are useful for exploring host preference of test plant species identified as potential hosts in no-choice tests.

To mitigate the effects of factors like prior learning or feeding experience which can influence the host selection behaviour of the test insect, in all host specificity trials only newly emerged adults were used.

3.4.2 South African no-choice trial

Based on previous field observation, a preliminary screening trial was undertaken to assess the acceptability of the Australian and southern African subspecies of *V. nilotica*. *Acaciothrips ebneri* was collected in Ethiopia and exported into a quarantine facility at the Agricultural Research Council – Plant Protection Research Institute at Pretoria, South Africa in July 2014. *V. nilotica* subsp. *indica* plants were grown from seeds sourced from Australia. *V. nilotica* subsp. *kraussiana* plants were grown from seeds sourced locally in South Africa.

During the preliminary screening, 20 adults (unknown sex) of *A. ebneri* were released onto potted *V. nilotica* subsp. *indica* and *V. nilotica* subsp. *kraussiana* in insect-proof cages (one plant per cage) with five replications of each subspecies. The trial was continued for four weeks. At the end of the trial, the number of galls induced by *A. ebneri* was recorded.

3.4.3 Australian no-choice trial

Based on results from preliminary host specificity tests in South Africa, *A. ebneri* from Ethiopia was imported into a high security quarantine facility (28 ± 2 ºC; 60% RH) at Brisbane, Australia for colony establishment and host specificity tests. A colony of gall-inducing thrips was established and maintained on *V. nilotica* subsp. *indica* grown from seeds collected from north Queensland. Test plants were sourced either as seeds and potted plants from nurseries or sourced as field collected seeds (Table 6). No choice tests were conducted by releasing 10 pairs (10 males and 10 females) of newly emerged mating adult thrips into insect-proof cages containing one potted test plant or a control prickly acacia plant. A minimum of five replications were conducted for each test species and at least one *V. nilotica* subsp. *indica* plant was included as a control for each replicate (Figure 13). For each test plant species, the testing can take from four to eight weeks, depending on how long the adults survived on the plants. When no living thrips were found (i.e. when all adults had died) on the test plant, the trial was continued for an additional 2 weeks. During each trial, adult survival, gall induction (number of galls) and development (size of galls) were monitored and recorded. At the end of testing, the number of galls were harvested from the target plant and stored in a freezer. A hand magnifier was used to check for feeding damage if any on shoot tips of non-target plants. Representative galls of different sizes [small (1×1 cm - 2×1 cm), medium (3×2 cm) and large (5×3 cm)] from control plants were selected and dissected to count the thrips population in each gall. In total 59 test species were screened for host specificity testing (Table 6).

As there was no visible adult feeding, gall induction, egg laying and larval development on any of the non-target test plant species, choice tests were not conducted.

3.5 Statistical analysis

For the no-choice trials, adult survival duration was recorded on both target and non-target plants on Mondays, Wednesdays and Fridays during the host-specificity trial. One-way ANOVA was used to compare the duration of survival of adults on all test species. All analyses were conducted using R4.0.2 via the RStudio Version 1.3.1058. Tukey's Post Hoc Test was used to analyse any pairwise significant differences among the test species.

3.6 Host specificity testing results

3.6.1 South African no-choice trial

In the preliminary study, *A. ebneri* only induced galls on Australian exotic prickly acacia (*V. nilotica* subsp. *indica*) with moniliform fruit pods and not on the southern African prickly acacia (*V. nilotica* subsp. *kraussiana*) with non-moniliform fruit pods. No adults survived and there were no galls induced on the southern African endemic prickly acacia (*V. nilotica* subsp. *kraussiana*).

3.6.2 Australian no-choice trial

Adult feeding, gall induction, egg laying and larval development only occurred on Australian prickly acacia (*V. nilotica* subsp. *indica*), and all replicates of control plants showed gall formation by *A. ebneri*. There were no visible feeding symptoms, gall induction, egg laying and larval development on any of the non-target species during the host specificity testing (Table 7). An average of 25 ± 2.1 galls were found on the target plant (Table

7). The number of galls formed was dependent on the number of shoots/shoot tips on the control plants. The variability in the number of galls in control plants was due to the use of prickly acacia plants with varying number of shoots and shoot tips. It was not possible to use prickly acacia with same or similar number of shoots and shoot tips, due to natural variability in plant growth and branching under glasshouse conditions.

It was difficult to count the number of progeny as the entire life cycle is completed inside the gall, therefore larval development was quantified by periodically opening samples of galls (Table 7). On prickly acacia, approximately 1891 ± 41 eggs were laid and 1785 ± 35 progeny developed during the four to eight weeks host specificity testing period (Table 7). The longevity of adults on prickly acacia was 76 ± 10 days. In contrast, on non-target test plant species, the average adult longevity was an order of magnitude and significantly lower than on prickly acacia (Figure 14; Appendix 2). On non-target plants, adult longevity was the same or lower than on the negative controls (no test plants, only water or honey; Figure 14).

