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Prepared by: Dr K Dhileepan

Department of Agriculture,
Fisheries and Forestry

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New biocontrol opportunities for prickly acacia: exploration in India

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Abstract

Prickly acacia (*Vachellia nilotica* ssp. *indica*), a multipurpose tree native to the Indian subcontinent, is a Weed of National Significance and is widespread throughout the grazing areas of northern Australia. Biological control of *V. nilotica* ssp. *indica* has been in progress since the early 1980s, but with limited success to date. Based on genetic and climate matching studies, native surveys for potential biological control agents were conducted in India during 2008-2011. A total of 72 sites were surveyed in southern India and 60 sites in north-western India. Surveys yielded 33 species of phytophagous insects and two rust fungi. Based on host records, 20 insect species that are crop pests or polyphagous, and all plant pathogens other than the two rust fungi, were excluded from the list of potential biological control agents. Using field host range, geographic range, seasonal incidence, damage potential, and preliminary host-specificity test results in India, as filters, the following agents were prioritised for detailed host specificity tests: a scale insect (*Anomalococcus indicus*), two leaf-webbers (*Phycita* sp. A and *Phycita* sp. B), a leaf weevil (*Dereodus denticollis*), a leaf beetle (*Pachnephorus* sp.), one gall-inducing rust (*Ravenelia acacia-arabicae*) and a leaf rust (*Ravenelia evansii*). The two rusts were sent to CABI-UK for preliminary host-specificity testing. Import permits for the brown leaf-webber (*Phycita* sp. A), the green leaf-webber (*Phycita* sp. B), the scale insect (*A. indicus*), the leaf-weevil (*D. denticollis*) and the leaf-beetle (*Pachnephorus* sp.) have been obtained from relevant regulatory authorities in Australia. So far, 11 importations, containing several thousands of insects in total have been exported from India into a quarantine facility in Brisbane, Australia. Based on these importations, host specificity tests for the brown leaf-webber (*Phycita* sp. A) have been completed, and the tests for the scale insect (*A. indicus*) and the green leaf-webber (*Phycita* sp., B) are in progress. Additional importations of the leaf-weevil (*D. denticollis*) and the leaf-beetle (*Pachnephorus* sp.) are planned for later in the year, when conditions are more conducive for field collections.

Executive summary

Prickly acacia (*Vachellia nilotica* ssp. *indica*), a multipurpose tree native to the Indian subcontinent, is a Weed of National Significance, and is widespread throughout the grazing areas of northern Australia. Biological control of *V. nilotica* ssp. *indica* has been in progress since the early 1980s, but with limited success to date. Based on genetic and climate matching studies, native surveys for potential biological control agents were initiated in India in July 2008 and continued until June 2011.

In southern India, a total of 64 sites in Tamil Nadu and eight sites in Karnataka were surveyed. In north-western India, surveys were conducted at 22 sites in Rajasthan and 48 sites in Gujarat. Surveys were conducted throughout the year, and each site was visited at least once every three months.

In southern India, a total of 33 species of phytophagous insects were collected from 72 sites over three years**.** Based on host records, 20 insect species that are crop pests or polyphagous, and all plant pathogens other than the two rust fungi, were excluded from the list of potential biological control agents. Using field host range, geographic range, seasonal incidence, damage potential, and preliminary host-specificity test results in India, as filters, the following agents were prioritised for detailed host specificity tests: a scale insect (*Anomalococcus indicus*), two leaf-webbers (*Phycita* sp. A and *Phycita* sp. B), a leaf weevil (*Dereodus denticollis*), a leaf beetle (*Pachnephorus* sp.), one gall-rust (*Ravenelia acacia-arabicae*) and a leaf-rust (*Ravenelia evansii*).

In north-western India, 14 species of insects (all known to be polyphagous pests), and 11 diseases (all known to have wide host ranges, except for the leaf-rust (*Ravenelia evansii*)) have been documented.

Permissions to export the prioritised agents from India (National Biodiversity Authority of India and the Indian Council for Forestry Research and Education) and permits to import the prioritised insects into a quarantine facility in Brisbane, Australia (Biosecurity Australia and the Department of Sustainability, Environment, Water, Population and Communities) were obtained in 2010 and 2013.

The rust gall (*R. acacia-arabicae*) and the leaf rust (*R. evansii*) were exported to CABI-UK and the preliminary host-specificity tests for both rust species have been completed.

So far, 11 importations of various prickly acacia insects have been made from India to Australia since 2011. Included in this are 218 leaf-weevil (*D. denticollis*) adults, 140 brown leaf-webber (*Phycita* sp., A) larvae, pupae and adults, 219 green leaf-webber (*Phycita* sp. B) larvae and pupae and thousands of scale insect (*A. indicus*) adults and nymphs.

Host specificity testing of the brown leaf-webber (*Phycita* sp. A) has been terminated midway due to unacceptable non-target feeding. Tests for the scale insect (*A. indicus*) and the green leaf-webber (*Phycita* sp., B) are in progress. A field choice trial for the scale insect (*A. indicus*) is underway in India. Additional importations of the leaf-weevil (*D. denticollis*) and the leaf-beetle (*Pachnephorus* sp.) are planned for later in the year, when conditions are more conducive for field collections.

The leaf-feeding *Chiasmia assimilis* introduced from Africa became well established at coastal sites in northern Queensland, but not widely in the arid inland regions. In an exclusion study in a coastal site, defoliation by *C. assimilis* significantly reduced the vigour of prickly acacia seedlings. The study suggests that in the coastal areas, periodic defoliation events by *C. assimilis* will cause significant negative impact on immature prickly acacia plants, resulting in reduced prickly acacia recruitment.

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1 Background

Prickly acacia (*Vachellia nilotica* ssp. *indica* (L.) Wild. Ex De.), a Weed of National Significance, is widespread throughout the grazing areas of western Queensland and has the potential to spread throughout northern Australia (www.weeds.org.au/WoNS/pricklyacacia). In the natural grasslands of western Queensland, over 7 million hectares, including 2000 km of bore drains, are infested with this weed (Mackey, 1997). The weed is also present in the coastal regions of Queensland, in the Northern Territory and Western Australia (Mackey, 1997). Prickly acacia infestations cost primary producers A$9 million/year in lost pasture production (Dhileepan, 2009). In such areas, prickly acacia forms impenetrable thorny thickets; competes with native pasture species; facilitates the replacement of native grasses with less stable, short-lived plants; prevents the growth of native plants beneath the canopy; restricts stock access to watercourses and poses a threat to nearly 25 rare and threatened animal species and two endangered plant communities by displacing native grasses (Spies and March, 2004). Prickly acacia has also been identified as one of the woody weeds responsible for land degradation, soil erosion and the inability to maintain biodiversity in riparian vegetation and wetlands (Dhileepan, 2009).

Biological control of prickly acacia was initiated in the early 1980s, with native range surveys conducted in Pakistan (Mohyuddin, 1986), Kenya (Marohasy, 1992) and South Africa (Stals, 1997). Among the 43 phytophagous arthropods collected on *V. nilotica* ssp. *indica* in Pakistan, two were introduced into Australia, but only the seed-feeding bruchid *Bruchidius sahlbergi* Schilsky established. Three of the 90 phytophagous insects collected on V. *nilotica* ssp. *subalata* (Vatke) Brenan and *V. nilotica* ssp. *leiocarpa* Brenan in Kenya were introduced into Australia. A leaf-feeding geometrid *Chiasmia assimilis* (Warren) that was introduced from Kenya was re-introduced again, after being collected from *V. nilotica* ssp. *kraussiana* (Benth) Brenan in the later South African surveys. Of the three African insects, only *C. assimilis* established (Dhileepan, 2009). So far, the impact of *B. sahlbergi* on *V. nilotica* ssp. *indica* has been insignificant (Radford *et al*., 2001). *Chiasmia assimilis* became well established at coastal sites causing severe defoliation in northern Queensland (Lockett *et al*., 2012), but not widely in the arid inland regions (Palmer *et al*., 2007). As a result, more effective biological control agents are needed for arid inland Australia, where the introduced agents have either not established or established but are ineffective.

Vachellia nilotica (L.) Willd. Ex Del., with nine recognised subspecies (Brenan, 1983), has a broad native range including much of Africa, the Middle East and the Indian subcontinent (Dwivedi, 1993). The invasive prickly acacia (*V. nilotica* ssp. *indica*) in Australia is native to the Indian subcontinent. Based on genetic (Wardill *et al*., 2005) and climate matching (Dhileepan *et al*., 2006) studies, native range surveys were initiated in north-western (Gujarat and Rajasthan) and southern (Tamil Nadu and Karnataka) India (Dhileepan *et al*., 2010). A simulated herbivory study indicated that a combination of leaf- and shoot-feeding insects would be more effective than root-feeding insects alone, as biological control agents for prickly acacia (Dhileepan *et al*., 2009). Since prickly acacia populations are not seed limited, flower and seed feeding agents are believed to have only a minor impact as weed biological control agents (Marohasy, 1992; Kriticos *et al*., 1999). Hence, less priority was given for flower and seed feeding insects during surveys in India. In this report, I list the potential biological control agents that were prioritised for further study, based on host plant records from the literature, field host range, geographic range, seasonal activities, and damage levels.

2 Project objectives

1. Survey and catalogue insects and pathogens associated with prickly acacia (*V. nilotica*) in India (**MET**).

   - In southern India, 33 insects and two rust pathogens were collected from 72 sites. These included 16 leaf-feeders, eight stem-feeders, four species with leaf-feeding adults and root-feeding larvae, two stem-borers and bark-feeders and three flower-feeders. In north-western India, 14 insects and 11 plant pathogens were collected from 22 sites.

2. Assess host range of insects and pathogens based on host plant use in the field (**MET**).

   - In southern India, eight species of insects and two rust fungi were observed only on prickly acacia and not on any non-target plants co-occurring in the survey sites. In north-western India, all insects and pathogens collected all have wide host ranges, except for the two rust pathogens.

3. Confirm primary host of *V. nilotica* for prioritised agents through preliminary host-range testing (**MET**).

   - Based on field host range, no-choice host specificity tests were conducted for four insect species in India and two rust fungi in UK.

4. Prioritise potential biocontrol agents on the basis of likely impacts on the weed (**MET**).

   - Using a scoring system based on field host range, geographic range, seasonal incidence and damage levels, a scale insect, two leaf-webbing, a leaf weevil, a leaf beetle, a gall-inducing rust and a leaf rust were prioritised for detailed host specificity tests.

5. Seek and obtain approval from the Indian government through its biodiversity act to export prioritised biocontrol agents to Australia for further host specificity tests (**MET**).

   - The gall rust southern India and the leaf rust from north-western India were exported to CABI (UK). So far, 11 importations of various prickly acacia insects have been made from India to Australia.

3 Methodology

3.1 Study organisms

In India, *V. nilotica* is a multipurpose tree that occurs naturally and is cultivated throughout the country. It is used widely in agroforestry, social forestry, reclamation of wastelands, and rehabilitation of degraded forests. The subspecies of *V. nilotica* that are native to India are *V. nilotica* ssp. *indica*, *V. nilotica* ssp. *cupressiformis* (Stewart) Ali & Faruqi and *V. nilotica* ssp. *hemispherica* Ali & Faruqi (Dwivedi, 1993). Among these, *V. nilotica* ssp. *indica* is the most prominent and widespread subspecies. In southern India, *V. nilotica* ssp. *tomentosa* (Benth.) Brenan, a native of central Africa, co-occurs with *V. nilotica* ssp. *indica* in Tamil Nadu, while *V. nilotica* ssp. *cupressiformis* co-occurs with *V. nilotica* ssp. *indica* in Karnataka. In addition, other species of *Acacia* including species native to Australia, occur widely in India, and in some areas co-occur with *V. nilotica* ssp. *indica*. In north-western India, *V. nilotica* ssp. *hemispherica* is prevalent in protected nature reserves and national parks (e.g. Gir Forest) and along the southern coast in Gujarat; and *V. nilotica* ssp. *cupressiformis* is widespread and co-occurs with *V. nilotica* ssp. *indica* throughout the state of Rajasthan.

3.2 Native range surveys

The project was initiated in India after approval from the Indian Government was received in November 2007. Contracts with the two collaborating research agencies in India, the Institute of Forest Genetics and Tree Breeding (IFGTB), Coimbatore and the Arid Forest Research Institute (AFRI), Jodhpur were signed in February 2008 and the surveys commenced in July 2008 (Dhileepan *et al*., 2010). Rajasthan and Tamil Nadu represent areas with hot and dry conditions similar to those of western Queensland, while Gujarat and Karnataka represent areas with hot and humid conditions (Dhileepan *et al*., 2006). In southern India, survey sites were predominantly forestry plantations, in tank beds, isolated plants on the roadsides and on bunds on agricultural lands. In contrast, survey sites in north-western India were predominantly natural groves and forestry plantations.

In southern India, a total of 64 sites across 20 districts (administrative areas within States) in Tamil Nadu and eight sites across two districts in Karnataka were surveyed (Figure 1; Table 1). In Tamil Nadu and Karnataka, surveys were conducted throughout the year, with all sites visited at least once in every three months, from November 2008 to December 2011 (Table 2).

