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ORIGINAL CONTRIBUTION

Life cycle and host range of *Phycita* sp. rejected for biological control of prickly acacia in Australia

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Abstract

Prickly acacia (*Vachellia nilotica* subsp. *indica*), a native of the Indian subcontinent, is a serious weed of the grazing areas of northern Australia and is a target for classical biological control. Native range surveys in India identified a leaf webber, *Phycita* sp. (Lepidoptera: Pyralidae) as a prospective biological control agent for prickly acacia. In this study, we report the life cycle and host-specificity test results *Phycita* sp. and highlight the contradictory results between the no-choice tests in India and Australia and the field host range in India. In no-choice tests in India and Australia, *Phycita* sp. completed development on two of 11 and 16 of 27 non-target test plant species, respectively. Although *Phycita* sp. fed and completed development on two non-target test plant species (*Vachellia planifrons* and *V. leucophloea*) in no-choice tests in India, there was no evidence of the insect on the two non-target test plant species in the field. Our contention is that oviposition behaviour could be the key mechanism in host selection of *Phycita* sp., resulting in its incidence only on prickly acacia in India. This is supported by paired oviposition choice tests involving three test plant species (*Acacia baileyana*, *A. mearnsii* and *A. deanei*) in quarantine in Australia, where eggs were laid only on prickly acacia. However, in paired oviposition choice trials, only few eggs were laid, making the results unreliable. Although oviposition choice tests suggest that prickly acacia is the most preferred and natural host, difficulties in conducting choice oviposition tests with fully grown trees under quarantine conditions in Australia and the logistic difficulties of conducting open-field tests with fully grown native Australian plants in India have led to rejection of *Phycita* sp. as a potential biological control agent for prickly acacia in Australia.

Introduction

Prickly acacia, *Vachellia nilotica* subsp. *indica* (Benth.) Kyal. & Boatwr. (previously known as *Acacia nilotica* subsp. *indica*), is a serious weed of the grazing areas of western Queensland and has the potential to spread throughout northern Australia (Mackey 1997; Kriticos et al. 2003; Dhileepan 2009). Prickly acacia infests over 7 million hectares of natural grasslands and over 2000 km of bore drains (artificial channels of permanent flowing water from artesian bores) in western Queensland (Mackey 1997). Infestations also occur in the coastal regions of Queensland, in the Northern Territory and Western Australia (Mackey 1997). Prickly acacia infestations in Queensland cost primary producers Au$ 9 million/year in lost pasture production (Dhileepan 2009). In such areas, prickly acacia forms impenetrable thorny thickets, competes with native pasture species, prevents the growth of native plants beneath the canopy, restricts stock access to watercourses and poses a threat to nearly 25 rare and threatened animal species and two endangered plant communities (Spies and March 2004).

Vachellia nilotica (L.) P.J.H.Hurter & Mabb is a multipurpose tree native to Africa, the Middle East and the Indian subcontinent (Dwivedi 1993). It is a polytypic species with nine recognized subspecies in its native range, each subspecies having a distinct geographic range (Brenan 1983). Three subspecies, *V. nilotica* subsp. *indica* (prickly acacia), *V. nilotica* subsp. *cupressiformis* (J.L. Stewart) Ali & Faruqi and *V. nilotica* subsp. *hemispherica* Ali & Faruqi, are native to India and Pakistan (Dwivedi 1993).

Prickly acacia was introduced from India into Australia in the late 1890s (Dhileepan 2009). It is the only subspecies of *V. nilotica* introduced into Australia. It is a large thorny tree growing up to 10 m high. Seedling recruitment in Australia is linked to rainfall pattern, and under favourable conditions, young plants attain maturity in 2–5 years. When mature, prickly acacia forms dense thorny thickets (~900 plants/ha), and mature plants live for *c*. 40 years. The trees have distinct flat sickle-shaped pods, each with 8–15 seeds. A mature tree can produce up to 300 000 seeds per year, and seeds, when buried in soil, can remain viable up to 7 years (Dhileepan 2009). Prickly acacia seedlings and juvenile trees are considered the best life stage to target for control (Kriticos et al. 1999). Simulated herbivory study suggests that prickly acacia seedlings are susceptible to defoliation and shoot damage (Dhileepan et al. 2009).