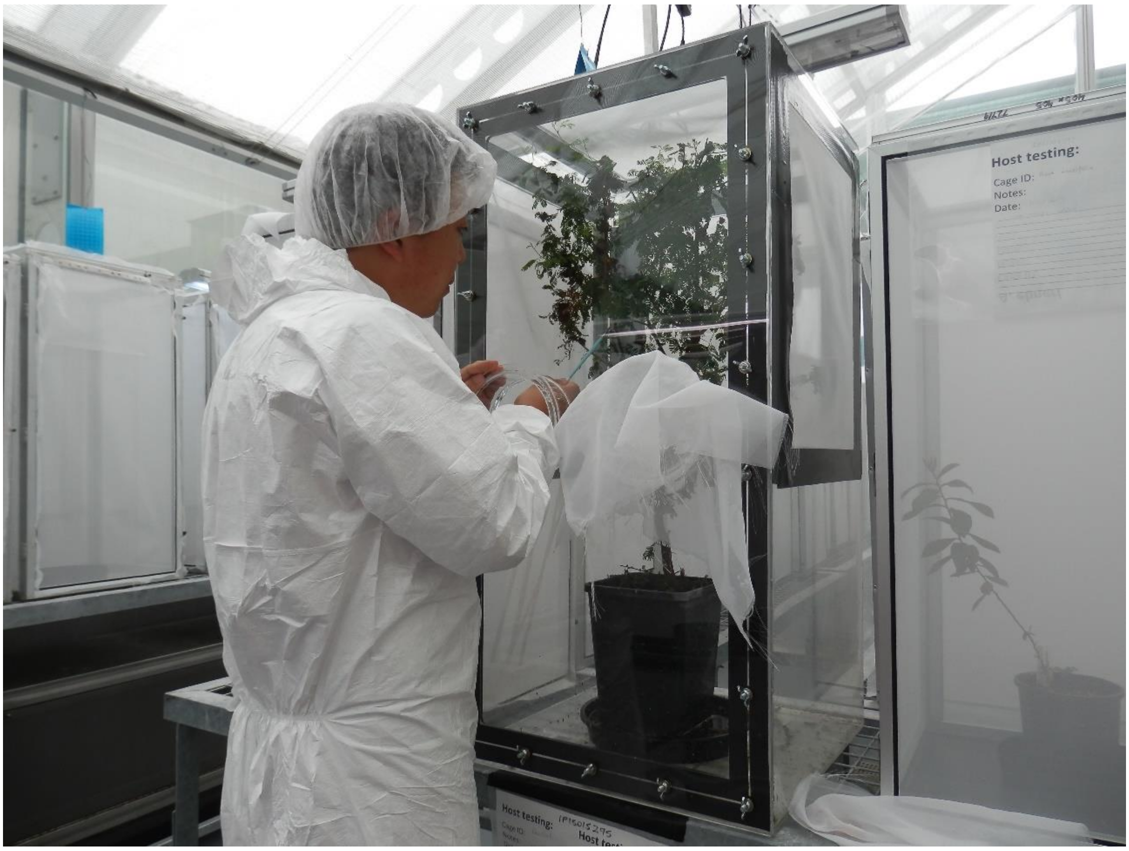

Figure 13. Host specificity testing trials within the quarantine facility, Ecosciences Precinct, Brisbane.

Table 6. List of test plant species involved in the host specificity testing of *Acaciothrips ebneri* under quarantine conditions in Australia. **Status: TW = target weed, N = native, I = invasive, O = ornamental, C = crop, E = exotic, non-invasive.**

Species	Status	Degree of phylogenetic separation	Sources
Order Fabales			
Family Fabaceae Lindl.			
Subfamily Caesalpinioideae DC			
Mimosoid clade (= Mimosoideae de Candolle)			
Genus *Vachellia*			
V. nilotica subsp. *indica* (Benth.) Kyal. & Boatwr	TW	0	Bowen, QLD
V. bidwillii (Benth.) Kodela	N	0	Nindethana Seed Service
V. clarksoniana (Pedley) Kodela	N	0	Einasleigh (QLD),Ngukurr
V. ditricha (Pedley) Kodela	N	0	Georgetown, QLD
V. douglasica (Pedley) Kodela	N	0	Douglas-Daly, NT
V. farnesiana (L.) Wight & Arn.	I/N	0	Nindethana Seed Service
V. pachyphloia (W.Fitzg.) Kodela	N	0	Katherine, NT
V. pallidifolia (Tindale) Kodela	N	0	Mataranka, NT
V. sutherlandii (F.Muell.) Kodela	N	0	Nindethana Seed Service
V. valida (Tindale & Kodela) Kodela	N	0	Katherine, Mataranka, NT
Basal 'Mimoseae'			
Dichrostachys spicata (F.Muell.) Domin	N	1	Australian Seed
Leucaena leucocephala (Lam.) de Wit	I	1	Progressive Seeds
Neptunia dimorphantha Domin	N	1	B & T World Seeds
N. monosperma F.Muell. ex Benth.	N	1	B & T World Seeds
N. major (Benth.) Windler	N	1	B & T World Seeds
Derived 'Mimoseae'			
Adenanthera abrosperma F.Muell.	N	2	Nindethana Seed Service
Entada phaseoloides (L.) Merr.	N	2	Herbalistics
Genus *Senegalia*			
Senegalia senegal (L.) Britton	E	3	Unknown
'Ingeae'			
Albizia lebbeck (L.) Benth	N	4	Australian Seed
Parachidendron pruinosum (Benth.) I.C.Nielsen	N	4	Burringbar Nursery
Genus *Acacia*			
Section *Botrycephalae*			
A. baileyana F.Muell.	N	5	Burringbar Nursery
A. cardiophylla A.Cunn. ex Benth.	N	5	Nindethana Seed Service
A. chinchillensis Tindale	N	5	Nindethana Seed Service
A. deanei subsp. *deanei* (R.T.Baker) M.B.Welch, Coombs & McGlynn	N	5	Nindethana Seed Service
A. decurrens Willd.	N	5	Nindethana Seed Service
A. glaucocarpa Maiden & Blakely.	N	5	Nindethana Seed Service
A. irrorata Sieber ex Spreng.	N	5	Burringbar Nursery
A. oshanesii F.Muell. & Maiden	N	5	Nindethana Seed Service
A. spectabilis A.Cunn. ex Benth.	N	5	Nindethana Seed Service
Section *Juliflorae*			
A. aneura F.Muell. ex Benth.	N	5	Nindethana Seed Service
A. cambagei R.T.Baker	N	5	AustraHort Seeds

A. chisholmii F.M.Bailey	N	5	Nindethana Seed Service
A. holosericea A.Cunn. ex G.Don	N	5	Nindethana Seed Service
A. shirleyi Maiden	N	5	AustraHort Seeds
Section *Lycopodiifoliae*			
A. spondyllophylla F.Muell.	N	5	Nindethana Seed Service
Section *Phyllodineae*			
A. conferta A.Cunn. ex Benth.	N	5	Nindethana Seed Service
A. falcata Willd.	N	5	Burringbar Nursery
A. fimbriata A.Cunn. ex G.Don	N	5	Nindethana Seed Service
A. podalyriifolia A.Cunn. ex G.Don	N	5	Burringbar Nursery
A. salicina Lindl.	N	5	Nindethana Seed Service
A. victoriae Benth.	N	5	Nindethana Seed Service
Section *Plurinerves*			
A. complanata A.Cunn. ex Benth.	N	5	Burringbar Nursery
A. coriacea DC.	N	5	Nindethana Seed Service
A. excelsa Benth.	N	5	Nindethana Seed Service
A. flavescens A.Cunn. ex Benth.	N	5	Nindethana Seed Service
A. simsii A.Cunn. ex Benth.	N	5	Nindethana Seed Service
A. stenophylla A.Cunn. ex Benth.	N	5	Nindethana Seed Service
Section *Pulchellae*			
A. drummondii Lindl.	N	5	Nindethana Seed Service
A. pulchella R.Br.	N	5	AustraHort Seeds
***Peltophorum* clade**			
Delonix regia (Boj. ex Hook.) Raf.	OI	6	AustraHort Seeds
Cassieae clade			
Senna artemisioides subsp. *helmsii* (Symon) Randell	N	7	Australian Seed
Subfamily Faboideae Rudd			
Hardenbergia violacea (Schneev.) Stearn	N	8	Nindethana Seed Service
Castanospermum australe A.Cunn & C.Fraser ex Hook.	N	8	Nindethana Seed Service
Swainsona galegifolia (Andrews) R.Br	N	8	Nindethana Seed Service
Hovea acutifolia A.Cunn. ex G.Don	N	8	Nindethana Seed Service
Phaseolus vulgaris L.	C	8	Mr Fothergill's Seeds
Pisum sativum L.	C	8	Mr Fothergill's Seeds
Vigna unguiculata L.	C	8	Mr Fothergill's Seeds
Subfamily Dialioideae LPWG			
Petalostylis labicheoides R.Br.	N	9	Herbalistics
Subfamily Cercidoideae LPWG			
Bauhinia hookeri F.Muell.	N	10	Nindethana Seed Service

Table 7. Results for no-choice host specificity testing conducted under quarantine conditions in Australia for *Acaciothrips ebneri*.

