# final report

Project code: B.NBP.0638

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Date published: 4 August 2016

PUBLISHED BY
Meat and Livestock Australia Limited
Locked Bag 991
NORTH SYDNEY NSW 2059

# Prickly acacia biocontrol phase II: host-specificity testing of agents from India

Meat & Livestock Australia acknowledges the matching funds provided by the Australian Government to support the research and development detailed in this publication.

# 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 prickly acacia 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, resulting in the prioritisation of several species. The brown leaf-webber, *Phycita* sp. A, was imported into quarantine in January 2011 and host specificity testing commenced in June 2011. In no-choice larval development trials the leaf-webber completed development on 16 out of 27 non-target plant species tested. Despite occurring only on prickly acacia in the field, testing of the brown leaf-webber was terminated in December 2012 due to unacceptable non-target feeding. The second leaf-webber, *Phycita* sp. B, was imported into quarantine in October and December 2013 and host-specificity testing commenced in May 2014 after colony establishment, though maintaining the colony has proved difficult due to issues with stimulating reproduction. Host-specificity testing of a scale insect, *Anomalococcus indicus*, commenced in July 2011. The scale insect completed development on 17 of the 83 non-target plant species tested during no-choice trials. 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, choice trials under field conditions in India involving non-target test plants on which the scale insect completed development in no-choice tests in quarantine are in progress. Results to date suggest that *Neptunia major* and *Vachellia sutherlandii* are likely to be susceptible to the scale insect attack under field conditions. Tests for the susceptibility of other non-target plants for the scale insect under field conditions in India are in progress. The green leaf-webber larvae completed development on 10 out of 19 test plant species under no-choice conditions but in no-choice oviposition trials egg have been laid only on prickly acacia and *N. major*. However, in paired choice oviposition trials, eggs were laid only on prickly acacia and not on *N. major*. No further progress on screening the remaining test plants for the green leaf-webber was made due to difficulties in maintaining a culture of the insect in quarantine. A colony of the leaf-weevil *Dereodus denticollis* could not be established in the quarantine due to difficulties with its oviposition and larval feeding. Three promising gall-inducing biocontrol insects (a thrips gall, a mite gall and a midge gall) have been identified in Ethiopia. Based on damage potential, field host range and geographic range, the gall thrips, *Acaciothrips ebneri* (Karny) (Thysanoptera: Phlaeothripidae), inducing rosette galls in shoot tips and sprouting axillary buds resulting in shoot tip dieback, was imported into high-security quarantine in Brisbane, Australia and host specificity tests are in progress. No-choice tests on 14 non-target test plant species so far suggest that the gall thrips is highly host specific with no galls on any of the non-target plant species.

# 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 prickly acacia 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 and resulted in the prioritisation of the following agents for detailed host specificity tests: a scale insect (*Anomalococcus indicus*), two leaf-webbers (*Phycita* sp. A and *Phycita* sp. B) and a leaf weevil (*Dereodus denticollis*).

The brown leaf-webber (*Phycita* sp. A) was imported from India into a quarantine facility at the Ecosciences Precinct in Brisbane in January 2011. Under quarantine conditions adult moths lived for 8 ± 0.5 days and laid 78 ± 8 eggs. Eggs hatched in 6 ± 0.8 days and the larval stage lasted 41 ± 1.2 days, during which they consumed prickly acacia leaves. Fully-grown larvae pupated for 13 ± 0.4 days within the larval silk tunnel or in the soil. On prickly acacia, 80% of neonate larvae became adults.

Host-specificity testing of the brown leaf-webber commenced in June 2011. In no-choice larval development trials, the leaf-webber completed development on 16 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.

The scale insect (*A. indicus)* was initially imported into Australia in June 2011. Females have three instars, developing into sexually mature nymphs after 52 days under quarantine conditions. The generation time from egg to egg was 89 days. Females can produce more than 1200 eggs which they oviposit into a cavity underneath their body. Emerging crawlers are the only life stage at which the scale can disperse, though most settle close to the parental female. Males took 62 days to develop through five instars into winged adults.

Host-specificity testing of the scale insect commenced in July 2011. The scale insect completed development on 17 of the 83 non-target plant species tested during no-choice trials. Development on *Acacia falcata, Vachellia sutherlandii*, *V. bidwillii*, *Neptunia major* and *N. monosperma* was comparable to the scale's development on prickly acacia. In contrast to the brown leaf webber, testing of host preference in the field is feasible because host selection is not dependent on oviposition behaviour.

In view of the field host specificity of the scale insect in India, choice trials under field conditions in India, involving non-target test plants on which the scale insect completed development in no-choice tests in quarantine are in progress. Results to date suggest that *N. major* and *V. sutherlandii* are likely to be susceptible to the scale insect attack under field conditions. Tests for the susceptibility of other non-target plants for the scale insect under field conditions in India are in progress.

The green leaf webber (*Phycita* sp. B) was established in quarantine in early 2014. The larvae completed development on 10 out of 19 test plant species under no-choice conditions but in no-choice oviposition trials egg have been laid only on prickly acacia and *N. major*. However, in paired choice oviposition trials, eggs were laid only on prickly acacia and not on *N. major*. No further progress on screening the remaining test plants for the green leaf-webber was made due to difficulties in maintaining a culture of the insect in quarantine.

A colony of the leaf-weevil (*D. denticollis*) could not be established in the quarantine due to difficulties with its oviposition and larval feeding. Further work will be conducted using freshly field collected adults from India.

Three promising gall-inducing biocontrol insects (a thrips gall, a mite gall and a midge gall) have been identified in Ethiopia. Based on damage potential, field host range and geographic range, the gall thrips, *Acaciothrips ebneri* (Karny) (Thysanoptera: Phlaeothripidae), inducing rosette galls in shoot tips and sprouting axillary buds resulting in shoot tip dieback, was imported into high-security quarantine in Brisbane, Australia in December 2015. A colony of the gall thrips has been established and host specificity tests are in progress. Preliminary no-choice host specificity testing has been conducted on 14 non-target test plant species and to date no galls have been recorded on any of these species.

To MLA 10 Jan 2017:

The MLA agreement is closed, QDAF have progressed investigations in India current being hopeful that the scale insect will prove sufficiently host specific to be approved for release in Australia.

Update on field susceptibility studies in India (10 Jan 2107): Seeds of all the 16 test plant species have been exported to India, on multiple occasions, for inclusion in the field choice trails in India. Many of the Australian native plant species are not growing/surviving well under field conditions in India. As a result, field susceptibility data are available only for some of the non-target test plant species from India. Among the test plant species that grew well under Indian conditions, the scale insect attack was evident (though only very few adults) on only one species (Neptunia major, a plant that sustained high scale insect population under no-choice conditions in quarantine tests in Australia), only when they are physically in contact with or in close proximity (less than 60 cm) the prickly acacia plants with scale insects. In the trial in progress since January 2016, there was no evidence of the scale insect on any of the surviving Australian native test plant species, including N. major, Acacia cardiophylla, A. irrorata, A. decurrens, A. deanei, A. filicifolia and A. oshanseii (the test plants are about 90 cm from each other). It is likely that only one of the non-target test plant species, N. major, identified as ‘susceptible to scale insect’ in the no-choice tests in quarantine, could sustain the scale insect attack (at a very low level) only when they are in direct physical contract with or in close proximity (less than 60 cm) to the prickly acacia plants with scale insects. However, a self-sustaining population of the scale insect on N. major was never observed in the Indian trials.

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# 1 Background

*Vachellia nilotica* ssp. *indica* (Benth.) Kyal. & Boatwr (formerly known as *Acacia nilotica* subsp. *indica* (Benth.) Brenan and hereon referred to as prickly acacia) is a Weed of National Significance in Australia (http://www.weeds.org.au/WoNS/pricklyacacia). Introduced from India in the late 1890s as an ornamental tree, and promoted as a shade tree for sheep in western Queensland, prickly acacia now infests over seven million hectares of the Mitchell Grass Downs in western Queensland, as well as scattered coastal infestations in Queensland, the Northern Territory and Western Australia (Mackey 1997). Prickly acacia infestations cost primary producers AU$9 m/year in lost pasture production (Dhileepan 2009). It forms dense thickets, reducing pasture, interfering with mustering and stock access to water, threatens grassland biodiversity and facilitates soil erosion. It has the potential to infest most of northern Australia (Kriticos et al. 2003; Spies and March 2004). Biological control of prickly acacia was initiated in Australia in the early 1980s and of the six agents released, two have established, but with limited success (Dhileepan 2009).

*Vachellia nilotica* (L.f.) P.J.H. Hurter & Mabb. consists of nine morphologically and ecologically distinct subspecies (Brenan 1983). Biochemical and molecular studies suggest that the prickly acacia populations in Australia are the subspecies *indica* (Benth.) Brenan, which is native to India and Pakistan (Wardill et al. 2005). Native surveys for potential biological control agents were initiated in India in July 2008 and continued until June 2011. 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) and a leaf weevil (*Dereodus denticollis*). Permits to import the prioritised insects into a quarantine facility in Brisbane, Australia were obtained from relevant regulatory authorities in 2010 (*Phycita* sp. A, *A. indicus* and *D. denticollis)* and 2013 *(Phycita* sp. B).

To be approved for release in Australia, candidate biocontrol agents need to be assessed in quarantine for their ability to damage and complete their life cycle on non-target native, ornamental and agricultural plants. Host-specificity tests usually involve no-choice (agent in a container with only the non-target host species) and choice (non-targets plus target) trials. Continuation trials assess the population viability of an agent on a non-target host by allowing it to try and pass through multiple generations. An agent may feed and even develop on a non-target host in no-choice trials, but this may be an artefact of laboratory conditions, such as indiscriminate oviposition seen in lepidopterans, and would not be seen in the wild. Choice trials can be used to further assess these potential non-target host plants. The results of these may still be inconclusive, with only an incomplete preference for the target host over the non-target host. 'False negatives' (i.e. incorrectly concluding no non-target attack) are also possible, because the choice may not be available in the wild. Trials under field conditions, if feasible, can resolve some uncertainty. Consideration must be given to the biology of the agent and whether non-target hosts will be encountered and selected in the wild (van Klinken 2000).

There are no clear-cut rules for rejecting or accepting agents. Lists of host plants for testing cannot be exhaustive and laboratory host-specificity assessments are inherently overly conservative (Marohasy 1998, van Klinken 2000). An assessment of the non-target risk posed by an agent must be made case-by-case, considering experimental laboratory and field data, host and agent biology, field host range and consequences of any non-target attack.

# 2 Projective Objectives

1. **Imported two insects, the brown leaf-webber (*Phycita* sp. A) and the scale insect (*A. indicus*) from India into a quarantine facility at the Ecosciences Precinct in Brisbane (MET).**

   The brown leaf-webber and the scale insect were imported into quarantine on multiple occasions.

2. **Conduct host specificity tests for the brown leaf-webbier (*Phycita* sp. A) and the scale insect (*A. indicus*) with over 80 species of test plants including Australian native *Acacia* species (MET).**

   For the brown leaf-webber no-choice host specificity tests have been completed for 27 test plant species. Due to no-target feeding on multiple test plant species, screening of other test plants in no-choice test was suspended. Further studied have focussed on no-choice continuation trials and choice oviposition preference tests. Due to potential non-target, further screening of test plants was suspended and the colony in quarantine was destroyed.

   For the scale insect, no-choice tests were conducted with 81 plant species, 78 species with four or more replicates and three species with fewer than four replicates. Based on the results from the no-choice trials, choice trials were conducted under quarantine conditions and in the field in India.

3. **Complete detailed studies on the lifecycle of the two insects, including mating patterns, sexual dimorphism, oviposition, feeding patterns, diapause tendencies, adult longevity and temperature tolerance (MET).**

   Studies on the lifecycle including mating patterns, oviposition behaviour, feeding patterns and longevity of brown leaf-webber and the scale insect have been completed.

4. **Assess the risk to non-target plants in Australia based on the field host range in India and no-choice, multiple choice and demographic tests in quarantine in Brisbane (MET).**

   Non-target risk assessment for brown leaf-webber based on no-choice larval feeding, no-choice continuation trials and choice oviposition tests completed. Based on the risk assessment, further screening of test plants was suspended and the colony in quarantine was destroyed.

   For the scale insect, no-choice and choice trials have been completed in quarantine. Field choice test in India are in progress. Non-target risk assessment will be conducted when the field choice tests in India are completed.

5. **Submit regulatory approval to release the insects in Australia if the test results indicate that the agents are host specific and do not pose any non-target risk (NOT MET).**

A decision on submitting release application will be made based on results from the choice trials in India.

# 3 Methodology

## 3.1 Test plants

The test plant list was based on the list approved by the regulatory authorities for testing *Chiasmia* spp. (Palmer and Senaratne 2007), but has been revised following recent taxonomic changes to *Acacia sensu lato.* Where substitutions to the host test list were made, species from the same phylogenetic group were used (Table 1). Test plants species were sourced either as potted plants from nurseries or grown from seed and were maintained in a shade house or heated glasshouse at the Ecosciences Precinct (ESP), Brisbane, Australia. Attempts to source some test species (notably *Vachellia* species) were largely unsuccessful, and as such, a reduced number of these species have been tested.

**Table 1. Test plant species used in host specificity tests for *Phycita* sp. A and *A. indicus* in Australia. N = Native; I = Invasive; C = Crop; NC LD = no-choice larval development test; PC O = paired-choice test – oviposition; CT = continuation test; MC = multiple-choice test.**

| Order/Family/Subfamily/Tribe/Genus/Section/Species | Status | *Phycita* sp. A | *Phycita* sp. B | *A. indicus* |
|---|---|---|---|---|
| Order Fabales | | | | |
| Family Fabaceae | | | | |
| Subfamily Mimosoideae | | | | |
| Tribe Acacieae | | | | |
| Genus *Vachellia* | | | | |
| *V. bidwillii* Benth. | N | NC LD | | NC LD |
| *V. farnesiana* (L.) Willd.* | I | NC LD | | NC LD; MC LF |
| *V. sutherlandii* (F. Muell.) F. Muell | N | NC LD | NC LD; NC O | NC LD |
| *V.valida* (Tindale & Kodela) Kodela | N | | | NC LD |
| Genus *Acacia* | | | | |
| Section *Alatae* | | | | |
| *A. alata* R. Br. | N | NC LD | | NC LD |
| Section *Botrycephalae* | | | | |
| *A. baileyana F. Muell.* | N | NC LD; PC O, CT | NC LD; NC O | NC LD |
| *A. cardiophylla* A. Cunn. ex Benth. | N | NC LD | NC LD | NC LD |
| *A. dealbata* Link | N | | | NC LD |
| *A. deanei* (R. T. Baker) Welch, Coombs & McGlynn | N | NC LD | NC LD | NC LD |
| *A. decurrens* Willd. | N | | | NC LD |
| *A. elata* A.Cunn. ex Benth. | N | | NC LD | |
| *A. filicifolia* Cheel & Welch | N | NC LD | NC LD; NC O | |
| *A. glaucocarpa* Maiden & Blakely | N | | | NC LD |
| *A. irrorata* Seib. ex Streng. | N | NC LD, CT | NC LD | NC LD |
| *A. mearnsii* De Wild. | N | NC LD; PC O, CT | NC LD; NC O | NC LD |
| *A. oshanesii* F. Muell. & Maiden | N | NC LD | NC LD | NC LD |
| *A. parramattensis* Tind. | N | NC LD | | NC LD |
| *A. spectabilis* A. Cunn. ex Benth. | N | NC LD | NC LD; NC O | NC LD |
| *A. terminalis* (Salisb.) J.F.Macbr. | N | NC LD | | NC LD |
| Section *Juliflorae* | | | | |
| *A. aneura* F. Muell. ex Benth. | N | | NC LD | NC LD |

| | | | | |
|---|---|---|---|---|
| *A. cincinnata* F.Muell. | N | | | NC LD |
| *A. hemsleyi* Maiden | N | | | NC LD |
| *A. holosericea* A. Cunn. ex G. Don | N | NC LD | | NC LD |
| *A. leiocalyx* (Domin) Pedley | N | | | NC LD |
| *A. mangium* Willd. | N | NC LD | | NC LD |
| *A. plectocarpa* A. Cunn. ex Benth. | N | NC LD | | NC LD |
| *A. shirleyi* Maiden | N | | | NC LD |
| Section *Phyllodineae* | | | | |
| *A. conferta* A. Cunn. ex Benth. | N | NC LD | | NC LD |
| *A. falcata* Willd. | N | | | NC LD; MC LF |
| *A. macradenia* Benth. | N | NC LD | | NC LD |
| *A. peuce* F. Muell. | N | | | NC LD |
| *A. podalyriifolia* A. Cunn. ex G. Don | N | NC LD | | NC LD |
| *A. salicina* Lind. | N | | NC LD | NC LD |
| *A. tetragonophylla* F. Muell. | N | | NC LD | NC LD |
| *A. victoriae* Benth | N | NC LD | | NC LD |
| Section Plurinerves | | | | |
| *A. complanata* A. Cunn. Ex Benth. | N | | | NC LD |
| *A. coriacea* DC. | N | | NC LD | NC LD |
| *A. excelsa* Benth. | N | | | NC LD |
| *A. harpophylla* F. Muell. Ex Benth. | N | NC LD | | NC LD |
| *A. flavescens* A. Cunn. ex Benth | N | NC LD | | |
| *A. melanoxylon* R. Br | N | | | NC LD |
| *A. simsii* A. Cunn. ex Benth. | N | | NC LD | NC LD |
| *A. stenophylla* A. Cunn. ex Benth. | N | | | NC LD |
| Section *Pulchellae* | | | | |
| *A. drummondii* Lindley | N | NC LD | | NC LD |
| *A. lasiocarpa* Benth. | N | NC LD | NC LD; NC O | |
| *Acacia pulchella* R.Br. | N | | NC LD; NC O | NC LD |
| Tribe Mimoseae | | | | |
| *Adenanthera pavonina* L. | N | NC LD | | |
| *Dichrostachys cinerea*(L.) Wight & Arn. | N | | | NC LD |
| *Leucaena leucocephala* (Lam.) de Wit * | C/I | NC LD | | NC LD |
| *Neptunia dimorphantha* Domin | N | | | NC LD |
| *N. major* (Benth.) Windler | N | | NC LD; NC O; PC O | NC LD |
| *N. monosperma* F. Muell. Ex. Benth. | N | | | NC LD |
| Tribe Ingeae | | | | |
| *Albizia procera* (Roxb.) Benth. | N | | | NC LD |
| *Archidendron lucyi* F.Muell. | N | | | NC LD |
| *Cathormion umbellatum* (Vahl) Kosterm. | N | | | NC LD |
| *Inga edulis* Mart.* | E | | | NC LD |
| *Pararchidendron pruinosum* (Benth.) I.C.Nielsen | N | | NC LD | NC LD; MC LF |
| *Paraserianthes lophantha* (Willd.) I.C.Nielsen | N | | | NC LD |
| Subfamily Caesalpinaceae | | | | |
| Tribe Caesalpinieae | | | | |
| *Caesalpinia ferrea* Tulasne* | O | | | NC LD |
| *Delonix regia* (Bojer ex Hook.) Raf. * | O | | | NC LD |
| Tribe Cassieae | | | | |
| *Cassia brewsteri* (F.Muell.) Benth. | N | | | NC LD |
| *Ceratonia siliqua* L.* | E | | | NC LD |
| *Senna acclinis* (F.Muell.) Randell | N | | | NC LD |
| Tribe Cercideae | | | | |
| *Barklya syringifolia F.Muell.* | N | | | NC LD |

| | | | | |
|---|---|---|---|---|
| *Bauhinia hookeri* F.Muell. | N | | | NC LD |
| Tribe Detarieae | | | | |
| *Tamarindus indica* L.* | O | | | NC LD |
| Subfamily Papilionoideae | | | | |
| Tribe Abreae | | | | |
| *Abrus precatorius* ssp. *precatorius* L. | N | | | NC LD |
| Tribe Bossiaeeae | | | | |
| *Hovea acutifolia* A.Cunn. ex G.Don | N | | | NC LD |
| *Platylobium formosum* Sm. | N | | | NC LD |
| Tribe Cercideae | | | | |
| *Barklya syringifolia (F.Muell.) Wund.* | N | | | NC LD |
| *Bauhinia hookeri* F.Muell. | N | | | NC LD |
| Tribe Dalbergieae | | | | |
| *Arachis hypogaea* L. | C | | | NC LD |
| Tribe Desmodieae | | | | |
| *Desmodium macrocarpum* Domin. | N | | | NC LD |
| Tribe Galegeae | | | | |
| *Swainsona galegifolia* (Andrews) R.Br | N | | | NC LD |
| Tribe Indigofereae | | | | |
| *Indigiofera australis* Willd. | N | | | NC LD |
| Tribe Millettieae | | | | |
| *Callerya megasperma* (F.Muell.) Schot | N | | | NC LD |
| *Millettia* sp. | N | | | NC LD |
| Tribe Mirbelieae | | | | |
| *Pultenaea villosa* Andrews | | | | NC LD |
| Tribe Phaseoleae | | | | |
| *Cajanus cajan* (L.) Millsp. | C | | | NC LD |
| *Clitoria ternatea* L. | I | | | NC LD |
| *Erythrina vespertilio* Benth. | N | | | NC LD |
| *Hardenbergia violacea* (Schneev.) Stearn | N | | | NC LD |
| *Phaseolus lunatus* L. | C | | | NC LD |
| *Vigna unguiculata* var. *sesquipedalis* (L.) Verdc. | C | | | NC LD |
| Tribe Sophoreae | | | | |
| *Castanospermum australe* A.Cunn. & C.Fraser ex Hook. | N | NC LD | | NC LD |
| Tribe Tephrosieae | | | | |
| *Tephrosia grandiflora* (L'Hér. ex Aiton) Pers.* | E | | | NC LD |
| Order Malpighiales | | | | |
| F amily Euphorbiaceae | | | | |
| *Mallotus claoxyloides* | N | | | NC LD |
| Order Malvaceae | | | | |
| Family Malvaceae | | | | |
| *Brachychiton acerifolius* (A.Cunn. ex G.Don) Macarthur & C.Moore | N | | | NC LD |

