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Life cycle, host specificity and potential impact of a gall-inducing thrips *Acaciothrips ebneri*, a biological control agent for prickly acacia (*Vachellia nilotica* subsp. *indica*) in Australia

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Abstract Prickly acacia (*Vachellia nilotica* subsp. *indica* (Benth.) Kyal. & Boatwr.; Fabales: Fabaceae) is a Weed of National Significance and a target for biological control in Australia. Currently there are no effective biological control agents for the weed in Australia. Based on genetic and climate matching, a gall thrips (*Acaciothrips ebneri* Karny; Thysanoptera: Phlaeothripidae) inducing rosette galls resulting in shoot tip dieback, was identified as a prospective biological control agent from Ethiopia. No-choice host-specificity tests were conducted on 59 test plant species in a high security quarantine in Brisbane, Australia. *Acaciothrips ebneri* is host-specific, inducing galls and reproducing only on prickly acacia. *Acaciothrips ebneri*, as predicted by the CLIMEX model, is suited to hot and arid western Queensland where major prickly acacia infestations occur. The Australian Government approved *A. ebneri* for field release in October 2022. This is the first time a true gall-inducing thrips has ever been approved as a weed biological control agent. Field releases commenced in January 2023 and are in progress. There are early signs of field establishment resulting in shoot tip die back in the field, and field release and monitoring will continue.

Keywords Weed biocontrol · Rosette gall · Field release · Establishment · Shoot tip dieback · Ethiopia

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Introduction

Prickly acacia (*Vachellia nilotica* subsp. *indica* (Benth.) Kyal. & Boatwr.; Fabales: Fabaceae), native to the Indian subcontinent and East Africa, is a Weed of National Significance and a declared weed in Australia. *Vachellia nilotica* subsp. *indica* is a thorny tree, growing 4–5 m high, occasionally reaching up to 10 m high. *Vachellia nilotica* subsp. *indica*, a serious weed of the rangelands of northern Australia, forms dense thorny thickets resulting in restricted water access for livestock, reduced pasture production, and is a threat to rare and endangered native plant and animal species (Spies and March 2004). *Vachellia nilotica* subsp. *indica* infests over six million hectares of natural pastures in northern Australia, resulting in over $AU nine million losses annually to the grazing industry due to lost pasture production (Dhileepan 2009).

Mechanical and herbicide methods utilised to manage *V. nilotica* subsp. *indica* (Spies and March 2004;

DAF 2018) are not economical (Mooy et al. 1992). Biological control, a sustainable long-term control option for *V. nilotica* subsp. *indica*, commenced in Australia in the early 1980s (Mohyuddin 1981). Based on native range surveys in Pakistan, Kenya, South Africa and India (Dhileepan et al. 2009, 2014), six biological control agents have been released in Australia since 1985. Among them, two agents, a seed-feeding *Bruchidius sahlbergi* Schilsky (Lepidoptera: Bruchidae) from Pakistan and a leaf-feeding geometrid *Chiasmia assimilis* Warren (Lepidoptera: Geometridae) from Kenya and South Africa, have become established (Dhileepan et al. 2009). The impact of *B. sahlbergi* on *V. nilotica* subsp. *indica* 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 *V. nilotica* subsp. *indica* occur (Palmer et al. 2007). The other agents did not establish (Palmer et al. 2012).

Biological control remained a priority for *V. nilotica* subsp. *indica*. Hence, native range studies have focussed on other unexplored areas in Africa. Literature (Dwivedi 1993) and herbarium records (Dhileepan et al. 2018) indicated that *V. nilotica* subsp. *indica* and other *V. nilotica* subspecies with moniliform (resembling a string of beads) fruit pods occur naturally in Ethiopia. This was further confirmed by morphological observations (Dhileepan et al. 2019) and genetic studies (Comben et al. 2021) on *V. nilotica* subsp. *indica* and other *V. nilotica* subspecies in Ethiopia. A CLIMEX model also suggested that areas in northern and eastern Ethiopia are climatically similar to the arid inland regions of western Queensland, Australia where *V. nilotica* subsp. *indica* is a major problem and are climatically suitable areas to source biological control agents (Senaratne et al. 2006; Dhileepan et al. 2018). Hence, the search for new biological control agents was redirected to Ethiopia. Potential survey sites were identified in Ethiopia based on herbarium records and the CLIMEX model. Based on field host range and damage potential, a gall-inducing thrips *Acaciothrips ebneri* (Karny) (Thysanoptera: Phlaeothripidae) inducing shoot-tip rosette galls in Ethiopia was identified as a prospective biological control agent for *V. nilotica* subsp. *indica* in Australia (Dhileepan et al. 2018).

Gall-inducing insects are widely used in weed biological control programs due to their host specificity (Harris and Shorthouse 1996; Raman 2011; Winston et al. 2014). Hence, the gall-inducing thrips was prioritised for further studies in Australia, in view of its field host range and damage potential in Ethiopia (Dhileepan et al. 2018). In this study, we report on the life cycle, host specificity, climatic suitability and potential impact of *A. ebneri* as a potential biological control agent for *V. nilotica* subsp. *indica* in Australia. This is the first time that a true gall-inducing thrips (rosette galls) has been approved as a weed biological control agent. The study also reports on the introduction and early stages of the establishment of *A. ebneri* in Australia.

Materials and methods

Acaciothrips ebneri

Acaciothrips ebneri is native to northern Africa and has been reported only on *V. nilotica* (Dhileepan et al. 2018). Mature galls containing adults of *A. ebneri* were imported (IP15015295) twice from Ethiopia (Shewa Robit, 10° 00′ 13.0" N; 39° 54′ 04.2" E) in 2015 and 2016. *Acaciothrips ebneri* sourced from Ethiopia were identified by L. Mound (CSIRO, Canberra). A colony of the gall thrips was established and maintained in the high security quarantine facility (Biosecurity insectary containment level 3, generally plant pathogens and smaller arthropods, mean temperature ± SE of 28 ± 2 °C; RH 60%; natural photoperiod) in insect-proof cages (300-micron mesh cages, 50 × 50 × 100 cm) containing potted *V. nilotica* subsp. *indica* plants (40–60 cm tall) for life cycle studies and host-specificity tests.