Species	No. of Reps	Gall induction (Y/N, mean ± SE of galls)	Egg laying (Y/N, mean ± SE of eggs)	Larval development (Y/N, mean ± SE of progeny)	Adult emergence (Y/N. mean ± SE of adults)
V. nilotica subsp. *indica* (Benth.) Kyal. & Boatwr.	65	Y, 25 ± 2.1	Y, 1891 ± 41	Y, 1785 ± 35	Y, 1236 ± 55
V. bidwillii (Benth.) Kodela	5	N	N	N	N
V. clarksoniana (Pedley) Kodela	5	N	N	N	N
V. ditricha (Pedley) Kodela	7	N	N	N	N
V. douglasica (Pedley) Kodela	5	N	N	N	N
V. farnesiana (L.) Wight & Arn.	6	N	N	N	N
V. pachyphloia (W.Fitzg.) Kodela	5	N	N	N	N
V. pallidifolia (Tindale) Kodela	5	N	N	N	N
V. sutherlandii (F. Muell.) Kodela	5	N	N	N	N
V. valida (Tindale & Kodela) Kodela	6	N	N	N	N
Dichrostachys spicata (F. Muell.) Domin	5	N	N	N	N
Leucaena leucocephala (Lam.) de Wit	5	N	N	N	N
Neptunia dimorphantha Domin	5	N	N	N	N
Neptunia major (Benth.) Windler	5	N	N	N	N
Neptunia monosperma F. Muell. ex Benth.	5	N	N	N	N
Adenanthera abrosperma F. Muell.	5	N	N	N	N
Entada phaseoloides (L.) Merr.	5	N	N	N	N
Senegalia senegal (L.) Maslin	5	N	N	N	N
Albizia lebbeck (L.) Benth.	5	N	N	N	N
Parachidendron pruinosum (Benth.) I. C. Nielsen	5	N	N	N	N
A. baileyana F. Muell.	5	N	N	N	N
A. cardiophylla A. Cunn. ex Benth.	5	N	N	N	N
A. chinchillensis Tindale	5	N	N	N	N
A. deanei subsp. *deanei* (R. T. Baker) M. B. Welch, Coombs & McGlynn	5	N	N	N	N
A. glaucocarpa Maiden & Blakely	6	N	N	N	N
A. irrorata Sieber ex Spreng.	5	N	N	N	N
A. oshanesii F. Muell. & Maiden	5	N	N	N	N
A. spectabilis A. Cunn. ex Benth.	7	N	N	N	N
A. decurrens Wild.	5	N	N	N	N
A. aneura F.Muell. ex Benth.	7	N	N	N	N
A. cambagei R.T.Baker	6	N	N	N	N
A. chisholmii F.M.Bailey	5	N	N	N	N
A. holosericea A.Cunn. ex G.Don	6	N	N	N	N
A. shirleyi Maiden	6	N	N	N	N
A. spondyllophylla F.Muell.	5	N	N	N	N
A. conferta A.Cunn. ex Benth.	6	N	N	N	N
A. falcata Willd.	5	N	N	N	N
A. podalyriifolia A.Cunn. ex G.Don	6	N	N	N	N
A. salicina Lindl.	6	N	N	N	N
A. victoriae Benth.	5	N	N	N	N
A. fimbriata A.Cunn. ex G.Don	5	N	N	N	N
A. complanata A.Cunn. ex Benth.	5	N	N	N	N
A. coriacea DC.	6	N	N	N	N
A. excelsa Benth.	5	N	N	N	N
A. simsii A.Cunn. ex Benth.	7	N	N	N	N
A. stenophylla A.Cunn. ex Benth.	5	N	N	N	N
A. flavescens A.Cunn. ex Benth.	5	N	N	N	N
A. drummondii Lindl.	5	N	N	N	N
A. pulchella R.Br.	7	N	N	N	N
Delonix regia (Boj. ex Hook.) Raf.	5	N	N	N	N
Senna artemisioides subsp. *helmsii* (Symon) Randell	5	N	N	N	N
Hardenbergia violacea (Schneev.) Stearn	5	N	N	N	N
Castanospermum australe A.Cunn & C.Fraser ex Hook.	5	N	N	N	N
Swainsona galegifolia (Andrews) R.Br	5	N	N	N	N
Hovea acutifolia A.Cunn. ex G.Don	5	N	N	N	N
Phaseolus vulgaris L.	5	N	N	N	N
Pisum sativum L.	5	N	N	N	N
Vigna unguiculata (L.) Walp.	5	N	N	N	N
Petalostylis labicheoides R.Br.	5	N	N	N	N
Bauhinia hookeri F.Muell.	5	N	N	N	N