Biological control of prickly acacia in Australia was initiated in the early 1980s, with native range surveys conducted on *V. nilotica* subsp. *indica* in Pakistan (Mohyuddin 1986), on *V. nilotica* subsp. *subalata* (Vatke) Kyal. & Boatwr. and *V. nilotica* subsp. *leiocarpa* (Brenan) Kyal. & Boatwr. in Kenya (Marohasy 1992) and on *V. nilotica* subsp. *kraussiana* (Benth.) Kyal. & Boatwr. in South Africa (Stals 1997). These surveys resulted in the introduction of two agents from Pakistan and four agents from South Africa and Kenya into Australia. Among them, only a seed-feeding bruchid *Bruchidius sahlbergi* Schilsky introduced from Pakistan and a leaf-feeding geometrid *Chiasmia assimilis* (Warren) introduced from Kenya and South Africa have become established (Dhileepan 2009). The impact of *B. sahlbergi* on prickly acacia has been insignificant (Radford et al. 2001), while *C. assimilis* has established only at coastal sites and not widely in the arid inland regions where the major infestations occur (Palmer et al. 2007). As a result, more effective biological control agents are needed for arid inland Australia.

Native range surveys were refocussed in India (Dhileepan et al. 2010, 2013), where the invasive Australian prickly acacia populations (subsp. *indica*) originated (Wardill et al. 2005). Areas climatically similar to the arid inland regions of northern Australia in India were targeted (Dhileepan et al. 2006). Based on field host range, geographic range and damage potential, a leaf webber, *Phycita* sp. (Lepidoptera: Pyralidae), was prioritized for detailed host-specificity tests (Dhileepan et al. 2013). No-choice larval development tests were conducted in India and Australia to determine the fundamental host range (species on which the agent can complete its life cycle) of *Phycita* sp. As *Phycita* sp. larvae completed development on several non-target plants under no-choice conditions, field host range studies in India and oviposition tests in Australia were also conducted to try and predict the realized host range (plant species that will support the agent population in the field) of the moth. In this study, we report the life cycle, fundamental host range in India and Australia and field host range in India for *Phycita* sp., a prospective biological control agent for prickly acacia in Australia.

Materials and Methods

Study species

Members of the Phycitinae have been exploited as weed biological control agents (e.g. Dodd 1940; Coombs et al. 2004), including species in the genus *Phycita* (Sakalasooriya et al. 2000). The majority of species in the genus *Phycita* for which host records are available are crop pests (e.g. Brues 1936; Butani 1970; Ponnuswami 1971; Aina 1983; Ram and Pathak 1987; Rani and Sridhar 2002). Host records for other species are not available.

The leaf webber collected on prickly acacia in India was initially identified as *Phycita leuconeurella* Ragonot (syn. *Hyalospila leuconeurella* Ragonot) by Dr George Mathew at the Kerala Forest Research Institute in India. A literature search found 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). However, no larval development occurred on either mango or cashew under no-choice conditions in India, suggesting that the species is not *P. leuconeurella*. Specimens were then sent to the Natural History Museum (NHM) in the United Kingdom for identification. As the species status of the *Phycita* sp. could not be confirmed by NHM, we treated the species as *Phycita* sp.

In surveys conducted in India (Dhileepan et al. 2013), *Phycita* sp. was found only in southern India (Tamil Nadu and Karnataka) and not in north-west

India (Rajasthan and Gujarat). In Tamil Nadu and Karnataka, *Phycita* sp. caused severe defoliation in prickly acacia trees throughout the year. *Phycita* sp. was observed in the majority of the survey sites (76%) in Tamil Nadu and Karnataka throughout the year, with higher incidences from September to January, coinciding with the north-east monsoon. *Phycita* sp. was found on all three subspecies of *V. nilotica* (subsp. *indica*, subsp. *cupressiformis* and subsp. *tomentosa*), but more often on larger trees than on juvenile plants. Females lay eggs on prickly acacia trees and the emerging neonate larvae construct a leaf web by tying the leaves. The larvae feed and complete development within the leaf web on the same host tree. Thus, *Phycita* sp. behaves more like leaf miners or gall insects where the adult moths choose the host tree for the larvae.