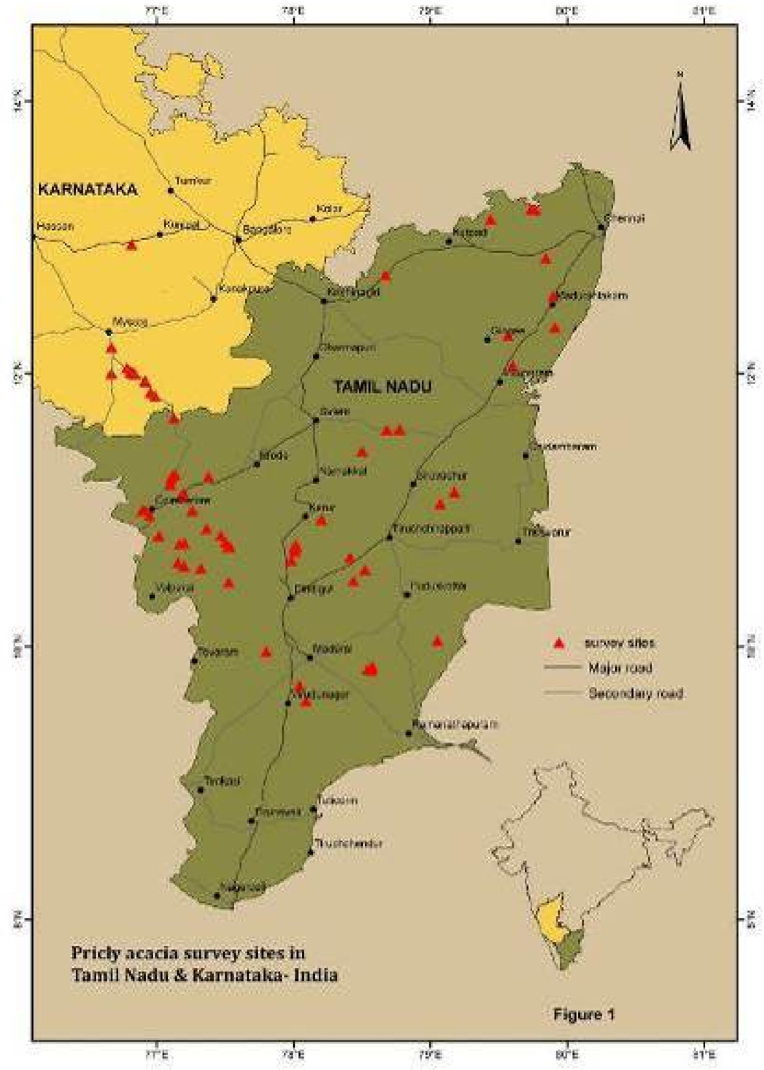

Figure 1. Survey sites in Tamil Nadu and Karnataka states in southern India.

In north-western India, surveys were conducted at 22 sites across 13 districts in Rajasthan and 48 sites across 11 sites in Gujarat (Figure 2). Among them, eight permanent sites, Jodhpur (26°14'20.21"N; 73°1'27.51"E), Pali (29°30.746'N; 77°30.632'E), Hanumangar (29°30.746'N; 74°20.289'E) and Bharatpur (27°11.305'N; 77°30.632'E) in Rajasthan, and Gandhinagar (23°15.046'N; 72°41.375'E), Nadiad (22°45.069'N; 72°51.572'E), Junagarh (21°26.892'N; 70°29.239'E) and Bhuj (23°25.307'N; 69°10.340'E) in Gujarat, were surveyed at quarterly intervals, while other sites, many of them plantations and natural groves along roadside (Figure 2), were surveyed opportunistically while visiting the eight permanent sites.

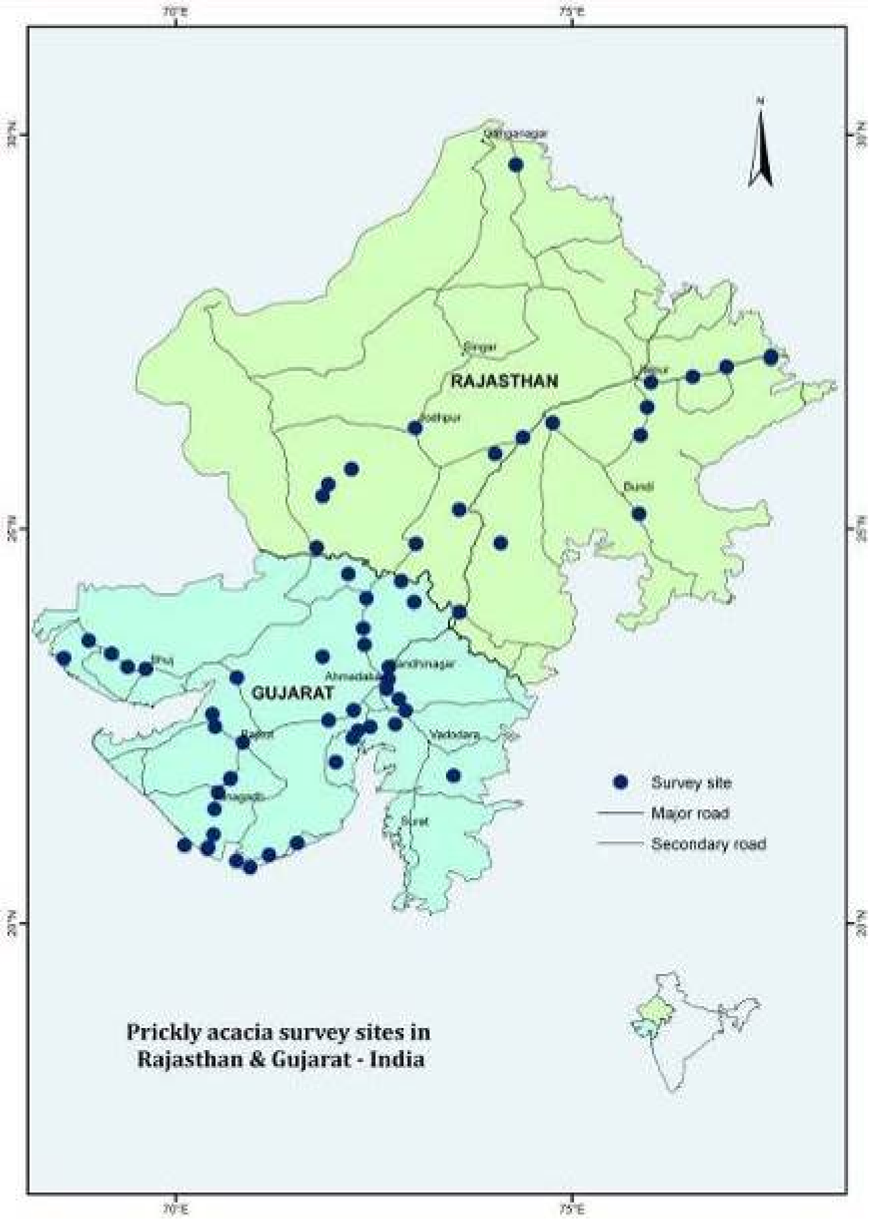

Figure 2. Survey sites in Gujarat and Rajasthan states in north-western India.

At survey sites with juvenile and young trees, the entire tree canopy was inspected visually for the presence of insects, or signs of feeding damage or disease symptoms. At sites with mature trees, branches of trees that could be accessed from the ground were sampled visually for insects and diseases. At each site, the incidence of insects or insect damage and disease symptoms were recorded, together with details of which subspecies of *V. nilotica* were present, the plant life stages (seedlings, juveniles or mature trees) found, co-occurring vegetation (e.g. other *Acacia* spp. and *Vachellia* spp.) and weather details (temperature and relative humidity) at the time of sampling. At each site, two or three research staff spent a minimum of one hour surveying for insects and diseases. Immature stages of insects collected in southern India were brought to IFGTB (Coimbatore), while those collected in north-western India were brought to AFRI (Jodhpur), and reared to adults for identification.

3.3 Agent identification

All insects, mites and rust fungi collected on *V. nilotica* were first matched locally with previously identified specimens at IFGTB (Coimbatore) and AFRI (Jodhpur) within India.

If necessary, the specimens were then sent to taxonomic experts and agencies within India (Indian Agricultural Research Institute, Kerala Forest Research Institute, Tamil Nadu Agricultural University, Indian Forest Research Institute, University of Agricultural Sciences - Bangalore and Zoological Survey of India) and overseas (British Museum of Natural History, CABI – UK, CSIRO, Queensland Plant Pathology Herbarium and Queensland Museum) to confirm the identification.

3.4 Ecological host range

In southern India, *V. nilotica* ssp. *indica* and *V. nilotica* ssp. *tomentosa* were both present at 51 of the 72 survey sites, while *V. nilotica* ssp. *indica* and *V. nilotica* ssp. *cupressiformis* occurred together at only six sites (Table 1). There were 12 survey sites with only *V. nilotica* ssp. *indica* and three sites with only *V. nilotica* ssp. *cupressiformis*, and these sites were all found in Karnataka (Table 1). There was no site with only *V. nilotica* ssp. *tomentosa*. Where two subspecies co-occurred, both were sampled with equal time spent on each subspecies. Field specificity of various phytophagous insects and mites, and rust pathogens at subspecies level was documented. Other *Vachellia, Senegalia* and *Acacia* species, *V. leucophloea* (Roxb.) Maslin, Siegler & Ebinger, *Senegalia ferruginea* (DC.) Pedley, *S. senegal* (L.) Britton & P. Wilson (natives of India), *V. horrida* (L.) Kyal. & Boatwr. (native of Africa) and other non-target trees (e.g. *Prosopis juliflora* (Sw.) DC., *Pongamia pinnata* (L.) Panigrahi, *Azardirachta indica* A. Juss., *Ziziphus mauritiana* Lam) occurring at the survey sites were also surveyed to ascertain if the ecological host range of insects found on *V. nilotica* extended to these species. Sampling of the non-target *Acacia* spp. and other tree species at each survey site was restricted to only the agents that were collected on *V. nilotica*.

In north-western India, the two subspecies, *V. nilotica* ssp. *indica* and *V. nilotica* ssp. *cupressiformis* co-occurred at most of the survey sites in Rajasthan. In Gujarat, *V. nilotica* ssp. *indica* was the predominant subspecies at all survey sites, except in protected nature reserves and national parks (e.g. Gir Forest) and southern coast where *V. nilotica* ssp. *hemispherica* was the dominant subspecies (Dhileepan *et al*., 2010). Other *Acacia* species, *V. leucophloea, S. catechu* (L.f.) P.J.Hurter & Mabb.*, S. senegal, V. tortilis* (Forssk.) Galasso & Banfi., *A. jacquemontii* Benth, and the leguminous species, *Prosopis cineraria* (L.) Druce, occurring at the survey sites were also surveyed.

3.5 Potential impacts (exclusion studies)

The impact of native insect herbivores (all insects combined) on the survival and growth of potted juvenile plants under field conditions was evaluated in exclusion trials conducted over two years at four sites, each in Rajasthan (Hanumangar, Desuri, Bharatpur and Jodhpur) and Gujarat (Gandhinagar, Nadiad, Junagarh and Bhuj), and over 18 months at two sites in Tamil Nadu (Coimbatore and Thoppur). At each site, potted prickly acacia juvenile plants, with half of the plants protected from insect herbivores (by spraying insecticides at fortnightly intervals) and the remaining half exposed to insect herbivores (by spraying with water) were maintained. In Rajasthan and Gujarat, there were 25 potted prickly acacia plants for each treatment (insecticide treated under canopy, insecticide treated under open sun; no-insecticide treatment under canopy and no-insecticide treatment under direct sun) in each site (25 plants x 4 treatments x 4 sites x 2 states). In Tamil Nadu, there were five blocks of 12 potted prickly acacia plants for each treatment (insecticide treated and no-insecticide) in each site (2 sites) (12 plants x 5 blocks x 2 treatments x 2 sites). All plants were sampled at quarterly intervals, with the incidence and abundance of various insects along with details on several plant parameters (e.g. defoliation levels, plant height, number of leaves, number of shoots, basal stem diameter, etc.) were recorded. This information was used for prioritising agents for more detailed studies, including host-specificity tests.

3.6 No-choice host specificity tests

Only those insects and pathogens that showed field-level specificity for *V. nilotica* were selected for preliminary host specificity tests. For phytophagous insects and diseases, for which species-level identification could be made, host records were checked through a literature search, and those agents recorded with a wider range of hosts were excluded from preliminary host specificity tests. Likewise, phytophagous insects and diseases that were observed on non-target *Acacia*, *Vachellia* and *Senegalia* species during the field surveys were also excluded from the no-choice host specificity tests.

Based on field host range, no-choice host specificity tests were conducted for four insect species, *Anomalococcus indicus* Ramakrishna Ayyar (Hemiptera: Lecanodiaspidae), *Phycita* sp. A (Lepidoptera: Pyralidae), *Isturgia disputaria* (Guenée) (Lepidoptera: Geometridae), and *Dereodus denticolis* Boheman (Coleoptera: Curculionidae), and two rust fungi, *Ravenelia acaciae-arabicae* Mundk. & Thirum (Uredinales: Raveneliaceae) and *R. evansii* Syd. & P. Syd (Uredinales: Raveneliaceae). The insect species, together with the gall rust (*R. acaciae-arabicae*), were tested at the IFGTB campus (11°1'36.68"N; 76°56'39.17"E), Coimbatore, India, while testing of the leaf rust (*R. evansii*) was conducted at AFRI, Jodhpur, India.

No-choice host specificity tests for the five species of insects at IFGTB included *Acacia*, *Vachellia* and *Senegalia* species from Asia (*Acacia planiferans,* Wight & Arn., *S. ferruginea*, *V. leucophloea*, *S. catechu*), Africa (*Senegalia mellifera* (M. Vahl) Seigler & Ebinger, *V. tortilis*), Australia (*A. auriculiformis* A. Cunn. ex. Benth., *A. deanei* (R.T. Bajer) M.B. Welch, Coombs & McGlynn) and tropical America (*V. farnesiana* (L.) Wright et Arn.). The tests were conducted in the laboratory using cut foliage, and under field conditions using potted plants enclosed in insect-proof cages. Four additional non-acacia test plants were included in the no-choice host specificity tests because they were mentioned in previous host plant reports: *Delonix regia* (Bojer ex Hook.) Raf. against *I. disputaria*; *Anacardium occidentale* L. and *Mangifera indica* L. against *Phycita* sp.A; and *Piper nigrum* L. against *A. indicus*.

No-choice larval feeding and development tests for *I. disputaria* and *Phycita* sp. A were conducted using cut foliage as a bouquet with shoots immersed in a glass jar with water. In all tests, *V. nilotica* ssp. *indica* was included as a control, and the test was repeated at least five times. The cut foliage was replaced on alternate days and the percentage of larvae completing development (% pupation and % adult emergence) and duration of larval and pupal development were recorded. For *A. indicus*, a scale-infested shoot of *V. nilotica* ssp. *indica* collected in the field (10-15 cm long and with at least 20 gravid female scales) was tied on each potted test plant or on field plants. A scale-infested stem was tied to potted *V. nilotica* ssp. *indica* juvenile plants as a control. Test and control plants were sampled at monthly intervals for four months and the proportion of female nymphs becoming gravid females per plant were recorded. For *D. denticollis* no-choice adult feeding tests were conducted using cut-foliage of test plants as bouquets (bouquets replaced on alternate days) and the proportion of leaf area consumed was recorded. Tests of no-choice larval survival and development for this agent were not undertaken as the oviposition behaviour and larval habits of the agent are not known.