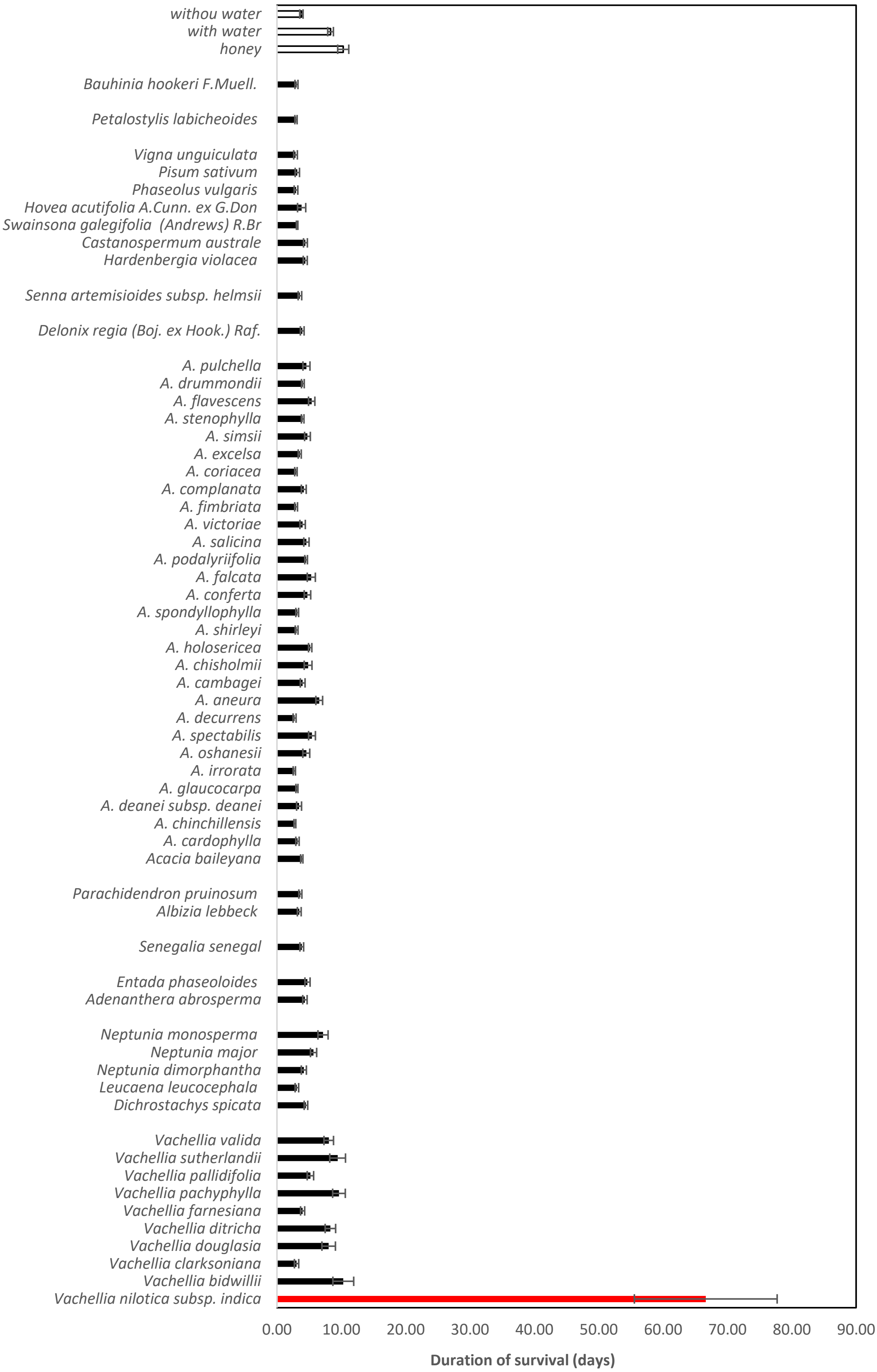

Figure 14. Duration of survival (days; mean ± sem) (i.e., longevity) of adult gall-inducing thrips on target (red bar) and non-target plants (black bars) during no-choice host specificity testing. Transparent bars represent negative control treatments, (i.e. no plant presented to the thrips, either honey, water, or no water).

4 Discussion

The risk of *A. ebneri* feeding, inducing galls and developing on representative non-target plant species of increasing phylogenetically distance to the target weed prickly acacia was assessed using no-choice tests in a quarantine glasshouse (Table 6). These confirmed field observations in the native range that *A. ebneri* is highly host specific. Gall induction, egg laying and larval development of *A. ebneri* occurred only on *V. nilotica* subsp. *indica*. No other test species were suitable hosts for *A. ebneri*. Adult survival on non-target species was substantially lower than on the target weed. In its native range, *A. ebneri* is only observed on *V. nilotica* subsp. *indica* and *Vachellia nilotica* subsp. *tomentosa*, not on any other co-occurring *Vachellia* species (Dhileepan et al., 2018). During host-specificity testing in the quarantine glasshouse, *A. ebneri* adults fed and induced galls, laid eggs and the emerging larvae completed development only on prickly acacia, the target weed. No adult feeding (no visible feeding damage), gall induction, egg laying and progeny development occurred on any non-target test plant species. Not surprisingly, adult survival was poor on all non-target plant species. As there was no non-target feeding (no visible feeding damage), gall induction, egg laying or larval development on any of the non-target plants tests, choice tests were not conducted.

A total of 59 non-target species were tested in no-choice trials in the quarantine glasshouse, based on a recent host test list developed using an updated centrifugal-phylogenetic method (Taylor and Dhileepan, 2019). Notably, the species tested included 10 *Vachellia* species present in Australia, comprising eight native species and two naturalised species. There was no visible adult feeding damage, gall induction, egg laying or larval development by the thrips on these species, indicating that it does not pose a risk to any of these non-target Australian congeners of target weed.

There was also no adult visible feeding, gall induction, egg laying and larval development on any of the Australian native *Acacia* species tested. Adult survival of *A. ebneri* on the Australian native *Acacia* species was also poor (Figure 8), although some adults lived for a similar amount of time as adults provided with just honey, which was likely due to them feeding on the extrafloral nectaries (Boughton, 1981; Gonzalez and Marazzi, 2018).

This gall-inducing thrips, both adults and larvae, feeds within the galls on the nutritive cells that form the inner-most layer of the galls. Gall-induction on shoot terminals and axillary meristems results in the telescoping of the shoot axis, which inhibits normal shoot growth within 1-3 weeks. The overall colour changes from green to purple red. The developing galls act as nutrient sinks drawing nutrients from other parts of the plant, thereby imposing physiological stresses on the plant. Due to gall development, the terminal buds of shoots are completely destroyed, often resulting in shoot-tip dieback.

Phytophagous thrips have been successfully used as weed biological control agents previously (Cock, 1982; Cuda et al., 2009; McConnachie and McKay, 2015; Memmott et al., 1998; Reimer and Beardsley Jr, 1989; Wheeler et al., 2018). Gall-inducing thrips are known to be highly host specific (Raman 1984). The gall-inducing thrips (*A. ebneri*) was prioritised based on its suitability for arid northern Australian climatic conditions, field host specificity and damage potential (Dhileepan et al., 2018). This is the first time a rosette-gall inducing thrips has been evaluated as a weed biological control agent for any weed around the world.

Native range surveys in Ethiopia, recorded *A. ebneri* only on *V. nilotica* subsp. *indica* and subsp. *tomentosa* with moniliform pods, and not on *V. nilotica* subsp. *leiocarpa* with non-moniliform pods (Dhileepan et al., 2018). This observation further highlights that the gall-inducing thrips is highly host specific at the subspecies level.

This was confirmed under quarantine glasshouse conditions in Pretoria, South Africa, where *A. ebneri* induced galls on *V. nilotica* subsp *indica*, but not on the South Africa endemic species *V. nilotica* subsp. *kraussiana* with non-moniliform fruit pod. Globally, there are four *V. nilotica* subspecies with moniliform fruits (all native to Africa and Asia) – *V. nilotica* subsp. *indica*, *V. nilotica* subsp. tomentosa, *V. nilotica* subsp. *nilotica*, and *V. nilotica* subsp. *cupressiformis* (Table 1). *Vachellia nilotica* subsp. *indica* (target weed) is the only subspecies occurring in Australia. All *V. nilotica* subspecies are prohibited species in Australia, and hence not likely to be introduced into Australia (Comben et al., 2021).

Based on the evidence, it seems reasonable to conclude that the gall-inducing thrips (*A. ebneri*) is host specific and that the risk of non-target damage appears very low in Australia. A CLIMEX model predicts that the entire inland region of northern Australia, including the Mitchell grass downs area where prickly acacia is a serious problem, is climatically suitable for *A. ebneri* (Figure 11). The gall-inducing thrips has also shown to be highly damaging to prickly acacia seedlings and juvenile plants, as observed in the field in Ethiopia and in quarantine studies. If approved for field release, *A. ebneri* will complement the two existing biological control agents, by reducing the vigour of prickly acacia seedlings and juvenile plants and stop flowering and fruit production in galled shoots.