Vachellia nilotica subsp. *indica* plants

Seeds of *V. nilotica* subsp. *indica* were field collected from north Queensland, Australia. Seeds were soaked in hot water for 24–48 h before planting. Once seeds were swollen, they were then transferred into a germination tray (48 × 30 cm) filled with potting mix (Centenary Landscaping, Darra, Queensland, Australia). All individual seedlings were transferred into single pots (25 cm diameter) filled with potting mix and maintained in a glasshouse set at 27 ± 2 °C (mean ± SE) with a L:D 12:12 photoperiod for one to three years, before being used in host-specificity testing. Slow-release fertilizer (Scotts Osmocote,

Evergreen Garden Care Australia Pty Ltd, New South Wales, Australia) was applied in early spring, summer and autumn. The plants were checked every month for generalist insect herbivores, and any insects found on them were removed manually. The potted *V. nilotica* subsp. *indica* plants were pruned periodically (every 2–4 months), to induce new shoot growth, before being used in all studies.

Test plants

The test plant list for host specificity was developed by Taylor and Dhileepan (2019). The host test list contained 59 species and reflected currently accepted phylogenetic relationships while considering the species richness of the various groups in Australia. Eight native *Vachellia* species were included in the list along with the naturalised *Vachellia farnesiana* (L.) Wight & Arn. Similar to past test lists (e.g., Palmer et al., 2007; Taylor and Dhileepan 2018), species from each of the four native Mimoseae genera were included in the test. Three species of *Neptunia* were also included due to their close relationship to *Vachellia*. Two species of other basal Mimoseae were included in the list (*Leucaena leucocephala* (Lam) de Wit, which has become widely naturalised in Queensland and is an important pasture forage plant and *Dichrostachys spicata* (F. Muell.) Domin) as well as two derived Mimoseae (*Adenanthera abrosperma* F.Muell. and *Entada phaseoloides* (L.) Merr.). The genus *Acacia* is approximately five degrees (degrees of phylogenetic separation as described in Table 1) 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 (sections are not considered natural groupings as there is not phylogenetically based classification for this *Acacia* group) represented. Outside of the former Mimosoideae, the test list was 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.*) were 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 also included several species from other legume subfamilies including the large subfamily Faboideae (syn. Papilionoideae). No non-leguminous species were included in the test list. Non-target test plant species were grown from seeds, which were either 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) or collected from the field in northern Queensland.

Life cycle studies

Both males and females are macropterous, with duplicated cilia absent in forewings (personal observation). The entire life cycle of *A. ebneri* 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 *V. nilotica* subsp. *indica* plant are unable to feed or induce galls, and so die. Hence, observations of the different life stages had to be made by destructive sampling of the galls at regular intervals. Destructive sampling of the galls was carried out periodically to study the morphometrics and duration of life stage of eggs, larvae, prepupae and pupae in a Petri dish. A newly emerged mating pair was released in an insect-proof mesh cage containing a potted *V. nilotica* subsp. *indica* plant and all galls induced by the pair were cut off every two weeks in order to prevent emergence of any new adult progeny. The mature adults were collected from destructed galls every two weeks and released onto new a *V. nilotica* subsp. *indica* plant until all the adults died. The experiment was conducted in a high security quarantine glasshouse (mean temperature 28 ± 2 °C; RH 60%.; natural photoperiod) and was repeated a minimum five times, and the longevity of adults, fecundity and duration of various life stages were recorded.

No-choice host specificity tests

Based on results from preliminary host specificity tests in South Africa (Dhileepan et al. 2018), *A. ebneri* from Ethiopia was imported into a high security quarantine facility at Brisbane, Australia (December 2015 and November 2016) for colony establishment and host specificity testing. A colony of gall-inducing thrips maintained on *V. nilotica* subsp. *indica* was used in all host specificity tests.

Table 1 Results for no-choice host specificity testing conducted under quarantine conditions in Australia for *Acaciothrips ebneri*