No-choice host specificity tests for the gall rust (*R. acaciae-arabicae*) at IFGTB included *V. tortilis*, *A. auriculiformis*, *A. deanei* and *V. farnesiana* as test plants with *V. nilotica* ssp. *indica* plants as controls. A suspension of field collected urediniospores of *R. acaciae-arabicae* was applied to the undersides of young leaves of control and treatment plants. The rust-inoculated plants were transferred to a shade-house and monitored for macroscopic rust symptoms.

No-choice host specificity tests for the leaf rust (*R. evansii*) at AFRI included three potted plants each of *A. planiferans, A. auriculiformis*, *V. tortilis*, *V. farnesiana*, *S. catechu* (as test plants) and *V. nilotica* ssp. *indica* (as control). The experiment was conducted in a shade-house at AFRI between 24 February 2010 and 3 March 2010 using urediniospores of *R. evansii* collected from Nadiad in the previous week. A suspension of urediniospores of field-collected *R. evansii* was sprayed on the leaves of test and control plants. Inoculated test and control plants were monitored daily for macroscopic rust symptoms and the proportion (%) of rust-infected leaflets were recorded along with the temperature and humidity levels in the shade-house.

3.7 Prioritisation process

For all insects and rust pathogens collected during the survey that were identified to species level, previous host records and pest status were used to first eliminate known crop pests and polyphagous insects and pathogens. Based on a literature search, a score between '1' and '5' was given (1 = pest of crops; 2 = host records across diverse plant families; 3 = host records restricted within Mimosaceae; 4 = host records limited to *Vachellia*/*Acacia* species; 5 = host records limited to *V. nilotica*) for each insect and rust fungus. Insects and rust pathogens with a score of '3' or less were eliminated from the prioritisation process.

Based on the field host range recorded during the survey, a score between '1' and '5' was given (1 = hosts across diverse plant families; 2 = occur on multiple genera, within a plant family; 3 = occur on a wide range of *Vachellia*/*Acacia* species; 4 = limited to few closely related *Vachellia* species; 5 = restricted to *V. nilotica*) for each insect and rust fungi. Insects and rust pathogens with a score of '3' or less were eliminated from the prioritisation process.

For geographic range of each agent, a score between '1' and '5' was given (1 = collected from less than 20% of the survey sites; 2 = collected from 20 to 40% of the survey sites; 3 = collected from 40 to 60% of the survey sites; 4 = collected from 60 to 80% of survey sites; 5 = collected from more than 80% of survey sites).

For seasonal incidence, a score between '1' and '5' was given (1 = occur less than three months in a year; 2 = occur between three and five months in a year; 3 = occur between six and eight months in a year; 4 = occur between nine and 10 months in a year; 5 = occur more than 10 months in a year) for each insect and rust fungus.

For damage levels, a score between '1' and '5' was given (1 = no visible symptoms; 2 = visible, but minor symptoms; 3 = seasonal damage - defoliation, shoot dieback, etc.; 4 = loss of vigour – complete defoliation, shoot dieback, etc; 5 = field mortality), based on visual field observations.

Results from the preliminary host specificity tests conducted for selected insects in India were also used as filters in the prioritisation process. Scores between '1' and '5' were given (1 = feeding and development on host plants across diverse plant families; 2 = feeding and development on multiple genera, within a plant family; 3 = feeding and development on a wide range of *Vachellia*/*Acacia* species; 4 = feeding and development on only a few closely related *Vachellia* species; 5 = restricted to *V. nilotica*), and insects with scores below '4' were eliminated from the list.

For each insect species, a priority score combining scores from 'host plant records', 'field host range', 'geographic range', 'seasonal incidence' and 'damage levels' was computed and the species were ranked based on the priority scores.

3.8 Effectiveness of established agents in Australia

The leaf-feeding geometrid (*Chiasmia assimilis*) has become established in infestations in coastal areas between the townships of Ayr and Bowen where the larvae caused severe, periodic defoliation at some localities during summer and autumn. Defoliation by *C. assimilis* has also been reported from other sites near Hughenden. Sampling for incidence of and damage caused by this agent at the original coastal release site (Ashfield) near Guthalungra in north Queensland was conducted between March 2005 and September 2010. Visits to the site were made at 6-8 weekly intervals. Twenty tip cuttings were taken from each of 10 trees. The numbers of larvae present were recorded together with estimates of the per cent defoliation by the insect. Percentage of normal leaf cover present, irrespective of insect feeding, as well as number of seed pods and the number of filled seeds per pod, were also recorded.

The impact of this herbivory on a number of plant parameters, including shoot length, basal stem diameter, root length, number of leaves, number of branches, and above and below ground biomass was investigated at a second coastal site (Inkerman) through an insect exclusion trial using potted seedlings and regular spray applications of a systemic insecticide to exclude the biological control agent. Half the seedlings, both sprayed and unsprayed, were placed beneath the prickly acacia canopy, the other half in full sunlight. A total of 160 potted prickly acacia seedlings were used in the 2 x 2 factorial experiment containing four replicates with 10 seedlings per replication. Factor A comprised two biological control treatments: exposure to *C. assimilis* or exclusion from *C. assimilis*. Factor B was the location of seedlings within the infestation: in shade beneath the prickly acacia canopy or in full sunlight away from the canopy. For the insect-excluded treatment, seedlings were sprayed on a fortnightly basis using the insecticide Folimat (2 g/kg omethoate). Treatment and control plants were inspected every two weeks and the numbers of larvae and eggs on each plant were recorded, together with estimates of the per cent defoliation caused by insect feeding. Measurements of shoot length and basal stem diameter were recorded and counts of the number of leaves and number of branches were also made. At the end of the trial, final counts and measurements were taken and all surviving seedlings were collected from the site for biomass estimation.

4 Results

4.1 Phytophagous insects and plant pathogens

A total of 33 species of phytophagous insects were collected from 72 sites over three years (2008-2011) in southern India (Tables 1-3). These included 17 leaf-feeders, five species with leaf-feeding adults and root-feeding larvae, five sap-feeders, two stem-borers and bark-feeders, two flower-feeders and a seed-feeder. Species-level identification was made for 28 insects (Table 1). The two rust pathogens (Table 1) were identified to species level. The average number of species recorded at each survey site differed significantly between districts ($F_{17,54}$ = 1.898; P = 0.039), but the difference was not significant between Tamil Nadu (10.4 ± 0.6) and Karnataka (10.1 ± 1.3) states (t = 0.217, df =70, P = 0.829). The number of species recorded at each survey site varied significantly depending on the number of times the site was surveyed ($F_{7,54}$ =-8.45; P < 0.001) and the number of species collected at survey sites increased with an increase in the number of times the sites were surveyed (Figure 3). There was no significant difference in the number of phytophagous species collected from survey sites with ssp. *cupressiformis* alone (14.7 ± 0.88), with ssp. *indica* alone (10.5 ± 0.89), with ssp. *indica* + *cupressiformis* (8.5 ± 1.06), and with ssp. *indica* + *tomentosa* (10.3 ±-0.67) ($F_{3,69}$ = 0.939; P = 0.424).

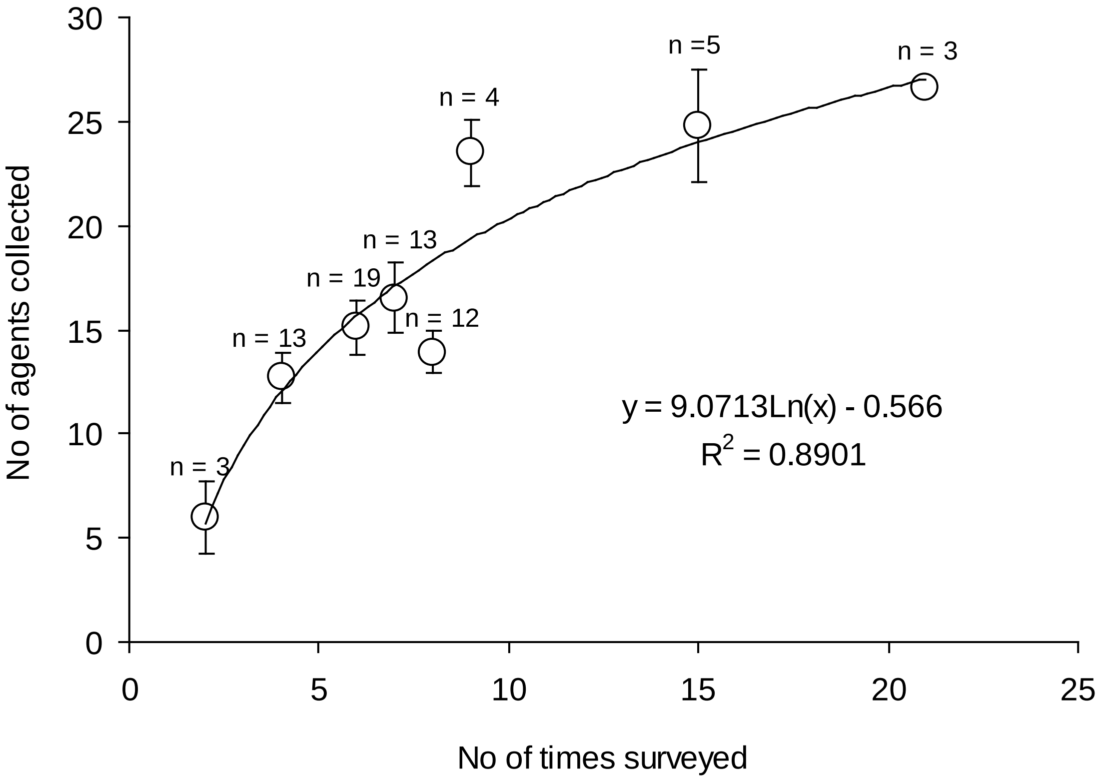

Figure 3. Relationship between the number of times each site was surveyed and the number of agents recorded (mean ± se) at the survey sites over a three-year period.

In north-western India, 14 species of phytophagous insects and mites, and 11 diseases were documented. However, all of the insect species (e.g. *Oxyrchachis tarandus* Fab., *Achaea janata* (L.), *Cerosterna scabrator* (F.), *Selepa celtis* Moore, *Indarbela quadrinotata* Walk., *Eumeta crameri* (Westwood), *Mylloceros* spp., *Pteroma plagiophleps* Hampson, *Florithrips traegardhi* (Trybom), *Catopsilia pomona* Fab. and *Acaudaleyrodes rachipora* (Singh)) and all plant diseases (e.g. *Ganoderma lucidum* (Curtis) P. Karst, *Macrophomina phaseolina* (Tassi) Gold., *Lasiodiplodia theobromae* (Pat.) Griffon & Maubl., *Rhizoctonia solani* J.G. Kühn, *Phellinus fastuosus* (Lév.) Ryv., *Formes* spp., *Fusarium solani* (Mart.) Sacc. and *Oedium* sp.), except the leaf rust (*R. evansii*) are known to have wide host ranges. The host range of the two species of mites (*Tenuipalpus* sp. and *Oligonynchus* sp.) collected on *V. nilotica* leaves in Rajasthan could not be ascertained as they were unable to be identified to species level. Three species of nematodes, *Hoplolaimus indicus* Sher. (plant parasitic nematode associated with roots), *Aphelenchus avenae* Bastian (fungal-feeding nematode associated with plant roots), and *Helicotylenchus* sp (root-feeding nematode) were also collected from root zones of stunted *V. nilotica* trees in the Bhuj region of Gujarat. The leaf-rust (*R. evansii*) was collected only in Gujarat and not in Rajasthan.

4.2 Host records from the literature

Twenty of the 33 insect species collected during the survey were either known crop pests or polyphagous insects (Table 3), and were excluded from the prioritisation list. Among the remaining insects, *I. disputaria*, *D. denticollis* and *D. mastos* Herbst. have been reported only on *V. nilotica* in India. There are no host records for the chrysomelid beetle, *Cryptocephalus rufofemoratus* Jacoby. The geometrid *I. disputaria*, a major pest of *V. nilotica* in India, has been recorded on *V. tortilis, A. mollissima and A. decurrens* in Africa (Kruger, 2001). The babul scale (*A. indicus*), a pest of *V. nilotica*, has been reported on *V. farnesiana, V. leucophloea* (Ben-Dov 2006), *S. catechu, Ziziphus mauritiana* Lam. (Rhamnaceae) (Beeson 1941) and *Piper nigrum* L. (Piperaceae) (Koya *et al*., 1996). The leaf-feeding chrysomelid, *Diapromorpha turcica* Fab., has been reported on other hosts like *Mikania micrantha* Kunth ex H.B.K. (Abraham *et al*., 2002), *Leucaena leucocephala* (Lam.) de Wit (Nayar, 2001), *Lagerstroemia* sp. (David and Ananthakrishan, 2004) and *S. catechu* (Beeson, 1919). Adults of *Clytra succincta* Lacordaire have been reported feeding on leaves of *Prosopis cineraria* (L.) Druce in India. Host record searches were not possible for insects not identified to species (e.g. *Phycita* spp., *Pachnephorus* sp. and *Nipaecoccus* sp.).