## 3.2 *Phycita* sp. A (Lepidoptera: Pyralidae)

Field collected brown leaf-webber larvae and pupae were imported from India into a quarantine facility at ESP in January 2011 (Table 2). A colony was established and maintained in insect-proof cages (90 x 80 x 75 cm) on both whole plants and cut foliage of prickly acacia in a quarantine glasshouse (22-27ºC; 65% RH and natural photoperiod). Newly emerged moths were either released directly into insect-proof cages containing potted prickly acacia plants or were placed in pairs in glass / plastic oviposition containers with prickly acacia cut foliage for egg laying. An isotonic liquid (Gatorade® or Powerade®) was supplied to adults to enhance egg production and adult longevity. Newly emerged

larvae in oviposition containers were transferred onto potted prickly acacia plants in insect-proof cages for larval development and pupation. Pupae were collected from potted plants and maintained in plastic containers for adult emergence. Newly emerged larvae and adults were used in all experiments.

**Table 2. 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 |
| 12/12/2014 | *Phycita* sp. B | IP13013814 | 137 | larvae and pupae |
| 12/12/2014 | *Dereodus denticollis* | IP13013814 | 103 | adults |
| 24/05/2015 | *Phycita* sp. *B* | IP13013814 | 85 | larvae and pupae |
| 24/05/2015 | *Dereodus denticollis* | IP13013814 | 104 | adults |
| 15/08/2015 | *Phycita* sp. *B* | IP15012580/ IP1501222 | 93 | larvae |
| 23/10/2015 | *Phycita* sp. *B* | IP15012580/ | 128 | larvae |
| 23/10/2015 | *Dereodus denticollis* | IP15012580 | 98 | adults |

### 3.2.1 Life cycle

To study the lifecycle, a pair of newly emerged and mating adults (n = 10) were transferred onto a potted plant enclosed in a cylindrical transparent Perspex tube (34 cm high and 12 cm diameter) with a gauze cap. The adults were transferred on to a fresh plant each week, and the longevity, pre-oviposition period, and the number of eggs laid per female per week were recorded.

### 3.2.2 Host-specificity tests

Host-specificity testing commenced in June 2011 and was completed in December 2012. All tests were conducted in a temperature (22-27°C), light (14 h light: 10 h dark) and humidity (60-70% RH) controlled quarantine insectary at ESP in Brisbane, Queensland.

#### *3.2.2.1 No-choice larval feeding and development tests*

Batches of test plants were screened as they became available and in each batch potted prickly acacia plants were included as positive controls. Ten newly emerged larvae were added to each potted plant. Plants with larvae were placed in insect-proof cages and were

checked two to three times per week for evidence of larval feeding and webbing, larval development, pupation and adult emergence. A minimum of five replicates of each test plant was used.

*3.2.2.2 No-choice continuation trials*

No-choice larval development continuation trials to ascertain the suitability of non-target plant species to sustain continuous multiple generations of the leaf-webber, commenced in April 2012. Three non-target test plant species, *Acacia baileyana, A. mearnsii*, and *A. irrorata* were chosen, as these had supported the development of the leaf-webber to adults, in the no-choice larval feeding trials. Each test was replicated a minimum of three times and commenced with the placement of 60 newly emerged first instar larvae onto both test and control plants. Additional plants were added to cages as required, to feed developing larvae until pupation. The total number of adults emerging per test cage was recorded, together with the development period (in days) from first instar larva to adult. When sufficient numbers of males and females were collected together, pairs were placed in oviposition containers to allow mating and oviposition. The number of eggs laid by pairs were recorded. Newly hatched larvae were then used to set up subsequent generations on the same test plant species. Individual test replicates were continued for a maximum of three subsequent generations, if sufficient eggs and larvae were produced.

*3.2.2.3 Choice oviposition tests*

Test plant species on which there was any evidence of feeding and development in no-choice tests were subjected to choice oviposition tests. A choice oviposition trial involving prickly acacia and four non-target test plant species (*A. baileyana*, *A. mearnsii*, *A. oshanesii* and *A. macradenia*) within a single, large, walk-in cage was conducted (three replications). Despite using large and similar-sized test plants and multiple pairs of the leaf-webber moth, adults failed to lay eggs on any plant – all eggs were laid on the gauze walls of the cage. One hypothesis for the lack of oviposition on any tests plants in the test arena was that one or some of the test plants may repulse the ovipositing moths. Hence paired choice oviposition tests involving only two test plants (prickly acacia + one test plant species) were conducted with prickly acacia and *A. mearnsii* (seven replications) and prickly acacia and *A. baileyana* (three replications). Adults were left in the choice oviposition arena for five days and then the numbers of eggs laid on individual test plants and on the cage walls were counted.

## 3.3 *Anomalococcus indicus* (Hemiptera: Lecanodiaspididae)

Identification of the insect was made by a scale taxonomy expert Dr Gillian Watson, then working for Commonwealth Agricultural Bureaux International (CABI, UK). Infested prickly acacia stems collected in the field in Tamil Nadu, India were initially shipped to Brisbane on January 2011 (Table 2). A colony was established within the quarantine facility at ESP on potted prickly acacia plants contained in large gauze cages (61 x 61 x 183 cm) and housed in a temperature-controlled quarantine glasshouse maintained at 22-27$^{o}$C, 60-70% RH and natural photoperiod. Gravid females were removed from the imported stems and attached to uninfested plants in a vegetable gel-cap glued or pinned to the main trunk of the plant. All females were examined for the presence of predators and parasitoids, which were destroyed.

### 3.3.1 Lifecycle

The size of individuals was determined by measuring their length and width (widest part) using a stereo microscope with a calibrated micrometre.

#### *3.3.1.1 Egg and crawler biology*

To determine the time to death of crawlers without feeding, 100 newly emerged crawlers were confined in a glass petri-dish on a moistened filter paper. As the crawlers were adversely affected by handling, monitoring was restricted to once a day. Nymphal development was studied by transferring 100 newly emerged crawlers to a new potted prickly acacia plant using a fine paint brush and following their development. The number, sex and developmental stage of the nymphs were recorded on a weekly basis for a minimum of 100 days, at which time crawlers were emerging from beneath mature females.

The distribution of nymphs in relation to the parent female was examined by gluing gravid females, still attached to a piece of bark from the plant on which they developed, to trunks of uninfested prickly acacia plants.

#### *3.3.1.2 Female biology*

Gravid females were carefully removed from prickly acacia stems so that no bark was attached. Each female was placed in a glass petri-dish on a piece of filter paper and checked daily for eggs and crawlers until they died. Gravid females were also attached to uninfested prickly acacia plants. Two methods of attachment were used. Some were attached as mentioned earlier, while others were placed into vegetable gel-cap halves which were pinned to the trunk.

To investigate whether *A. indicus* reproduces parthenogenetically, newly emerged crawlers were placed on potted prickly acacia plants (see above). To reduce extraneous variation, plants were infested in pairs, each with crawlers from the same female. Plant pairs were kept in separate cages. All males were removed from the plants in one of the cages as soon as they pupated. Once individuals became adults, female size (length, width and height) was measured on a weekly basis and the presence of newly emerged crawlers noted.

#### *3.3.1.3 Male biology*

Due to the difficulty of locating adult males, second-instar males were labelled and the date of pupation noted. Pupae were removed from plants and confined in a petri dish until adult males emerged, the date of which was noted. To determine the survival rates of adult males, groups of at least four newly emerged adults were confined in a petri-dish on a piece of filter paper and checked periodically.

### 3.3.2 Host specificity

#### *3.3.2.1 No-choice nymphal development*

Plants up to 1.5 m were used for testing. Gravid females were placed in vegetable gel-cap halves which were attached to the main stem/s of test and control plants. Initial tests used field collected *A. indicus* females imported from India. These females were first screened for parasitoids and predators. Four capsule halves, containing five gravid females each, were attached to each test plant and control plant. Later tests used females from the laboratory

colony. Due to the known absence of parasitoids and predators, only three capsule halves (each containing five gravid females), were attached to each plant. Test and control plants were arranged in 175 $cm^3$ collapsible gauze cages so that there was no contact between plants. Test and control plants were checked on a monthly basis. The capsules containing the adult females were removed after one month. The number and developmental stage of any scale found was recorded. Each trial ran for a minimum of three months. After this time, test plants with no scale were removed. Those with scale were monitored for crawler emergence together with the control plants, until either crawlers were observed or scale development had ceased. Due to non-target feeding and development, screening of test plants was suspended in 2013 but recommenced in late 2014.

The *V. valida* plants available for testing were very small (< 10 cm tall) so similar sized prickly acacia plants were used for comparisons. Using a fine paint brush, 50 newly emerged crawlers were carefully placed on each plant. The number, sex and developmental stage of the nymphs were recorded periodically for a minimum of three months until the writing of this report.

*3.3.2.2 Comparative nymphal development*

Two of the native species on which *A. indicus* completed development (*Neptunia major* and A*. falcata*) were included in a trial to compare the survival and development of the scale on prickly acacia and the non-target species. Using a fine paint brush, 100 newly emerged crawlers were carefully placed on each plant. The number, sex and developmental stage of the nymphs were recorded periodically for a minimum of three months, until crawlers were seen emerging from beneath gravid females. Gravid females were then removed from their host plant. Each was placed into a glass petri-dish on a piece of moistened filter paper and checked daily for eggs and crawlers (until they died).

*3.3.2.3 Nymphal host preference*

To examine the host preference of newly emerged crawlers, one plant each of prickly acacia, *V. farnesiana, A. falcata*, and *Parachidendron pruinosum* were arranged around an infested prickly acacia plant with gravid females, so that each was in contact with the infested plant and each other. Given that crawlers tend not to travel far from the parental female, an attempt was made to ensure the point of contact between the test plants and the infested plant occurred close to a gravid female. The infested prickly acacia plant was removed after two weeks. Several weeks after this the number of scale on each plant was counted and the proportion on each species determined. A second set of nymphal host preference trials was conducted with *Vachellia sutherlandii, A. falcata, N. major* and prickly acacia.

*3.3.2.4 Field choice trials*

Over the duration of this project hundreds of seeds of the following test plants have been exported to India so that potential non-target species which supported complete development of the scale could be tested under field conditions; species include *A. falcata, A. terminalis, A. plectocarpa, A. irrorata, A. complanata, A. coriacea, A. farnesiana, Paraserianthes lophantha, N. monosperma, N. major, V. sutherlandii, Ceratonia siliqua* and *Platylobium formosum.* For reasons unknown there have been a lot of difficulties in germinating these seeds in India, so few species have been available for testing in the field. A final shipment of seeds was exported to India in October 2015. Seeds were germinated and grown in pots at the Institute of Forest Genetics and Tree Breeding (IFGTB), Coimbatore, Tamil Nadu, India. Potted plants (about a meter tall) were used in all field choice trials. Field choice trials were conducted either at the IFGTB campus or at the farmer's field in Coimbatore, India.

Trial 1: The first field choice trial involving *N. major* (n = 8) as test plants and prickly acacia (n = 8) and control plants commenced in January 2014 and continued till July 2014 (when all prickly acacia control plants died due to scale insect attack). Scale insect infested prickly acacia plants (as source plants for the scale insect) was placed in the middle (about 6 scale insect infested plants). Prickly acacia and *N. major* plants were placed in concentric circles at a distance of 60 cm (4 plants for each of prickly acacia and *N. major*) and 90 cm (4 plants for each of prickly acacia and *N. major*) from the source plants. Any source plants which died due to scale insect damage were replaced with a fresh scale insect source plant. Plants were sampled at monthly intervals and the number of plants with scale insect and the number of scales per plant were recorded.

Trial 2: A similar field choice trial involving *N. major* (n = 11) as test plants and prickly acacia (n = 11) as control plants (Fig. 1) commenced in August 2014 and continued till Jan 2015 (when all prickly acacia control plants died due to scale insect attack). Scale insect infested prickly acacia plants (as source plants for the scale insect) were placed in the middle (about 4 scale insect infested plants). Prickly acacia and *N. major* plants were placed in concentric circles at a distance of 60 cm (4 prickly acacia plants and 7 *N. major* plants) and 120 cm (4 prickly acacia plants and 7 *N. major* plants) from the source plants. Any source plants which died due to scale insect damage were replaced with a fresh scale insect source plant. Plants were sampled at monthly intervals and the number of plants with scale insect and the number of scales per plant were recorded.

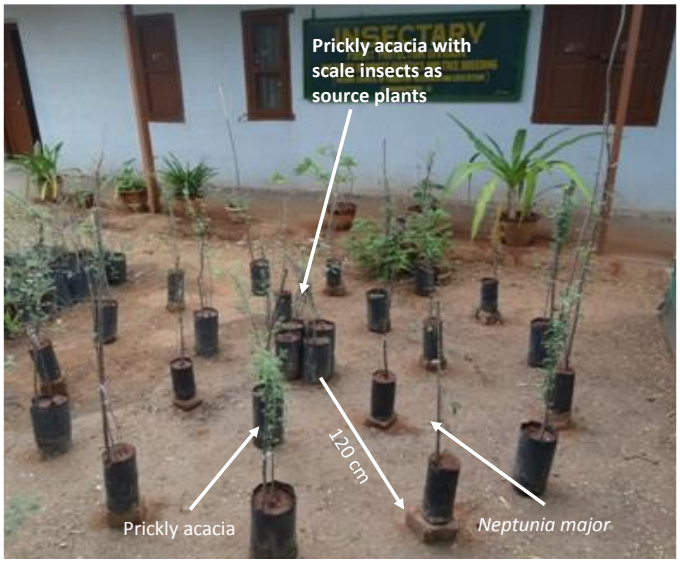


**Fig. 1. Field susceptibility trial for the scale insect in India involving prickly acacia (control) and *N. major* (test plant) with scale-insect infested prickly acacia plants as source plants for the scale insect in the middle.**

Trial 3: A field choice trial involving prickly acacia (n=15) as control plants and *N. major* (n=15) as test plants was conducted from December 2014 to May 2015, under a prickly acacia tree naturally infested with the scale insect at the IFGTB campus (Fig. 2). The plants were arranged alternately around the naturally infested tree and observations were made at weekly intervals.

**Fig. 2. Field susceptibility trial for the scale insect in India involving prickly acacia (control) and *N. major* (test plant) below a prickly acacia tree naturally infested with the scale insect.**

Trial 4: A more robust field susceptibility trial for the scale insect, involving six species of test plant species from Australia (on which the scale insect completed development in no-choice tests in quarantine) commenced in a farmer's field in Devarayapuram (N 10°59'51.54"; E 76°48'57.12"), a village near Coimbatore in December 2014 (Fig.3) and continued till May 2015. Ten potted plants each of *N. major, Vachellia farnesiana, Acacia falcata, A. terminalis, Parachidendron pruinosum, V. nilotica* ssp. *indica* (control-scale free) and *V. nilotica* ssp. *indica* (source plant – scale infested) were arranged in a split-plot design (10 replications, each with a single scale infested prickly acacia plant in the centre, with the six test plant species [including a control prickly acacia plant] placed 90 cm from the scale infested source plant in a circle) were arranged below scale-infested prickly acacia trees in the farmer's field. Arrangements were made with the owner of the property to water the experimental plants on alternate days.

**Fig. 3. Field susceptibility trail for the scale insect in a farmer's field in Coimbatore, India involving prickly acacia (control) and five test plant species below scale-insect infested prickly acacia trees and with scale-insect infested prickly acacia plants as source plants as well.**

Trial 5: A field choice trial involving 10 test plant species (*N. major*, *Paraserianthes lopentha*, *V. farnesiana*, *V. tortilis*, *V. sutherlandii*, *Acacia auriculiformis*, *A. falcata, A. terminalis*, *A. planifrons* and *Senegalia ferruginea*) commenced in March 2015 (Table 3; Fig. 4). The trial included 10 rows of potted prickly acacia plants alternating with 10 rows of test plant species (10 replications for each test plant species + 50 replications for prickly acacia + 50 scale-insect infested prickly acacia plants as source plants for scale insect). In each row of 10 prickly acacia plants, 5 were scale-infested (source plants for the scale insect) alternating with 5 scale-insect free plants. In each row of test plant species, there was one potted plant each of 10 test plant species (*N. major*, *Paraserianthes lophantha*, *V. farnesiana*, *V. tortilis*, *V. sutherlandii*, *A. auriculiformis*, *A. falcata, A. terminalis*, *A. planifrons* and *S. ferruginea*). The test and control plants are being monitored at monthly intervals for scale incidence.