Test plant species and degree of phylogenetic separation	No. of replicates	Gall induction (Y/N, mean ± SE of number of galls)	Egg laying (Y/N, mean ± SE of number of eggs)	Larval development (Y/N, mean ± SE of number of progeny nymphs and pupae)	Adult emergence (Y/N. mean ± SE of number of progeny adults)
Vachellia nilotica subsp. *indica* (Benth.) Kyal. & Boatwr. [0]	65	Y, 25 ± 2	Y, 1891 ± 41	Y, 1785 ± 35	Y, 1236 ± 55
V. bidwillii (Benth.) Kodela [0]	5	N	N	N	N
V. clarksoniana (Pedley) Kodela [0]	5	N	N	N	N
V. ditricha (Pedley) Kodela [0]	7	N	N	N	N
V. douglasica (Pedley) Kodela [0]	5	N	N	N	N
V. farnesiana (L.) Wight & Arn. [0]	6	N	N	N	N
V. pachyphloia (W.Fitzg.) Kodela [0]	5	N	N	N	N
V. pallidifolia (Tindale) Kodela [0]	5	N	N	N	N
V. sutherlandii (F. Muell.) Kodela [0]	5	N	N	N	N
V. valida (Tindale & Kodela) Kodela [0]	6	N	N	N	N
Dichrostachys spicata (F. Muell.) Domin [1]	5	N	N	N	N
Leucaena leucocephala (Lam.) de Wit [1]	5	N	N	N	N
Neptunia dimorphantha Domin [1]	5	N	N	N	N
N. major (Benth.) Windler [1]	5	N	N	N	N
N. monosperma F. Muell. ex Benth. [1]	5	N	N	N	N
Adenanthera abrosperma F. Muell. [2]	5	N	N	N	N
Entada phaseoloides (L.) Merr. [2]	5	N	N	N	N
Senegalia senegal (L.) Maslin [3]	5	N	N	N	N
Albizia lebbeck (L.) Benth. [4]	5	N	N	N	N
Parachidendron pruinosum (Benth.) I. C. Nielsen [4]	5	N	N	N	N
Acacia baileyana F. Muell. [5]	5	N	N	N	N
A. cardiophylla A. Cunn. ex Benth. [5]	5	N	N	N	N
A. chinchillensis Tindale [5]	5	N	N	N	N
A. deanei subsp. *deanei* (R. T. Baker) M. B. Welch, Coombs & McGlynn [5]	5	N	N	N	N
A. glaucocarpa Maiden & Blakely [5]	6	N	N	N	N
A. irrorata Sieber ex Spreng. [5]	5	N	N	N	N
A. oshanesii F. Muell. & Maiden [5]	5	N	N	N	N
A. spectabilis A. Cunn. ex Benth. [5]	7	N	N	N	N
A. decurrens Wild. [5]	5	N	N	N	N
A. aneura F.Muell. ex Benth. [5]	7	N	N	N	N
A. cambagei R.T.Baker [5]	6	N	N	N	N
A. chisholmii F.M.Bailey [5]	5	N	N	N	N
A. holosericea A.Cunn. ex G.Don [5]	6	N	N	N	N
A. shirleyi Maiden [5]	6	N	N	N	N
A. spondyllophylla F.Muell. [5]	5	N	N	N	N
A. conferta A.Cunn. ex Benth. [5]	6	N	N	N	N
A. falcata Willd. [5]	5	N	N	N	N
A. podalyriifolia A.Cunn. ex G.Don [5]	6	N	N	N	N
A. salicina Lindl. [5]	6	N	N	N	N
A. victoriae Benth. [5]	5	N	N	N	N
A. fimbriata A.Cunn. ex G.Don [5]	5	N	N	N	N
A. complanata A.Cunn. ex Benth. [5]	5	N	N	N	N
A. coriacea DC. [5]	6	N	N	N	N
A. excelsa Benth. [5]	5	N	N	N	N
A. simsii A.Cunn. ex Benth. [5]	7	N	N	N	N
A. stenophylla A.Cunn. ex Benth. [5]	5	N	N	N	N
A. flavescens A.Cunn. ex Benth. [5]	5	N	N	N	N
A. drummondii Lindl. [5]	5	N	N	N	N
A. pulchella R.Br. [5]	7	N	N	N	N
Delonix regia (Boj. ex Hook.) Raf. [6]	5	N	N	N	N
Senna artemisioides subsp. *helmsii* (Symon) Randell [7]	5	N	N	N	N

Table 1 (continued)

Test plant species and degree of phylogenetic separation	No. of replicates	Gall induction (Y/N, mean ± SE of number of galls)	Egg laying (Y/N, mean ± SE of number of eggs)	Larval development (Y/N, mean ± SE of number of progeny nymphs and pupae)	Adult emergence (Y/N. mean ± SE of number of progeny adults)
Hardenbergia violacea (Schneev.) Stearn [8]	5	N	N	N	N
Castanospermum australe A.Cunn & C.Fraser ex Hook. [8]	5	N	N	N	N
Swainsona galegifolia (Andrews) R.Br [8]	5	N	N	N	N
Hovea acutifolia A.Cunn. ex G.Don [8]	5	N	N	N	N
Phaseolus vulgaris L. [8]	5	N	N	N	N
Pisum sativum L. [8]	5	N	N	N	N
Vigna unguiculata (L.) Walp. [8]	5	N	N	N	N
Petalostylis labicheoides R.Br. [9]	5	N	N	N	N
Bauhinia hookeri F.Muell. [10]	5	N	N	N	N

Numbers in square brackets for test plant species refers to degree of phylogenetic separation from the target weed: 0 = genus *Vachellia*; 1 = 'basal' Mimoseae; 2 = 'derived' Mimoseae; 3 = genus *Senegalia*; 4 = tribe Ingeae; 5 = genus *Acacia*; 6 = *Peltophorum* clade; 7 = *Cassieae* clade; 8 = subfamily Faboideae; 9 = subfamily Dialioideae; 10 = subfamily Cercidoideae (Taylor and Dhileepan 2019)

No choice tests were conducted by releasing 20 randomly selected newly emerged *A. ebneri* adults (mating pairs were preferred) into insect-proof mesh cages (60 × 60 × 90 cm) containing one potted test plant or control *V. nilotica* subsp. *indica* plant. With each replicate, at least one *V. nilotica* subsp. *indica* plant was included as a control, and a minimum of five replications were conducted for each test species. The control and test plants were monitored for a minimum of four weeks to record adult survival, gall induction and development and/or an additional two weeks after all adults died. At the end of testing, the number of galls were counted and all galls harvested from the control 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 from control plants were selected and dissected to count the *A. ebneri* (eggs, larvae, pupae and adults) population in each gall. In total, 59 test species were screened during host-specificity testing (Table 1).

Potential impact

The potential impacts of *A. ebneri* on potted three-year old *V. nilotica* subsp. *indica* plants were studied in a temperature (27 ± 2 °C), humidity (65% RH) and photoperiod-controlled (L:D 12:12) glasshouse. Randomly collected *A. ebneri* adults (20 adults of unknown sex) were released into an insect-proof cage (45 × 45 × 90 cm) containing an individual 40 – 60 cm tall *V. nilotica* subsp. *indica* plant with ten replicates. The number of shoot tips were counted at the beginning of the trial, and, subsequent to the release of *A. ebneri*, the size of representative galls and number of galls per plant were recorded along with the number of shoot tips dying at weekly intervals. The trial was continued for 20 weeks (or when the plant died). An equal number of plants without *A. ebneri* were maintained as control plants.