All plant pathogens (e.g. *G. lucidum*, *F. solani*, *L. theobromae*, *Fomes* spp., *R. solani,* and *P. fastuosus*) other than the two rust fungi (*R. acacia-arabicae* and *R. evansii*) were also excluded due to their wide range of hosts. *Ravenelia acaciae-arabicae* was originally described by Mundkur & Thirumalachar (1946) on *V. nilotica* (misapplied syn *A. arabica* Willd.) from Mysore, Karnataka State in India. Later, Kapoor & Agarwal (1974) and Bagyanarayana and Ravinder (1988) treated *R. acaciae-arabicae*, as a synonym of *R. evansii*. In Africa, *R. evansii* has been reported from *V. sieberiana (*DC.), *A. macrothyrsa* Harms, *A. gerrardii* Benth., *A. rehmanniana* Schinz, *V. robusta* (Burch.) Kayl. & Boatwr, and *V. seyal (*Del.) P.J.H. Hurter (Cannon, 2008). Recent surveys in India found that the two rusts, *R. acaciae-arabicae* and *R. evansii*, were distinct species that could be easily separated by morphology of the urediniospores (Shivas *et al*., 2012).

4.3 Field host range

In southern India, 13 species of insects and two rust fungi that progressed through the ‘host record’ filter (Table 3) were observed intensively on hosts in the field. Eight species of insects (*A. indicus*, *Phycita* sp. A, *Phycita* sp. B., *I. disputaria*, *D. denticollis*, *D. mastos*, *Pachnephorus* sp. and *C. rufofemoratus*) and two rust fungi (*R. acacia-arabicae* and *R. evansii*) were observed only on *V. nilotica*, and not on *S. catechu*, *V. leucophloea*, *V. horrida* or *S. ferruginea* co-occurring with *V. nilotica* in the survey sites. *Pachnephorus* sp. and *R. acacia-arabicae* were restricted to *V. nilotica* ssp. *indica*, while *A. indicus*, *Phycita* sp. A, *Phycita* sp. B., *I. disputaria*, *D. denticollis*, *D. mastos* and *C. rufofemoratus* were observed on all three subspecies of *V. nilotica*. *Nipaecoccus* sp., *Clytra succincta* Lacord., and *Mylloceros* spp. were observed on *Prosopis juliflora* (Sw.) DC and hence excluded.

In north-western India, all insects identified to species level were known polyphagous pest species. Likewise all plant diseases (*G. lucidum*, *F. solani*, *L. theobromae*, *Fomes* spp., *R. solani,* and *P. fastuosus*) other than the leaf-rust (*R. evansii*) were also known to have a wide host range.

Table 1. Incidence of phytophagous insects and rust fungi on various subspecies of *Vachellia nilotica* in survey sites in southern India. Values represent proportion (%) of time each agent was collected at each site over the three year period. Values for prioritised agents presented in dark background and the rest of the insects are shaded grey. Unshaded areas indicate that the agent was collected at the corresponding site.

				Prioritised insects and rusts								Other insects																										
State	District	Survey sites	*Acacia nilotica* subspecies#	***Anomalococcus indicus*** **R. Ayyer**	***Phycita sp. A***	***Phycita sp. B***	***Isturgia disputaria*** **(Guenee)**	***Dereodus denticollis*** **Boheman**	***Pachnephorus sp.***	***Ravenelia acacia-arabicae*** **Mundk. & Thirumalachari**	***Ravenelia evansii*** **Syd. & P.**	*Diapromorpha turcica* Fab.	*Selepa celtis* Moore	*Ascotis infiraria* Walker	*Hyposidra successaria* Walker	*Clyda succinata* Lacord	*Myllocerus* sp.	*Inderbela quadrinotata* Walker	*Oxyrhachis tarandus* Fab.	*Homoeocerus signatus* Walker	*Eumeta crameri* (Westwood)	*Pteroma plagiophleps* Hampson	*Hypolixus truncatulus* (Fab.)	*Nippococcus* sp.	*Paracoccus marginatus* Williams and Granara de Willink	*Oxytonia versicolor* (Fab.)	*Euproctis scintillans* Walk.	*Cryptocephalus* sp.	*Dereodus mastos* Herbst.	*Mylabris* sp.	*Steblote siva* (Lefebvre)	*Euproctis lutana* Walk.	*Dasychira mentosa* (Hubner)	*Chrysocoris purpureus* (Westwood)	*Psiloptera fastuosa* (Fab.)	*Sthenias* sp.	*Iedra mutica* Fab.	*Flata ferrugata* (Fab.)
Tamil Nadu	Coimbatore	1	*I + T*	100	38	48	76		52	38	29		43	29	38	14	48	43	95	33	86	38	14	29				29			48		38	19		19	24	38
		2	*I + T*	100	29	24	62		29	29	29	14	57	19	10	24	29	57	100	14	57	29		10				24			38		29	14		10	10	19
		3	*I + T*	100	100	24	86		62	43	29		48	19	19	10	29	24	100	38	100	48	14	29							57		33	29		24	14	29
		4	*I + T*	100	27	40	67	100	100	53	40	40	40		13	13	33	73	100	40	67	33							13	33	47		33	13		40	33	13
		5	*I + T*	100	73	53	80	100	100	40	40	67	60	13		27	40	53	40	47	53	40	33					40	20	40	53		53	33		40	40	7
		6	*I + T*	100	40	20	60	100	100	60	60		53		20	20	20	67	80	33	60	33	20	27		20	27	13	40	27	40		40	13	13	13	53	60
		7	*I + T*	80	20		80	50	80			25	10	30		10	20	50	80	20	80												10	10			20	
	Madurai	1	*I*	50	17		83	50	67	33	33		17						50	33	67						33					83	33			17		17
		2	*I + T*	67	33		50	33	50								33	33	67		50				17		17										17	
		3	*I + T*	67	50	50	67		17	17	17		50				17	17	67		67	17	17				17	33			33		17		17	33		33
	Sivagangai	1	*I*	67		67	50	83	67	17	17	25	17				17	17	67	33	33							50	33	17	33		33			33		50
		2	*I*	50	33	50	83	50	50	17	17	33	33			17	17		17	33	50	17								17	17		17				50	17
		3	*I + T*	67	17		33	67	33			25	33			17	50		33	17	50	33	17								50		17			17		
		4	*I + T*	50	33	33	33	67	17	17	17		33		33		50	33	50	33	67	33				33				33	33		50			17		33
	Tuticorin	1	*I + T*	33	50			33	17			50	33			17			17	17	50	17				17				17	17						17	
		2	*I*	33	17		33	33	33				17								50	33									33							
		3	*I + T*	67	50		33	67	50	50	50		33			33	17	50	50	17	50	17		17	17		33				33		17			17		
	Tirunelveli	1	*I + T*	50	33		50	67	33	33	33	67	50		17	17	33	33	50	17	67	33	17		17	50			17		33		17				50	
		2	*I + T*	33	17			33	17	33	33					33					50																	
		2	*I + T*	33	17		33	33	17	17	17										17																17	
	Tanjore	1	*I + T*	88	25	25	38	38	13	38	38										38												13			25		
		2	*I + T*	50	25	25	25	75		25	25					13	25				75		25					38			25					25		25
		3	*I + T*	25				38	13								13				38	13							13									
		4	*I + T*	38			25	25		25	25		13				38	25			38	13					13				25		25			13	25	13
		5	*I + T*	50	25	38	25			25	25		25				50	38	38	25	50	38					25	25					25			38	50	38
	Erode	1	*I + T*	60	20		50	70		30	30		30				30		50	10	20		10					40	20	10	20					20	10	20
		2	*I + T*	50	25		25						25				50		50		50		25								25							
	Salem	3	*I + T*	43	14		14						29				29		14	29	43	14		14		14	29				14	14	14			14	29	43
		4	*I + T*	43			29	14	29	14	14			14			14		29		29	14									14		14				29	
		5	*I + T*	29	14		29		29	14	14		14				29		14		14	14		14	14		14					14	29			29		14
	Villupuram	1	*I + T*	29	14		29	29	29	14	14		14				14	14	29	29	29					29	14	29							43	14	14	
		2	*I + T*	71	43	29	57	71	43	29	14	40	29		29	29	57	14	71	14	43	14		29				71			14		14	14		29	43	43
		3	*I + T*	57	57	29	29	43	29	14	14		14				43	29	43	14	57	14						43					14			14	14	
		4	*I + T*	29	14	43	14	43					29				14		43		43							29			14							
	Thiruvanamalai	1	*I + T*	86	86	57	71	86	43	29	29		57	14	29	43	57		71	43	100	14					43	14			43	14	29					
		2	*I + T*	100	86	43	71	100	86	57	29	43	57		14	29	57	71	100	43	100	29					43				29		29	29		14	57	43
		3	*I + T*	29	14		14	14											29		29										14			14				
	Kanjipuram	1	*I + T*	100	29	57	43	100	29	29	29		43		29	14	43		57		71	43									29		14					
		2	*I + T*	86	14	43	29	86					14				29		71		43										29							
		3	*I + T*	43				43					14						29	14	43	14									14					14		
	Thiruvallur	1	*I + T*	78	44	22	44	33	56	33	33	29	22		22		56	67	56	22	67	33		22			44				33		44	22			11	11
		2	*I*	33	22	22	56	33	56	33	33		44		11	11	56	33	56	11	56	33		11			11				33	11	22	11		22		22
		3	*I*	44	33	22	44	33	33	22	33		22		22		33	44	33	22	44	22	11	22							22		11	22		11	22	
		4	*I*	33	22	22	22	11		22	22		22		11		33	22	33	11	33						22	11			22		33					11
	Pudukottai	1	*I + T*	100			75	125	50	50	50						50	25	100	50	50	75	50															
		2	*I*	50				50									50				50													25			25	
		3	*I + T*	50			50	75	25							25	50				50						25				50						25	
		4	*I + T*	50				75								25		25		25	50		25	25														
	Theni	1	*I + T*	50	50			50											50		50	50		50							50							50
		2	*I + T*	50	50			50					50				50				50																	
		3	*I + T*	50																	50			50														
	Ramanathapuram	1	*I + T*	67	33		33	17	50	50	50		33			33	17	50	50	17	50	17		17	17		33				33		17					
		2	*I + T*	50	17	33	50		50	17	17		33			33		33	33	17	50	17	17	17							50					17	17	
	Trichy	1	*I + T*	25	25		25	25		25	25		25				25				38												25			13		25
		2	*I + T*	25	25		25	25	25						25	25	38		25	25	25																25	
		3	*I*	25	25			13	25	13	13		13		13	13	13	13	25		13										13							
		4	*I + T*	38			38	38	25	13	25	67			25		38		25	25	38										25					25	38	38
		4	*I + T*	50			25	50	25	25	25		25				25		38	13	38															25	13	
	Ariyalur	1	*I + T*	25		25	25	25	25	25	25	100	13				25	25			38	13									13					13	25	25
		2	*I + T*	50	25		25	38	25	25							38	25		25	38	38	13								25					25	38	25
	Dindigul	1	*I*	33				33	17								33				17	17																
		2	*I*	33	33			33	17	17	17			17					50	33	17	17	17								17	17	17					
		3	*I + T*	33	33			33	17										17		17																	
	Karur	1	*I*	67			83		67	17	33						17		67	17	33	50	33				33				17					17	33	17
Karnataka	Chamraj Nagar	2	*I + C*	100			25	25		25			50			25	75		50	25	50	50	50		25			50	25									25
		3	*I + C*	75	25		50				25						75	50	75	50	25																	
		4	*I + C*	50	25		50	25		25							50	75	75	50	50						25	25									25	
		5	*C*	75	100	25	50	25		50	25	67	25			25	##	50	100	75	75	25						75			25		25					25
	Nanjangud	1	*C*	75	75		50			50	25	67	50			25	25	25	25	50	25	25	25					50			25		25		25		25	
		2	*I + C*	75			50	50				33				25	25	25			25							75			25							
		3	*I + C*	75	75		50			50	25		75						75									50			25		25		50		25	
		4	*I + C*	75	50		75			50	75		50			25	50		25	75	25	25					50	25					75		50		50	25

Vachellia nilotica subspecies: *I* = '*indica*'; *T* = '*tomentosa*'; *C* = '*cupressiformis*'

Table 2. Seasonal variations in the incidence (% of survey sites) of phytophagous insects and rust fungi on *V. nilotica* in survey sites in Tamil Nadu and Karnataka states in southern India

			Prioritised insects and rusts								other insects																										
year	Season	No of sites surveyed	*Anomolococcus indicus*	*Phycita* sp. A	*Phycita* sp. B	*Isturgia disputaria*	*Dereodus denticollis*	*Pachnephorus* sp.	*Ravenelia acacia-arabicae*	*Ravenelia evansii*	*Diapromorpha turcica*	*Selepa celtis*	*Ascotis infiraria*	*Hyposidra successaria*	*Clyda succinata*	*Mylloceros* sp.	*Inderbela quadrinotata*	*Oxyrhachis tarandus*	*Homoeocerus signatus*	*Eumeta crameri*	*Pteroma plagiophlephs*	*Hypolixus truncatulus*	*Nippococcus* sp.	*Paracoccus marginata*	*Oxytonia versicolor*	*Euproctis scintillans*	*Cryptocephalus* sp.	*Dereodus mastos*	*Mylabris* sp.	*Steblote siva*	*Euproctis lunata*	*Dasychira mendosa*	*Chrysocoris purpureus*	*Psiloptera fastuosa*	*Sthenias* sp.	*Iedra mutica*	*Flata ferrugata*
2008	Oct-Dec	32	88	53	6	44	22	6	0	0	0	34	4	2	6	34	13	53	22	47	34	6	9	0	0	13	0	0	0	38	0	22	6	0	0	2	0
2009	Jan-Mar	41	76	39	7	22	17	17	20	17	5	32	1	6	2	22	12	46	7	66	12	0	5	0	0	10	0	0	0	37	4	22	2	1	0	6	10
	Apr-Jun	52	67	12	0	50	44	23	4	0	0	13	0	1	6	25	8	31	10	73	50	0	19	4	10	23	0	10	2	33	3	21	1	2	0	0	4
	Jul-Sep	56	48	13	0	36	29	36	29	18	9	9	0	2	13	20	7	34	18	46	18	5	0	7	4	9	0	14	23	27	6	29	3	2	3	2	14
	Oct-Dec	51	59	41	24	45	37	55	37	18	22	24	10	12	16	29	4	31	20	45	27	2	4	0	2	18	4	4	2	20	4	20	10	0	6	9	4
2010	Jan-Mar	48	88	40	17	33	38	44	46	35	25	27	5	5	21	25	15	50	25	40	17	6	10	4	4	15	0	0	0	33	2	29	7	2	2	7	19
	Apr-Jun	44	82	25	11	36	52	45	64	18	11	36	0	0	20	34	18	50	36	55	14	32	0	0	5	11	5	16	2	11	3	7	3	3	0	2	11
	Jul-Sep	42	67	26	10	52	43	62	48	7	7	19	2	0	21	33	5	40	19	48	29	2	5	0	0	5	10	21	26	14	5	7	0	1	2	12	14
	Oct-Dec	16	69	25	0	75	50	44	63	19	25	25	6	10	0	38	6	69	13	63	0	0	6	0	0	13	25	13	0	19	2	19	6	0	4	10	13
2011	Jan-Mar	32	69	31	0	47	50	59	41	28	9	31	0	3	16	41	9	59	25	63	19	9	0	3	0	6	0	13	0	22	3	16	3	1	0	5	13
	Apr-Jun	21	57	29	24	52	57	43	38	14	0	0	0	0	0	57	0	48	0	38	5	5	14	0	0	0	0	19	10	14	0	5	0	0	0	0	0
	Jul-Sep	20	60	30	10	50	55	45	40	0	5	50	3	5	10	35	5	70	15	50	20	0	0	5	0	10	5	25	15	10	1	10	1	2	1	1	0
	Oct-Dec	20	65	50	20	45	60	40	70	20	10	25	3	3	10	55	10	50	15	40	10	5	10	5	0	20	20	15	0	20	3	20	9	0	0	2	15

Table 3. Scores for prioritisation of potential biological control agents for *V. nilotica* from southern India based on host records, field host range, geographic range, seasonal incidence, and damage levels. For scoring criteria, refer to materials and methods.