Insect cultures

A colony of *Phycita* sp. was established in an insectary at the Institute of Forest Genetics and Tree Breeding (IFGTB), Coimbatore, Tamil Nadu, in southern India in March 2010, using field-collected larvae and pupae from Coimbatore, Pollachi, Tiruchirappalli, Madurai and Thanjavur regions in Tamil Nadu, India. The colony was maintained either on cut foliage of prickly acacia held in glass jars (30 cm × 15 cm) with the cut ends of the shoots inserted in a glass vial with tap water and the mouth of the glass jar covered with white muslin cloth, or on potted prickly acacia plants in insect-proof cages (60 × 60 × 10 cm). Adults were fed on diluted honey. Newly emerged moths were released directly into glass oviposition containers with prickly acacia cut foliage for egg laying. Newly emerged larvae collected from oviposition containers were used in the life cycle and host-specificity tests in India.

Field-collected *Phycita* sp. larvae and pupae from Coimbatore, Pollachi, Tiruchirappalli, Madurai and Thanjavur regions in Tamil Nadu, India, were exported to a quarantine facility at the Ecosciences Precinct (ESP), Brisbane, Australia, in January 2011. A colony of *Phycita* sp. was maintained in insect-proof cages (90 × 80 × 75 cm) on both whole plants and cut foliage of prickly acacia in a quarantine glasshouse (22–27°C; 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 oviposition containers with prickly acacia cut foliage for egg laying. Food (sports drink containing water, carbohydrates and electrolytes; Gatorade®; PepsiCo Australia, Chatswood, Australia) was supplied in 30-ml transparent plastic cups with a sponge as a wick to adults used in colony maintenance and in oviposition tests, to enhance egg production and adult longevity. Newly emerged larvae collected from 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 kept in plastic containers for adult emergence. Newly emerged larvae and adults were used in all experiments.

Life cycle

The life cycle was studied using potted prickly acacia plants in a quarantine glasshouse at ESP under controlled climatic conditions (night temperature: 20°C; day temperature: 27°C; RH 65%; and photoperiod: 12 h dark: 12 h light). Pairs of newly emerged and mating adults (n = 10 pairs) were transferred on to potted prickly acacia plants enclosed in cylindrical transparent Perspex tubes (34 cm high and 12 cm diameter) with an insect-proof gauze cap at the top and the bottom end of the tube inserted in to the pot. The adults were transferred onto a fresh plant each week. Adult longevity and pre-oviposition period were recorded together with the number of eggs laid per female per week and the duration of larval and pupal stages.

Test plants

The host-specificity test list comprising 74 plant species that was used for previous agents (e.g. Palmer et al. 2007) was revised (Dhileepan et al. 2014), based on recent taxonomic changes to *Acacia sensu lato* (Maslin 2001; Orchard and Maslin 2003; Kodela and Wilson 2006). The genus *Acacia sensu lato*, the largest genus (with over 950 endemic species) of flowering plants in Australia (Orchard and Wilson 2001), has recently been split into five genera: *Acacia* Mill., *Vachellia* Wright & Arn., *Senegalia* Raf., *Acaciella* Britton & Rose and *Mariosousa* Seigler & Ebinger (Orchard and Maslin 2003; Kodela and Wilson 2006). Within the tribe Acaciae, representatives of *Vachellia* (six species), *Senegalia* (three species) and *Acacia* (36 species) species were included in the test list. Representatives from subfamilies Mimosoideae (tribes Mimoseae and Ingeae), Caesalpinoideae (tribes Cesalpinieae, Cassieae and Detarieae), Faboideae (tribes Bossiaaeeae, Cercideae, Mirbellieae, Millettieae, Phaseoleae and Sophoreae) in the order Fabales and representatives of other closely related orders Malpighiales (Euphorbiaceae), Malvaes (family Malvaceae), Sapinales (family

Anacardiaceae) and Piperales (family Piperaceae) were also included in the test plant list (Dhileepan et al. 2014). For *Phycita* sp., tests were completed only for 37 plant species (Table S1) and testing of the remaining test plants was not continued due to feeding and development on multiple non-target test plant species.

In India, 10 test plant species from the tribes Acaciae and Mimoseae (Table S1) that either co-occur with prickly acacia in India or are endemic to Australia (exported to India as seeds) were included in the no-choice tests. Two phylogenetically unrelated, but economically important plants, mango (*M. indica*) and cashew (*A. occidentale*) (both Anacardiaceae) were also included in the no-choice tests, as a *Phycita* species has been reported as a pest of both crops (Hutson 1939; Ponnuswami 1971). All test plants and prickly acacia used in host-specificity tests were grown in pots under direct sunlight.