CLIMEX model

A CLIMEX 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. These data were collected as part of native range explorations in Ethiopia (103 sites) from 2014 to 2017 (Dhileepan et al. 2018). The climate profile of *A. ebneri* was determined by recursively testing various sets of parameter values until the model's distribution matched its recorded distribution in Ethiopia. The estimated parameters (Supplementary Table S1) were then used to estimate its potential distribution in Australia. The suitability of an area was expressed in terms of its Eco-climatic Index (EI) with areas having an EI of > 30 considered as climatically suitable and

those with an index of < 10 being unlikely to sustain a population over a sustained period (Kriticos et al. 2003).

Rearing and field release

Acaciothrips ebneri was approved for release in October 2022. Field releases commenced in December 2022 and are currently in progress. Prospective release sites have been identified in consultation with Local Government Authorities (LGAs), Natural Resource Management (NRM) groups and landholders in both coastal and inland sites in Queensland, Australia. Four sites (Giru, Guthalungra, Whitsunday Paradise and Bowen) in coastal far north Queensland, six sites in central Queensland (Clermont, Capella, Sapphire, Gracemere, and Rockhampton), and 14 sites (two release locations within each property in Isabel Downs, Channel Downs, Wayangerie, Afton Downs, Limbri, Aldingham and Bernfels, and one release location each in Minamere and Channel Downs properties) in inland western Queensland were selected for field releases. At each site, around *ca.* 20–50 mature/old galls with approximately 5,000 *A. ebneri* adults per tree were released (galls were tied onto five branches with actively growing shoot tips). In some sites, multiple releases were made. Follow up surveys were conducted at majority of release sites, between three and 12 months after initial release. During follow up visits, the proportion of shoots with galls, and the number of galls per tree were documented at the release sites. Hence, follow up surveys was not possible in some release sites due to flooding.

Data analysis

For the no-choice tests, a repeated measures ANOVA was used to compare the duration of adult survival on all test species including target and non-target plant species. A repeated measures ANOVA was also used to compare number of galls per plant and proportion of shoot-tip die back over the weeks (number of galls per plant and proportion of shoot-tip die back was repeated in the analysis). For the potential impact study, the weekly averages of the growth parameters (number of galls and proportion of shoot-tip die back) were selected as the dependent and weeks was the independent variables. All analyses were conducted using R4.0.2 via the RStudio Version 1.3.1058 (R Core Team 2021). For the potential impact study, the number of galls per plant and proportion of shoot-tip dieback were recorded on individual plants repeatedly over many weeks. Hence one-way repeated measures ANOVA was used to analyse the data followed by pairwise multiple comparison. Post-hoc LSD (Least Significant Difference) test at $P < 0.05$ was used to analyse any pairwise significant differences among the test species, number of galls per plant and proportion of shoot-tip die back per plant. All results in the text are represented as means ± SE.

Results

Life cycle

The life cycle of *A. ebneri* consists of five stages − egg, larvae, prepupae, pupae and adults (Table 2). Under quarantine conditions (28 ± 2 °C; 60% RH) *A. ebneri* reproduced sexually throughout the year and the total duration of the life cycle (egg to adult) was about 25.58 ± 1.36 days. Adults fed externally on the axillary and terminal buds of *V. nilotica* subsp. *indica* and induced rosette galls. Once a gall was initiated (after 2–3 days), the adults entered the gall and continued to feed on the nutritive tissue lining the inner surface of the galls. Adults (1.81 ± 0.09 mm long) lived for up to 16 weeks, with females living significantly longer (111.25 ± 26.65) than males (41.14 ± 4.03 days) ($F_{1,12} = 159.32$; $P < 0.01$). Without any food (water alone), adults lived for 8.33 ± 0.44 days. Eggs were laid in clusters, attached to the surface of the nutritive tissues, lining walls inside the developing galls. Eggs were small (0.36 ± 0.18 mm long), pale green to yellowish in colour, and hatched approximately 6.42 ± 0.16 days after oviposition. The emerging larvae fed on the nutritive tissues lining the inner walls of the galls and developed through two larval instars. Newly emerged larvae (0.54 ± 0.03 mm long) were almost colourless (transparent) and then became yellowish to reddish (Table 2). The duration of first instar larvae was 3.25 ± 0.07 days. The second instar larvae (1.14 ± 0.09 mm long) were slightly larger and bright red with a dark black head, notum, and abdominal tip. The duration of the second instar larvae was 6.88 ± 0.23 days. The development through the non-feeding prepupal and the two pupal stages were completed within the gall. The pre-pupae

were dark red with no wing buds and greatly reduced antennae pointing backward. The duration of development of first and second prepupae were 3.20 ± 0.32 and 3.83 ± 0.38 days, respectively (Table 2). Females produced 200.22 ± 28.54 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.

No-choice host specificity tests

In quarantine in Australia, adult feeding, gall induction, oviposition and larval development only occurred on *V. nilotica* subsp. *indica*. There was no gall induction, oviposition and larval development on any of the non-target species tested (Table 1). An average of 25.10 ± 2.12 galls were induced on *Vachellia nilotica* subsp. *indica* (Table 1). On control *V. nilotica* subsp. *indica* plants, *A. ebneri* laid 1891.90 ± 41.14 eggs and produced 1785.64 ± 34.56 progeny (egg, larvae, pupae and adults) during the no-choice host specificity testing period (Table 1). Under no-choice conditions, adults lived for 76.64 ± 10.09 days (n = 14) on *V. nilotica* subsp. *indica* plants, significantly ($F_{11,58} = 2.91$, $P < 0.01$) longer than on any of the non-target plants (< ten days; Fig. 1). Adult survival on the non-target test plants was either similar or significantly ($F_{62,267} = 106.13$, $P < 0.01$) lower than on the negative controls (no test plants and no water, or no test plants and only water, or no test plants and honey) (Fig. 1).