Species	Order: Family	Habit	Filters						Priority score	Rank
			Host records	Field host range	Geographic range	Seasonal incidence	Damage levels	Preliminary host		
Insects										
Anomalococcus indicus	Hemiptera: Laecanodiaspidae	Stem	4	5	5	5	5	5	**29**	1
Flata ferrugata	Hemiptera: Flatidae	Leaf	0							
Ledra mutica	Hemiptera: Cicadellidae	Leaf	0							
Oxyrhachis tarandus	Hemiptera: Membracidae	Stem	1	1						
Homoeocerus signatus	Hemiptera: Coreidae	Stem	1	1						
Nipaecoccus sp.	Hemiptera: Pseudococcidae	Stem	N/A	1						
Paracoccus marginatus	Hemiptera: Pseudococcidae	Stem	0							
Chrysocoris purpureus	Hemiptera: Scutelleridae	Stem	0							
Phycita sp. A	Lepidoptera: Pyralidae	Leaf	N/A	5	5	5	5	4	**24**	2
Phycita sp. B	Lepidoptera: Pyralidae	Leaf	N/A	5	2	2	4	4	**17**	5
Isturgia disputaria	Lepidoptera: Geometridae	Leaf	3	4	3	3	2	0	15	
Selepa celtis	Lepidoptera: Noctuidae	Leaf	0							
Ascotis selenaria imperata	Lepidoptera: Geometridae	Leaf	0							
Steblote siva	Lepidoptera: Lasiocampidae	Stem/bark	2							
Hyposidra successaria	Lepidoptera: Geometridae	Leaf	0							
Inderbela quadrinotata	Lepidoptera: Metarbelidae	Stem	0							
Eumeta crameri	Lepidoptera: Psychidae	Leaf	0							
Pteroma plagioplephs	Lepidoptera: Psychidae	Leaf	1							
Euproctis scintillans	Lepidoptera: Lymantridae	Leaf	0							
Euproctis lunata	Lepidoptera: Lymantridae	Leaf	0							
Dasychira mendosa	Lepidoptera: Lymantridae	Leaf	0							
Dereodus denticollis	Coleoptera: Curculionidae	Leaf/root	5	5	4	4	1	4	**23**	3
Dereodus mastos	Coleoptera: Curculionidae	Leaf/root	0							
Myllocerus sp.	Coleoptera: Curculionidae	Flower	N/A	1						
Hypolixus truncatulus	Coleoptera: Curculionidae	Leaf	0							
Pachnephorus sp.	Coleoptera: Chrysomelidae	Leaf/root	N/A	5	5	4	2	N/A	**16**	7
Diapromorpha turcica	Coleoptera: Chrysomelidae	Leaf/root	2	1	1	2	1	N/A	7	
Clytra succinct	Coleoptera: Chrysomelidae	Leaf	3	N/A	1	1	1	N/A	6	
Cryptocephalus sp.	Coleoptera: Chrysomelidae	Leaf	N/A	5	4	3	2	N/A	14	
Psiloptera fastuosa	Coleoptera: Buprestidae	Stem	0							
Oxycetonia versicolor	Coleoptera: Scarabaeidae	Flower	0							
Mylabris spp.	Coleoptera: Meloidea	Flower	1							
Sthenias sp.	Coleoptera: Cerambycidae	Stem/bark	N/A	N/A	2	1	1	N/A	4	
Rust fungi										
Ravenlia acacia-arabicae	Uredinales: Raveneliaceae	Stem/leaf/fruit	5	5	3	5	4	N/A	**22**	4
Ravenilia evansii	Uredinales: Raveneliaceae	Leaf	3	5	2	2	4	N/A	**16**	6

4.4 Geographic range, seasonal incidence and damage levels

There was a significant variation in the percentage of survey sites where various insects and rust fungi were observed over the three-year period ($F_{30,\ 341} = 16.59$; $P < 0.001$). *Anomalococcus indicus* was the most widespread, occurring in 100% of the survey sites (Table 1) throughout the year (Table 2). Severe infestations of *A. indicus* caused defoliation, wilting and death of affected branches or the entire tree. Other agents that are distributed widely and occur throughout the year include *D. denticollis*, *Eumeta crameri* (Westwood), *I. disputaria*, *Phycita* sp. A., *O. tarandus*, *R. acacia-arabicae* and *Pachnephorus* sp. (Tables 1 and 3). *Phycita* sp. A. caused severe defoliation in young and mature trees throughout the year. Defoliation by *I. disputaria* was observed at all survey sites, predominantly from September to January, coinciding with the south-west monsoon. For other insects, their distribution was limited (Table 1), they were only active seasonally (Table 2), or they caused only minor feeding damage (Table 3). *Phycita* sp. B collected from only 38% of the survey sites (Table 1), was active only during three to six months of the year (Table 2).

Ravenelia acaciae-arabicae (gall rust) was observed at 68% of the survey sites (Table 1) covering both Tamil Nadu and Karnataka. It produces uredinia and telia on leaflets, predominately on the upper surface. Associated spermogonia and aecia occur on fruits, inflorescences and shoot tips, causing hypertrophy that result in galls (Figure 4). Rust infection on leaves resulted in premature yellowing and leaf shedding. No seed development occurred from galled fruits or inflorescences, and galling in shoot tips arrested the shoot development. The spermogonial and aecial stages are found from December to March whereas the uredinia and telia are found during most of the year. *Ravenelia evansii* (leaf rust) was recorded at all sites with *R. acaciae-arabicae*, often co-occurring along with telial stages of *R. acaciae-arabicae*.

In north-western India, the leaf rust (*R. evansii*) was collected from Nadiad (22°41'22.31"N; 72°51'26.08"E), Tarapur (22°29'19.69"N; 72°39'28.75"E), Talala (21° 3'18.27"N; 70°31'54.45"E) and Veraval (20°54'43.21"N; 70°21'12.15"E). In Gujarat the leaf-rust was observed in the field from November to March, following the wet season. In the field, only uredinia and telia were observed on the upper leaflet surface. The rust was not seen in Rajasthan in north-western India.

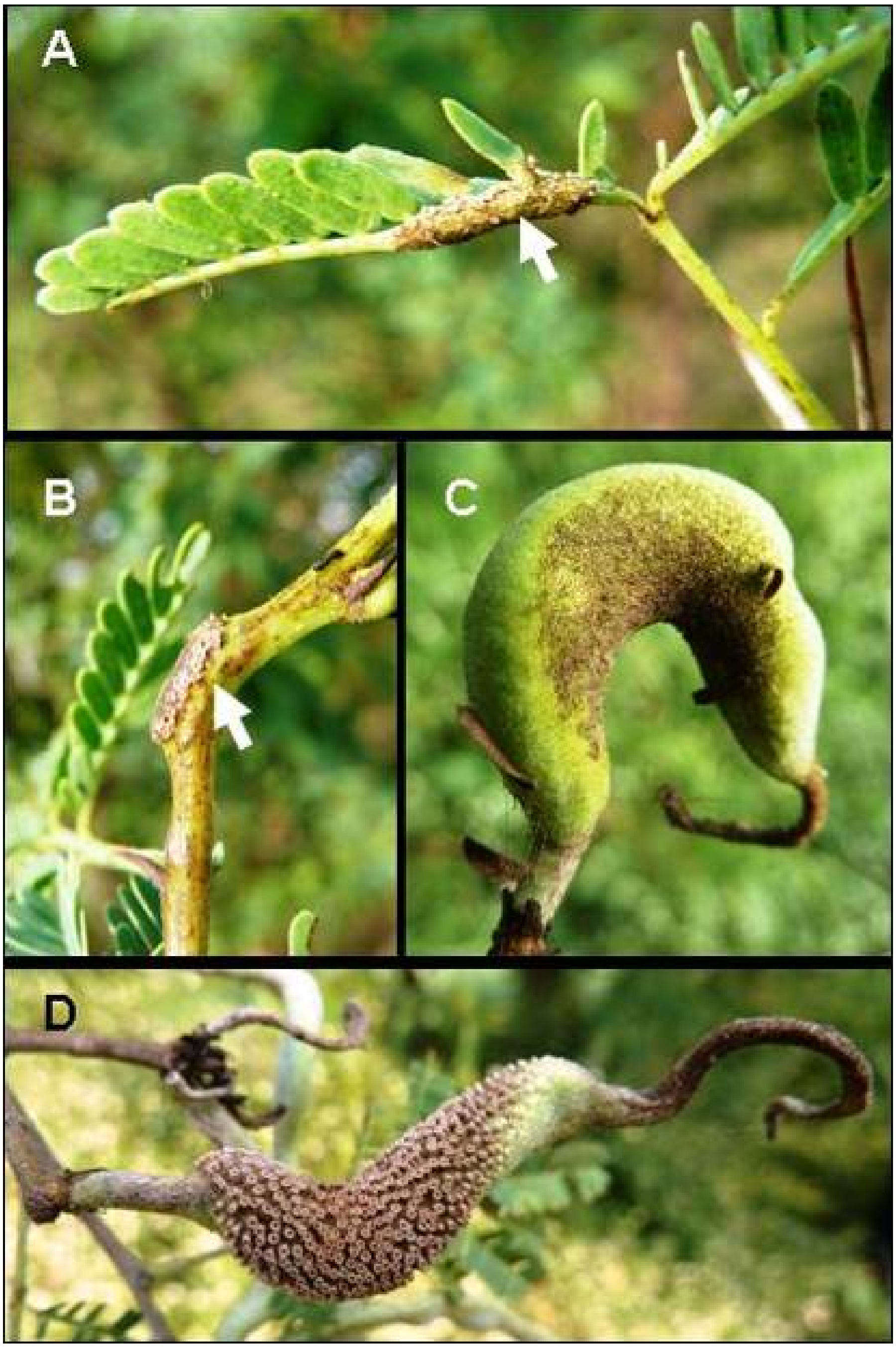

Figure 4. Rust galls of *Ravenelia acaciae-arabicae* causing hypertrophy on leaf rachis (A), stem (B), and immature (C) and mature (D) fruit pods on *Vachellia nilotica* ssp. *indica*.

4.5 Potential impact (exclusion studies)

In Tamil Nadu, exclusion studies were conducted over 18 months at Thoppur and Coimbatore. At both sites, native insect herbivores reduced the plant height, basal stem diameter, number of branches, number of leaflets, leaf biomass, stem biomass and root biomass (Figures 5 and 6). Reductions in plant vigor at both sites were primarily due to the scale insect *A. indicus* infesting the shoot.

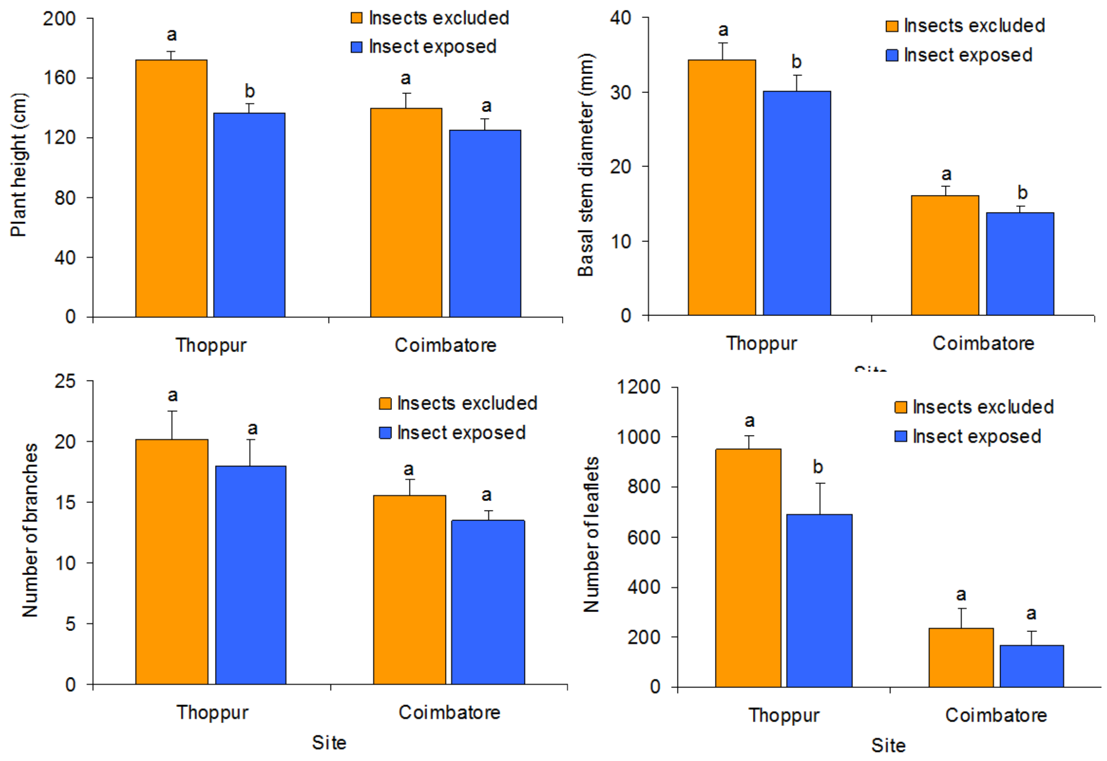

Figure 5. Impact of insect herbivores on plant height, basal stem diameter, number of branches and number of leaflets of prickly acacia juvenile plants in Tamil Nadu, India. For each plant parameter in each site bars marked with the same letters are not significantly different (P<0.05).