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Appendices

Appendix 1. Longevity trials comparing females and males of *Acaciothrips ebneri*.

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Female	7	778	111.1429	101.4762
Male	7	288	41.14286	113.8095

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	17150	1	17150	159.3232	2.75E-08	4.747225
Within Groups	1291.714	12	107.6429			
Total	18441.71	13				

Appendix 2. Survival duration of *Acaciothrips ebneri* on both non-target and target species during no-choice host specificity testing.

Analysis of Variance Table

```
Analysis of Variance Table

Response: log10(longevity)
           Df Sum Sq  Mean Sq F value    Pr(>F)
Species    62 14.902 0.240349  5.0209 < 2.2e-16 ***
Residuals 267 12.781 0.047869
Signif. Codes : 0 '***' 0.01'**' 0.01 '*' 0.05 '.' 0.1 '' 1

Study: m2 ~ "Species"

LSD t Test for log10(longevity)

Mean Square Error:  0.04786935

Species,  means and individual ( 95 %) CI

                        log10.longevity.        std  r       LCL       UCL
A.aneura                      0.7641342 0.23960744  7 0.6013167 0.9269516
A.baileyana                   0.5652191 0.16171294  5 0.3725709 0.7578673
A.cambagei                    0.5630689 0.19761968  6 0.3872060 0.7389318
A.cardophylla                 0.4654800 0.21375082  5 0.2728318 0.6581281
A.chinchillensis              0.4355529 0.10241972  5 0.2429047 0.6282011
A.chisholmii                  0.6177298 0.28855456  5 0.4250816 0.8103779
A.complanata                  0.5698302 0.24744640  5 0.3771820 0.7624784
A.conferta                    0.5865620 0.32858806  6 0.4106991 0.7624250
A.coriacea                    0.3995729 0.25812094  6 0.2237100 0.5754359
A.deanei                      0.4900770 0.22831739  5 0.2974288 0.6827252
A.decurrens                   0.3992643 0.21794655  6 0.2234014 0.5751272
A.drummondii                  0.5629266 0.20766388  5 0.3702785 0.7555748
A.excelsa                     0.5313920 0.14499642  5 0.3387439 0.7240402
A.falcata                     0.7182854 0.10245067  5 0.5256372 0.9109336
A.fimbriata                   0.4253898 0.23085381  5 0.2327417 0.6180380
A.flavescens                  0.7189617 0.12334037  5 0.5263135 0.9116099
A.glaucocarpa                 0.5060568 0.09768708  5 0.3134087 0.6987050
A.holosericea                 0.6932613 0.14190025  6 0.5173984 0.8691243
A.irrorata                    0.3869431 0.23715030  5 0.1942949 0.5795913
```

A.oshanesii	0.6330340	0.16489032	5	0.4403858	0.8256821
A.podalyriifolia	0.6244297	0.19058496	6	0.4485668	0.8002926
A.pulchella	0.5509398	0.31331982	7	0.3881223	0.7137572
A.salicina	0.6381390	0.15229613	6	0.4622761	0.8140019
A.shirleyi	0.4612160	0.17544352	6	0.2853530	0.6370789
A.simsii	0.5733562	0.34458363	7	0.4105387	0.7361736
A.spectabilis	0.6985504	0.22595826	7	0.5357330	0.8613678
A.spondyllophylla	0.4888739	0.10230868	5	0.2962257	0.6815221
A.stenophylla	0.5654739	0.19912528	5	0.3728257	0.7581220
A.victoriae	0.5708183	0.17721103	5	0.3781701	0.7634665
Adenanthera_abrosperma	0.6099862	0.18749394	5	0.4173380	0.8026344
Albizia_lebbeck	0.5213932	0.14298128	5	0.3287450	0.7140413
alone	0.5294613	0.05136090	3	0.2807535	0.7781690
Bauhinia_hookeri	0.4592835	0.16193606	6	0.2834206	0.6351464
Castanospermum_australe	0.5802470	0.27918534	5	0.3875988	0.7728951
Delonix_regia	0.5785557	0.13366394	5	0.3859076	0.7712039
Dichrostachys_spicata	0.6469925	0.09048466	5	0.4543443	0.8396407
Entada_phaseoloides	0.6664449	0.10845068	5	0.4737967	0.8590931
female	2.0448445	0.04072464	4	1.8294573	2.2602317
Hardenbergia_violacea	0.6277664	0.13393823	5	0.4351182	0.8204145
honey	0.4903523	0.76928370	3	0.2416446	0.7390600
Hovea_acutifolia	0.5083158	0.27057440	5	0.3156677	0.7009640
Leucaena_leucocephala	0.4782748	0.12813023	3	0.2295670	0.7269825
N_monosperma	0.8157883	0.21019444	5	0.6231401	1.0084365
Neptunia_dimorphantha	0.6014466	0.14362789	5	0.4087984	0.7940948
Neptunia_major	0.7087304	0.22852384	5	0.5160822	0.9013786
Parachidendron_pruinosum	0.5505105	0.10751592	5	0.3578623	0.7431587
Petalostylis_labicheoides	0.4550781	0.13007093	5	0.2624299	0.6477262
Phaseolus_vulgaris	0.4526079	0.14234280	5	0.2599597	0.6452561
Pisum_sativum	0.4789925	0.15568115	5	0.2863444	0.6716407
Senegalia_pennata	0.5734060	0.12565594	5	0.3807578	0.7660541
Senna_artemisioides	0.5271546	0.16677462	5	0.3345065	0.7198028
Swainsona_galegifolia	0.4777623	0.15171071	5	0.2851141	0.6704105
Vachellia_bidwillii	0.9805956	0.18848278	5	0.7879474	1.1732438
Vachellia_clarksoniana	0.4185974	0.28256894	5	0.2259493	0.6112456
Vachellia_ditricha	0.8053271	0.34280605	10	0.6691042	0.9415499
Vachellia_douglasia	0.8589120	0.24834966	5	0.6662638	1.0515602
Vachellia_farnesiana	0.5980174	0.05722421	6	0.4221545	0.7738803
Vachellia_pachyphylla	0.9238471	0.26233358	5	0.7311989	1.1164952
Vachellia_pallidifolia	0.6148628	0.36141867	5	0.4222146	0.8075110
Vachellia_sutherlandii	0.9510181	0.16870974	5	0.7583699	1.1436663