Table 2 Life cycle and size and mean development time of *Acaciothrips ebneri* under quarantine conditions with five replicates

Life stage	Length (mm)	Duration of development/longevity (days)
	Mean ± SE	Mean ± SE
Eggs	0.36 ± 0.01	6.42 ± 0.16
1st instar larvae	0.54 ± 0.03	3.25 ± 0.07
2nd instar larvae	1.13 ± 0.09	6.88 ± 0.23
Pre-pupae	1.37 ± 0.08	2.00 ± 0.20
1st pupae	1.43 ± 0.06	3.20 ± 0.32
2nd pupae	1.53 ± 0.04	3.83 ± 0.38
Adult—males	1.62 ± 0.57	41.14 ± 4.03
Adult—females	1.81 ± 0.35	111.25 ± 21.38

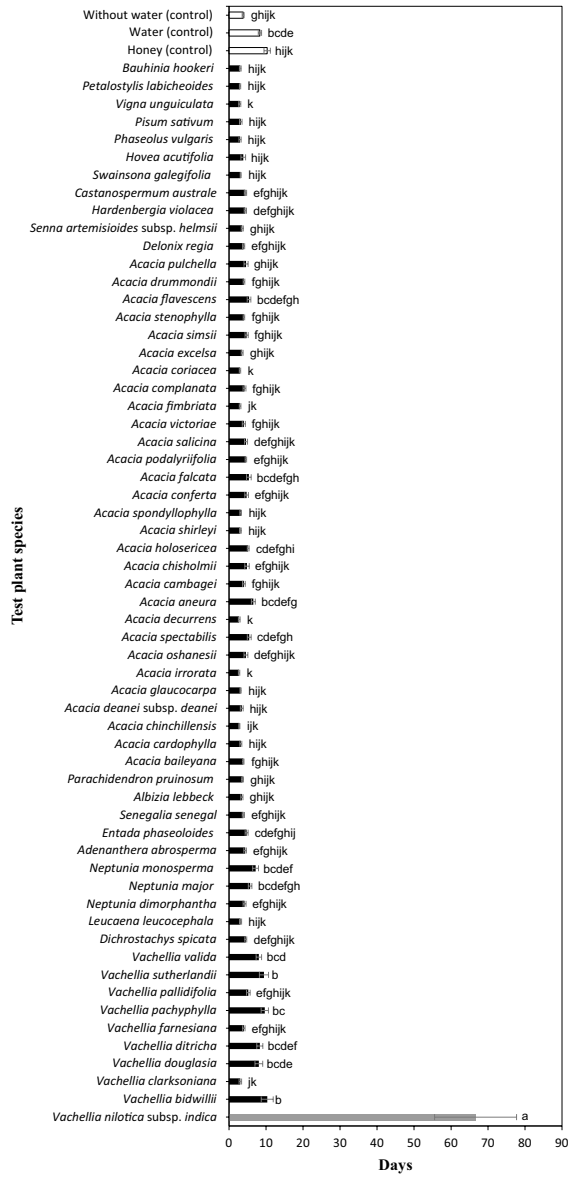

Fig. 1 Duration of survival (days; mean ± SE) (i.e., longevity) of *Acaciothrips ebneri* adult on target (grey bar) and non-target plants (black bars) during no-choice host-specificity testing. Transparent bars represent negative control treatments, (i.e., no plants presented to the thrips, either honey, water, or no water). Different species with different letters are significant different ($F_{62,267} = 106.13$, P < 0.01). (Color figure online)

Potential impact

Acaciothrips ebneri induced galls on all *V. nilotica* subsp. *indica* plants exposed to it. Evidence of gall initiation was observed three days after the

introduction of *A. ebneri* and there were no galls found on control plants (with *A. ebneri* absent). The number of galls per plant increased over time (Fig. 2a) with 5.00 ± 0.78 galls per plant observed during the first week of exposure. There was no significant difference between the mean number of galls per plant in the first and second weeks ($F_{1,17} = 1.75$, $P > 0.05$) or after three and four months ($F_{3,22} = 1.00$, $P > 0.05$). However the mean number of galls per plant showed a significant increase ($F_{3,31} = 3.01$, $P < 0.01$) after four weeks (Fig. 2a). The total number of galls per plant was more than 100, depending on the size of the plant, the number of branches and actively growing shoot tips available. Additionally, the proportion of shoot-tip die back increased over time and an average of 98% of shoot-tips dying four months post-exposure (Fig. 2b). There was no shoot-tip die back observed on any of the control plants. A significant proportion of die back was observed two months post-*A. ebneri* exposure (Fig. 2b; $F_{11,32} = 6.32$, $P < 0.01$).

CLIMEX model

The estimated climatic parameters for the potential distribution of *A. ebneri* in Australia are presented in Supplementary Table S1. The CLIMEX model based on the native range of *A. ebneri* in Ethiopia suggested that central and western Queensland, central Northern Territory and coastal areas in Western Australia, which have major infestations of *V. nilotica* subsp. *indica*, are climatically suitable for *A. ebneri* establishment (Fig. 3). In contrast, only limited areas along coastal areas in Queensland would be climatically suitable for *A. ebneri* where *V. nilotica* subsp. *indica* is also a major problem.