In Australia, no-choice larval feeding tests for *Phycita* sp. were completed for only 28 test plant species (Table S1). These included two test plant species, *Vachellia farnesiana* (L.) Willd. and *Acacia deanei* (R. T. Baker) Welch, Coombs & McGlynn, that were also tested in India. Testing of the remaining species was suspended due to non-target feeding and development on several test plant species. Instead, oviposition tests were conducted to predict the realized host range of *Phycita* sp. Test plant species used in host-specificity tests in Australia were sourced either as potted plants from nurseries or grown from seeds. The potted plants used in host-specificity tests were either grown or maintained in glasshouse (27°C day temperature, 22°C night temperature, 65% relative humidity and UV-excluded sunlight) or in greenhouse (under 50% shade).

Host-specificity tests

No-choice tests

In India, no-choice tests were conducted on 12 potted plant species (including prickly acacia as control). Tests were conducted from June 2010 to March 2011 and from September 2011 to December 2011. Additional plants or cut foliage as bouquets (for *M. indica*) was added to cages, when required, and the larvae were allowed to move onto the fresh foliage by themselves. In each test, 10 unfed neonate larvae were placed on potted test plants within insect-proof cages placed outside under direct sun at IFGTB. There were five replicates for each test plant species. All inoculated test plants were monitored daily to determine the duration of larval and pupal stages and the proportion of larvae and pupae developing into pupae and adults, respectively.

In Australia, 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 the ESP in Brisbane, Queensland. The potential host range of *Phycita* sp. was evaluated initially using no-choice tests. Batches of test plants, predominantly seedlings or juveniles, were screened as they became available, and in each batch potted, prickly acacia plants were included as positive controls. Ten newly emerged larvae were placed on each potted test plant, as well as a prickly acacia control plant. Plants with larvae were placed in groups in insect-proof cages and were checked 2–3 times per week for evidence of larval feeding and webbing. Fresh test plants were added as required to feed developing larvae. When there was larval feeding, the duration of larval survival, proportion of larvae developing into pupae, pupal duration and proportion of pupae emerging as adults were recorded. A minimum of five replicates of each test plant was used.

No-choice continuation trials

To ascertain the suitability of non-target plant species to sustain continuous generations of *Phycita* sp., no-choice continuation trials were commenced in April 2012 under quarantine conditions in Australia. Three non-target test plant species, *Acacia baileyana* F.Muell., *A. mearnsii* De Wild. and *A. irrorata* Sieber ex Spreng., were chosen for no-choice continuation trials, as they supported higher survival and development of *Phycita* sp. larvae to adults in the no-choice larval feeding trials. Trials were conducted using potted test and control plants placed separately in insect-proof cages (90 × 80 × 75 cm). Each test was replicated a minimum of three times and commenced with the placement of 60 newly emerged first instar larvae onto both test (*A. baileyana*, *A. mearnsii* or *A. irrorata*) and control (prickly acacia) 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 numbers of eggs laid by each female 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.

Choice oviposition tests

All oviposition tests were conducted in Australia under quarantine conditions. The initial oviposition trial was a paired-choice test simultaneously exposing prickly acacia and a non-target plant (*A. mearnsii* or *A. deanei*). A single pair of adults was placed in a cage (90 × 80 × 75 cm) with the two plants (one prickly acacia and one non-target plant), and the number of eggs laid was counted after 1 week. No non-target egg laying was observed through six replicates with *A. deanei* and through three replicates with *A. mearnsii*. However, egg laying was very erratic, and in the majority of replicates, no eggs were laid on any plant, thus making the results unreliable and statistically difficult to draw any conclusion.

We hypothesized that the initial trial had failed to give clear results because plants were an unacceptable size for females. To test this, we ran paired-choice tests with two prickly acacia plants: one the size used in the initial trial (~30 cm tall) and another a larger (~60–90 cm tall) plant in insect-proof cages (90 × 80 × 75 cm). These trials revealed a clear preference for larger plants. Preliminary trials also showed that the use of multiple pairs of adults (two females + two males per cage) that were pre-mated (pairs that were allowed to mate over a day before releasing them to the experimental oviposition cages) produced more consistent oviposition than using single pairs of adults and newly emerged adults.