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Vachellia_valida          0.8782562 0.16874380  6 0.7023933 1.0541191
Vigna_unguiculata         0.3973185 0.27521110  5 0.2046703 0.5899667
water                     0.8765514 0.07430217  3 0.6278437 1.1252591
                           Min       Max
A.aneura                  0.36172784 1.0718820
A.baileyana               0.30103000 0.6812412
A.cambagei                0.36172784 0.8512583
A.cardophylla             0.17609126 0.6627578
A.chinchillensis          0.34242268 0.5910646
A.chisholmii              0.20411998 0.8920946
A.complanata              0.21748394 0.7853298
A.conferta                0.07918125 0.9493900
A.coriacea                0.06069784 0.8450980
A.deanei                  0.20411998 0.7671559
A.decurrens               0.00000000 0.6232493
A.drummondii              0.30103000 0.8573325
A.excelsa                 0.30103000 0.6483600
A.falcata                 0.56820172 0.8195439
A.fimbriata               0.14612804 0.7160033
A.flavescens              0.53147892 0.8692317
A.glaucocarpa             0.36172784 0.6020600
A.holosericea             0.53147892 0.8750613
A.irrorata                0.00000000 0.6334685
A.oshanesii               0.44715803 0.8692317
A.podalyriifolia          0.30103000 0.8450980
A.pulchella               0.16136800 1.1238516
A.salicina                0.48429984 0.8543060
A.shirleyi                0.11394335 0.5797836
A.simsii                  0.11394335 0.9395193
A.spectabilis             0.20411998 0.8228216
A.spondyllophylla         0.38021124 0.6020600
A.stenophylla             0.30103000 0.8450980
A.victoriae               0.36172784 0.8356906
Adenanthera_abrosperma    0.31175386 0.7481880
Albizia_lebbeck           0.38021124 0.6989700
alone                     0.47712125 0.5797836
Bauhinia_hookeri          0.17609126 0.6020600
Castanospermum_australe   0.17609126 0.9216865
Delonix_regia             0.42324587 0.7160033
Dichrostachys_spicata     0.50514998 0.7242759
Entada_phaseoloides       0.49831055 0.7671559
```

female 2.00860017 2.1003705

Hardenbergia_violacea 0.45484486 0.7442930

honey -0.39794001 0.9344985

Hovea_acutifolia 0.30103000 0.9395193

Leucaena_leucocephala 0.38021124 0.6232493

N_monosperma 0.53781910 1.0273496

Neptunia_dimorphantha 0.47712125 0.8027737

Neptunia_major 0.47712125 0.9566486

Parachidendron_pruinosum 0.41497335 0.6901961

Petalostylis_labicheoides 0.30103000 0.6532125

Phaseolus_vulgaris 0.30103000 0.6384893

Pisum_sativum 0.30103000 0.6766936

Senegalia_pennata 0.36172784 0.6812412

Senna_artemisioides 0.30103000 0.7558749

Swainsona_galegifolia 0.23044892 0.6020600

Vachellia_bidwillii 0.78175537 1.2317244

Vachellia_clarksoniana 0.00000000 0.7442930

Vachellia_ditricha 0.36172784 1.2540645

Vachellia_douglasia 0.43933269 1.0549959

Vachellia_farnesiana 0.52504481 0.6720979

Vachellia_pachyphylla 0.63346846 1.2068259

Vachellia_pallidifolia 0.11394335 0.9637878

Vachellia_sutherlandii 0.68124124 1.1055102

Vachellia_valida 0.65321251 1.1254813

Vigna_unguiculata 0.00000000 0.7032914

water 0.80617997 0.9542425

Alpha: 0.05 ; DF Error: 267

Critical Value of t: 1.968889

Groups according to probability of means differences and alpha level(0.05)

Treatments with the same letter are not significantly different.

```
                           log10(longevity)   groups
female                            2.0448445        a
Vachellia_bidwillii               0.9805956        b
Vachellia_sutherlandii            0.9510181        b
Vachellia_pachyphylla             0.9238471       bc
Vachellia_valida                  0.8782562      bcd
water                             0.8765514     bcde
```

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Vachellia_douglasia                0.8589120     bcde
N_monosperma                       0.8157883    bcdef
Vachellia_ditricha                 0.8053271    bcdef
A.aneura                           0.7641342   bcdefg
A.flavescens                       0.7189617  bcdefgh
A.falcata                          0.7182854  bcdefgh
Neptunia_major                     0.7087304  bcdefgh
A.spectabilis                      0.6985504   cdefgh
A.holosericea                      0.6932613  cdefghi
Entada_phaseoloides                0.6664449 cdefghij
Dichrostachys_spicata              0.6469925 defghijk
A.salicina                         0.6381390 defghijk
A.oshanesii                        0.6330340 defghijk
Hardenbergia_violacea              0.6277664 defghijk
A.podalyriifolia                   0.6244297  efghijk
A.chisholmii                       0.6177298  efghijk
Vachellia_pallidifolia             0.6148628  efghijk
Adenanthera_abrosperma             0.6099862  efghijk
Neptunia_dimorphantha              0.6014466  efghijk
Vachellia_farnesiana               0.5980174  efghijk
A.conferta                         0.5865620  efghijk
Castanospermum_australe            0.5802470  efghijk
Delonix_regia                      0.5785557  efghijk
Senegalia_pennata                  0.5734060  efghijk
A.simsii                           0.5733562   fghijk
A.victoriae                        0.5708183   fghijk
A.complanata                       0.5698302   fghijk
A.stenophylla                      0.5654739   fghijk
A.baileyana                        0.5652191   fghijk
A.cambagei                         0.5630689   fghijk
A.drummondii                       0.5629266   fghijk
A.pulchella                        0.5509398    ghijk
Parachidendron_pruinosum           0.5505105    ghijk
A.excelsa                          0.5313920    ghijk
alone                              0.5294613    ghijk
Senna_artemisioides                0.5271546    ghijk
Albizia_lebbeck                    0.5213932    ghijk
Hovea_acutifolia                   0.5083158     hijk
A.glaucocarpa                      0.5060568     hijk
honey                              0.4903523     hijk
A.deanei                           0.4900770     hijk
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A.spondyllophylla              0.4888739     hijk
Pisum_sativum                  0.4789925     hijk
Leucaena_leucocephala          0.4782748     hijk
Swainsona_galegifolia          0.4777623     hijk
A.cardophylla                  0.4654800     hijk
A.shirleyi                     0.4612160     hijk
Bauhinia_hookeri               0.4592835     hijk
Petalostylis_labicheoides      0.4550781     hijk
Phaseolus_vulgaris             0.4526079     hijk
A.chinchillensis               0.4355529      ijk
A.fimbriata                    0.4253898       jk
Vachellia_clarksoniana         0.4185974       jk
A.coriacea                     0.3995729        k
A.decurrens                    0.3992643        k
Vigna_unguiculata              0.3973185        k
A.irrorata                     0.3869431        k
```

Appendix 3. Raw data for comprehensive no-choice host specificity testing under quarantine conditions.

a. Life cycle study

Duration (number of days) of development of different life stages of *Acaciothrips ebneri* on the target species (*Vachellia nilotica* subsp. *indica*).