**Table 3. Schematic experimental plan for the field susceptibility trial for the scale insect at the IFGTB campus, Coimbatore, India involving prickly acacia (control) and 10 test plant species. PAI = prickly acacia – scale infested (source plants); PAC = prickly acacia – control; NM = *Neptunia major*; VF = *Vachellia farnesiana*; AA = *Acacia auriculiformis*; AT = *Vachellia tortilis*; Ater: *Acacia terminalis*; AP = *Acacia planifrons*; SF = *Senegalia ferruginea*; AF = *Acacia falcata*; VS = *Vachellia sutherlandii*; PL = *Paraserianthes lophantha*.**

| | Line 1 | Line 2 | Line 3 | Line 4 | Line 5 | Line 6 | Line 7 | Line 8 | Line 9 | Line 10 |
|---|---|---|---|---|---|---|---|---|---|---|
| **Row 1** | PAC | **PAI** | PAC | **PAI** | PAC | **PAI** | PAC | **PAI** | PAC | **PAI** |
| **Row 2** | NM | VF | AA | AT | Ater | AP | SF | AF | VS | PL |
| **Row 3** | **PAI** | PAC | **PAI** | PAC | **PAI** | PAC | **PAI** | PAC | **PAI** | PAC |
| **Row 4** | PL | NM | VF | AA | AT | Ater | AP | SF | AF | VS |
| **Row 5** | PAC | **PAI** | PAC | **PAI** | PAC | **PAI** | PAC | **PAI** | PAC | **PAI** |
| **Row 6** | VS | PL | NM | VF | AA | AT | Ater | AP | SF | AF |
| **Row 7** | **PAI** | PAC | **PAI** | PAC | **PAI** | PAC | **PAI** | PAC | **PAI** | PAC |
| **Row 8** | AF | VS | PL | NM | VF | AA | AT | Ater | AP | SF |
| **Row 9** | PAC | **PAI** | PAC | **PAI** | PAC | **PAI** | PAC | **PAI** | PAC | **PAI** |
| **Row 10** | SF | AF | VS | PL | NM | VF | AA | AT | Ater | AP |
| **Row 11** | **PAI** | PAC | **PAI** | PAC | **PAI** | PAC | **PAI** | PAC | **PAI** | PAC |
| **Row 12** | AP | SF | AF | VS | PL | NM | VF | AA | AT | Ater |
| **Row 13** | PAC | **PAI** | PAC | **PAI** | PAC | **PAI** | PAC | **PAI** | PAC | **PAI** |
| **Row 14** | Ater | AP | SF | AF | VS | PL | NM | VF | AA | AT |
| **Row 15** | **PAI** | PAC | **PAI** | PAC | **PAI** | PAC | **PAI** | PAC | **PAI** | PAC |
| **Row 16** | AT | Ater | AP | SF | AF | VS | PL | NM | VF | AA |
| **Row 17** | PAC | **PAI** | PAC | **PAI** | PAC | **PAI** | PAC | **PAI** | PAC | **PAI** |
| **Row 18** | AA | AT | Ater | AP | SF | AF | VS | PL | NM | VF |
| **Row 19** | **PAI** | PAC | **PAI** | PAC | **PAI** | PAC | **PAI** | PAC | **PAI** | PAC |
| **Row 20** | VF | AA | AT | Ater | AP | SF | AF | VS | PL | NM |

Due to high mortality among the native Australian test plant species in India, additional fresh seeds of Australian native test plant species (*Acacia irrorata, A. terminalis, A. plectocarpa, A. falcata, V. sutherlandii, A. coriacea, A. complanata, N. major*, *Platylobium formosum* and *P. lophantha* - species on which the prickly acacia scale insect completed development under no-choice conditions in quarantine in Australia) were hand delivered for inclusion in field choice tests in October 2015.

**Fig. 4. Field susceptibility trial for the scale insect at IFGTB campus involving prickly acacia and 10 test plant species (see table 3 for details).**

Trial 6: An additional field choice trial involving 13 test plant species (*N. major, A. falcata*, *A. terminalis, V. sutherlandii, C. siliqua, Platylobium formosum, A. filicifolia, A. cardiophylla, A. irrorata, A. deanei, A. parramattensis, A. mearnsii,* and *A. decurrens*) commenced in January 2016 in the IFGTB campus and is in progress. Each species was replicated 10 times and were arranged in the trial in Latin square design. Ten scale infested prickly acacia plants were also included in the experiment as source of infestation. A 45 cm gap between the plants in column and a 60 cm gap between the rows were maintained. Observations on the incidence and spread of the scale insect on each test plant in terms of number of nymphs and adults and growth parameters such as height and basal diameter of the plants have been recorded at monthly intervals.

*3.3.2.5 Field host range*

Field host specificity of the scale insect at subspecies level was documented in 72 survey sites in southern India. Various non-target species, *Vachellia leucophloea*, *S. ferruginea*, *Senegalia senegal* (natives of India) and *Vachellia horrida* (native of Africa) occurring at the survey sites were surveyed to ascertain if the ecological host range of insects found on prickly acacia extended to these species.

## 3.4 *Dereodus denticollis* (Coleoptera: Curculionidae)

The weevil was identified by Dr. V.V. Ramamurthy, a specialist coleopteran taxonomist based at the Indian Agricultural Research Institute, New Delhi, India.

In India, studies on the life cycle and preliminary host specificity testing for *D. denticollis* were conducted under laboratory conditions at the IFGTB using field collected adults (Coimbatore, India). Adult no-choice feeding tests were conducted on six test plant species (*A. falcate*, *A. terminalis*, *V. sutherlandii*, *V. tortilis, N. major* and *Paraserianthes lophantha*) with prickly acacia as control, with 10 replications (potted plants) for each test plant species.

Ten field collected *D. denticollis* adults (of unknown age) starved for 48 hours were released on each plant, enclosed in an insect-proof cage, and monitored daily for feeding and survival, till all adults died.

In Australia, imported adults were kept within the quarantine facility at ESP on potted prickly acacia plants contained in insect-proof cages (90 x 80 x 75 cm). It has yet to be successfully cultured in quarantine.

New culturing practises were implemented in May 2015 following importation of new insect material and advice from Indian counterparts. Medium sized glass jars (15 cm diameter and 25 cm high) were used to house two mating pairs of *D. denticollis* weevils (n=16). Jars were supplied with two 20 cm sprigs of prickly acacia submerged into saturated floral foam. Jars were checked periodically and foliage changed every two to three days.

Eggs were removed from glass jars once they began to darken in colour and placed on moistened filter paper within plastic petri dishes. Newly hatched larvae were placed on various feeding substrate and periodically assessed for survival. Larval feeding substrates tested to date include; freshly cut stem semi-submerged in moistened sand, fresh root material buried in moistened sand, fresh root material within a petri dish lined with moistened paper towel, potted seedlings, potted plants and a semi-artificial diet consisting of ground up root material from a mature prickly acacia tree excavated from Gayndah.

## 3.5 *Phycita* sp. B (Lepidoptera: Pyralidae)

Field-collected green leaf-webber larvae and pupae were imported from India into a quarantine facility at ESP in 2012 and 2013 (Table 2). A colony was established and maintained in insect-proof cages (90 x 80 x 75 cm) in a quarantine glasshouse (22-27$^{o}$C; 65% RH and natural photoperiod). Newly emerged moths were released directly into insect-proof cages containing potted prickly acacia plants. An isotonic liquid (Gatorade® or Powerade®) was supplied to adults to enhance egg production and adult longevity.

The colony failed to reproduce in early 2015 and consequently died out. Additional importations were thus made in May and August 2015.

### 3.5.1 Life cycle

Lifecycle of *Phycita* sp. B was studied under laboratory conditions in India and under quarantine conditions in Australia. To study the lifecycle, eggs were collected and placed into glass petri-dishes until larvae hatched. Newly emerged larvae were transferred either onto potted prickly acacia plants or placed in a glass petri dish containing cut prickly acacia foliage (foliage added as required) in insect-proof cages for larval development and pupation. Pupae were collected from potted plants and maintained in plastic containers for adult emergence. Adult moths were transferred to new cages either every day or every few days.

### 3.5.2 Host-specificity tests

Host specificity tests for *Phycita* sp. B were conducted under laboratory conditions in India and under quarantine conditions in Australia.

In India, no choice host specificity tests were conducted on 10 non-target test plant species (*Acacia planifrons, A. auriculiformis, A. deanii*, *Vachellia leucophloea, V. farnesiana, V. tortilis, Senegalia mellifera, S. catechu, S. ferruginae* and *Neptunia major*) (on live potted plants) with a minimum of five replications for each species. Ten newly emerged larvae were released on each plant kept inside the rearing jars, covered by transparent muslin cloth, and monitored daily for larval feeding, survival and development.

In Australia, host-specificity testing commenced in May 2014 and continued till December 2015. All tests were conducted in a temperature (22-27°C), light (14 h light: 10 h dark) and humidity (60-70% RH) controlled quarantine insectary at ESP. Newly emerged larvae and adults were used in all experiments. Batches of test plants were screened as they became available and in each batch potted prickly acacia plants were included as positive controls. Ten newly emerged larvae were added to each potted test plant, as well as prickly acacia. Plants with larvae were placed in insect-proof cages and were checked two to three times per week for evidence of larval feeding and webbing, larval development, pupation and adult emergence. A minimum of five replicates of each test plant have been conducted for 12 species (Table 1).

No-choice oviposition trials are being conducted with non-target species in which larval development occurred. In these tests, five male and five female moths are confined to insect-proof cages containing non-target test plants and a control cage containing a prickly acacia plant. Cages are checked for eggs periodically.

Oviposition choice tests have commenced for the non-target test species that received egg lay during no-choice oviposition tests. Five pairs of moths are released into an insect proof cage containing one non-target test plant and one prickly acacia plant.

# 4 Results

## 4.1 Host test list

The majority of the species in the test list belong to *Acacia sensu lato* (*s.l.).* Morphological and molecular studies have demonstrated that *Acacia s.l.* is polyphyletic and the genus has been split into five genera (*Acacia*, *Vachellia*, *Senegalia*, *Acaciella* and *Mariosousa). Acacia sensu stricto (s.s.)* has been retypified so that the majority of species within *Acacia s.l.* (i.e. *Acacia* subg. *Phyllodineae)* retain this name (see Maslin et al. 2003; Miller and Seigler 2012). *Acacia s.s.* contains nearly 1000 species and most of these are Australian. Sections within *Acacia s.s*. are not considered natural groupings (see Maslin et al. 2003), but are retained here. Species within *Acacia* subgenus *Acacia* (including prickly acacia) have been transferred to the genus *Vachellia* Wight & Arn. Fourteen *Vachellia* species are found in Australia, of which nine are endemic and five (*V. nilotica, V. farnesiana, V. karroo* (Hayne) Banfi & Galasso, *V. gerrardii* (Benth.) P.J.H.Hurter and *V. xanthophloea* (Benth.) Banfi & Galasso) are exotic (Kodela and Wilson 2006; University of Queensland 2011; AVH 2015). *Senegalia* is represented in Australia by two rare endemic species and two naturalised species (Maslin 2012), and *Acaciella* is represented in Australia only by the naturalised species *Acaciella angustissima* (Mill.) Britton & Rose and *Acaciella glauca* (L.) L. Rico (University of Queensland 2011). Sourcing specimens of these genera was either difficult (*Vachellia*) or not possible (*Senegalia* and *Acaciella*). Ideally more *Vachellia* species would have been tested.

Two of the three other tribes in the Mimosaceae are represented in Australia. Mimoseae contains four genera (12 species) native to Australia *(Neptunia, Dichrostachys, Entada* and

*Adenanthera)* and four naturalized genera (*Prosopis*, *Mimosa, Leucaena* and *Desmanthus*). The tribe Ingeae is represented in Australia by eight genera including 20 native species and three naturalised species. Recent molecular work suggests that *Vachellia* is nested with tribe Mimoseae (Bouchenak-Khelladi et al. 2010). As such, *Vachellia nilotica* is now believed to be more closely related to species within Mimoseae than it is to the majority of Australian acacia species (*Acacia s.s*). *Vachellia* is also more closely related to *Senegalia*, *Acaciella* and *Mariosousa* (all formerly within *Acacia s.l*.) than *Acacia s.s.* which forms a clade with the Ingeae tribe.

## 4.2 *Phycita* sp. A

### 4.2.1 Life cycle

Images of several of the life stages are provided (Fig. 5). Under quarantine conditions, adult moths lived for 8.8 ± 0.5 days (range: 6 to 21 days) and laid eggs within 2-10 days of adult emergence. Females laid 78 ± 8 eggs (range: 55 to 350 eggs), on the leaves and stems of host plants, cage walls or the gauze covers on oviposition containers. Eggs hatched in 6 ± 0.8 days (range: 6 to 10 days). Newly emerged larvae feed almost immediately, tying leaves together with silk webs, forming tunnels as they mature. The larval stage lasted for 41 ± 1.2 days (range: 27 to 48 days). Fully grown larvae pupated for 13 ± 0.4 days (range: 6 to 19 days) within the larval silk tunnel or in the soil. On prickly acacia, 80% of the neonate larvae became adults.

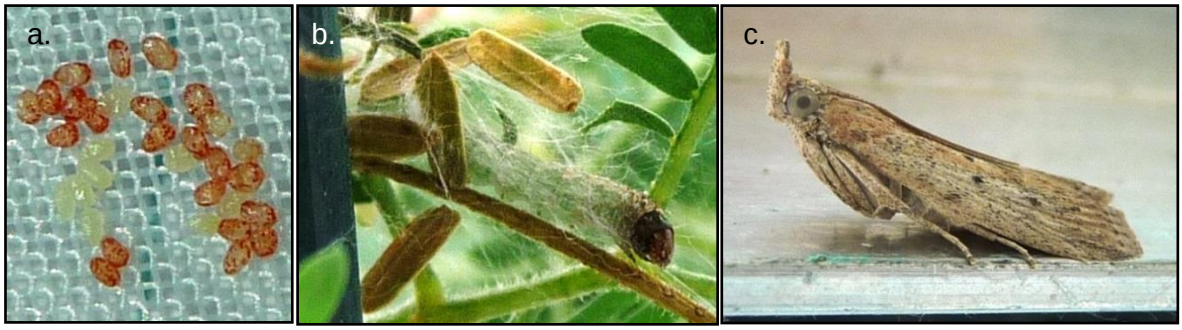


**Fig. 5. *Phycita* sp. A a. eggs laid on gauze; b. larva; c. adult.**

### 4.2.2 Host-specificity tests

#### *4.2.2.1 No-choice tests*

No-choice larval feeding and development tests were completed for 27 test plant species (Table 2). Non-target feeding and development through to adults occurred on 16 of the 27 non-target plant species (Figures 6 and 7). The durations of larval, pupal and total (larva to adult) development, pupal weight, and proportion of larvae that developed into adults differed significantly between the test plant species (Figures 6 and 7). On four out of the 17 non-target test plant species (*V. sutherlandii*, *A. deanei*, *A. mearnsii* and *A. lasiocarpa*), the durations of development and rates of survival were not significantly different to the target weed. On the remaining test plant species, the durations of development and survival rates varied greatly, but were significantly lower than on prickly acacia (Figures 6 and 7). There was a significant negative correlation between the duration of larval survival and the proportion of larvae that developed into adults ($R$ = 0.663, $F$ = 11.8, $P$ = 0.004; Fig. 8),

indicating that many of the non-target plant species that sustained complete development of *Phycita* sp. A are less favourable than prickly acacia.

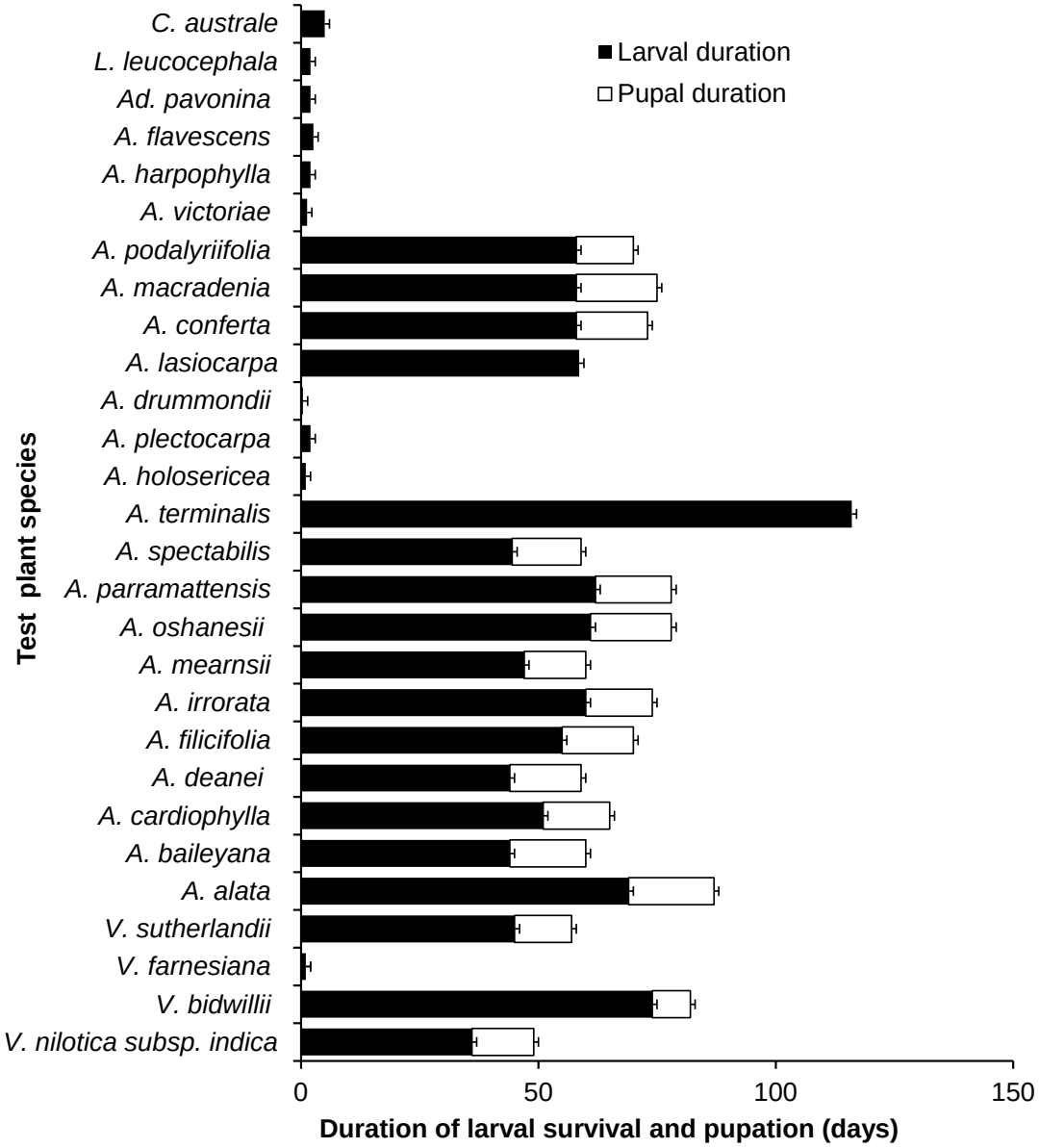


**Fig. 6. Duration (mean ± se) of *Phycita* sp. A larval (solid bars) and pupal survival (empty bars) on various test plants in no-choice tests under quarantine in Australia.**