Rearing and field release

More than 1,100 galls, equating to approximately 100,000 adults, have been released across 24 field sites between December 2022 and October 2023. Field releases have been made at 24 sites so far, with additional releases continuing. The CLIMEX model indicated that *A. ebneri* is climatically better suited to the hot and arid inland regions of Australia compared to the coastal areas. In an effort to validate the model, field releases have been made at coastal (seven sites) and inland sites (17 sites) (Fig. 4). Releases were also made at sites close to permanent water bodies (e.g., dams and lakes) as well as in areas not close to any permanent water bodies. Field releases involved tying mature galls (containing adult and immature *A. ebneri*) on shoots of *V. nilotica* subsp. *indica* trees with actively growing shoot tips/meristems. Preliminary monitoring at most release sites has indicated evidence of gall induction and development (Fig. 5). However, the incidence and intensity of gall development varied widely across sites. At some of the sites close to permanent water bodies, galling was evident on the majority of released shoots, resulting in >90% shoot-tip dieback. At the time of publication, most *A. ebneri* dispersal has been limited within the release tree (from inoculated shoot tips to surrounding shoots), and neighbouring trees (by wind). Interestingly, dispersals have been found more than 40 and 100 m at the Afton Downs (Inland) and Giru release sites, respectively (Fig. 4).

Discussion

Phytophagous thrips have been successfully used as weed biological control agents globally (Cock 1982; Reimer and Beardsley Jr 1989; Memmott et al. 1998; Ireson et al. 2008; Cuda et al. 2009; McConnachie and McKay 2015; Wheeler et al. 2018). Gall-inducing thrips are highly host-specific (Raman 1984), and hence *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 true gall-inducing thrips has been evaluated as a weed biological control agent for any weed around the world.

Acaciothrips ebneri adults induced the galls in the shoot tips of *V. nilotica* subsp. *indica* in laboratory studies. Once galls were formed, females laid eggs within them. Both adults and larvae, fed within the galls on the nutritive cells that formed their innermost layer. The entire life cycle was completed within the galls, with *A. ebneri* having a short developmental period of three to four weeks under glasshouse conditions. Under optimal conditions, *A. ebneri* was able to complete more than 12 generations per year.

The risk of *A. ebneri* feeding, inducing galls and developing on representative non-target plant species of increasing phylogenetically distance to the target weed *V. nilotica* subsp. *indica*, was assessed using no-choice tests in a quarantine glasshouse. No-choice

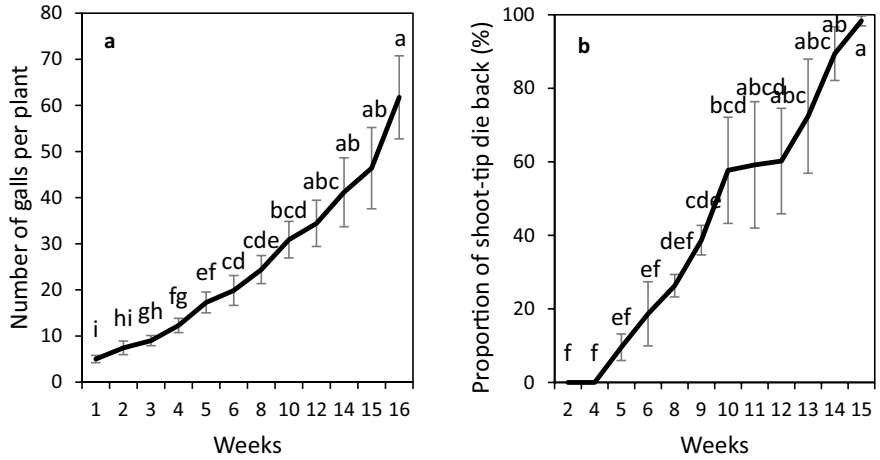

Fig. 2 The mean (± SE) number of galls per plant and proportion of shoot-tip die back per plant over time. Weeks with different letters are significantly different (LSD test; **a** number of galls per plant, $F_{11,78} = 14.48$, $P < 0.01$; **b** proportion of shoot-tip die-back per plant, $F_{11,32} = 6.32$, $P < 0.01$)