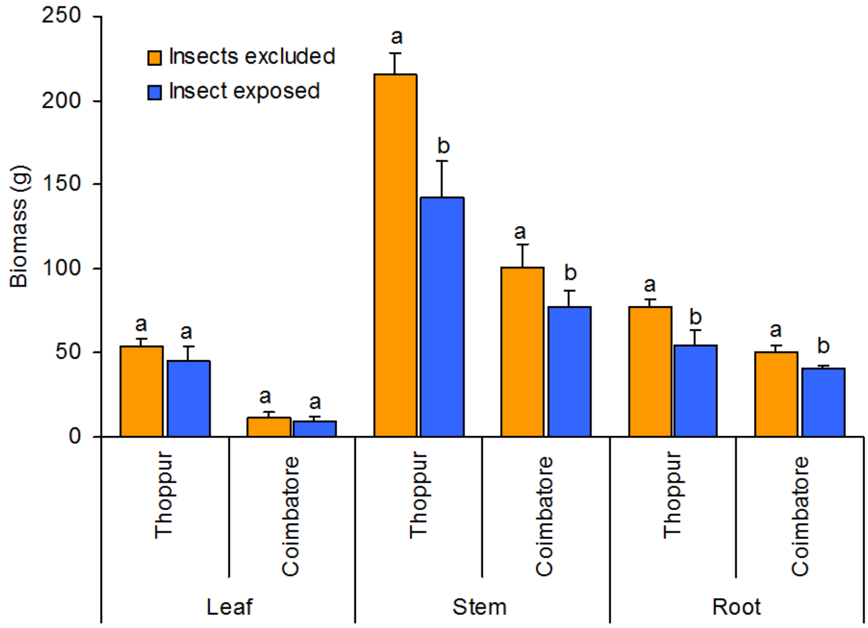

Figure 6. Impact of insect herbivores on biomass of prickly acacia juvenile plants at two trial sites in Tamil Nadu, India. For each plant parameter in each site bars marked with the same letters are not significantly different (P<0.05).

In Gujarat and Rajasthan, plant height, basal stem diameter, root length, and above ground biomass were lower in plants exposed to insect herbivores than in plants excluded from insect herbivores, and the reductions varied between 'open' and 'shade' conditions (Figure 7). There were no reductions in the number of branches per plant, the number of leaves per plant, or the below-ground biomass due to insect herbivores in both Gujarat and Rajasthan.

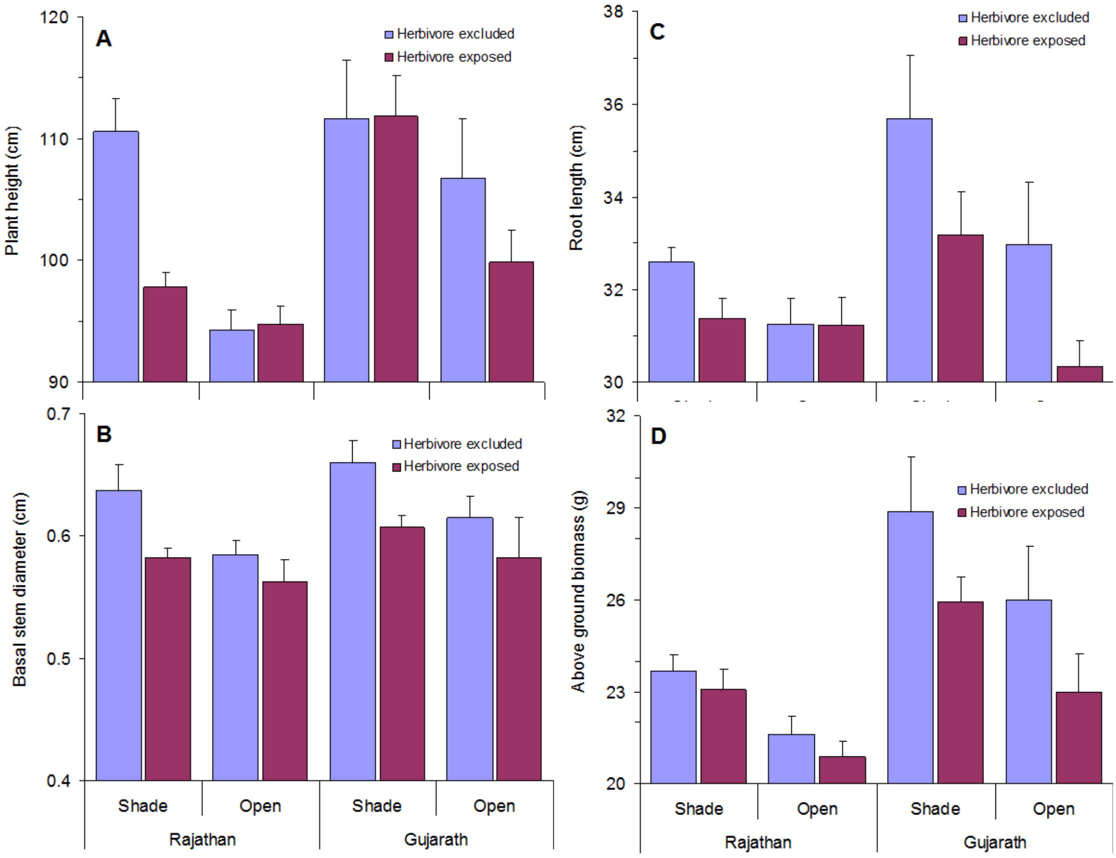

Figure 7. Impact of insect herbivores on plant vigour of prickly acacia juvenile plants over two years in north-western India.

4.6 Preliminary no-choice host specificity tests

Anomalococcus indicus (Hemiptera: Lecanodiaspidae)

On potted *V. nilotica* seedlings, *A. indicus* crawlers established and nymphs developed into mature females 35 to 45 days after inoculation (Figure 8). Female nymphs attained reproductive maturity (462.2 ± 30.8 females per plant) after 70 to 90 days (Figure 8). Both crawler establishment and reproductive maturation of female nymphs (2.2 ± 1.4 females/plant) were significantly lower ($F_{1,14}$ = 110.5, P < 0.001) on *V. tortilis* than on *V. nilotica*, and none of the females on *V. tortilis* produced progeny (Figure 8). No crawler establishment or development occurred on *V. farnesiana, A. auriculiformis, A. planifrons, V. leucophloea, S. catechu* or *P. nigrum*.

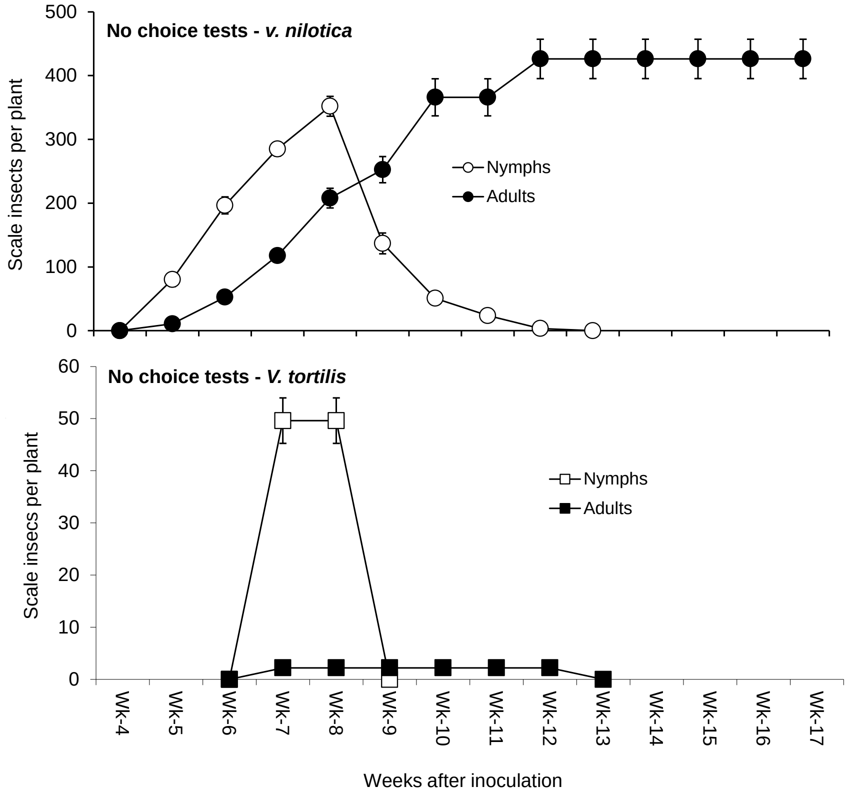

Figure 8. Establishment, survival and development and maturation of females of *Anomalococcus indicus* (mean ± se) on *Vachellia nilotica* and *Vachellia tortilis* under no-choice conditions.

<u>*Phycita* sp. A (Lepidoptera: Pyralidae)</u>

For *Phycita* sp. A, larval feeding and development occurred on two non-target plants, *A. planifrons and V. leucophloea* (Table 4). However, the percentage of larvae that completed development to pupae ($F_{2,13}$ = 90.7, P < 0.001) was significantly higher on *V. nilotica* ssp. *indica* (98 ± 2%) than on *A. planiferons* (40 ± 6%) and *V. leucophloea* (larva: 30 ± 3%). No larval feeding or development occurred on other non-target plants, *A. deanei, V. tortilis, A. auriculiformis*, *V. farnesiana*, *M. indica* or *A. occidentale* (Table 4).

Table 4. Larval and pupal survival and development (mean ± se) of the leaf-webber (*Phycita* sp. A) on target and non-target plants under no-choice conditions.

Test plant	Larval duration (days)	Larvae developing to pupae (%)	Pupal duration (days)	Pupae developing to adults (%)
Vachellia nilotica ssp. *indica*	22.5 ± 0.4	98 ± 2	8.5 ± 0.2	76 ± 0.9
Acacia planiferens	16.4 ± 0.9	52 ± 5.8	9.6 ± 0.2	34 ± 4.3
V. leucophloea	11.8 ± 0.8	30 ± 3.1	10.5 ± 0.3	26 ± 4.1
Senegalia mellifera	5.7 ± 1.1	Nil	Nil	Nil
S. cathechu	5.6 ± 1.3	Nil	Nil	Nil
S. ferruginea	4.8 ± 0.4	Nil	Nil	Nil
A. auriculiformis	3.0 ± 0.0	Nil	Nil	Nil
V. fernesiana	3.8 ± 0.2	Nil	Nil	Nil
V. tortilis	3.3 ± 0.2	Nil	Nil	Nil
A. deanei	1 ± 0	Nil	Nil	Nil
Mangifera indica	2.7 ± 0.6	Nil	Nil	Nil
Anacardium occidentale	1.6 ± 0.5	Nil	Nil	Nil

Isturgia disputaria (Lepidoptera: Geometridae)

Neonates of *I. disputaria* fed and completed development on *V. tortilis*, *A. planifrons* and *V. leucophloea* (Figure 9). The percentage of *I. disputaria* larvae that developed into adults was significantly higher ($F_{4,20}$ = 522.9, $P < 0.001$) on *V. nilotica* ssp. *indica* (99 ± 1%) and *V. nilotica* ssp. *tomentosa* (97 ± 2%) than on *V. tortilis* (31 ± 1.8%), *A. planiferons* (33 ± 2%) and *V. leucophloea* (11 ± 2%), suggesting that the three non-target plants are less suitable hosts (Figure 9).

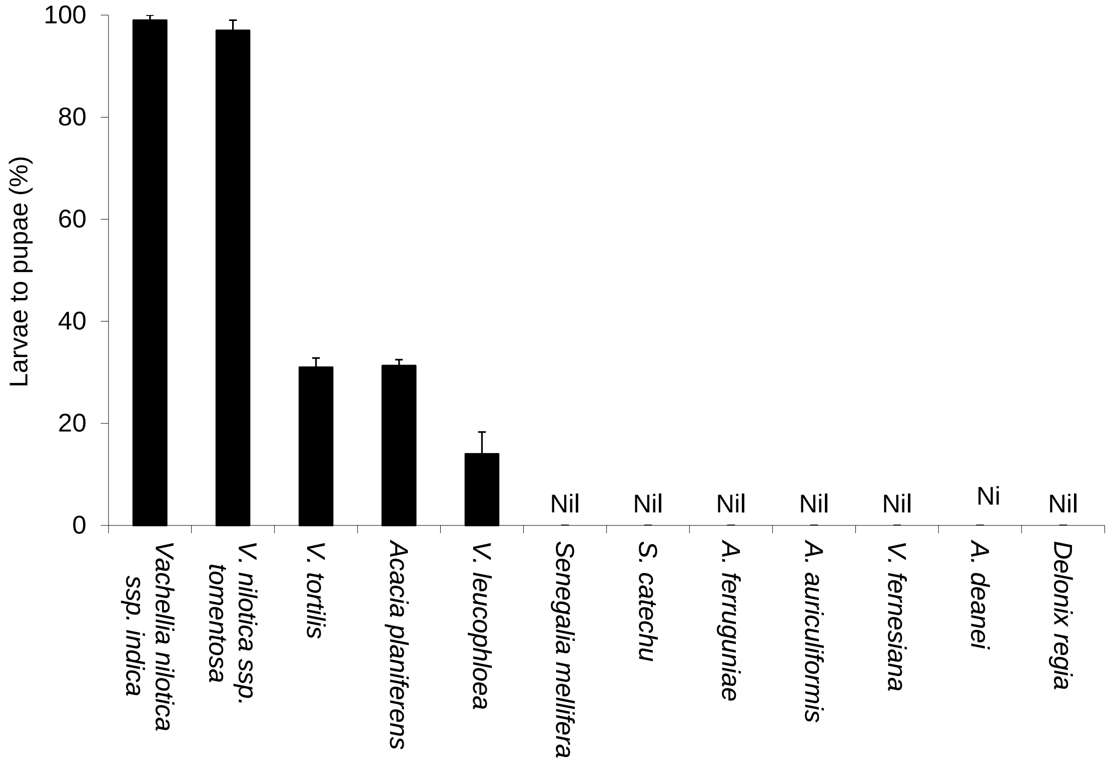

Figure 9. Larval survival and development (mean ± se) of *Isturgia disputaria* on target and non-target plants under no-choice conditions.