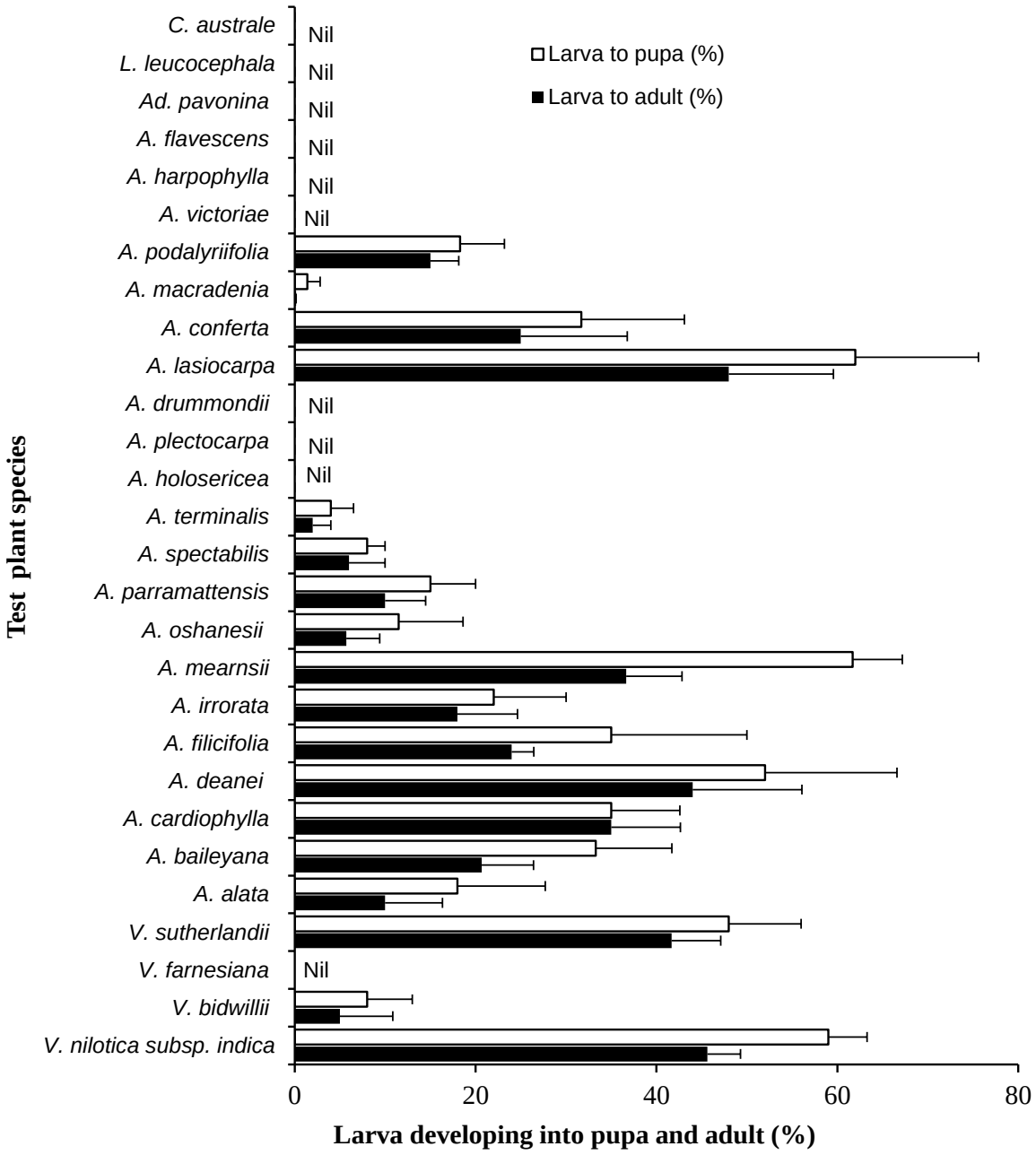


**Fig. 7. Proportion (mean ± se) of *Phycita* sp. A larvae that developed into pupae (empty bars) and adults (solid bars) on various test plant species in no-choice tests under quarantine in Australia.**

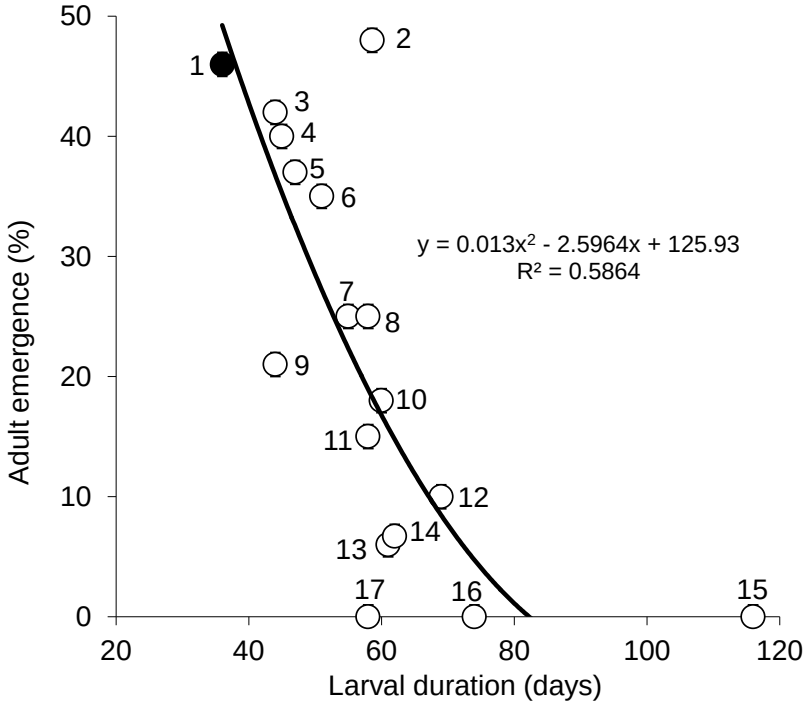


**Fig. 8. Relationship between the duration of *Phycita* sp. A larval development (x- axis) and proporiton of larvae that developed into adults (y-axis) in no-choice tests under quarantine conditions in Australia.** Target (solid circle), non-targets (empty circles); 1 = prickly acacia; 2 = *A. lasiocarpa*; 3 = *A. deanei*; 4 = *V. sutherlandii*; 5 =*A. mearnsii*; 6 = *A. cardiophylla*; 7 = *A. filicifolia*; 8 = *A. conferta*; 9 = *A. baileyana*; 10 = *A. irrorata*; 11 = *A. podalyriifolia*; 12 = *A. alata*; 13 = *A. oshanesii*; 14 = *A. parramattensis*; 15 = *A. terminalis*; 16 = *V. bidwillii*; 17 = *A. macradenia*.

*4.2.2.2 No-choice continuation trials*

The leaf-webber completed up to three generations on the non-target species *A. baileyana* and at least two generations on the non-target species *A. mearnsii* (Fig. 9). The proportion of larvae that developed into adults did not differ significantly between prickly acacia and *A. baileyana* in the first two generations (generation 1: $F_{1,4}$ = 6.94, $P$ = 0.058; generation 2: $F_{1,4}$ = 6.99, $P$ = 0.057; Fig. 9), and between prickly acacia and *A. mearnsii* in the first generation ($F_{1,8}$ = 0.354, $P$ = 0.568; Fig. 9). However, on *A. baileyana,* adults developed in only one of the replications in the third generation and no adults developed in the fourth generation (Fig. 9). On *A. mearnsii*, adults developed in only one replication in the second generation, and hence the trial could not be continued. The development time from neonate larva to adult on *A. baileyana* ($H$ = 23.457, $P$ < 0.001) and *A. mearnsii* ($H$ = 4.392, $P$ = 0.036), was significantly longer than on prickly acacia (Fig. 9). In fecundity trials, significantly fewer eggs were laid by females that developed on *A. baileyana* (64 ± 29 eggs per female) and *A. mearnsii* (117 ± 25 eggs per female) than on prickly acacia (156 ± 63) ($F_{2, 21}$ = 7.65, $P$ = 0.004).

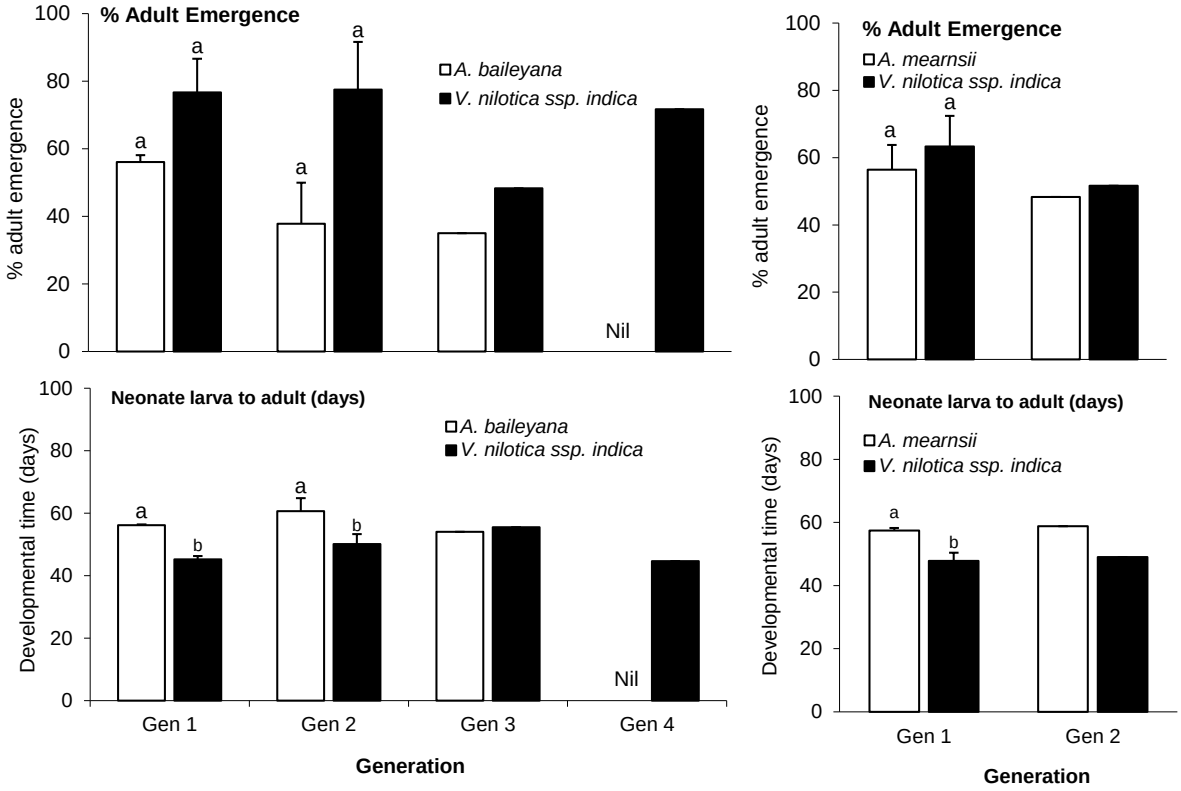


**Fig. 9. Duration of *Phycita* sp. A larval development (mean ± se) and proportion of larvae that developed into adults (mean ± se) on two non-target test plant species, *A. meansii* and *A. baileyana* (solid bars) and prickly acacia (empty bars) over multiple generations under no-choice conditions in quarantine.** Within each generation, treatment means with the same letter are not significantly different (Dunn's Method, P > 0.05).

#### *4.2.2.3 Choice oviposition tests*

In paired-choice tests all eggs were laid only on prickly acacia (5.9 ± 3.8 eggs in paired choice trials with *A. mearnsii* and 2.0 ± 0.58 eggs in paired choice trials with *A. baileyana*) with no eggs laid on the non-target plants.

## 4.3 *Anomalococcus indicus*

### 4.3.1 Lifecycle

The life cycle of *A. indicus*, from egg through to adult, is shown in Fig. 10. The two sexes undergo different developmental cycles (Taylor and Dhileepan 2013). There are three instars in the female (1st, 2nd and adult) and five in the male (1st, 2nd, pre-pupa, pupa and adult).

#### *4.3.1.1 Egg and crawler biology*

Fertile eggs are deep-green speckled (Table 3; Fig. 11). Eggs are oviposited into a cavity under the parent female's body (Taylor and Dhileepan 2013). Crawlers emerged from eggs

within one or two hours of being oviposited. Newly hatched crawlers remain in the cavity beneath the female for an indeterminate length of time.

In the absence of food, newly emerged crawlers survived for up to 208 hours but 75% died within 54 hours. Regular handling adversely affected crawlers' survival ($F_{2,17}$ = 32.11, $P$ < 0.001) and 95% died within 96 hours (Fig. 12).

Most crawlers settled close to the parent female, with more than 70% of those successfully settled doing so within 10 cm of her (Fig. 13). The average mortality of newly emerged crawlers transferred to new host plants was 31.7% (± 2.9). A further 5.6% (± 1.2) died during the stationary phase of the first instar and another 5% died before reaching adulthood.

Sexual dimorphism becomes obvious during the second instar, 36 days (range 22-47 days) after crawlers have hatched (Fig. 11). The ratio of male to female progeny produced by adult females varied greatly, averaging 2.6 (± 0.5) and ranging from 0.6 to 3.9.

#### *4.3.1.2 Female biology*

The duration of the female's juvenile stage is 52 days (Fig. 10), after which they moult into adults (Taylor and Dhileepan 2013). Adult females morphologically resemble nymphs. Fertilised adult females become distended, with eggs visible through the membranous derm (Fig. 11). Under quarantine conditions, oviposition began when females were an average of 89 days old (Fig. 10), and continued for up to 16 days. The female body shrivels as eggs are discharged from the body and the female dies when oviposition has finished. An average of 802 ± 114 crawlers emerged per reproductive female (range: 167-1647). The size of the parent female (volume) had a significant impact on the number of crawlers produced ($F_{1,8}$ = 40.06, P < 0.001; y = 34.54x-329; Fig. 14). A significantly lower number of nymphs established where females were attached to prickly acacia plants using gel capsules compared to those that were glued with a section of stem from the plant on which they developed (108 ± 94 and 471 ± 94 respectively; $t_7$ =-3.64, $P$ = 0.008).

Under quarantine conditions, 5.7% (± 1.2%) of crawlers developed into reproductive females. Based on the mean number of crawlers produced per female, an average gravid female is therefore likely to produce 45 reproductive females. The proportion of adult females on a plant that did not become gravid varied from none to all of them.

In the absence of adult males, females did not produce eggs or nymphs. Unmated females as old as 150 days were observed producing honey dew, indicative of feeding.

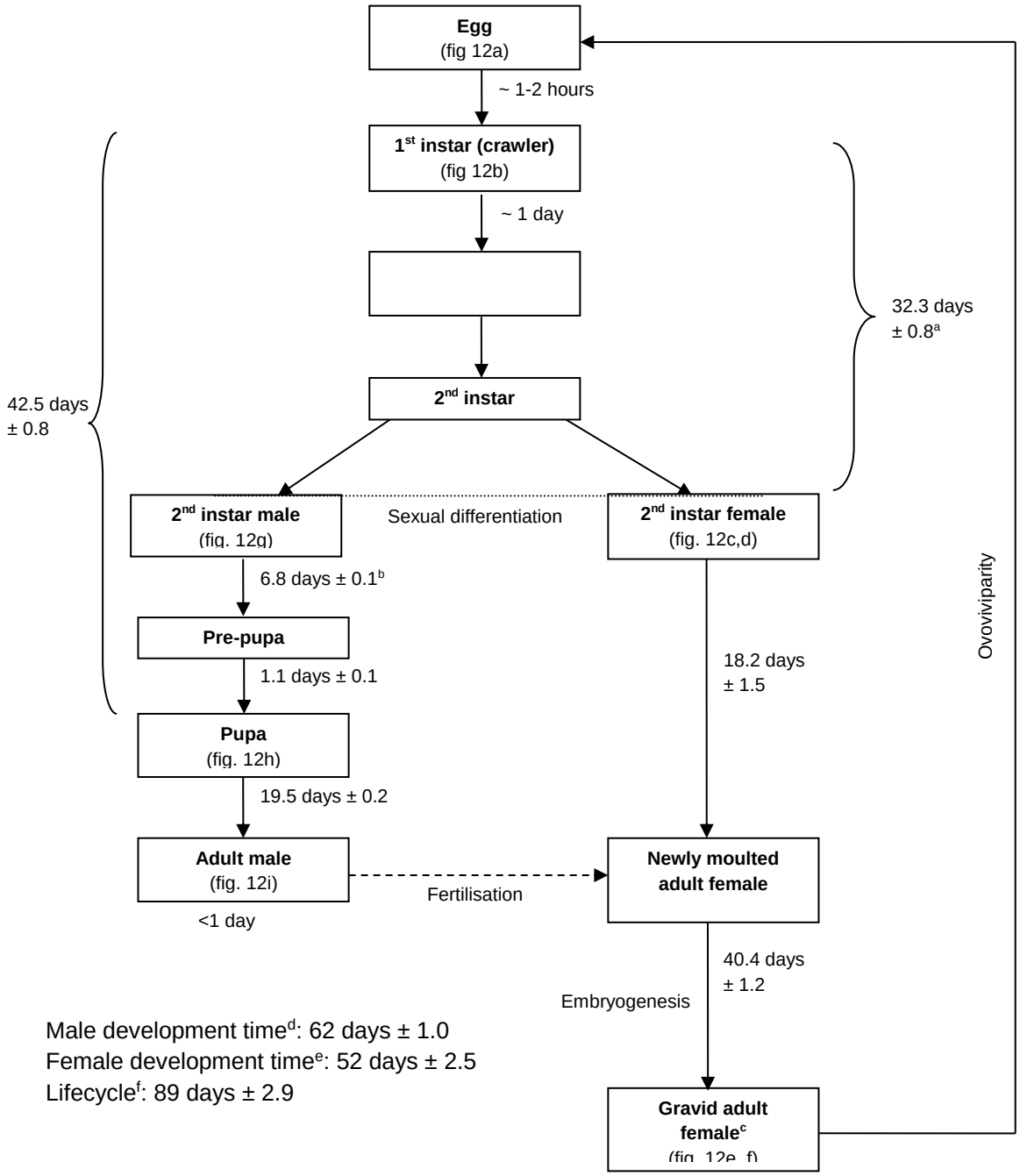


**Fig. 10. Development of *A. indicus*, from egg through to adult.** [a]time from newly emerged crawler to obvious sexual differentiation; [b]time from obvious sexual differentiation; [c]first sign of crawlers; [d]from newly emerged crawler to emergence of adult male; [e]from newly emerged crawler to newly moulted adult; [f]from newly emerged crawler to ovipositing adult female.