Having established a more reliable method for conducting oviposition trials using larger plants, multiple-choice oviposition trials, exposing one prickly acacia plant together with four non-target species (*A. baileyana*, *A. mearnsii*, *A. oshanesii* F. Muell. & Maiden and *A. macradenia* Benth,) in the large walk-in cage (200 cm × 200 cm × 200 cm), were conducted. Despite following the new procedure, and repeating three replicates, moths failed to lay eggs on any plant and all eggs were laid on the gauze walls of the cage. Hence, multiple-choice oviposition trials in large walk-in cages were discontinued.

Selected test plant species on which there was higher larval survival and development in no-choice tests (*A. baileyana*, *A. mearnsii* and *A. deanei*) were subjected to paired-choice oviposition tests (one test plant and one prickly acacia plant per cage) in insect-proof cages (90 × 80 × 75 cm). Larger test plants (~60–90 cm tall) were used with a minimum of two pairs of pre-mated, 1-day-old adults. There were seven replicates for paired-choice trials involving *A. mearnsii*, six replicates for paired-choice trials involving *A. deanei* and three replicates for paired-choice trials involving *A. baileyana*. Adults were left in the choice oviposition arena for 5 days and then the numbers of eggs laid on individual test plants and on the cage walls were counted a week later.

Field host range – India

In India, a total of 72 sites (64 sites in Tamil Nadu and eight sites in Karnataka) were surveyed at quarterly intervals from November 2008 to December 2011 (Dhileepan et al. 2013, Table S2). At each site, two or three research staff spent a minimum of 1 h surveying for insects. Incidence and severity of damage by *Phycita* sp. were recorded, along with plant age (seedling, juvenile tree or mature tree) and the subspecies of the *V. nilotica* (subsp. *indica*, subsp. *cupressiformis* and subsp. *tomentosa*) present. Among the survey sites, 13 had only subsp. *indica*, two had only subsp. *cupressiformis*, 51 sites had both subsp. *indica* and subsp. *tomentosa,* and six sites had both subsp. *indica* and subsp. *cupressiformis* (Table S2). On all visits, co-occurring vegetation (other *Acacia*, *Vachellia* and *Senegalia* species) was also surveyed for the presence of *Phycita* sp. larvae. At sites with juvenile and young plants, the entire plant canopy was surveyed, while at sites with mature trees, only branches accessible from the ground were sampled.

Data analysis

One-way ANOVA was used to compare (i) the duration of larval and pupal survival, the proportion of larvae that developed into pupae and the proportion of pupae that developed into adults on various test plant species in no-choice larval feeding tests; (ii) the duration of larval development, the proportion of larvae that developed into pupae and adults and the number of eggs per female in no-choice continuation trials; and (iii) the number of eggs laid in choice oviposition tests. The data sets that did not meet underlying assumptions of normality and homogenous variances were analysed using Kruskal–Wallis test. The means were compared using Dunn's test. All results in the text are presented as means ± standard error.

Results

Life cycle

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) during their life, on the leaves and stems of host plants, cage walls or the gauze covers on oviposition containers. Eggs hatched in 6 to 10 days and the newly emerged larvae fed almost immediately, tying leaves together with silk webs and forming tunnels as they matured. The larval stage lasted for 41 ± 1.2 days (range: 27–48 days). Fully grown larvae pupated for 13 ± 0.4 days (range: 6–19 days) within the larval silk tunnel or in the soil. On prickly acacia, 80% of the neonate larvae became adults.

Host-specificity tests

No-choice tests

In no-choice trials in India, the duration of larval survival was significantly lower on the non-target plants than on the target weed ($F_{11,44}$ = 106.2, P < 0.001; fig. 1). The larvae completed development and became adults on only two non-target test plant species, *Vachellia leucophloea* (Roxb.) Maslin, Seigler & Ebinger and *V. planifrons* Ragupathy, Seigler, Ebinger & Maslin (fig. 1). However, on both non-target species, the proportion of larvae that developed into pupae (*V. nilotica* subsp. *indica* = 98%, *V. planifrons* = 52%, *V. leucophloea* = 30%; $F_{2,12}$ = 75.3, P < 0.001) and adults (*V. nilotica* subsp. *indica* = 76%, *V. planifrons* = 34%, *V. leucophloea* = 26%; $F_{2,12}$ = 16.9, P < 0.001) was significantly lower than on the target weed (fig. 1). Larvae did not complete development on the other non-target test plant species tested (fig. 1).