Dereodus denticollis (Coleoptera: Curculionidae)

The adult leaf weevils (*D. denticollis*) fed on *A. auriculiformis* leaves and nibbled on *V. farnesiana and V. tortilis* leaves, but did not survive on them for more than three days. In contrast, on *V. nilotica*, adult weevils fed and survived more than a year. However, the larval host range of the weevil is unknown.

Ravenelia acaciae-arabicae (Raveneliaceae)

A preliminary host-range testing involving the susceptibility of 17 non-target test plant species comprising representatives of the genus *Vachellia* and *Acacia* were conducted under quarantine at CABI (UK). With the exception of *Vachellia sutherlandii* (Australian native), the rust pathogen was not able to sporulate on any of the evaluated test plant species. The rust was able to produce uredinia bearing viable urediniospores on V. sutherlandii under quarantine conditions. However, sporulation on V. sutherlandii was less prolific compared to sporulation on prickly acacia. On V. sutherlandii, limited sporulation was always accompanied by strong plant defence responses. Thus, while shown to be susceptible to the rust, V. sutherlandii cannot be considered a natural host of the rust (Seier & Tanner, 2011; Seier *et al*., 2012).

Ravenelia evansii (Raveneliaceae)

Preliminary studies on the leaf rust were conducted at the Arid Forest Research Institute, Jodhpur, India. Rust infection was seen only on *V. nilotica* ssp. *indica*, with no macroscopic rust symptoms evident on the non-target test plants. The percentage of leaflets with rust in *V. nilotica* ssp. *indica* ranged from 67% to 78%. However, the percentage of leaflets with rust infection declined with the increasing temperature ($y = -1.378x^2 + 75.411x - 953.1$; $R^2 = 0.999$) and declining relative humidity (RH) ($y = 3.58x - 142.4$; $R^2 = 0.839$) in the shade-house and no rust infection was sustained when the temperature exceeded 33.5^o C and the RH was below 48.5% in March 2010.

A preliminary host-range testing was conducted under quarantine at CABI (UK). Initial specificity assessments conducted with the Queensland-native species *V. sutherlandii* showed *R. evansii* to be able to infect and sporulate on this non-target species. The disease development of the rust on *V. sutherlandii* resembled that noted for the natural host prickly acacia. Urediniospores produced on *V. sutherlandii* proved to be viable and infective as re-inoculation of spores ex *V. sutherlandii* onto prickly acacia and *V. sutherlandii* resulted in successful sporulation on both of these species. *Vachellia sutherlandii* is not a reported host of *R. evansii*. However, the lack of any macroscopically visible plant defence reactions in response to infection with *R. evansii* combined with successful “normal” sporulation of the rust on *V. sutherlandii* are indications for a compatible plant-pathogen interaction. Thus, the non-target species, *V. sutherlandii,* must be considered to be part of the fundamental host range of *R. evansii* and it is most likely that this Australian *Vachellia* species would become infected if it encounters the rust in the field (Seier & Pollard, 2012).

4.7 Prioritised agents

Based on host plant records, field host specificity, geographic range, seasonal incidence and damage levels (Table 3), the following insects and rust fungi have been prioritised in decreasing order for detailed host specificity tests in Australia (for insects) and UK (for rusts): *A. indicus* > *Phycita* sp. A > *D. denticollis* > *R. acaciae-arabicae* > *Phycita* sp. B > *R. evansii* > *Pachnephorus* sp. Since the host specificity tests for *I. disputaria* sourced from Africa, Pakistan and India have already been completed, this agent was not included in the priority list.

4.8 Agent exportation

Permission to export the prioritised agents from India was obtained from the National Biodiversity Authority of India and the Indian Council for Forestry Research and Education (ICFRE) in 2010. Permits to import the prioritised insects (*Phycita* sp. A, *Phycita* sp. B, *A. indicus*, *D. denticollis* and *Pachnephorus* sp.) into a quarantine facility in Brisbane for host specificity testing were obtained from the relevant regulatory authorities in Australia.

The gall rust (*R. acacia-arabicae)* from Coimbatore in Tamil Nadu and the leaf rust (*R. evansii*) from Talala and Tarapur in Gujarat were exported to CABI (UK) in October 2010. Seeds of 15 Australian *Acacia/Vachellia* species were also exported to CABI (UK) for inclusion in the preliminary host specificity tests. Preliminary host specificity tests for both rust species have been completed (Seier *et al*., 2012).

So far, 11 importations of various prickly acacia insects have been made from India to Australia since 2011 (Table 5). Included in this are 218 leaf-weevil (*D. denticollis*) adults, 140 brown leaf-webber (*Phycita* sp., A) larvae, pupae and adults, 219 green leaf-webber (*Phycita* sp. B) larvae and pupae and thousands of scale insect (*A. indicus*) adults and nymphs.

Table 5. Importations of prickly acacia biological control agents from India.

Date	Species	Permit	Number	Details
23/01/2011	*Dereodus denticollis*	IP10009416	10	adults
23/01/2011	*Phycita sp. A*	IP10009416	140	adults, larvae and pupae
23/01/2011	*Anomalococcus indicus*	IP10009416	numerous	adults and nymphs
4/05/2011	*Anomalococcus indicus*	IP10009416	numerous	adults and nymphs
29/07/2011	*Anomalococcus indicus*	IP11013070	70	adult females
24/09/2011	*Anomalococcus indicus*	IP11013070	400	adult females
24/12/2011	*Anomalococcus indicus*	IP11013070	520	adult females
16/01/2012	*Anomalococcus indicus*	IP11013070	275	adult females
9/07/2012	*Anomalococcus indicus*	IP11013070	600	adult females; 480 used
9/07/2012	*Dereodus denticollis*	IP11013070	14	dead adults
5/11/2012	*Anomalococcus indicus*	IP12018950	2000	adult females; 170 used
5/11/2012	*Dereodus denticollis*	IP12018950	88	adults
5/11/2012	*Phycita* sp B	IP12018950	4	larvae
20/01/2013	*Phycita* sp. B	IP12018950	37	30 larvae and 7 pupae
12/10/2013	*Dereodus denticollis*	IP13013814	106	adults
12/10/2013	*Phycita sp. B*	IP13013814	116	larvae and pupae
1/12/2013	*Phycita* sp. B	IP13013814	62	larvae and pupae

The scale insect (*A. indicus*), the brown leaf-webber (*Phycita* sp. A) and the leaf-weevil (*D. denticollis*) were imported first in January 2011. Subsequently, there have been seven more consignments of the scale insect (*A. indicus*) imported from India (March 2011, July 2011, September 2011, December 2011, January 2012, July 2012 and November 2012) (Table 5) to conduct the no-choice and choice host specificity tests in quarantine. The leaf-weevil (*D. denticollis*) adults were imported on four occasions (January 2011, July 2012, November 2012 and October 2013), but a viable colony of the leaf-weevil could not be established in the quarantine due to difficulties in standardising their oviposition. The green leaf-webber (*Phycita* sp. B) was imported on three occasions (November 2012, October 2013 and December 2013) and a colony has been successfully established in the quarantine. Life-cycle studies for the green leaf-webber have been completed, and host specificity tests involving no-choice larval development and no-choice oviposition tests are in progress. Additional larvae and pupae of the green leaf-webber will be imported in November/December 2014, if required. Additional importations of the leaf-weevil (*D. denticollis*) and the leaf-beetle (*Pachnephorus* sp.) are planned for later in 2014, when conditions are more conducive for field collections.

4.9 Effectiveness of established agents in Australia

In coastal areas of north Queensland, peaks in *C. assimilis* abundance were generally seen between March and May each year. Defoliation was generally more obvious on lower branches of prickly acacia than on upper branches, although differences were not significant (P = 0.136). Peaks in defoliation were seen in April 2008 (51.8 ± 8.04%), April 2009 (49.9 ± 6.64 %), September 2009 (52.2 ± 8.9 %) and April 2010 (80.7 ± 3.85 %).

In exclusion trials using insecticides, eggs and larvae of *C. assimilis* were found on unsprayed seedlings. The effects of herbivory, however, were significant only for seedlings grown beneath the canopy. At the end of the five month trial period, shoot length of these seedlings was reduced by 30%, basal stem diameter by 44%, root length by 15%, number of leaves by 97%, above ground biomass by 84%, and below ground biomass by 77% when compared to sprayed seedlings. Implications are that the insect, where established, can reduce seedling growth beneath existing canopies and in thin dense infestations (Lockett *et al.* 2012).

5 Discussion

Plant genotype and climate matching identified India as a suitable area for exploration for biological control agents for *V. nilotica* ssp. *indica* in arid inland regions of northern Australia. Potential agents have been prioritised for host specificity testing based on ecological host range, native range distribution and potential impacts. For effective biological control of *V. nilotica* ssp. *indica*, seedlings and juveniles need to be targeted (Kriticos *et al.*, 1999), using either leaf-feeding agents, shoot feeding agents, or a combination of both (Dhileepan *et al*., 2009). Hence, surveys focused more on juvenile plants, and on leaf and stem feeding agents than on root and seed feeding agents. Since *Acacia* is the largest genus (with over 950 endemic species) of flowering plants in Australia (Orchard & Wilson, 2001), field host range and preliminary host specificity test results involving Indian and Australian *Acacia/Vachellia* spp., were used while prioritising potential agents.

Not all of the insects, mites and pathogens collected during the survey could be identified due to a lack of taxonomic expertise. Among the species identified in southern India, it does appear that there is little overlap of insect species, particularly for prioritised species, with those collected from north-western India collected in this study and from Pakistan in earlier surveys (Mohyuddin, 1986). All of the insect species prioritised from India so far as potential biological control agents are from southern India. This was possibly due to more species being found in southern India, which, in turn, may be the result of more rigorous and systematic survey efforts there than in north-western India.

Several of the lepidopterans (e.g. *Phycita* spp.), coleopterans (e.g. *Pachnephorus* sp., *Sthenias* sp., *Myllocerus* sp., *Mylabris* sp., *Cryptocephalus* sp.) and hemipterans (e.g. *Nipaecoccus* sp.) could not be identified to species level, making it difficult to search for any existing host records. Hence, for insects with no species level identification, prioritisation has been based mainly on their field host range. While prioritising agents, more emphasis was given to shoot feeding agents, as experimentation has shown that *V. nilotica* ssp. *indica* is susceptible to shoot herbivory (Dhileepan *et al*., 2009).

Anomolococcus indicus, the only shoot-feeding agent that showed specificity for *V. nilotica* in the field, was widely distributed, active throughout the year, and caused severe damage to *V. nilotica* in the field. It is native to the Indian subcontinent and has been reported as a pest of *V. nilotica* in India (Pillai *et al*., 1995) and Bangladesh (Baksha & Islam, 1996). Hence, this agent was prioritised for host specificity testing. The scale insect was first imported into quarantine in Australia in January 2011 and host-specificity testing commenced in July 2011. The scale insect completed development on 13 of the 57 non-target plant species tested during no-choice trials. Development on *Acacia falcata, Vachellia sutherlandii*, *Neptunia major* and *N. monosperma* was comparable to the scale's development on prickly acacia. However, when provided with a choice, prickly acacia was the preferred host. In view of the field host specificity of the scale insect in India, a choice trial under field conditions in India, involving the four above-mentioned non-target test plants is in progress. Further work on the scale insect will depend on the results from the field choice tests under field conditions in India. Other shoot feeding insects collected during the survey (e.g. *Steblote siva* (Lefebvre), *Oxyrhachis tarandus* Fab., *Acalolepta cervina* (Hope) and *Inderbela quadrinotata* Wlk.) are polyphagous.

Among the leaf-feeding insects, *Phycita* sp. A, *Phycita* sp. B, *Pachnephorus* sp. and *D. denticollis* have been prioritised. For *Phycita* sp. A, it was difficult to determine potential non-target species at risk from literature searches due to uncertainty regarding its taxonomy. Hence, based on field host range, native geographic range, seasonal incidence, field defoliation levels and results from preliminary host specificity tests in India, *Phycita* sp. A was imported into a quarantine facility in Australia in January 2011 for detailed host specificity testing. Host-specificity testing of *Phycita* sp. A commenced in June 2011. In no-choice larval development trials the leaf-webber completed development on 13 of 27 non-target plant species tested, yet in the field the insect was observed only on prickly acacia. These results suggest that oviposition behaviour could be the key mechanism in host selection by the leaf-webber resulting in its incidence only on prickly acacia in India. However, oviposition preference could not be reliably determined under quarantine conditions. Hence testing of the brown leaf-webber was terminated in December 2012 due to unacceptable non-target feeding.

A colony of the green leaf-webber *Phycita* sp. B was established in quarantine in late 2013. Host specificity testing has recently commenced. A colony of the leaf-weevil *Dereodus denticollis* could not be established in the quarantine due to difficulties with its oviposition. Further work will be conducted using freshly field collected adults from India.