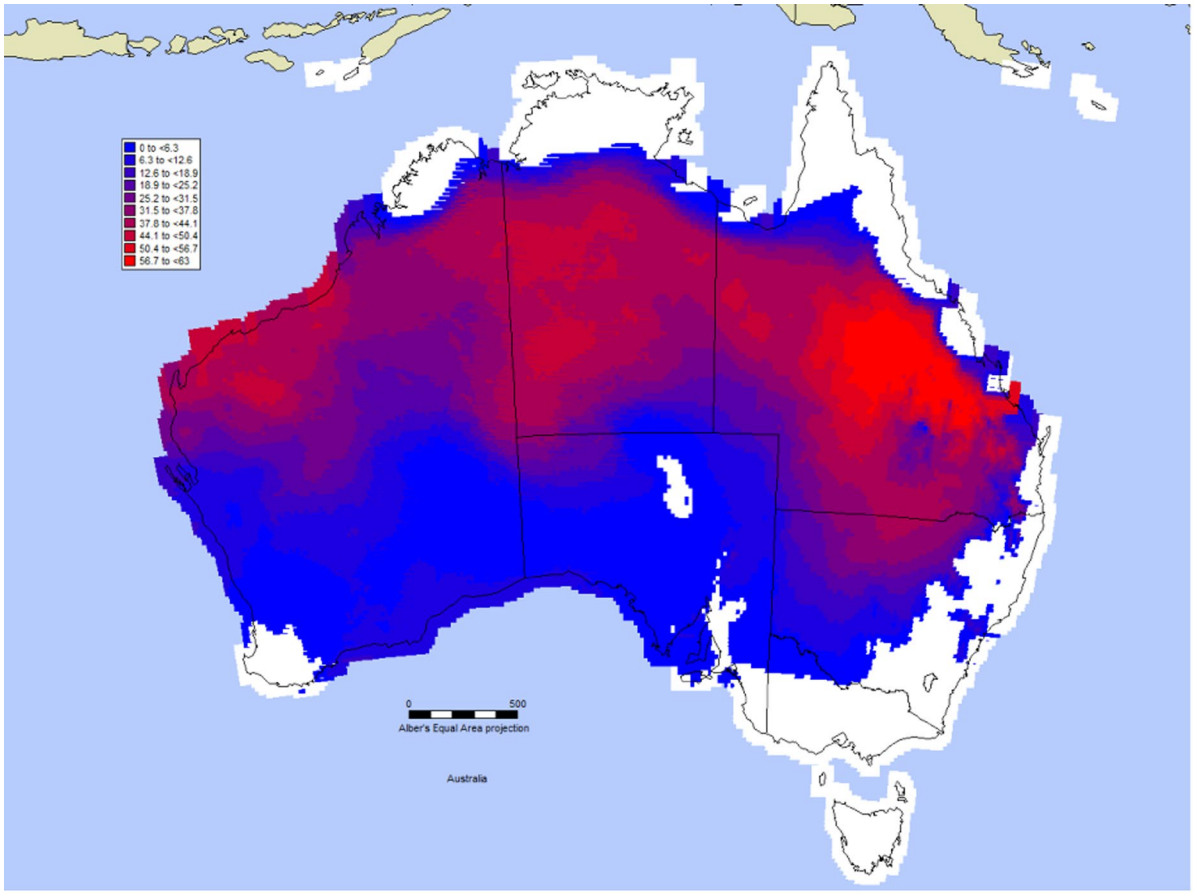

Fig. 3 Climate suitability prediction for *Acaciothrips ebneri* using the Composite Match Index in CLIMEX. (For full CLIMEX model parameters refer Supplementary Table S1)

host-specificity tests included 59 non-target species in four subfamilies, three clades and 21 genera (Table 1). This included ten *Vachellia* spp. present in Australia, comprising eight native and two naturalised species. In no-choice tests, *A. ebneri* adults induced galls, laid eggs and their emerging larvae completed development only on *V. nilotica* subsp. *indica*. There was also no gall induction, egg-laying and larval development on any of the Australian native *Acacia* spp. tested. Adult survival on non-target species was significantly lower than on the target weed, and none of the non-target species were suitable hosts for the development of *A. ebneri*. On Australian native *Acacia* spp., the survival of *A. ebneri* adults under no-choice conditions was very low (Fig. 1). However, on some of the test species, the duration of survival of *A. ebneri* adults was similar to the duration of survival on negative controls (e.g., only on honey), possibly due to

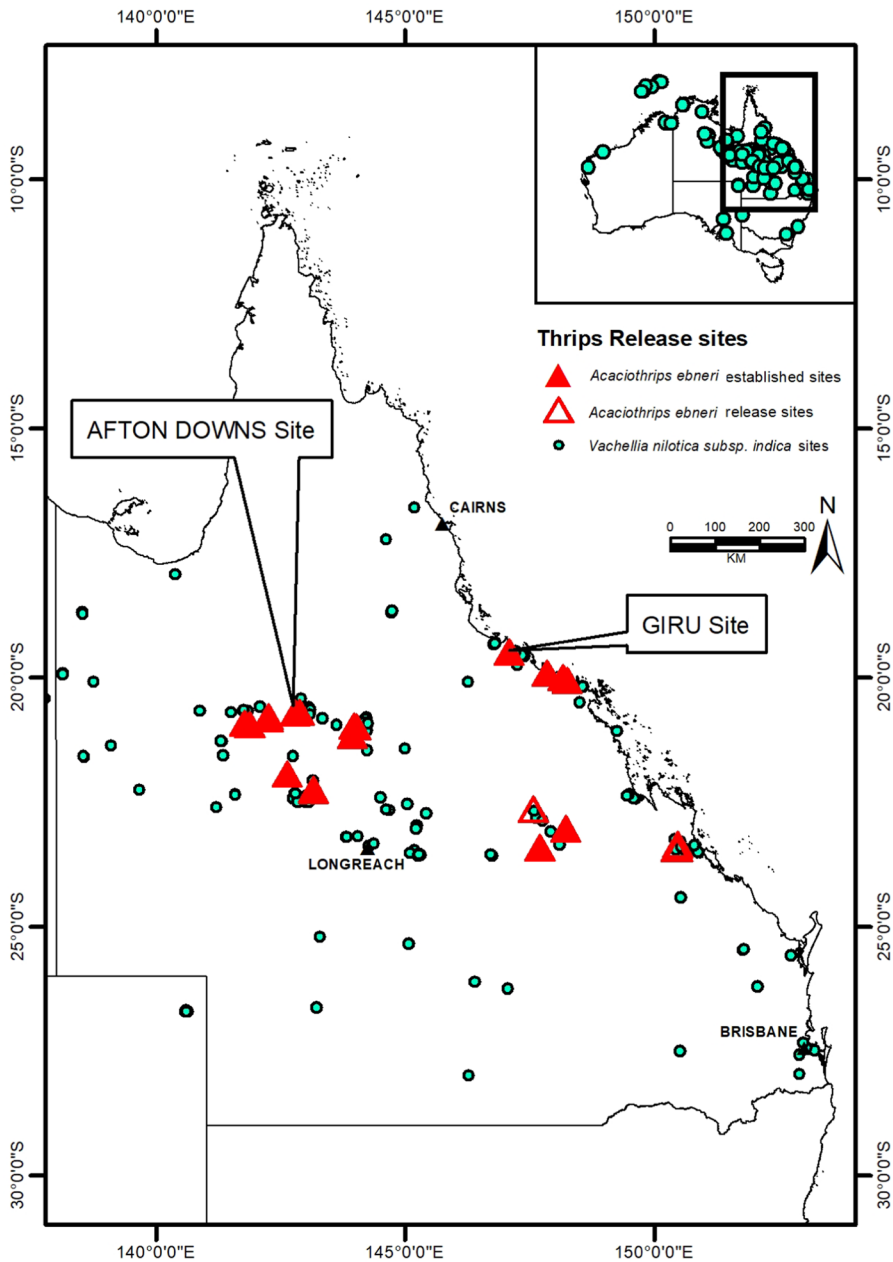

Fig. 4 *Vachellia nilotica* subsp. *indica* infestation and *Acaciothrips ebneri* release sites in Queensland, Australia (the submitted occurrence records from Atlas of Living Australia 2023), green circles showed the *Vachellia nilotica* subsp. *indica* infestation sites and red triangles indicated *A. ebneri* released and established sites