In Australia, no-choice larval feeding and development tests were completed for 28 test plant species (Table S1). Non-target feeding and development through to adults occurred on 16 of the 27 non-target plant species (figs 2 and 3). The durations of larval survival (Kruskal–Wallis test, *H* = 174.93; P < 0.001) and pupal survival (Kruskal–Wallis test, *H* = 89.32; P < 0.001) and the proportion of larvae that developed into pupae (Kruskal–Wallis test, *H* = 114.64; P < 0.001) and adults (Kruskal–Wallis test. *H* = 80.47; P < 0.001) differed significantly between the test plant species (figs 2 and 3). On all non-target test plant species on which the larvae developed into pupae, the duration of larval survival was significantly longer than on prickly acacia (Dunn's test, P < 0.05; fig. 2). On six of the 17 non-target test plant species (*V. sutherlandii* F.Muell., *A. cardiophylla* A. Cunn. ex Benth., *A. deanei*, *A. mearnsii*, *A. lasiocarpa* Benth. and *A. conferta* A. Cunn. ex Benth.), the rate of successful development (larvae to pupae and larvae to adults) was not significantly different to the target weed (Dunn's test, P > 0.05; fig. 3). On the remaining test plant species, the survival rates of larvae and pupae varied greatly, but were significantly lower than on prickly acacia (fig. 3).

No-choice continuation trials

Phycita sp. completed up to three generations on *A. baileyana* and at least two generations on *A. mearnsii*, although development time from neonate larva to adult on both species was significantly longer than on prickly acacia (fig. 4). On *A. irrorata*, *Phycita* sp. successfully completed one generation, but significantly fewer larvae developed into adults (41 ± 13%) than on prickly acacia (81 ± 4%) (*t* = 2.76, P = 0.05). Due to the low number of progeny adults in one of the replicates in the first generation, the second generation trial was not continued. In fecundity trials, fewer fertile eggs were laid by females that developed on *A. baileyana* (64 ± 29 eggs per female),

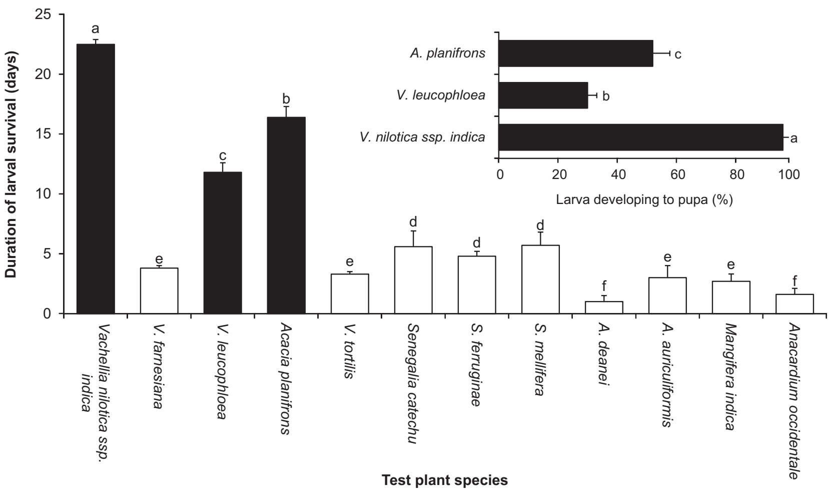

Fig. 1 Duration of larval survival (mean + SE) (main figure) and proportion of larvae developing into pupae (figure in inset) on various test plant species in no-choice tests in India. Solid bars represent test plants on which larvae developed into pupae and the empty bars represent test plants on which no larvae developed into pupae. Means with the same letter are not significantly different (Tukey's test, P > 0.05).