Replication	Incubation period	1st instar larvae	2nd instar larvae	Pre-pupae	Pupae 1	Pupae 2	Pre-oviposition period
1	5	3	6	3	4	1	2
2	7	3	8	2	2	2	2
3	10	3	6	1	5	4	2
4	7	3	7	3	1	6	3
5	7	3	3	1	4	7	4
6	7	3	7			3	1
7	6	6	10				2
8	7	2	8				2
9	8	3					2
10	6	3					
11	5	4					
12	2	4					
13		5					
14		3					
15		2					
16		2					

b. Female and male longevity

Number of days that both female and male of *Acaciothrips ebneri* survived on the target species (*Vachellia nilotica* subsp. *indica*).

Replications	Female	Male
1	112	60
2	126	31
3	105	29
4	102	38
5	115	39
6	98	49
7	120	42

c. No-choice trials

Number of days that *Acaciothrips ebneri* survived on non-target species, honey, with and without water.

Species	Rep1	Rep2	Rep3	Rep 4	Rep 5	Rep 6	Rep 7
Vachellia_bidwillii	6.05	11.05	17.05	6.35	11.05		
Vachellia_farnesiana	4.7	3.35	4.4	3.55	3.75	4.2	
Vachellia_sutherlandii	4.8	7.9	10.65	12.75	11.05		
Vachellia_valida	5.8	8.15	9.85	13.35	6.65	4.5	
Vachellia_clarksoniana	3.6	3.1	1	2	5.55		
Vachellia_douglasia	10.75	8.1	7.25	2.75	11.35		
Vachellia_pallidifolia	9.2	1.3	5.95	7.25	2.3		
Vachellia_pachyphylla	16.1	8.85	4.3	4.8	14.15		
Vachellia_ditricha	17.95	3.25	4.05	3.45	9.1	12.7	12.3
Adenanthera_abrosperma	5.6	2.05	5.5	5.15	3.45		
Neptunia_dimorphantha	3.05	6.35	3.5	3	5		
Dichrostachys_spicata	4.55	5.3	5.3	4.2	3.2		
Leucaena_leucocephala	2.7	2.4	4.2				
Entada_phaseoloides	5.85	4.8	4.3	3.15	5.65		
Neptunia_major	3	3	8.1	5.3	9.05		
N_monosperma	5.2	3.45	10.55	5.95	10.65		
Albizia_lebbeck	4.3	5	3.2	2.45	2.4		
Parachidendron_pruinosum	4.9	2.6	3.7	4	3		
Castanospermum_australe	4	8.35	3	1.5	5.3		
Swainsona_galegifolia	4	4	3	3	1.7		
Hovea_acutifolia	2	2.5	4	2	8.7		
Hardenbergia_violacea	2.85	5.15	5.2	3.25	5.55		
Vigna_unguiculata	1	5.05	4	2.4	2		
Phaseolus_vulgaris	2.95	4.35	3.4	2.1	2		
Pisum_sativum	3.3	3.6	4.75	2	2.2		
Bauhinia_hookeri	2.45	1.5	4	2.85	4	3.4	
Petalostylis_labicheoides	3.1	2	2.65	2.55	4.5		
A.baileyana	4.5	2	4.7	4.8	3.3		
A.cardophylla	4.6	4	3.85	2	1.5		
A.chinchillensis	2.6	3	2.2	2.25	3.9		
A.deanei	2.15	3.15	1.6	4.45	5.85		
A.glaucocarpa	3	3.9	4	2.3	3.15		
A.irrorata	3	2.3	4.3	2.9	1		
A.oshanesii	3.6	7.4	2.8	5.3	3.7		
A.spectabilis	6.4	4.5	1.6	6.4	6.65	6.65	5.95

A.decurrens	2.75	1	2.3	4.2	2.6	3.6	
A.aneura	4.45	6.3	10.65	5.2	11.8	5.3	2.3
A.cambagei	3.3	2.3	2.4	5.6	3.3	7.1	
A.chisholmii	2.8	1.6	5.05	6.95	7.8		
A.holosericea	4.4	4.1	4.3	7.5	3.4	7.3	
A.shirleyi	3.8	2.9	3.2	1.3	3.7	3.45	
A.spondyllophylla	2.4	2.4	3.45	4	3.5		
A.conferta	2.15	1.2	4	7.2	5	8.9	
A.falcata	6.6	5.65	4.6	6.15	3.7		
A.podalyriifolia	4	6.15	2	7	4	4.05	
A.salicina	7.15	3.35	3.45	6.15	3.05	4.35	
A.victoriae	2.3	3.6	3	4.2	6.85		
A.fimbriata	2.3	4	2	5.2	1.4		
A.complanata	1.65	6	6.1	4.5	2.6		
A.coriacea	2.35	7	3	2	1.15	2.2	
A.excelsa	2	4	4.25	4.45	3		
A.simsii	8.7	1.55	1.3	6.35	5.1	7.9	2.3
A.stenophylla	2	4	3	7	4		
A.flavescens	5	5.9	7.4	5.3	3.4		
A.drummondii	7.2	2.8	4.15	2	3.9		
A.pulchella	1.45	13.3	6.05	3.1	2.7	3.2	2.3
Senegalia_senegal	3.8	4.8	4.5	3.9	2.3		
Delonix_regia	4.55	4.45	2.8	5.2	2.65		
Senna_artemisioides	2	3	5.7	3.2	3.95		
Without water	3	3.8	3.4				
Water	9	6.4	7.4				
Honey	10.4	8.6	8.6				

d. The length (number of days) of each trial for non-target species, honey, with and without water.

Species	Rep1	Rep2	Rep3	Rep 4	Rep 5	Rep 6	Rep 7
Vachellia_bidwillii	39	33	52	47	51		
Vachellia_farnesiana	28	44	48	28	28	42	
Vachellia_sutherlandii	33	47	35	55	50		
Vachellia_valida	32	30	35	55	28	28	
Vachellia_clarksoniana	28	35	42	42	28		
Vachellia_douglasia	34	51	42	28	28		
Vachellia_pallidifolia	34	42	42	28	28		
Vachellia_pachyphylla	42	28	28	28	39		
Vachellia_ditricha	32	30	28	48	50	28	51
Adenanthera_abrosperma	28	28	33	32	30		
Neptunia_dimorphantha	28	50	28	28	51		
Dichrostachys_spicata	28	28	28	28	39		
Leucaena_leucocephala	47	51	28				
Entada_phaseoloides	28	28	28	28	28		
Neptunia_major	42	28	28	28	28		
N_monosperma	28	28	39	28	28		
Albizia_lebbeck	30	28	47	28	28		
Parachidendron_pruinosum	32	30	35	55	28		
Castanospermum_australe	28	28	28	44	32		
Swainsona_galegifolia	28	28	48	34	28		
Hovea_acutifolia	33	28	32	35	48		
Hardenbergia_violacea	28	28	28	42	32		
Vigna_unguiculata	56	28	35	28	28		
Phaseolus_vulgaris	56	35	28	28	28		
Pisum_sativum	28	28	28	28	28	28	
Bauhinia_hookeri	28	44	32	30	28		
Petalostylis_labicheoides	34	51	56	28	28		
A.baileyana	33	28	28	28	30		
A.cardophylla	31	30	28	28	28		
A.chinchillensis	33	30	30	28	28		
A.deanei	28	28	42	30	30		
A.glaucocarpa	30	28	28	28	28		
A.irrorata	52	28	28	28	28		
A.oshanesii	28	44	30	35	55		
A.spectabilis	52	28	28	28	28	42	32
A.decurrens	51	44	30	28	28	42	
A.aneura	28	28	42	35	32	31	28