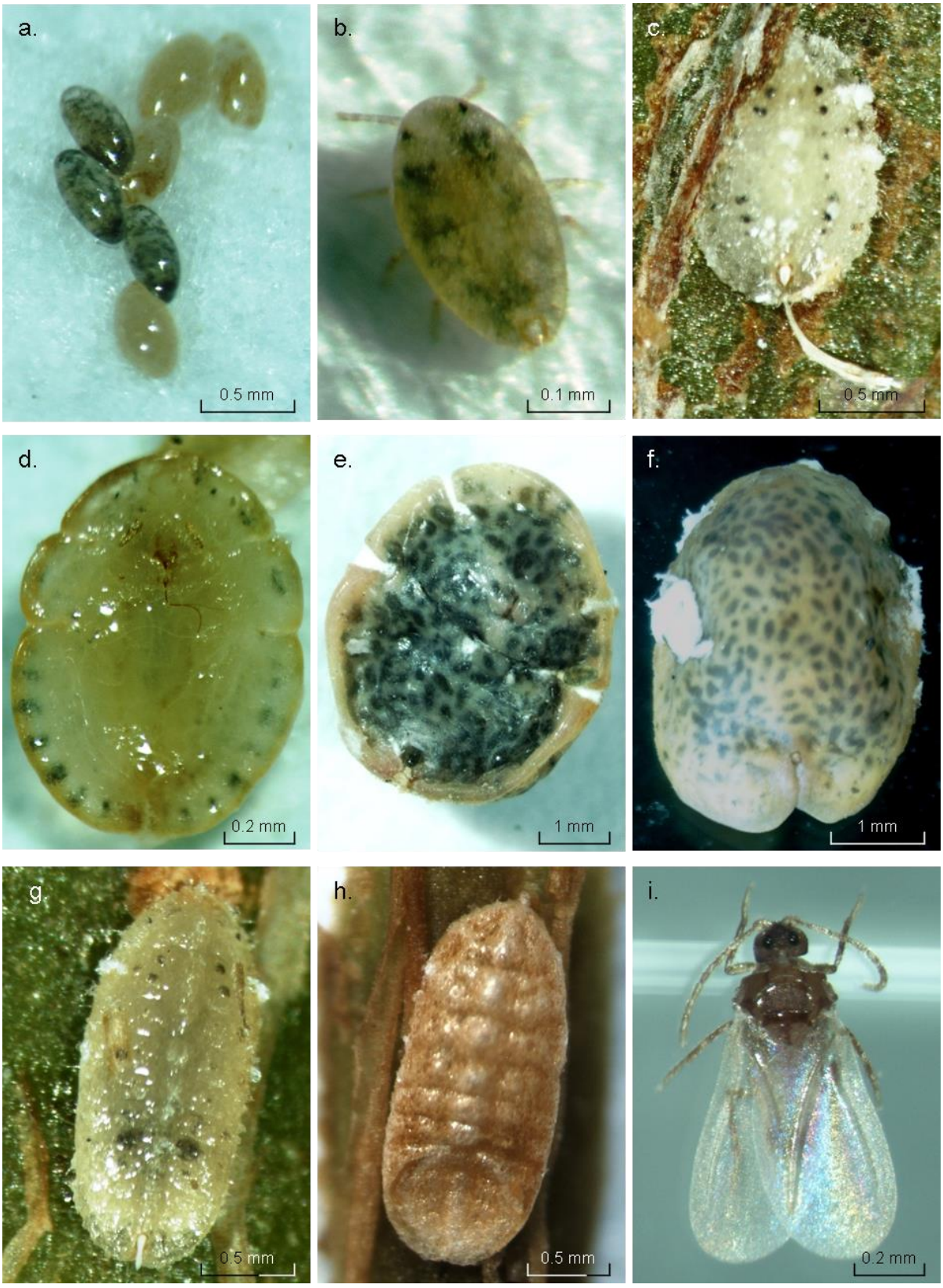


**Fig. 11. *A. indicus* life stages: a. eggs, fertile (green) and infertile (brown); b. crawler; c. dorsal view of a second instar nymph; d. ventral view of a second instar nymph with protruding stylets; e. ventral view of a gravid adult female ovipositing; f. dorsal view of a gravid female (wax removed); g. second instar male; h. male pupa; i. adult male.**

**Table 4. Mean (±SE) length, width and height (females only) of *A. indicus* life stages.** [a]measured shortly before pupation of males; [b]including the penile sheath; c single individuals were selected rather than those in groups with other females.

| Life stage | n | Length (mm) | Width (mm) | Height (mm) |
|---|---|---|---|---|
| Egg | 50 | 0.51 (0.01) | 0.26 (0.01) | - |
| Crawler | 50 | 0.46 (0.01) | 0.24 (0.01) | - |
| 2nd instar ♂[a] | 35 | 1.47 (0.02) | 0.72 (0.01) | - |
| ♂pupa | 110 | 1.54 (0.01) | 0.71 (0.01) | - |
| Adult ♂[b] | 23 | 1.03 (0.02) | | - |
| 2nd instar ♀[a] | 40 | 1.12 (0.01) | 0.77 (0.01) | 0.36 (0.01) |
| Newly moulted adult ♀[c] | 20 | 2.09 (0.03) | 1.59 (0.03) | 0.91 (0.03) |
| Unmated ♀[c] | 20 | 3.67 (0.10) | 3.09 (0.07) | 1.71 (0.05) |
| Gravid ♀[c] | 20 | 4.61 (0.08) | 3.71 (0.05) | 2.85 (0.07) |

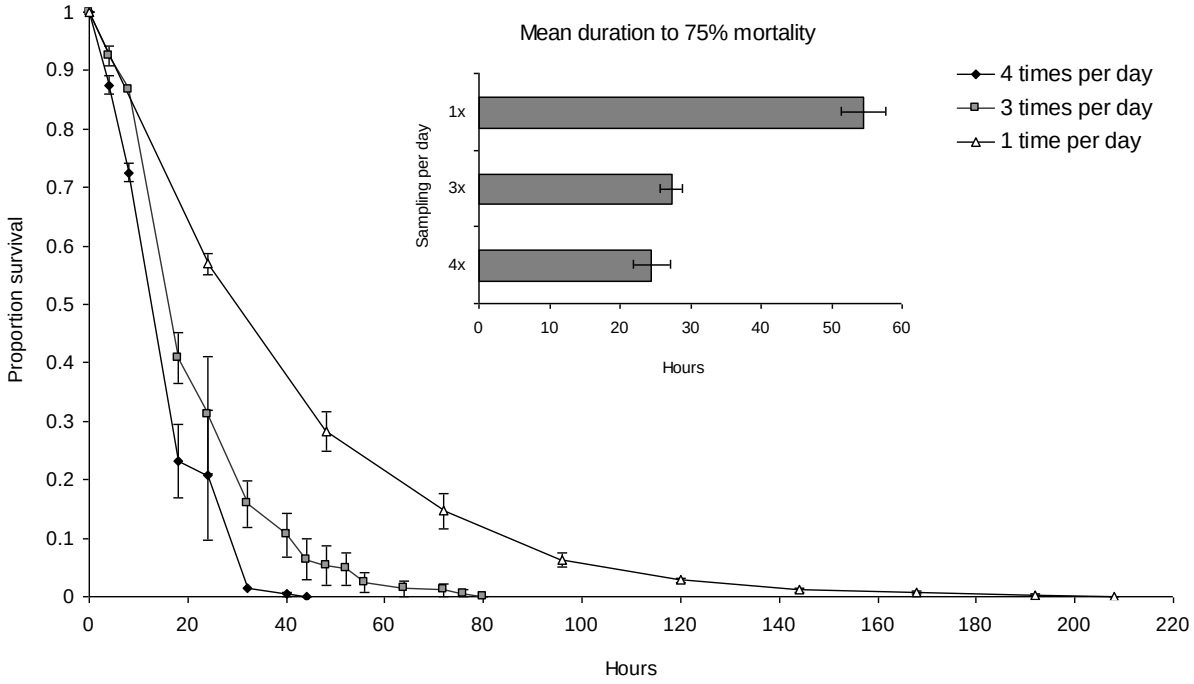


**Fig. 12. Survival of newly emerged crawlers in the absence of food in relation to sampling frequency (mean ± SE; n=5-11). Insert: Average time to 75% mortality. Means with the same letter are not significantly different (P<0.05).**

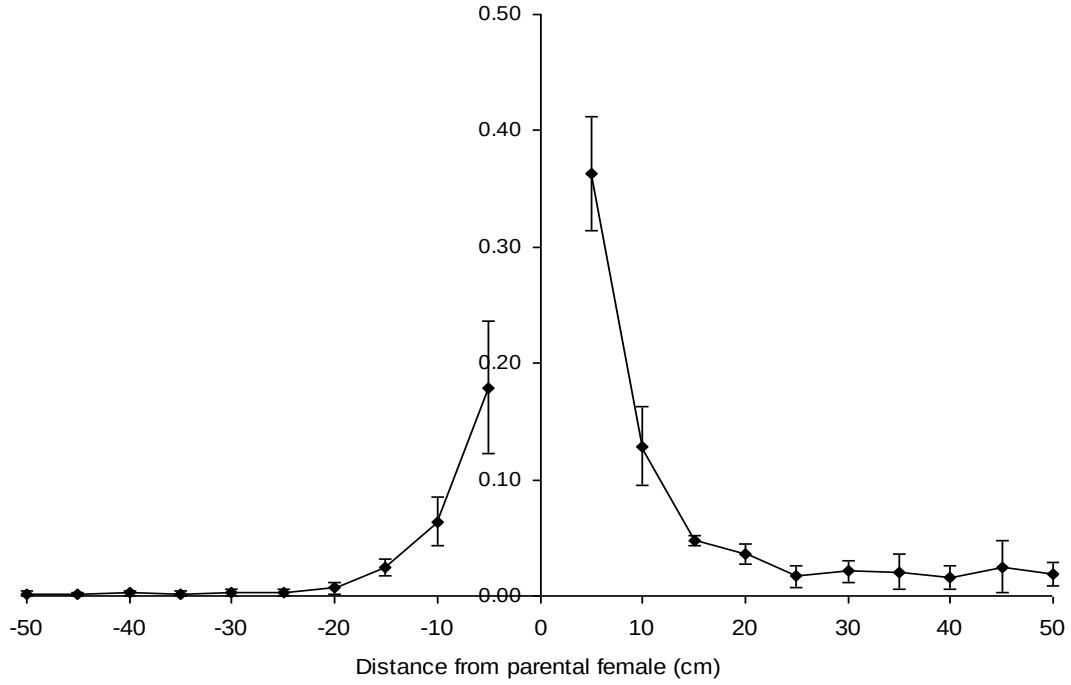


**Fig. 13. Proportional spread of 1st instar *A. indicus* nymphs in relation to the parental female (n=5).**

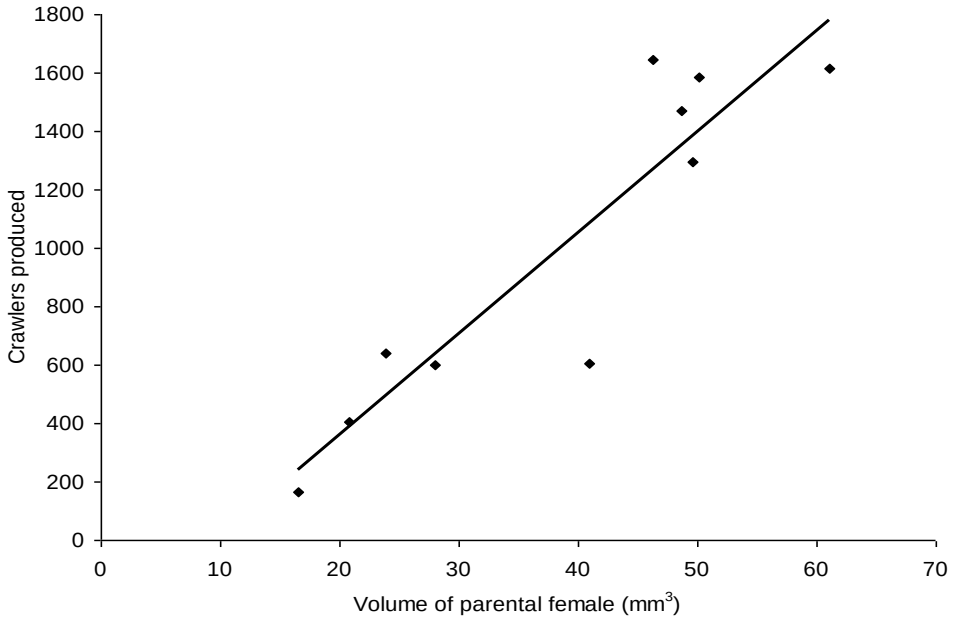


**Fig. 14. Relationship between gravid female volume and the number of crawlers produced.** The linear regression line fitted to the data is: y=34.54x-329; $R^2$=0.815.

#### *4.3.1.3 Male biology*

Towards the end of the second instar, males become elongated (Fig. 11). The average duration from the time that sexual dimorphism becomes obvious to becoming a pre-pupa was 7.4 days. Following a pupation period of 19 days, a tiny, brown, winged male emerges (Figures 10 and 11). Unlike adult females, adult males undergo a complete metamorphosis.

All adult males died within 24 hours of emergence (Fig. 10). Of males that began pupation, 29% did not survive to emerge as adults. The average time from crawler to adult male emergence took 62 days.

### 4.3.2 Host-specificity tests

#### *4.3.2.1 No-choice nymphal development*

The number of female *A. indicus* that developed to maturity on prickly acacia plants ranged from two to over 200, but was generally over 50 (mean = 87; Table 5). Testing of four or five replicates was completed for 81 non-target species, with one to three replicates completed for an additional four species (85 non-target species tested; Table 5). Numerous non-target species supported the development of male *A. indicus*. Development of *A. indicus* females to reproductive maturity was supported by 17 of the non-target species tested. *Acacia complanata, A. irrorata, A. pletocarpa, A. terminalis*, and *P. lophantha* supported low numbers of mature females in only one of five or six replicates. *Parachidendron pruinosum, C. siliqua, Platylobium formosum, A. coriacea* and *V. farnesiana* supported the development of mature females in several replicates. *Acacia falcata, V. bidwillii*, *V. sutherlandii, N. major* and *N. monosperma*, supported high numbers of mature females in all replicates. *N. dimorphantha* also supported mature females but (due to plant availability) were only subject to a single replicate. *Acacia attenuata* supported early development of high numbers of *A. indicus* nymphs (2 replicates), though plants died before complete development was possible. Figure 15 shows the scale on prickly acacia and three non-target species.

All three *V. valida* plants inoculated with scale crawlers supported complete development. The number of scale developing on similar sized *V. valida* and prickly acacia plants was similar (0.23 ± 0.06 and 0.19 ± 0.05 respectively), although a greater proportion developed into mature females on prickly acacia (0.05 ± 0.02 and 0.11 ± 0.02 respectively).

#### *4.3.2.2 Comparative nymphal development trial*

Nymphal survival was similar for prickly acacia and the two non-target species, with an average of 24% of crawlers completing development on prickly acacia and 17% and 20% completing development on *A. falcata* and *N. major* respectively (Fig. 16; $F_{2,20} = 1.61$, $P = 0.224$). The number of *A. indicus* crawlers that developed into gravid females was also similar on *N. major, A. falcata,* and the target prickly acacia (Fig. 17; $F_{2,13} = 1.21$, $P = 0.329$). Mature female *A. indicus* collected from these three species also produced comparable numbers of progeny (Fig. 17; $F_{2,26} = 0.11$, $P = 0.899$).

**Table 5. Development of *A. indicus* nymphs on non-target species during no-choice larval development trials in quarantine**. Species on which high numbers of gravid females developed are shown in red. Results for prickly acacia are shown in blue. #due to their fragility and limited lifespan, determining the presence of adult males was not feasible, but is assumed by the presence of pupae; @gravid – distended with or full of eggs.

| Test species | Plant status | Reps | Max. ♂ devel.# | Max. ♀ devel. | Reps w MA | Gravid females per rep.@ |
|---|---|---|---|---|---|---|
| **Order Fabales** | | | | | | |
| **Family Fabaceae** | | | | | | |
| **Subfamily Mimosoideae** | | | | | | |
| **Tribe Acacieae** | | | | | | |
| **Genus *Vachellia*** | | | | | | |
| *V. nilotica* ssp. *indica* (Benth.) Kyal. & Boatwr | TW | 55 | Pupae | Adult | all | 79.4 ± 7.5 |
| *V. bidwillii* (Benth.) Kodela | N | 4 | Pupae | Adult | all | 18.5 ± 7.8^ |
| ***V. farnesiana* (L.) Wight & Arn.** | I | 5 | Pupae | Adult | 3 | 17.8 ± 8.2 |
| *V. sutherlandii* (F.Muell.) Kodela | N | 5 | Pupae | Adult | all | 47 ± 4.1 |
| *V. valida* (Tindale & Kodela) Kodela | N | 3 | Pupae | Adult | all | 2.7 ± 0.9 |
| **Genus *Acacia*** | | | | | | |
| **Section *Alatae*** | | | | | | |
| *A. alata* R. Br | N | 4 | Pupae | 2nd | 0 | |
| **Section *Botrycephalae*** | | | | | | |
| *A. baileyana* F.Muell. | N | 5 | Pupae | 2nd | 0 | |
| *A. cardophylla* A.Cunn. ex Benth. | N | 5 | - | - | 0 | |
| *A. dealbata* Link | N | 5 | Pupae | - | 0 | |
| *A. deaneii* (R.T.Baker) M.B.Welch *et al.* | N | 5 | - | 1st | 0 | |
| *A. decurrens* Willd. | N | 5 | Pupae | 1st | 0 | |
| *A. glaucocarpa* Maiden & Blakely. | N | 5 | - | - | 0 | |
| *A. irrorata* Sieber ex Spreng. | N | 5 | Pupae | Adult | 1 | 1.8 ± 1.8 |
| *A. mearnsii* De Wild. | N | 6 | Pupae | Adult* | 1 | |
| *A. oshanesii* F.Muell. & Maiden | N | 6 | Pupae | 2nd | 0 | |
| *A. parramattensis* Tindale | N | 5 | Pupae | - | 0 | |
| *A. spectabilis* A.Cunn. ex Benth. | N | 5 | Pupae | 2nd | 0 | |
| *A. terminalis* (Salisb.) J.F.Macbr. | N | 5 | Pupae | Adult | 1 | 0.4 ± 0.4 |
| **Section *Juliflorae*** | | | | | | |
| *A. aneura* F.Muell. ex Benth. | N | 5 | - | - | 0 | |

| | | | | | | |
|---|---|---|---|---|---|---|
| *A. cincinnata* F.Muell. | N | 5 | Pupae | 2nd | 0 | |
| *A. hemsleyi* Maiden | N | 5 | Pupae | 2nd | 0 | |
| *A. holosericea* A.Cunn. ex G.Don | N | 9 | Pupae | 2nd | 0 | |
| *A. leiocalyx* (Domin) Pedley | N | 6 | - | 2nd | 0 | |
| *A. mangium* Willd. | N | 5 | Pupae | - | 0 | |
| *A. plectocaropa* A.Cunn. ex Benth. | N | 5 | Pupae | Adult | 1 | 1.4 ± 0.7 |
| *A. shirleyi* Maiden | N | 5 | 2nd | - | 0 | |
| **Section *Phyllodineae*** | | | | | | |
| *A. conferta* A.Cunn. ex Benth. | N | 5 | - | - | 0 | |
| *A. falcata* Willd. | N | 6 | Pupae | Adult | all | 66.0 ± 32.9 |
| *A. macradenia* Benth. | N | 5 | Pupae | 2nd | 0 | |
| *A. podalyriifolia* A.Cunn. ex G.Don | N | 6 | Pupae | - | 0 | |
| *A. peuce* F. Muell. | N | 2 | - | - | 0 | |
| *A. salicina* Lindl. | N | 5 | - | 2nd | 0 | |
| *A. tetragonophylla* F.Muell. | N | 5 | Pupae | 2nd | 0 | |
| *A. victoriae* Benth. | N | 5 | - | - | 0 | |
| **Section *Plurinerves*** | | | | | | |
| *A. complanata* A.Cunn. ex Benth. | N | 5 | Pupae | Adult | 1 | 1.0 ± 1.0 |
| *A. coriacea* DC. | N | 5 | Pupae | Adult | 3 | 2.2 ± 1.3 |
| *A. excelsa* Benth. | N | 6 | - | - | 0 | |
| *A. flavescens* A.Cunn. ex Benth. | N | 1 | - | - | 0 | |
| *A. harpophylla* F.Muell. ex Benth. | N | 9 | Pupae | Adult* | 1 | |
| *A. melanoxylon* R.Br. | N | 5 | Pupae | Adult* | 1 | |
| *A. simsii* A.Cunn. ex Benth. | N | 5 | Pupae | 2nd | 0 | |
| *A. stenophylla* A.Cunn. ex Benth. | N | 5 | Pupae | 2nd | 0 | |
| **Section *Pulchellae*** | | | | | | |
| *A. drummondii* Lindl. | N | 5 | Pupae | 2nd | 0 | |
| *A. pulchella* R.Br. | N | 5 | - | - | 0 | |
| **Tribe Mimoseae** | | | | | | |
| *Adenanthera pavonia* L. | N | 5 | - | - | | |
| *Dichrostachys cinerea* (L.) Wight & Arn. | N | 5 | - | 2nd | 0 | |
| *Entada phaseoloides* (L.) Merr. | N | 5 | - | - | 0 | |
| *Leucaena leucocephala* (Lam.) de Wit | I | 5 | - | - | 0 | |
| *Neptunia dimorphantha* Domin | N | 1 | Pupae | Adult | 1 | 1 |
| *N. major* (Benth.) Windler | N | 5 | Pupae | Adult | all | 84.3 ± 35.3^ |