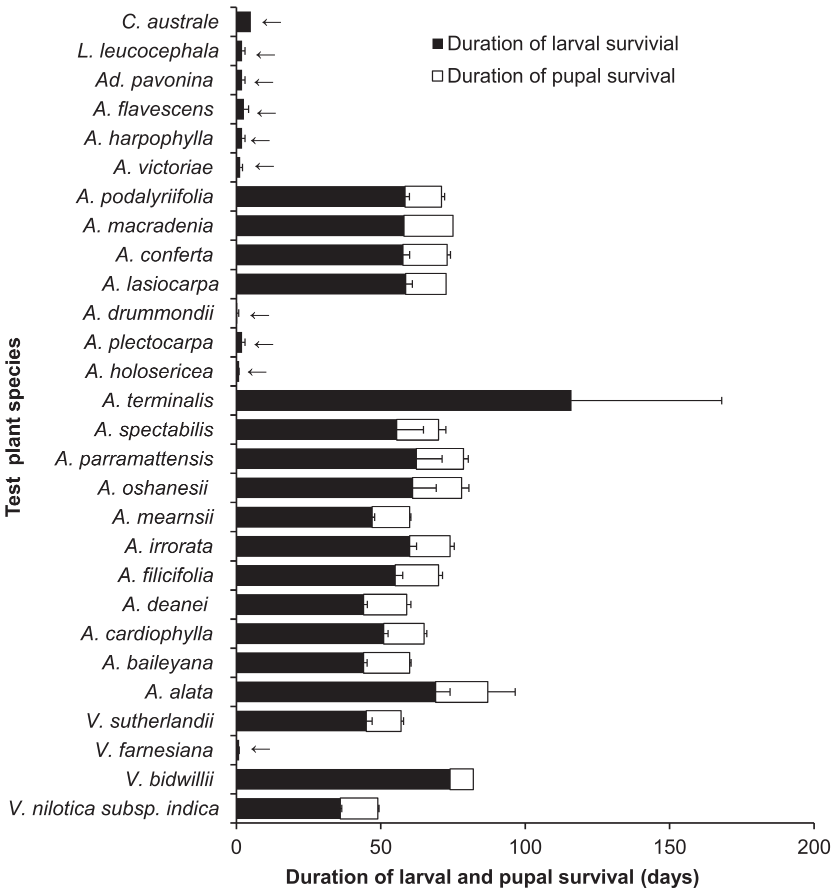

Fig. 2 Duration (mean + SE) of larval (solid bars) and pupal (empty bars) survival on various test plants in no-choice tests under quarantine in Australia. Arrows indicate the test plants on which the larvae did not develop into pupae.

A. mearnsii (117 ± 25 eggs per female) and *A. irrorata* (111 ± 90 eggs per female) than on prickly acacia (156 ± 63), but the differences were not significant ($F_{3,52}$ = 1.779, P = 0.163).

Choice oviposition tests

In paired-choice tests involving prickly acacia and *A. mearnsii,* no eggs were laid on the non-target plants, but only few eggs were laid on prickly acacia (5.9 ± 3.8 eggs). However, significantly more eggs were laid on the cage (30.7 ± 14.8 eggs) than on test plants (Kruskal–Wallis test, *H* = 13.818, d.f. = 2, P < 0.001). In paired-choice tests involving prickly acacia and *A. baileyana,* no eggs were laid on *A. baileyana*, but only few eggs were laid on prickly acacia (2.0 ± 0.58 eggs). There was no significant difference in the number of eggs laid on test plants and on the cage (2.67 ± 2.67 eggs) (Kruskal–Wallis test, *H* = 3.84, d.f. = 2, P = 0.254). In paired-choice trials involving prickly acacia and *A. deanei,* significantly more eggs were laid on prickly acacia (25.5 ± 19.8 eggs) than on the cage wall (0.5 ± 0.5 eggs) with no eggs laid on *A. deanei* (Kruskal–Wallis test, *H* = 7.1, d.f. = 2, P = 0.029).

Field host range – India

In India, *Phycita* sp. was collected from 47 of the 72 survey sites (Table S2). Survey sites with *Phycita* sp. varied widely between seasons, ranging from 10% to 55% (fig. 5). *Phycita* sp. caused widespread defoliation throughout the year on all three subspecies of *V. nilotica* – in 77% of the sites with only subsp. *indica*, in 100% of the sites with only subsp. *cupressiformis*, in 61% of sites with both subsp. *indica* and subsp. *tomentosa* and in 67% of the sites with both subsp. *indica* and subsp. *cupressiformis*. *Phycita* sp. was not observed on *V. horrida* (L.) Kyal. & Boatwr., *V. leucophloea*, *S. ferruginea* and *V. planifrons* co-occurring with *V. nilotica* in the field, except for a single collection of *Phycita* sp. larva on an *V. planifrons* tree at a single site (Ulakkudi kanmai) on one occasion (July 2010) (fig. 5).