A.cambagei	34	51	56	28	35	42	
A.chisholmii	28	28	42	32	30		
A.holosericea	33	28	28	28	42	35	
A.shirleyi	34	28	51	56	28	35	
A.spondyllophylla	28	35	28	28	28		
A.conferta	33	52	28	51	28	34	
A.falcata	28	28	28	44	30		
A.podalyriifolia	28	28	42	35	32	31	
A.salicina	32	31	30	28	35	28	
A.victoriae	30	48	50	28	28		
A.fimbriata	33	28	28	51	28		
A.complanata	28	30	28	28	28		
A.coriacea	42	35	31	47	28	28	
A.excelsa	28	32	55	28	48		
A.simsii	28	28	28	42	35	31	28
A.stenophylla	28	28	42	35	32		
A.flavescens	28	28	51	44	30		
A.drummondii	28	47	28	51	28		
A.pulchella	28	48	50	28	28	34	28
Senegalia_senegal	51	28	50	28	51		
Delonix_regia	32	31	28	47	28		
Senna_artemisioides	47	48	50	28	28		
Without water	28	28	28				
Water	28	28	28				
Honey	28	28	28				

e. Egg laying, larval development and adult emergence of *Acaciothrips ebneri* on target species *Vachellia nilotica* ssp. *indica* during the no-choice host specificity testing.

Replications	Target species	No. of galls	No. of eggs	No. of progenies	No. of adults	Duration of trial (No. of days)
1	*V. nilotica* subsp. *indica*	19	1550	1490	830	39
2	*V. nilotica* subsp. *indica*	25	1800	1750	990	33
3	*V. nilotica* subsp. *indica*	42	2000	1910	1440	55
4	*V. nilotica* subsp. *indica*	11	1580	1500	840	47
5	*V. nilotica* subsp. *indica*	15	1790	1730	990	28
6	*V. nilotica* subsp. *indica*	15	1390	1340	740	28
7	*V. nilotica* subsp. *indica*	18	1500	1440	780	28
8	*V. nilotica* subsp. *indica*	12	1580	1510	850	44
9	*V. nilotica* subsp. *indica*	48	2270	2050	1710	48
10	*V. nilotica* subsp. *indica*	21	2280	2110	1780	49
11	*V. nilotica* subsp. *indica*	77	2680	2340	2220	51
12	*V. nilotica* subsp. *indica*	6	1400	1340	750	28
13	*V. nilotica* subsp. *indica*	17	1480	1370	770	33
14	*V. nilotica* subsp. *indica*	10	1380	1260	690	35
15	*V. nilotica* subsp. *indica*	67	2930	2380	2330	50
16	*V. nilotica* subsp. *indica*	37	1900	1840	1250	32
17	*V. nilotica* subsp. *indica*	21	1910	1840	1310	30
18	*V. nilotica* subsp. *indica*	18	2030	1910	1440	35
19	*V. nilotica* subsp. *indica*	35	2380	2240	1970	42
20	*V. nilotica* subsp. *indica*	17	2290	2130	1790	28
21	*V. nilotica* subsp. *indica*	19	2100	1970	1580	35
22	*V. nilotica* subsp. *indica*	18	2050	1910	1450	42
23	*V. nilotica* subsp. *indica*	11	1880	1780	1210	28
24	*V. nilotica* subsp. *indica*	21	2150	1990	1680	51
25	*V. nilotica* subsp. *indica*	11	1590	1530	860	28
26	*V. nilotica* subsp. *indica*	15	1650	1570	890	28
27	*V. nilotica* subsp. *indica*	18	2110	1980	1620	28
28	*V. nilotica* subsp. *indica*	53	2390	2280	2020	39
29	*V. nilotica* subsp. *indica*	44	1810	1750	1000	30
30	*V. nilotica* subsp. *indica*	25	1820	1760	1010	47
31	*V. nilotica* subsp. *indica*	17	1940	1880	1340	28
32	*V. nilotica* subsp. *indica*	18	1880	1780	1020	28
33	*V. nilotica* subsp. *indica*	15	1680	1640	920	44
34	*V. nilotica* subsp. *indica*	22	1690	1640	930	32
35	*V. nilotica* subsp. *indica*	8	1510	1450	790	28
36	*V. nilotica* subsp. *indica*	13	1750	1660	940	28

37	*V. nilotica* subsp. *indica*	56	2300	2150	1820	42
38	*V. nilotica* subsp. *indica*	10	1660	1590	890	28
39	*V. nilotica* subsp. *indica*	8	1420	1350	760	30
40	*V. nilotica* subsp. *indica*	9	1610	1550	880	28
41	*V. nilotica* subsp. *indica*	63	2530	2340	2100	32
42	*V. nilotica* subsp. *indica*	19	1610	1570	880	30
43	*V. nilotica* subsp. *indica*	17	1950	1890	1390	31
44	*V. nilotica* subsp. *indica*	28	2090	1940	1560	33
45	*V. nilotica* subsp. *indica*	29	2140	1990	1680	51
46	*V. nilotica* subsp. *indica*	18	1530	1470	810	28
47	*V. nilotica* subsp. *indica*	17	1490	1370	770	42
48	*V. nilotica* subsp. *indica*	26	1890	1820	1220	30
49	*V. nilotica* subsp. *indica*	57	1980	1890	1400	28
50	*V. nilotica* subsp. *indica*	26	1890	1830	1230	28
51	*V. nilotica* subsp. *indica*	19	1990	1900	1420	44
52	*V. nilotica* subsp. *indica*	14	1770	1660	940	28
53	*V. nilotica* subsp. *indica*	20	1670	1620	890	28
54	*V. nilotica* subsp. *indica*	59	2430	2330	2020	42
55	*V. nilotica* subsp. *indica*	31	2350	2150	1900	32
56	*V. nilotica* subsp. *indica*	30	2220	2020	1680	31
57	*V. nilotica* subsp. *indica*	20	1670	1630	890	42
58	*V. nilotica* subsp. *indica*	16	1780	1680	950	28
59	*V. nilotica* subsp. *indica*	76	2350	2200	1910	47
60	*V. nilotica* subsp. *indica*	25	1880	1780	1020	51
61	*V. nilotica* subsp. *indica*	14	1780	1710	970	47
62	*V. nilotica* subsp. *indica*	12	1780	1710	980	48
63	*V. nilotica* subsp. *indica*	23	1770	1670	950	50
64	*V. nilotica* subsp. *indica*	9	1540	1480	820	28
65	*V. nilotica* subsp. *indica*	15	1710	1660	930	28