| | | | | | | |
|---|---|---|---|---|---|---|
| *N. monosperma* F.Muell. ex Benth. | N | 6 | Pupae | Adult | all | 15.0 ± 4.5^ |
| **Tribe Ingeae** | | | | | | |
| *Albizia procera* (Roxb.) Benth. | N | 5 | Pupae | 2nd | 1 | |
| *Archidendron lucyi* F.Muell. | N | 5 | Pupae | Adult* | 1 | |
| *Cathormion umbellatum* (Vahl) Kosterm. | N | 5 | - | - | 0 | |
| *Inga edulis* Mart. | E | 5 | - | 1st | 0 | |
| *Parachidendron pruinosum* (Benth.) I.C.Nielsen | N | 5 | Pupae | Adult | 2 | 9.8 ± 7.0 |
| *Paraserianthes lophantha* (Willd.) I.C.Nielsen. | N | 6 | Pupae | Adult | 1 | 5.0 ± 5.0 |
| **Subfamily Caesalpinaceae** | | | | | | |
| **Tribe Caesalpinieae** | | | | | | |
| *Caesalpinia ferrea* Mart. ex Tul. | O | 5 | - | - | 0 | |
| *Delonix regia* (Boj. ex Hook.) Raf. | O | 5 | - | - | 0 | |
| **Tribe Cassieae** | | | | | | |
| *Cassia brewsteri* (F.Muell.) Benth. | N | 6 | - | - | 0 | |
| *Ceratonia siliqua* L. (Carob tree) | E | 6 | Pupae | Adult | 3 | 7.8 ± 7.2 |
| *Senna acclinis* (F.Muell.) Randell | N | 5 | - | - | 0 | |
| **Tribe Cercideae** | | | | | | |
| *Barklya syringifolia* (F.Muell.) Wund. | N | 5 | - | - | 0 | |
| *Bauhinia hookeri* F.Muell. | N | 5 | - | - | 0 | |
| **Tribe Detarieae** | | | | | | |
| *Tamarindus indica* L. | CI | 5 | - | - | 0 | |
| **Subfamily Papilionoideae (Faboideae)** | | | | | | |
| **Tribe Abreae** | | | | | | |
| *Abrus precatorius* ssp. *precatorius* L. | N | 5 | - | 1st | 0 | |
| **Tribe Bossiaeeae** | | | | | | |
| *Hovea acutifolia* A.Cunn. ex G.Don | N | 5 | - | - | 0 | |
| *Platylobium formosum* Sm. | N | 6 | Pupae | Adult | 4 | 10.0 ±3.5^ |
| **Tribe Dalbergieae** | | | | | | |
| *Arachis hypogaea* L. | C | 8 | - | - | 0 | |
| **Tribe Desmodieae** | | | | | | |
| *Desmodium macrocarpum* Domin. | N | 5 | - | - | 0 | |
| **Tribe Galegeae** | | | | | | |
| *Swainsona galegifolia* (Andrews) R.Br | N | 5 | - | - | 0 | |
| **Tribe Indigofereae** | | | | | | |

| | | | | | | |
|---|---|---|---|---|---|---|
| *Indigofera australis* Willd. | N | 5 | - | - | 0 | |
| **Tribe Millettieae** | | | | | | |
| *Callerya megasperma* (F.Muell.) Schot | N | 5 | - | - | 0 | |
| *Millettia* sp. | N | 5 | - | - | 0 | |
| **Tribe Mirbelieae** | | | | | | |
| *Pultenaea villosa* Willd. | N | 5 | - | - | 0 | |
| **Tribe Phaseoleae** | | | | | | |
| *Cajanus cajan* (L.) Millsp. | C | 5 | - | - | 0 | |
| *Clitoria ternatea* L. | I | 5 | - | - | 0 | |
| *Erythrina vespertilio* Benth. | N | 5 | - | - | 0 | |
| *Hardenbergia violacea* (Schneev.) Stearn | N | 6 | Pupae | 2nd | 0 | |
| *Phaseolus lunatus* L. | C | 5 | - | - | 0 | |
| *Vigna unguiculata* var. *sesquipedalis* (L.) Verdc. | C | 5 | - | - | 0 | |
| **Tribe Sophoreae** | | | | | | |
| *Castanospermum australe* A.Cunn. ex Mudie | N | 5 | - | - | 0 | |
| **Tribe Tephrosieae** | | | | | | |
| *Tephrosia grandiflora* (L'Hér. ex Aiton) Pers. | E | 5 | - | - | 0 | |
| **Family Euphorbiaceae** | | | | | | |
| *Mallotus claoxyloides* (F.Muell.) Muell.Arg. | N | 5 | Pupae | 2nd | 0 | |
| **Family Malvaceae** | | | | | | |
| *Brachychiton acerifolius* (A.Cunn. ex G.Don) Macarthur | N | 5 | - | - | 0 | |

[#] Due to their fragility and limited lifespan, determining the presence of adult males was not feasible, but is assumed by the presence of pupae.

[@] Gravid - distended with or full of eggs.

[*] No evidence of egg production observed.

[^] Due to plant death, these figures includes all adult females with crawlers, not specifically fully gravid females for some reps.


**Fig. 15. *A. indicus* on a. prickly acacia; b. *N. major*; c. *V. sutherlandii*; d. *V. bidwillii*.**

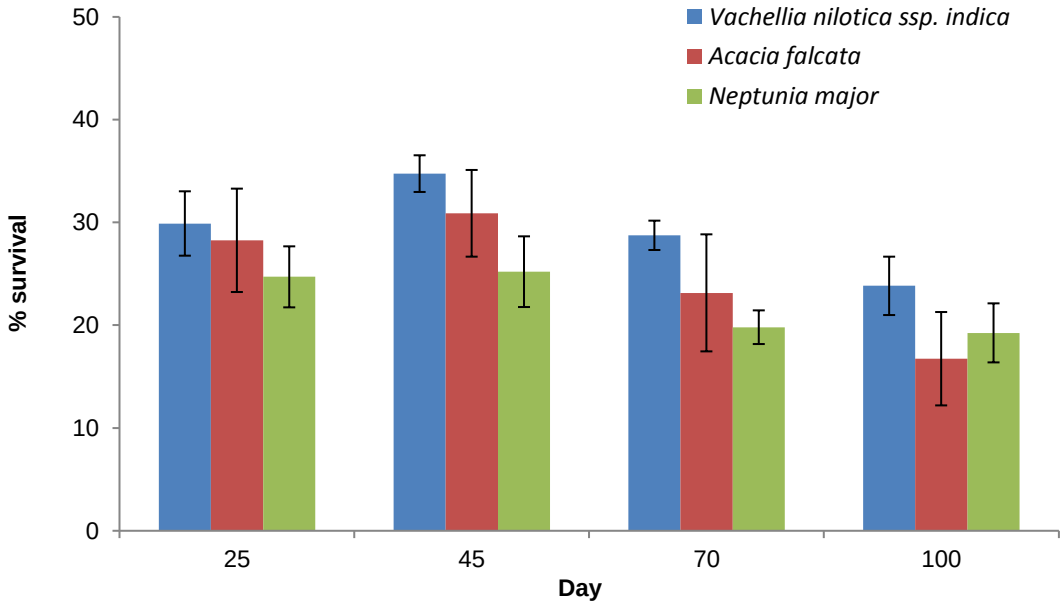


**Fig. 16. Survival of *A. indicus* nymphs on target and non-target species.**

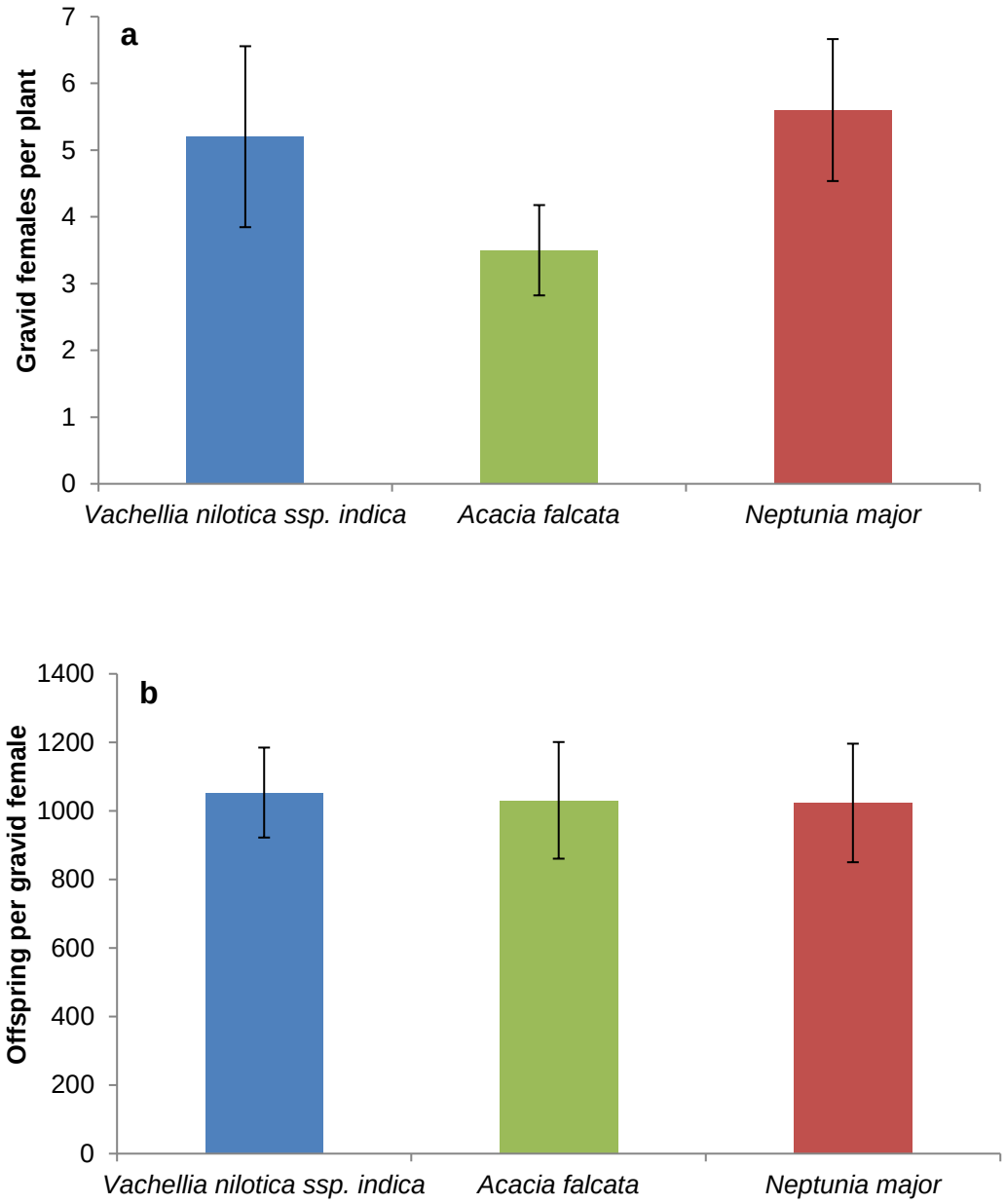


**Fig. 17. Development and fecundity of female *A. indicus* on target and non-target species; a. Number of scale that developed into gravid females per plant; b. Number of offspring per gravid female reared on different species.**

*4.3.2.3 Nymphal host preference*

When provided with a choice of hosts, 40-60% of *A. indicus* crawlers settled on prickly acacia, significantly more than on *V. farnesiana, A. falcata* and *Parachidendron pruinosum* (Fig. 18a.). Less than 10% of crawlers settled on *Parachidendron pruinosum,* the least preferred species tested.

Results from the second trial were much more variable with between 15 and 70% of crawlers settling on prickly acacia plants and between 7 and 55% settling on *V. sutherlandii* (Fig. 18b).

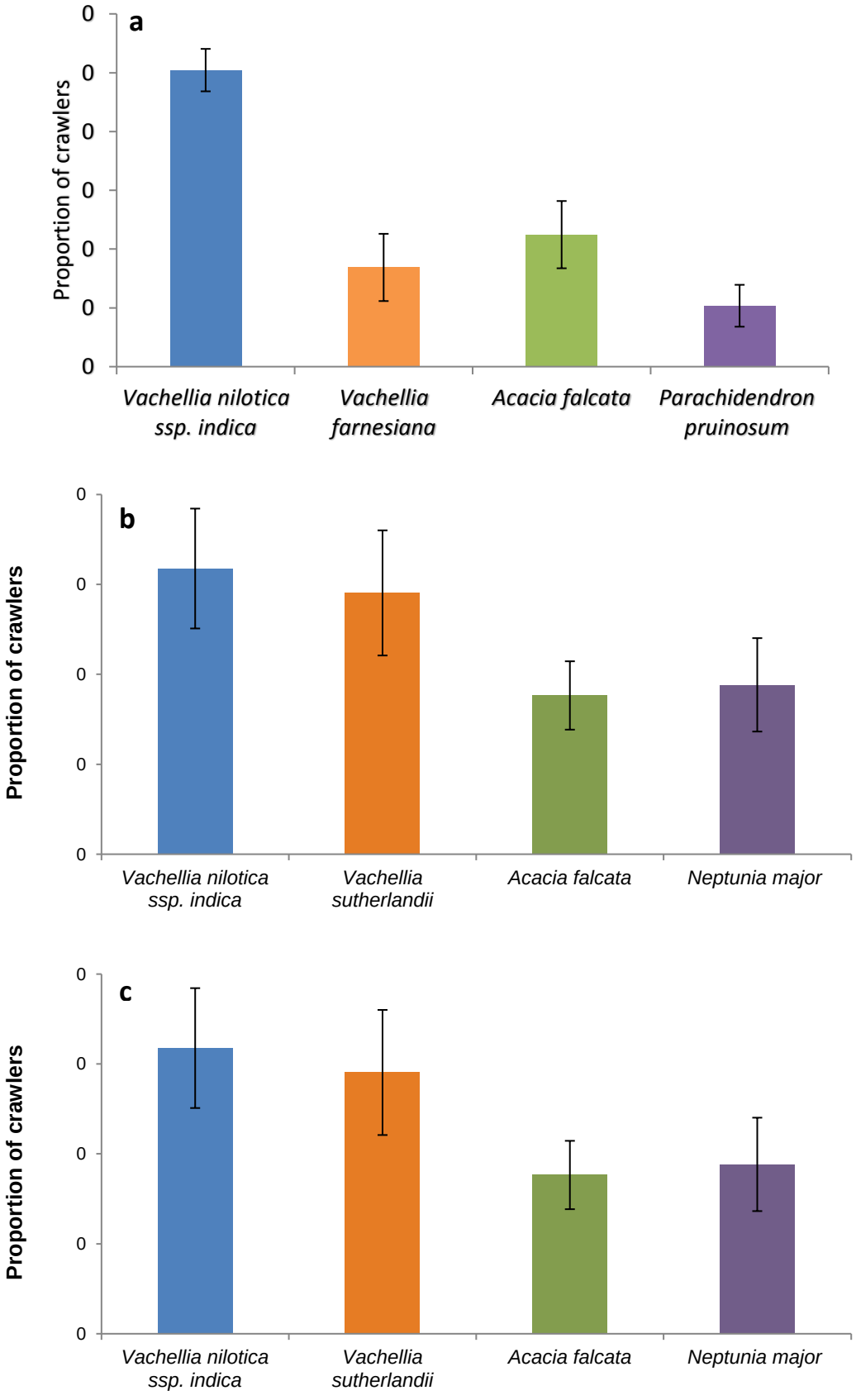


**Fig. 18. Host preference of *A. indicus* nymphs.**

In the third trial (Fig. 18c), which used small plants, up to 40% of crawlers settled on the two non-target *Vachellia* species (up to 30% per species). Prickly acacia was the preferred host in all replates (with 56-80% of crawlers settling).

The fourth trial included species that had supported low numbers of scale in the no-choice trials; *A. irrorata, A. plectocarpa, A. coriacea, C. siliqua* and *Platylobium formosum*. In three replicates only two crawlers settled on one *A. irrorata* plant and one crawler settled on one *A. coriacea* plant and one *Platylobium formosum* plant. The vast majority of crawlers (>99%) settled on the prickly acacia plants.

*4.3.2.4 Field choice trials*

Trial 1: All prickly acacia plants showed scale infestation (average of 32 scales per plant), and only one *N. major* plant showed scale insect, with one female in a plant. The trial could not be continued, as all source and prickly acacia control plants died due to scale insect attack.