The leaf-feeding geometrid (*I. disputaria*) was included in the list of prioritised agents in view of its field host specificity, wide occurrence, activity throughout the year, and damage potential. Although larval feeding and development occurred on three non-target *Acacia* spp. under no-choice conditions in India, higher larval mortality on non-target plants and absence of the agent on the three non-target *Acacia* spp. occurring with *V. nilotica* in the field, suggest that the non-target plants are not the preferred or natural hosts for the agent in the field. The contrasting results between field host range and host use under no-choice conditions was possibly related to the oviposition behaviour of the agent, which usually oviposits on mature trees with rough and fissured bark, rather than on seedlings and juveniles, with glabrous to subtomentose bark. Choice oviposition tests for this agent under quarantine conditions are of limited value, as the moth is known to oviposit on any rough or fissured surfaces including insect-proof cage walls. Based on earlier no-choice host specificity tests of the leaf-feeding geometrid (*I. disputaria*) from Pakistan, Kenya and India, conducted in quarantine in Australia (Palmer, 2004), this agent was not progressed further.

For other leaf-feeding insects (*Pachnephorus* sp., *D. denticollis* and *D. turcica*) it is necessary to understand the feeding habit of their larvae and standardise rearing methods so that no-choice host specificity tests can be initiated. Currently, attempts are being made to identify the feeding habits of the larvae of these insects in the field. Preliminary host specificity tests will be conducted for these insects in India over the next two years.

Host-range testing of the two rust species (*R. acaciae-arabicae* and *R. evansii*) using both urediniospore and aecidial spore accessions of *R. acaciae-arabicae* from Tamil Nadu, India and uredinospore accessions of *R. evansii* from Tamil Nadu and Gujarat, India, under quarantine conditions at CABI UK, revealed that both rust species infected and produced viable and infective urediniospores on an Australian native species, *Vachellia sutherlandii* (F. Mueller) F. Mueller (Seier & Tanner, 2011; Seier *et al*., 2012). Sporulation on *V. sutherlandii* by both rusts was always accompanied by dark necrotic lesions, indicating that this non-target species is not a natural host. Inoculation of prickly acacia and *V. sutherlandii*, using urediniospores of *R. acaciae-arabicae* produced on *V. sutherlandii* resulted in sporulation of the rust only on prickly acacia and not on *V. sutherlandii*, further confirming that *V. sutherlandii* is not a suitable host for the rust. However, in view of the potential risk posed by both rust species to Australian acacias, in particular *V. sutherlandii*, that grow sympatrically with the target weed in Australia (Seier & Tanner, 2011; Seier *et al*., 2012) no further work on the two rusts has been pursued in the United Kingdom. However, a field level host susceptibility tests for both rust species, involving both prickly acacia and *V. sutherlandii* in India would be worth pursuing.

6 Conclusion

Surveys in India resulted in the prioritisation of a scale insect (*A. indicus*), two leaf-webbers (*Phycita* sp. A and *Phycita* sp. B), a leaf weevil (*D. denticollis*), a leaf beetle (*Pachnephorus* sp.), a gall-rust (*R. acacia-arabicae*) and a leaf-rust (*R. evansii*), as prospective biological control agents for prickly acacia in Australia. Four of the prioritised insects (*A. indicus*, *Phycita* sp. A., *Phycita* sp. B and *D. denticollis*) were exported to Australia for detailed host specificity tests. The gall-rust and the leaf-rust were exported to CABI (UK). In view of the potential risk posed by the rust species to *V. sutherlandii*, an Australian native, in host range tests conducted at CABI-UK, no further work on the two rusts has been pursued. Host-specificity testing of *Phycita* sp. A under quarantine in Australia commenced in June 2011. In no-choice larval development trials the leaf-webber completed development on 13 of 27 non-target plant species tested, yet in the field the insect was observed only on prickly acacia. Testing of the brown leaf-webber was terminated in December 2012 due to unacceptable non-target feeding. Host-specificity testing of the scale insect *A. indicus* commenced in July 2011. The scale insect completed development on 13 of the 57 non-target plant species tested during no-choice trials. Development on *Acacia falcata, V. sutherlandii*, *Neptunia major* and *N. monosperma* was comparable to the scale's development on prickly acacia. However, when provided with a choice, prickly acacia was the preferred host. In view of the field host specificity of the scale insect in India, a choice trial under field conditions in India, involving the four above-mentioned non-target test plants will be undertaken. Further work on the scale insect will depend on the results from the field choice tests under field conditions in India. A colony of the leaf-weevil *Dereodus denticollis* could not be established in the quarantine due to difficulties with its oviposition. Further work will be conducted using freshly field collected adults from India. A colony of the green leaf-webber *Phycita* sp. B was established in quarantine in late 2013. Host specificity testing has recently commenced. Additional importations of the leaf-weevil (*D. denticollis*) and the leaf-beetle (*Pachnephorus* sp.) are planned for later in the year, when conditions are more conducive for field collections.

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8 Bibliography

Abraham, M., Abraham, C.T., Joy, P.J. (2002) Natural enemies of *Mikania micrantha* H.B.K. in Kerala. *Journal of Tropical Agriculture* **40**, 39-41.

Bagyanarayana, G. and Ravinder, E.J. (1988) Notes on two Indian species of *Ravenelia. Transaction of the British Mycological Society* **90**, 477-479.

Baksha, M.W. and Islam, M.R. (1996) Mortality of babul (*Acacia nilotica*) caused by the scale insect, *Anomalococcus indicus* and its control. *Indian Journal of Forestry* **19**, 355-360.

Beeson, C.F.C. (1919) The food plants of Indian forest insects. Part iii. *Indian Forester* **45**, 312-323.

Beeson, C.F.C. (1941) The ecology and control of forest insects of India and neighbouring countries. Vasant Press, Dehra Dun, India.1007 pp.

Ben-Dov, Y. (2006) A systematic catalogue of eight Scale Insect families (Hemiptera: Coccoidea) of the world Aclerdidae, Asterolecaniidae, Beesoniidae, Carayonemidae, Conchaspididae, Dactylopiidae, Kerriidae and Lecanodiaspididae. Elsevier, Amsterdam. 368 pp.

Brenan, J.P.M. 1983. Present taxonomy of four species of *Acacia* (*A. albida, A. senegal, A. nilotica* and *A. tortilis*). Manual on taxonomy of Acacia species. Food and Agricultural Organisation of the United Nations, Rome. pp. 20-35.

Cannon, P.F. (2008) *Ravenelia evansii*. IMI Descriptions of Fungi and Bacteria No. 1754. CABI, Bakeham Lane, Egham, Surrey, UK.

David, B.V. and Ananthakrishan, T.N. (2004) General and applied entomology. Second edition. Tata McGraw-Hill Publishing Company Ltd., New Delhi, India. pp. 1184.

Dhileepan, K. (2009) *Acacia nilotica* ssp. *indica*. (L.) Wild. Ex. Del. (Mimosaceae). In: Muniappan, R., Reddy, D.V.P., Raman, A (Eds.), Biological Control of Tropical Weeds Using Arthropods. Cambridge University Press, UK, pp. 17-37.

Dhileepan, K., Wilmot, K.A.D.W., Raghu, S. (2006) A systematic approach to biological control agent exploration and selection for prickly acacia (*Acacia nilotica* ssp. *indica*). *Australian Journal of Entomology* **45**, 302-306.

Dhileepan, K., Lockett, C.J., Robinson, M., Pukallus, K. (2009) Prioritising potential guild of specialist herbivores as biological control agents for prickly acacia through simulated herbivory. *Annals of Applied Biology* **154**, 97-105

Dhileepan, K., Balu, A, Ahmed, S.I., Singh, S, Srivastava, K.K., Senthilkumar, M., Murugesan, S., Senthilkumar, P., Gorain, M., Sharma, A., Sharma, N., Mahalakshmi, R., Shivas, R. (2010) New biological control opportunities for prickly acacia: exploration in India. In: Zydembos, S.M. (Ed.), Proceedings of the 17th Australasian Weeds Conference, New Zealand Plant Protection Society, Christchurch, New Zealand, pp. 231-234.

Dwivedi, A. P. (1993) Babul (*Acacia nilotica*): a multipurpose tree of dry areas. Arid Forest Research Institute, Indian Council of Forestry Research and Education, Jodhpur, India. 226 p.

Kapoor, J.N., Agarwal, D.K. (1974) Indian species of *Ravenelia* – I: on *Acacia*. *Indian Phytopathology* **27**, 666-669.

Koya, K.M.A., Devasahayam, S., Selvakumaran, S., Kalli, M. (1996) Distribution and damage caused by scale insects and mealy bugs associated with black pepper (*Piper nigrum* L.) in India. *Journal of Entomological Research* **20**, 129-136.

Kriticos, D., Brown, J., Radford, I., Nicholas, M. (1999) Plant population ecology and biological control: *Acacia nilotica* as a case study. *Biological Control*, **16**, 230-239.

Kruger M. (2001) A revision of the tribe Macariini (Lepidoptera: Geometridae: Ennominae) of Africa, Madagascar and Arabia. *British Natural History Museum* (*Entomology*) **70**, 1-502.

Lockett, C.J., Dhileepan, K., Robinson, M. and Pukallus, K.J. (2012) Impact of the biological control agent *Chiasmia assimilis* on prickly acacia (*Acacia nilotica* ssp. *indica*) seedlings. *Biological Control* **62**, 183-188.

Mackey, A.P. (1997) The biology of Australian weeds 29. *Acacia nilotica* ssp. *indica* (Benth.) Brenan. *Plant Protection Quarterly* **12**, 7-17.

Marohasy, J. (1992) Biocontrol of *Acacia nilotica* using insects from Kenya. Final Report to Australian Wool Corporation. Alan Fletcher Research Station, Queensland Department of Lands, 75 pp.

Mohyuddin, A.I. (1986) Investigations on the natural enemies of *Acacia nilotica* in Pakistan. Final Report, Commonwealth Institute of Biological Control, Rawalpindi, Pakistan, 116 p.

Mundkur, B.B., Thirumalachar, M.J. (1946) Revisions of and additions to Indian fungi. I. Mycol. Papers **16**, 1-27.

Nair, K.S.S. (2001) Pest outbreaks in tropical forest plantations: Is there a greater risk for exotic tree species? Centre for International Forestry Research, Jakarta, Indonesia. 74 pp.

Orchard, A.E., Wilson, A.J.G. (eds) (2001). Flora of Australia Volumes 11A & 11B, Mimosaceae, *Acacia* Parts 1 & 2. Australian Biological Resources Study (ABRS), Canberra, and CSIRO Publishing, Melbourne.

Palmer, W.A. (2004) Risk analysis of recent cases of non-target attack by potential biological control agents in Queensland, In: Cullen, J.M., Briese, D.T., Kriticos, D.J., Lonsdale, W.M., Morin, L., Scott, J. K. (Eds.), *Proceedings of the XI International Symposium on Biological Control of Weeds*, CSIRO Entomology, Canberra, Australia, pp. 305-309.

Palmer, W.A., Lockett, C.J., Senaratne, K.A.D.W., McLennan, A. (2007) The introduction and release of *Chiasmia inconspicua* and *C. assimilis* (Lepidoptera: Geometridae) for the biological control of *Acacia nilotica* in Australia. *Biological Control* **41**, 368-378.

Pillai, S.R.M., Gopi, K.C. (1990) Further records of insect pests of *Acacia nilotica* (Linn.) Willd. Ex. Del. in nurseries and young plantations and the need for control measures. *Indian Journal of Forestry* **13**, 8-13.

Pillai, S.R.M., Balu, A., Singh, R., Sasidharan, K.R., Raja Rishi, R, Salarkhan, A.M., Pankajam, S., Deeparaj, B., Sunitha, B. (1995) Pest problems of Babul (*Acacia nilotica* ssp. *indica*) and their management. Technical Bulletin No. 1, Institute of Forest Genetics and Tree Breeding, Indian Council of Forestry Research and Education, Coimbatore, Tamil Nadu, India. 16 pp.

Radford, I.J., Nicholas, D.M., Brown, J.R. (2001) Assessment of biological control impact of seed predators on the invasive shrub *Acacia nilotica* (Prickly acacia) in Australia. *Biological Control* **20**, 261-268.

Seier, M.K., Ellison, C.A., Corta, G., Day, M. and Dhileepan, K. (2012) How specific is specific enough? Case studies of three rust species under evaluation for weed biological control in Australia. In: Wu, Y., Johnson, T., Sing, S., Raghu, S., Wheeler, G., Pratt, P., Warner, K., Centre, T., Goolsby, J. and Reardon, R. (Eds.), *Proceedings of the XIII International Symposium on Biological Control of Weed*, Hawaii. USA, 11-16 September 2011, pp. 89-96.

Seier, M.K., Pollard, K.M. (2012) Preliminary host specificity testing of the prickly acacia rust *Ravenelia evansii* Syd. & P. Syd. Final Report submitted by CABI (UK) to Queensland Department of Agriculture, Fisheries & Forestry, October 2012, 17 pp.

Seier, M.K., Tanner, R.A. (2011) Host specificity testing of the prickly acacia rust *Ravenelia acacia-arabicae* Mund. & Thrum. (previously reported as *R. evansii* Syed. & Syd.). Final

Report submitted by CABI (UK) to Queensland Department of Employment, Economic Development & Innovation, June 2011, 30 pp.

Shivas, R.G., Balu, A., Singh, S., Ahmed, S.I. and Dhileepan, K. (2012) *Ravenelia acaciae-arabicae* and *Ravenelia evansii* are distinct species on *Acacia nilotica* subsp. *indica*. *Australasian Mycologist* **30**, (in press).

Spies, P., March, N. 2004. Prickly Acacia: National Case Studies Manual. Queensland, Australia: National Heritage Trust and Department of Natural Resources and Mines. 97 pp.

Stals, R. 1997. A survey of phytophagous organisms associated with *Acacia nilotica* in South Africa. Final Report to the Queensland Department of Natural Resources. ARC-Plant Protection Research Institute, Pretoria, South Africa. 113 pp.

Wardill, T.J., Graham, G.C., Manners, A., Playford, J., Zalucki, M., Palmer, W. A., Scott, K.D. (2005) The importance of species identity in the biocontrol process: identifying the subspecies of *Acacia nilotica* (Leguminosae: Mimosoideae) by genetic distance and the implications for biological control. *Journal of Biogeography* **32**, 2145-2159.