Fig. 5 Preliminary field observation of *Vachellia nilotica* subsp. *indica* plants with fresh galls (left: after 60 days) and dying galls (right: after 100 days) after *Acaciothrips ebneri* releases in Bowen, Queensland, Australia

the adults feeding on extrafloral nectaries (Boughton 1981; Gonzalez and Marazzi 2018). No-choice tests confirmed that *A. ebneri* is host-specific, and does not pose a risk to any of the non-target Australian congeners. This is confirmed by occurrence of *A. ebneri* only on *V. nilotica* subsp. *indica* and *Vachellia nilotica* subsp. *tomentosa* (Benth.) Kyal. & Boatwr., not on any other co-occurring *Vachellia* species in Ethiopia (Dhileepan et al. 2018). The majority of gall-inducing insects are very host-specific (Shorthouse et al. 2005), which has been validated in this host-range trial. Choice tests were not conducted as there was no gall induction, egg laying or larval development on any of the non-target test plant species.

In Ethiopia, *A. ebneri* was only found on *V. nilotica* subsp. *indica* and *V. nilotica* subsp. *tomentosa* with moniliform pods, and not on *Vachellia nilotica* subsp. *leiocarpa* (Brenan) Kyal. & Boatwr. with non-moniliform pods (Dhileepan et al. 2018). This observation further highlights that the gall-inducing thrips are 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 *Vachellia nilotica* subsp. *kraussiana* (Benth.) Kyal. & Boatwr. with non-moniliform fruit pod (Dhileepan et al. 2018). Globally, there are four *V. nilotica* subspecies with moniliform fruits (all native to Africa and Asia)—*V. nilotica* subsp. *indica*, *V. nilotica* subsp. *tomentosa*, *Vachellia nilotica* subsp. *nilotica* (L.) P.J.H.Hurter and Mabb., and *Vachellia nilotica* subsp. *cupressiformis* (J.L.Stewart) Ali & Faruqi. *Vachellia nilotica* subsp. *indica* is the only subspecies occurring in Australia. All *V. nilotica* subspecies are prohibited species in Australia, and hence not likely to be introduced (Comben et al. 2021). *Acaciothrips ebneri* are highly host-specific to *V. nilotica* subsp. *indica*, and do not pose a threat to any other plant species in Australia, which makes them a safe and sustainable control option for *V. nilotica* subsp. *indica*.

Field establishment of biological control agents for *V. nilotica* subsp. *indica* in Australia has so far been poor (Senaratne et al. 2006). All six of the agents released prior to the release of *A. ebneri* may not have been adapted to the harsh climatic conditions of western Queensland (Dhileepan et al. 2009). Hence, climatically suitable areas in the native range for exploration and sourcing of biological control agents were identified using a CLIMEX model based on an Ecoclimatic Index, to source biological control agents suited to the hot and arid Mitchell Grass Downs in the northwest of Queensland (Senaratne et al. 2006; Dhileepan et al. 2018). The CLIMEX model identified areas in northern and eastern Ethiopia were climatically similar to the hot and arid regions of western Queensland (Senaratne et al. 2006). Based on the CLIMEX model, *A. ebneri* was sourced from climatically comparable areas in Ethiopia. With a better eco-climatic match for western Queensland, compared to coastal Queensland, we anticipate that *A. ebneri* will establish and control *V. nilotica* subsp. *indica* in the hot and arid western parts Queensland, where currently there are no effective biological control agents.

Gall-induction on shoot terminals and axillary meristems resulted in inhibited shoot growth. Galling also resulted in leaf thickening due to hypertrophy, and the petioles and leaves curled inwards at the shoot terminals resulting in rosette galls (Dhileepan et al. 2017). The overall colour of the petioles, rachis and leaves changed from green to purple red. The developing galls are thought to 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 were completely destroyed, often resulting in shoot-tip dieback.

Seedlings and juvenile stages of *V. nilotica* subsp. *indica* are the most susceptible stages to target for effective biological control (Dhileepan et al. 2022). *Acaciothrips ebneri* has been shown to be highly damaging to *V. nilotica* subsp. *indica* seedlings and

juvenile plants, as observed in the field in Ethiopia and in quarantine studies. With the agent being approved for release by the Australian Government, several field releases have occurred at coastal and inland sites in Queensland. At each site, 20–50 galls with approximately 5,000 adults of *A. ebneri* were released. A number of new and developing galls were observed at all released sites checked, with numbers varying from site to site after only one release. Different proportion of shoot-tip die backs was also documented at the coastal and inland sites, possibly due to the effect of predators (e.g., ants), climate conditions, or to lack of actively growing shoot tips. Further monitoring and additional releases will continue at the selected sites over the next three years. *Acaciothrips ebneri* will complement the two existing biological control agents, by reducing the vigour of *V. nilotica* subsp. *indica* seedlings and juvenile plants, and reducing flowering and fruit production on galled shoots of mature trees. More field releases at new field sites will be required, and several releases will be attempted in the inland region of Queensland where main infestations are located. Alongside other control methods, such as mechanical, physical and chemical control, the release of *A. ebneri* as a biological control agent will be a valuable additional tool in the *Vachellia nilotica* subsp. *indica* management toolkit. Further research and monitoring will continue to assess the establishment, dispersal and impact of *A. ebneri* on *V. nilotica* subsp. *indica*.

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Declarations

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This article does not contain any studies involving human participants performed by any of the authors.

Informed consent None.

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