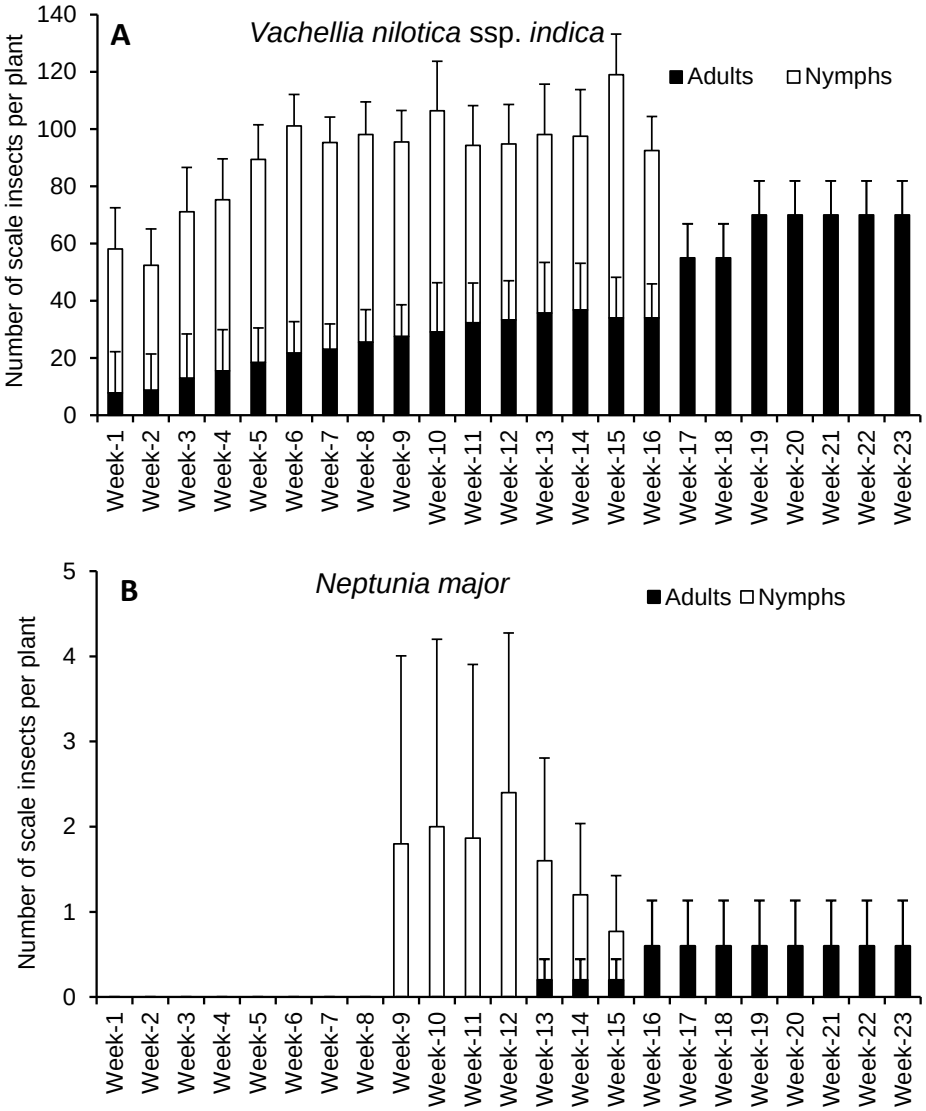


**Fig. 19. Scale insect population (nymphs and adults; mean ± SE) on prickly acacia and *N. major* plants in a field choice trial in Coimbatore, India.**

Trial 2: scale insect attack was evident on all prickly acacia plants (about 36 adults per plant) placed at a radius of 60 cm (4 out of 4 plants) and 120 cm (7 out of 7 plants) from the scale insect source plants. In contrast, only two out of four *N. major* plants placed at a radius of 60 cm from the scale insect source plants were infested with scale insects (3-6 adults per plant), while none of the plants (none of the 7 plants) placed at a radius of 120 cm from the source plants had any scale insects on them. At the end of the trial 100% of the prickly acacia plants were infested with scale insect

Trial 3: Evidence of scale infestation on prickly acacia plants was seen from the first week onwards. In contrast, scale insect on *N. major* was seen only from the ninth week of the trial (Fig. 15). At the end of the trial, only three of the 15 *N. major* test plants (20%) were found infested by the scale insect. Whereas in the same period 15 out of 15 prickly acacia control plants (100%) were infested by the scale. The population density of scale insect on *N. major* plants (0.6 ± 0.5) was much less compared to the control prickly acacia plants (55 ± 7.6) (Fig. 19). However, at the end of the trial, all scale-insect infested prickly acacia source plants and all control prickly acacia plants died due to severe scale infestation.

Trial 4: Observations on various plant parameters (e.g. plant height and basal diameter) and the scale infestation levels (number of scale nymphs and adults per plant) were documented on all test and control plants, at fortnightly intervals from December 2014 to May 2015. In the first four months, four of the 10 prickly acacia control plants (40%), and two out of 10 *N. major* test plants (20%), exhibited scale insect infestation (Fig. 20). The trial was abandoned in May 2015 as the trial site became submerged for several weeks, due to heavy unseasonal rains, resulting in the death of all test plants.

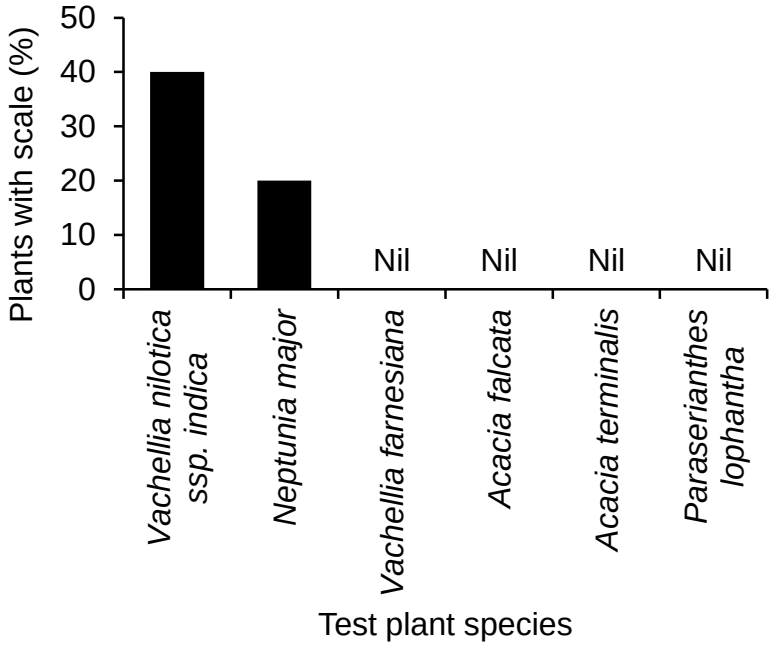


**Fig. 20. Incidence (% of plants with scale insect) of scale insect on prickly acacia and non-target test plants in a field choice trial in a farmer's field in Coimbatore, India.**

Trial 5: So far, scale insect attack was evident only on prickly acacia plants and not on any other test plant species. However, there has been very high mortality among the Australian native test plant species – 100% mortality in *Paraserianthes lophantha, A. falcata* and *A. terminalis*, and 80% mortality in *V. sutherlandii*. While five out of 50 (10%) control prickly acacia control plants have been found infested by the scale, none of the test plants was affected by the scale insect. Observations will continue till June 2017.

Trial 6: Due to high mortality among the native Australian test plant species in Trial 4 in India, fresh seeds of Australian native test plants species (*A. irrorata, A. terminalis, A. plectocarpa, A. falcata, V. sutherlandii, A. coriacea, A. complanata, N. major*, *Platylobium formosum* and *Paraserianthes lophantha*) were exported to India for inclusion in field choice tests. A new trial involving all these test plants commenced in January 2016. So far, 12 out of 50 (24%) control prickly plants were found infested by the scale. In contrast, two out of 13 *N. major* plants (15%) and one out of seven *V. sutherlandii* plants (14%) are showing the scale infestation. To date there is no evidence of scale insect on other test plant species. Monitoring of the test plants will continue till June 2017.

*4.3.2.5 Field host range*

The scale insect was observed on all the three subspecies of *V. nilotica* – ssp. *indica*, ssp. *cupressiformis* and ssp. *tomentosa*. The scale insect was not observed on *S. catechu*, *V. leucophloea*, *V. horrida* or *S. ferruginea* co-occurring with *V. nilotica* in the survey sites.

## 4.4 *Dereodus denticollis*

In India, studies on the life cycle and preliminary host specificity testing for *D. denticollis* were conducted under laboratory conditions at the Institute of Forest Genetics and Tree Breeding (IFGTB), Coimbatore, Tamil Nadu, using field collected adults. Adult no-choice feeding tests were conducted on six test plant species (*A. falcate*, *A. terminalis*, *V. sutherlandii*, *V. tortilis, N. major* and *Paraserianthes lophantha*) with prickly acacia as control, with 10 replications (potted plants) for each test plant species. Ten field collected *D. denticollis* adults (of unknown age) starved for 48 hours were released on each plant, enclosed in an insect-proof cage, and monitored daily for feeding and survival, till all adults died.

In Australia, *D. denticollis* has been imported into quarantine on six occasions, though none in the initial shipment survived (Table 2). Adults are long lived and are leaf feeders. Larvae are believed to be root feeders though feeding has yet to be observed.

Up until recently, very few eggs have been laid under quarantine conditions. However, a change in culturing practices has led to a dramatic increase in egg production. Weevils housed in glass jars, equipped with cut foliage, lay their eggs on the bottom of the jar once the foliage starts to wilt and dry (Fig. 21). Previous culturing methods utilised cages with potted plants, plants were always watered and maintained and therefore not under any stress. It is hypothesised that the process of the foliage drying acts as a cue to stimulate egg lay in this species.

Larvae have been introduced to prickly acacia roots in various mediums (sand, potting mix, dirt, filter paper) with no evidence of feeding and survival past a few days. A semi-artificial diet has been trialled, however no feeding was observed.


**Fig. 21. *Dereodus denticollis* a. eggs oviposited on floral foam; b. egg on a dried prickly acacia pinnule; c. larvae placed on a section of a prickly acacia root; d. larva (magnified)**

## 4.5 *Phycita* sp. B

The green leaf webber (*Phycita* sp. B) was first imported into quarantine in Brisbane in late 2012 and early 2013 (Table 2). Attempts to establish a colony following the methods used to rear *Phycita* sp. A, were unsuccessful due to a lack of egg laying. In late 2013 *Phycita* sp. B was again imported from India and after much trial and error we were able stimulate oviposition and thus establish a colony in early 2014.

In early 2015 the colony failed to reproduce. Dead female moths were recovered from the cages and dissected to discern probable cause for the lack of egg lay. Dissected female moths were found to have not reached reproductive maturity (no developed ovaries or matured eggs) and, in addition to this, none of the female moths showed signs of having mated (no spermatophore present). Advice was sought from Indian colleagues regarding the biology and methods they use to culture the insect. *Phycita* sp. B is a seasonal insect. This species does not appear to lay eggs in the off-season, with peak egg lay occurring from October to December, which is winter in India. While temperatures during winter in India are comparable to the conditions set up in quarantine in Australia, the lighting schedule is not. Quarantine has been set up to mimic Australian summer conditions with a 14 hour light and 10 hour dark cycle. In India during winter there are only 11.5 hours of light in a typical day cycle. The increased day length in quarantine may signal that summer is approaching and that conditions will be unsuitable for survival, leading the moth to cease reproductive maturity. Such physiological syndromes are apparent in other migratory insects both in

Australia and elsewhere. While no winter diapause was found to occur in the species, summer quiescence was not investigated.

**Fig. 22. "Blacked out" cage set up for shorter day length for *Phycita* sp. B colony.**

Several attempts were made to rear the moth under shorter day lengths (Fig. 22). There were ultimately unsuccessful with no eggs laid on any of the prickly acacia plants. However, in contrast to previous observations, in some instances newly emerged females showed developed eggs in the ovary. Yet, dead female moths recovered from the cages were dissected and found to have not mated.

### 4.5.1 Life history

In India, females laid 64.38 ± 5.33 green colour eggs on prickly acacia. About 53 ± 3.5% eggs hatched and the incubation period was 9.5 ± 2.8 days. The duration of larval

development was 28 ± 2 days and the pupal period was 11.2 ± 1.5 days. The adults lived for 13.3 ± 2.17 days

In Australia, females (Fig. 23a) only lay eggs on the foliage of live plants, not on cut foliage or gauze like *Phycita* sp. A. Mating and oviposition occur at night. Eggs are laid singly or in groups on the upper surface of pinnules or leaf rachis (Fig. 23b). Larvae begin to emerge from eggs five days after they are laid. Newly emerged larvae tie leaves together with silk webs, feed on the leaves (Fig. 23c). Larval development takes an average of 21 days and there are four instars. Pupation takes an average of 14 days. The average duration of adult moth survival is 3.5 days.

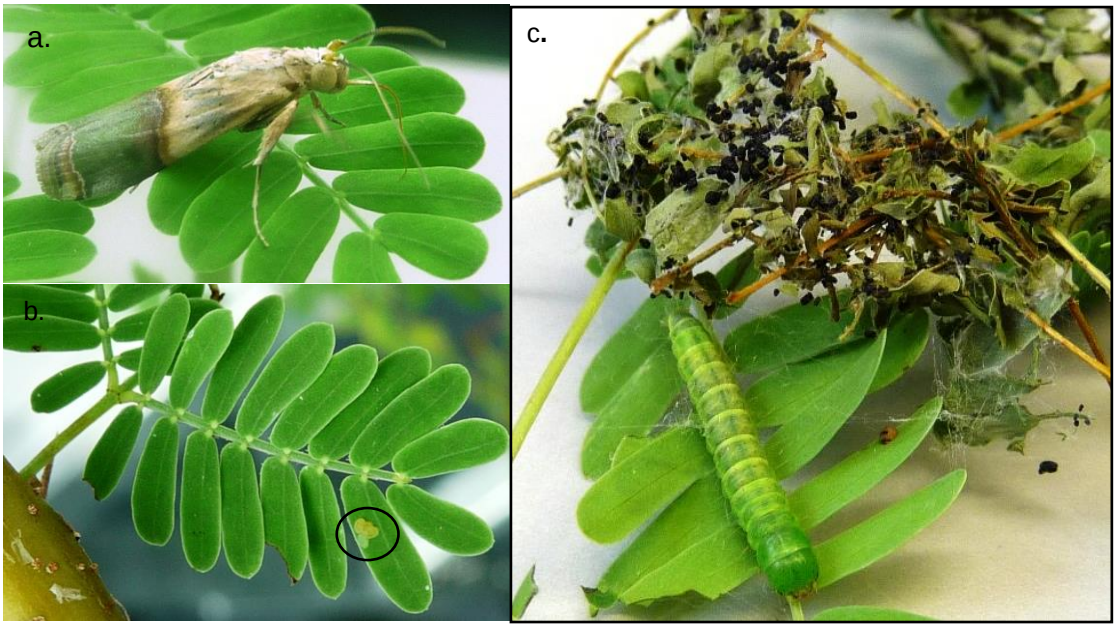


**Fig. 23. *Phycita* sp. B, a. adult; b. eggs; c. larva and feeding damage.**

## 4.5.2 Host specificity

### *4.5.2.1 No-choice larval feeding and development*

In India, *Phycita* sp. B larvae of the webber could feed and complete development on four non-targeted test plant species; *V. tortilis*, *V. farnesiana*, *A. planifrons* and *N. major*. Percentage of larval survival varied among the test plant species. On prickly acacia (control) 40% of the larvae developed into pupae. In contrast, 20% larvae developed into pupae on *A. planifrons* and 10% larvae developed into pupae on *V. tortilis*, *V. fernesiana* and *N. major.*

In Australia, no-choice larval development trials began in quarantine in May 2014. Nineteen species have been tested so far (Table 1). Feeding and development has occurred on 10 of these species: *N. major*, *V. sutherlandii*, *A. cardiophylla, A deanei, A. filicifolia, A. irrorata, A. mearnsii, A. spectabilis, A. pulchella, A. lasiocarpa* (Fig. 24).

*4.5.2.2 Oviposition tests*

During no-choice oviposition trials, eggs have only been laid on prickly acacia and *N. major* (Fig. 25). When provided with a choice between prickly acacia and *N. major*, no eggs were laid in *N. major* (Fig. 26).

During an unplanned and opportunistic paired choice oviposition trial with a *V. sutherlandii* plant and a prickly acacia plant, female moths laid eggs on both species.

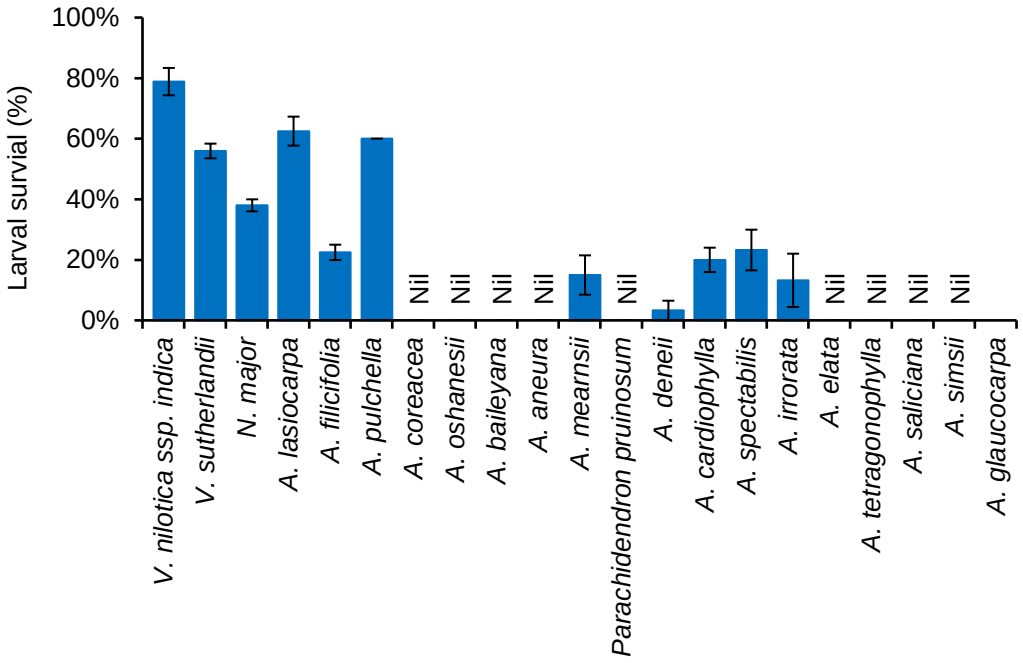


**Fig. 24. Percent survival of *Phycita* sp. B larvae in no choice development trials.**

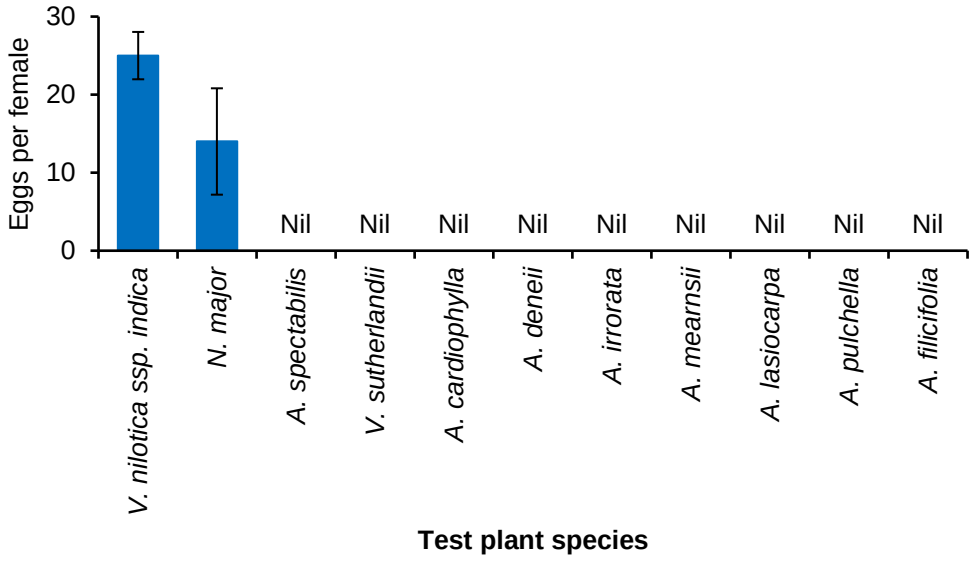


**Fig. 25. Number of *Phycita* sp. B eggs laid in no-choice oviposition trials.**

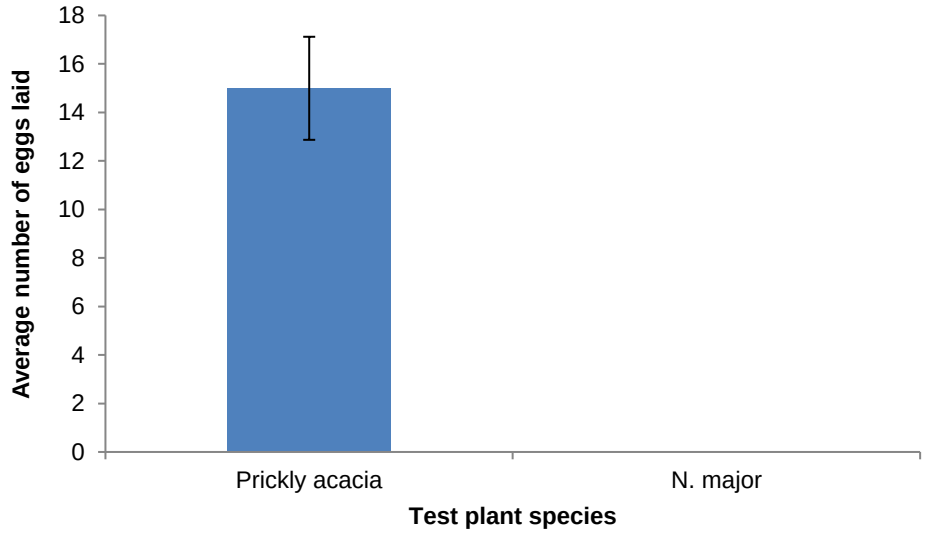


**Fig. 26. Number of *Phycita* sp. B eggs laid in paired-choice oviposition trials with prickly acacia and *N. major*.**

# 5 Discussion

## 5.1 *Phycita* spp.

The subfamily Phycitinae is a diverse group with many similar species that are poorly documented. There are about 112 species in the *Phycita* genus (www.zipcodezoo.com). The majority of species in this genus for which host records are available are crop pests (e.g. Brues 1936, Butani 1970, Ponnuswami 1971, Aina 1983, Ram and Pathak 1989, Rani and Sridhar 2002, Menzel 2002). Two leaf-webbing pyralid species were collected on prickly acacia in India, one with brown larvae (brown leaf-webber, *Phycita* sp. A) with wider geographic range and the second with green larvae (green leaf-webber, *Phycita* sp. B) with restricted geographic range (Dhileepan et al. 2013). The brown leaf-webber collected on prickly acacia in India was initially identified as *Phycita leuconeurella* Ragonot (syn. *Hyalospila leuconeurella* Ragonot) (Dr George Mathew, Kerala Forest Research Institute, India, personal communication). A literature search highlighted that *P. leuconeurella* has been reported as a pest of mango (*Mangifera indica* L.) in India (Ponnuswami 1971) and a pest of cashew (*Anacardium accidentale* L.) in Sri Lanka (Hutson 1939). Hence both mango and cashew were included as test plants in preliminary no-choice host-specificity tests. However, no larval development occurred on either of the hosts, suggesting that the species was not *P. leuconeurella.* The species status of both *Phycita* species could not be confirmed by British Natural History Museum (UK).

The no-choice host-specificity tests for the brown leaf-webber produced contrasting results to the observed field range. The leaf-webber was not seen on the two non-target test plant species (*V. leucophloea* and *A. planifrons*) in the field (Dhileepan et al. 2013), even though larvae fed and completed development on both of them under no-choice conditions in the quarantine glasshouse. No-choice tests are prone to false positive results because they identify the absolute host range and this is often wider than the field host range (due to factors including but not limited to life stage of the insect, phenology with the host, geographic isolation etc.) and hence may result in the rejection of potentially safe agents if results are taken at face value (van Klinken 2000). Although the brown leaf-webber completed development on 16 out of 27 non-target test plant species in no-choice tests in

quarantine, adults laid eggs only on prickly acacia under choice conditions. In the field in India, the brown leaf-webber was seen only on mature prickly acacia trees and not on seedlings. This was possibly due to the female moth using the silhouette of tall trees as a cue to locating host trees for oviposition (e.g. Cohen and Brower 1982, Wiklund 1984, Rabasa et al. 2005). Upon egg hatching, the neonate leaf- larvae feed on the same host tree, as the mobility of early larval instars is very limited (Zalucki et al. 2002). This suggests that oviposition behaviour could be the key mechanism in host selection of the brown leaf-webber, resulting in its incidence only on prickly acacia in India.

In many lepidopterans long-distance (visual and plant volatile) and short-distance (tactile, chemical stimulants and deterrents) cues are the principal mechanism for host-selection by adult females (e.g. Thompson and Pellmyr 1991, Keller 1999, Heard 2000, Singer 2004, Stefanescu et al. 2006). Within a restricted test arena, some of these cues may have been disrupted, resulting in false positives (e.g. Marohasy 1998, Withers and Barton-Browne 1998). The restricted test arena and small size of test plants used in the quarantine may have resulted in the indiscriminate oviposition on artificial surfaces (e.g. cage wall) in both no-choice and choice trials. Use of larger, more natural test arenas and open-field testing in the native range may alleviate this problem (Briese 1999, Heard 2000). However, since the field observations suggest that the female moth lays eggs on mature trees (the brown leaf-webber was seen only on mature trees and not on seedlings and juvenile plants; M. Murugesan, unpublished data), any field trial should be conducted using fully grown plants (those on which the larvae completed development in no-choice tests under quarantine conditions in Australia), but this is not a practical option. Given the diversity of acacia species in Australia (over 975 native species) and their iconic status (historically and culturally significant) the risk to non-target acacia species in particular was deemed to be too high to pursue as a potential agent. Thus, further screening of test plants was suspended and the colony of the brown leaf-webber in quarantine was destroyed.

*Phycita* sp. B. has proven to be more difficult to rear in quarantine than *Phycita* sp. A as adults lay eggs only on potted prickly acacia plants, and in early 2015 the colony failed to reproduce. Attempts to rear the moth under shorter day lengths was investigated, but was unsuccessful. While some females did appear to reach reproductive maturity, they did not mate and therefore laid no eggs. Feeding and development has occurred on 10 non-target species out of 19 non-target species tested. However, oviposition has only occurred on *N. major* and *V. sutherlandii.* No further work on *Phycita* sp. B will be pursued.

## 5.2 *Anomalococcus indicus*

### 5.2.1 Life history

Prior to this study, little was known about the life history of *A. indicus* (but see Baksha and Islam 1996). The life history of *A. indicus* is consistent with the pattern characteristic among soft scales, showing a distinct sexual dimorphism which becomes evident during the second instar (Ben-Dov et al. 2012). Crawlers emerged from eggs within one or two hours of being oviposited. We therefore consider the species to be ovoviviparous, a common form of reproduction in Coccoidea (Gordh and Headrick 2001, Gavrilov and Kuznetsova 2007, Howard et al. 2010). The egg is a common overwintering stage in scale insects, including the Lecanodiaspid *Lecanodiaspis prosopidis* (Maskell) (Howell and Kosztarab 1972).

Crawlers are the only life stage at which *A. indicus* can disperse. They generally settle within a day of emerging (Marotta 1997). The majority of *A. indicus* crawlers settled close to the female parent forming an aggregative distribution. A greater proportion of crawlers settle above the female rather than below her, suggesting that they, as with other Coccoidea

species, are positively phototrophic and negatively geotrophic (Greathead 1997, Marotta 1997). Crawlers therefore move towards younger tissue and to the top of plants from where those that have not settled can be easily dispersed via air currents (Greathead 1997). Long-distance dispersal is passive and is achieved predominantly using air currents, though phoretic dispersal has also been documented using insects such as ants and flies, birds and mammals (Washburn and Frankie 1981, Greathead 1997, Magsig-Castillo et al. 2010,). In the absence of food, most *A. indicus* crawlers die within two to three days, allowing time for passive dispersal to other potential hosts.

The development time of *A. indicus* males is shorter than that of females. Emergence of adult males occurs shortly after females have undergone their final moult and are sexually mature. Consistent with other adult male Coccoidea, *A. indicus* males are characteristically short lived (Marotta 1997), surviving for less than a day. As such, they do not feed and do not have functional mouthparts (Gullan and Kosztarab 1997). Although adult males are mobile, having a pair of functional wings, Coccoidea males are weak fliers (Hanks and Denno 1994). Studies of California red scale *Aonidiella aurantii* (Mask.) demonstrated that males were limited to passive downwind dispersal, unable to fly upwind, and generally mated with nearby females (Rice and Moreno 1970).

*Anomalococcus indicus* should prove to be a valuable biocontrol agent in Australia if released. In its native range, it is considered to be a major pest of prickly acacia, with severe infestations causing defoliation and ultimately death (Baksha and Islam 1996). Its high fecundity and the restricted short-distance dispersal promote substantial population build up on prickly acacia individuals, while long-distance spread is facilitated by air currents. The absence of this scale's indigenous parasitoids, which are prevalent in its native range, will further promote population increase.

### 5.2.2 Host specificity

Development of mature *A. indicus* females on the native non-target species *A. falcata, V. sutherlandii, V. bidwillii, N. major* and *N. monosperma* was comparable to the scale's development on prickly acacia. Five other native *Acacia* species, *N. dimorphantha*, *C. siliqua, Platylobium formosum*, *Parachidendron pruinosum* and *Paraserianthes lophantha* also supported low levels of scale development.

Scale insects have limited ability to disperse – crawlers generally settle close to the parental female (see 'Life history' Section 5.2.1). Dispersal beyond the host plant is achieved chiefly via passive movement of first instar crawlers via air currents and through phoretic dispersal, such as by ants. Scale insects are some of the slowest dispersing (and yet successful) weed biocontrol agents (Paynter and Bellgard 2011). If potential non-target species were geographically isolated from prickly acacia infestations, then the risk to these species would be minimal. However, five of the native non-target species, *V. sutherlandii, V. bidwillii, N. monosperma*, *N. dimorphantha* and *A. coriacea* occur in the Mitchell Grass Downs and are therefore likely non-target risks. The potential risk to these non-target plants when not in close proximity or when not in physical contact with prickly acacia is still to be assessed. The field choice trials in India involving some of the test plant species will help to resolve this.

Like prickly acacia, *V. sutherlandii,* and *V. bidwillii* are spiny shrubs or small trees, whereas *N. monosperma* and *N. dimorphantha* are small perennial herbs or shrubs (Hacker 1990). *N. major* (an erect slender shrub), *Platylobium formosum* and *A. falcata* are unlikely to occur sympatrically with the major prickly acacia infestation in western Queensland. However they may occur sympatrically with coastal infestations in Queensland and *N. major* may co-occur

with prickly acacia near Kununurra in Western Australia (in Western Australia prickly acacia is an eradication target). If *A. indicus* were to be released in these areas, these species may be at risk of attack when occurring in close proximity and in physical contact with prickly acacia. A species closely related to *A. falcata, A. attenuata* (Maslin 1995), supported early development of high numbers of *A. indicus* nymphs, though plants died before complete development was possible. Endemic to south-east Queensland, *A. attenuata* is classified as Vulnerable (Nature Conservation Act 1992). Attempts will be made to procure this plant so that more replications can be conducted.

During field surveys conducted over several years in southern India *A indicus* was only observed on three *V. nilotica* subspecies (Dhileepan et al. 2013). It was not observed on the previously reported hosts, *V. farnesiana, V. leucophloea, S. catechu, Z. mauritiana* and *P. nigrum*, nor was nymphal development recorded on these species during preliminary no-choice trials conducted in India. Yet in the no-choice trials conducted under quarantine conditions, *A. indicus* completed development on numerous species, including *V. farnesiana*. This raises the possibility that the quarantine results may be false positives, which can cause the unnecessary rejection of potentially safe agents (Marohasy 1998). Indeed, no-choice tests, which determine the absolute limit of host range, are known for producing false positive results (Heard 2000; van Klinken 2000). Further, determining host range under quarantine conditions can be difficult due to the absence of potentially critical steps in the host selection process (e.g. oviposition host range of many lepidopterans is particularly difficult to determine). However, host selection by *A. indicus* is largely determined by the passive dispersal of crawlers (e.g. wind) rather than a decision based process. For such insects, cage tests tend to be accurate (Heard 2000).

In view of the field host specificity of the scale insect in India, a choice trial under field conditions in India, involving the four non-target test plants *(A. falcata, V. sutherlandii, N. major,* and *N. monosperma)* on which the scale insect completed development in no-choice tests in the quarantine, is being undertaken. Further work on the scale insect in the quarantine in Australia will depend on the results from the field choice tests under field conditions in India.

## 5.3. *Dereodus denticollis*

Colony establishment of *Dereodus* weevil failed despite several attempts to facilitate mating and egg production. Male and female weevils remained paired, with occasional mating observed, however egg laying was sporadic on cage frames and the eggs were mostly infertile. Very few eggs were fertile and the larvae that hatched out burrowed into soil after hatching suggesting they could be root feeders as of other Entiminae (Marvaldi 1999). Since the larvae can be root feeders, and are typically less mobile, we suspected females lay eggs underneath soil. Hence, the colony cages were floored with pot mix and seedlings were raised to facilitate oviposition and larval feeding on fresh roots. Adults were also provided with supplemental protein and nectar sources such as bee pollen and fresh acacia flowers. However, all the attempts failed and yielded no fertile eggs. The adults used for colony establishment were probably old, at least one year old since collection from field sites in India.

A new batch of weevils was imported and new culturing practises implemented. Weevils housed in glass jars, equipped with cut foliage, laid their eggs on the bottom of the jar once the foliage started to wilt and dry. Previous culturing methods utilised cages with potted plants, plants were always watered and maintained and therefore not under any stress. While the issue with sporadic egg lay was resolved with new culturing methods, the larvae were not observed to feed on any of the substrate supplied to them and adult mortality

increased dramatically with the increased output of eggs. To prevent the colony from dying out completely, adults were moved back into cages equipped with potted prickly acacia plants. A small colony remains.

# 6 Acknowledgements

I thank Meat & Livestock Australia for funding the study; Di Taylor, Jason Callander, Cathy Lockett, Kumaran Nagalingam and David Perovic (Ecosciences Precinct, Boggo Road, Brisbane) for the scientific support in conducting the host specificity tests; A Balu, S. Murugesan, M Senthilkumar, and R. Mahalakshimi, (Institute of Forest Genetics and Tree Breeding, Coimbatore, Tamil Nadu, India) for supplying the insects for host specificity tests; and the Director, IFGTB, Coimbatore, India for the facilities.

# 7 Conclusions/Recommendations

## 7.1 Brown leaf-webber (*Phycita* sp. A)

Although in no-choice tests the leaf-webber fed and completed development on multiple non-target test plant species, there was no evidence of the insect on any non-target test plant species in the field. Although oviposition choice tests suggest that prickly acacia is the most preferred and natural host, difficulties in conducting choice oviposition tests involving fully grown trees under quarantine conditions in Australia and the logistical difficulties of conducting open-field testing with fully grown native Australian plants in India have led to rejection of the leaf-webber as a potential biological control agent for prickly acacia in Australia.

## 7.2 Scale insect (*A. indicus*)

The scale insect completed development on 17 of the 83 non-target plant species in no-choice trials. This may be an artefact of laboratory conditions, as this insect is known to be host specific under field conditions in India. Hence, choice trials involving non-target test plants on which the scale completed development in quarantine in Australia have commenced in India to ascertain the non-target risks of the Australian test plants under natural field conditions. Results to date suggest that *N. major* and *V. sutherlandii* are likely to be susceptible to the scale insect attack under field conditions. Tests for the susceptibility of other non-target plants for the scale insect under field conditions in India are in progress.

## 7.3 Green leaf-webber (*Phycita* sp. B)

The green leaf webber larvae completed development on 10 out of 19 test plant species under no-choice conditions but in no-choice oviposition trials egg have been laid only on prickly acacia and *N. major*. However, in paired choice oviposition trials, eggs were laid only on prickly acacia and not on *N. major*. No further progress on screening the remaining test plants for the green leaf-webber was made due to difficulties in maintaining a culture of the insect in quarantine.

### 7.4 Leaf weevil (*D. denticollis*)

Although difficulties with egg laying was resolved, a colony of the leaf-weevil could not be established in the quarantine due to continued difficulties with larval feeding and survival. Further research will need to focus on developing artificial diets for larval feeding and development.

### 7.5 New agents from Ethiopia

Surveys in Ethiopia in July 2014 and December 2015 discovered natural prickly acacia (ssp. *indica*) populations, along with populations of other *V. nilotica* subspecies (ssp. *leiocarpa* and ssp. *subalata*). So far, three promising gall-inducing biocontrol insects (a thrips gall, a mite gall and a midge gall) associated with ssp. *indica* were documented. This is the first time specific gall-insect associations with ssp. *indica* have been documented. Field observations in Ethiopia suggest that the three gall insects are likely to be specific to subspecies *indica* (invasive species in Australia). More intensive and systematic surveys in Ethiopia will help to understand the field host range and damage levels of the gall insects and may unravel other prospective agents as well.

#### 7.5.1 Gall thrips

Based on damage potential, field host range and geographic range, the gall thrips, *Acaciothrips ebneri* (Karny) (Thysanoptera: Phlaeothripidae), inducing rosette galls in shoot tips and sprouting axillary buds resulting in shoot tip dieback, was imported into high-security quarantine in Brisbane, Australia in December 2015. A colony of the gall thrips has been established and host specificity tests are in progress. Preliminary no-choice host specificity testing has been conducted on 14 non-target test plant species and to date no galls have been recorded on any of these species.

## 8 Key Messages

Surveys in India identified five insects and two rust fungi as prospective biocontrol agents. Host specificity tests found the two rusts (the gall rust and the leaf rust) and one insect (brown leaf-webber) to be not sufficiently host specific for release in Australia. Though no-choice host specificity tests for the scale insect have been completed, choice tests under natural field conditions for the test plants on which the scale completed development in quarantine tests will decide if the scale insect is adequately host specific for field release in Australia. Regardless of the outcome, additional agents are needed to increase the chances of effective control. There are no other prospective biological control agents identified in India. Future research will need to focus on Ethiopia for additional prospective biological control agents.

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# 10 Appendix

### Publications from the project


1. Taylor, D.B.J. and Dhileepan, K. 2013. Life history of babul scale *Anomalococcus indicus* (Hemiptera: Lecanodiaspididae), a potential biological control agent for prickly acacia in Australia. *Biocontrol Science and Technology* 23: 1373-1386.
2. Dhileepan, K., Taylor, D.B.J., Lockett, C.J., Balu, A., Seier, M., Murugesan, S., R.A. Tanner, K.M. Pollard, N. Kumaran and S, Neser. 2014. Biological control of prickly acacia (*Vachellia nilotica* subsp. *indica*): current research and future prospects. Pp. 21-30. In: Impson FAC, Kleinjan CA, Hoffmann JH (eds), *Proceedings of the XIV International Symposium on Biological Control of Weed*, Kruger National Park, South Africa, 2-7 March 2014.
3. Balu, A., Murugesan, S., Senthilkumar, P., Mahalakshmi, R. and Dhileepan, K. 2014. The leaf-feeding geometrid *Isturgia disputaria* – a potential biological control agent for prickly acacia (*Vachellia nilotica* subsp. *indica*) in Australia. *Journal of Biological Control* 28: 81-86.
4. Khan, A.N., Raman, A., Dhileepan, K. and Hodgkins, D.S. 2014. Feeding behaviour of *Anomalococcus indicus* (Hemiptera: Lecanodiaspididae) with supplementary biological notes and biological management of the invasive *Vachellia nilotica indica* (Fabales: Mimosoideae) in north-eastern Australia. *Annales de la Société entomologique de France* 49: 476-492.
5. Khan, A.N., Sharma, A., Raman, A., Dhileepan, K. and Hodgkins, D.S. 2014. Biological management of the invasive *Vachellia nilotica* ssp. *indica* (Fablales: Mimosoideae) in tropical Australia: stress-inducing potential of *Anomalococcus indicus* (Hemiptera: Lecanodiaspididae) an agent of promise. *Arboricultural Journal* 36: 63-75.
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7. Dhileepan, K., Lockett, C.J., Murugesan, M., Balu, A., Perovic, D. and Taylor, D.B.J. 2015. Life cycle and host range of *Phycita* sp. rejected for biological control of prickly acacia in Australia. *Journal of Applied Entomology* 139: 800-812.