Discussion

In classical weed biological control, potential agents are subjected to host-specificity testing to ensure that the agents are specific to target weeds and there is no risk to non-target plants. This primarily involves no-

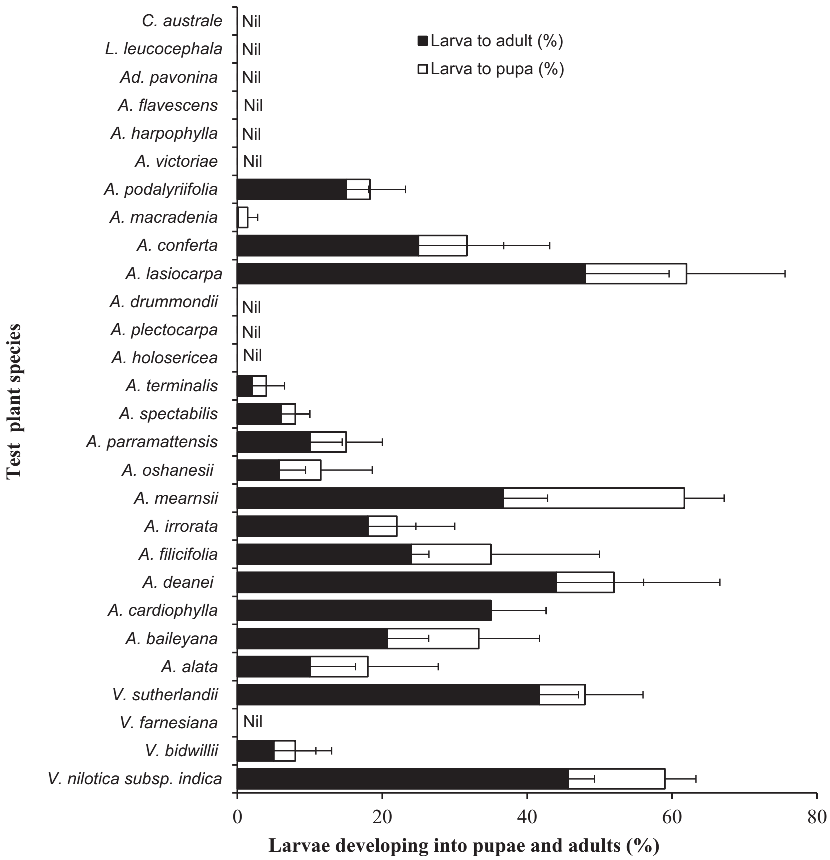

Fig. 3 Proportion (mean + SE) of larvae that developed into pupae (empty bars) and adults (solid bars) on various test plant species in no-choice tests under quarantine in Australia.

choice tests to predict the fundamental host range (Fowler et al. 2012). When there is non-target feeding and development in no-choice tests, choice tests and no-choice continuation trials are needed to predict the realized host range. Under the current risk-averse regulatory process, any feeding or development on a non-target plant in no-choice tests is often treated as 'risky' (e.g. Dhileepan et al. 2005). This may result in discarding some good agents which are known to have restricted or limited field (realized) host ranges in their native area (Heard 2000; Fowler et al. 2012). Host-specificity test results of *Phycita* sp. produced contradictory results between the fundamental host range and field host range studies, resulting in its rejection as a biological control agent for prickly acacia.

No-choice larval feeding and development tests were conducted first to determine the fundamental host range of *Phycita* sp. Under no-choice conditions, *Phycita* sp. larvae were able to feed and develop on many non-target test plant species. However, on most non-target test plants species, *Phycita* sp. performed either poorly or not as well as on prickly acacia, as evident from prolonged larval periods and lower rates of successful pupation and adult emergence (figs 1-3). On one of the test plant species (*A. deanei*), the no-choice larval development tests in India and Australia produced contradictory results – none of the larvae developed into adults in India and 44% larvae developed into adults in Australia. This was possibly due to difference in the testing methods used, the conditions under which the test plants were grown prior to testing, and the environmental conditions under which the tests were conducted. In India, test plants used were more field-hardened as they were grown under direct sun in the field, while in Australia, the test plants were grown either in a temperature-/humidity-controlled glasshouse or under shade in a greenhouse (with no temperature, humidity and photoperiod control). Also, the tests in India were conducted under natural field conditions including natural sunlight, whereas the tests in Australia were conducted in a quarantine glasshouse under optimum temperature and humidity, but under UV-excluded sunlight. The no-choice host-specificity tests for *Phycita* sp. in India produced contrasting results to the observed field host range. Although *Phycita* sp. completed larval development on two non-target test

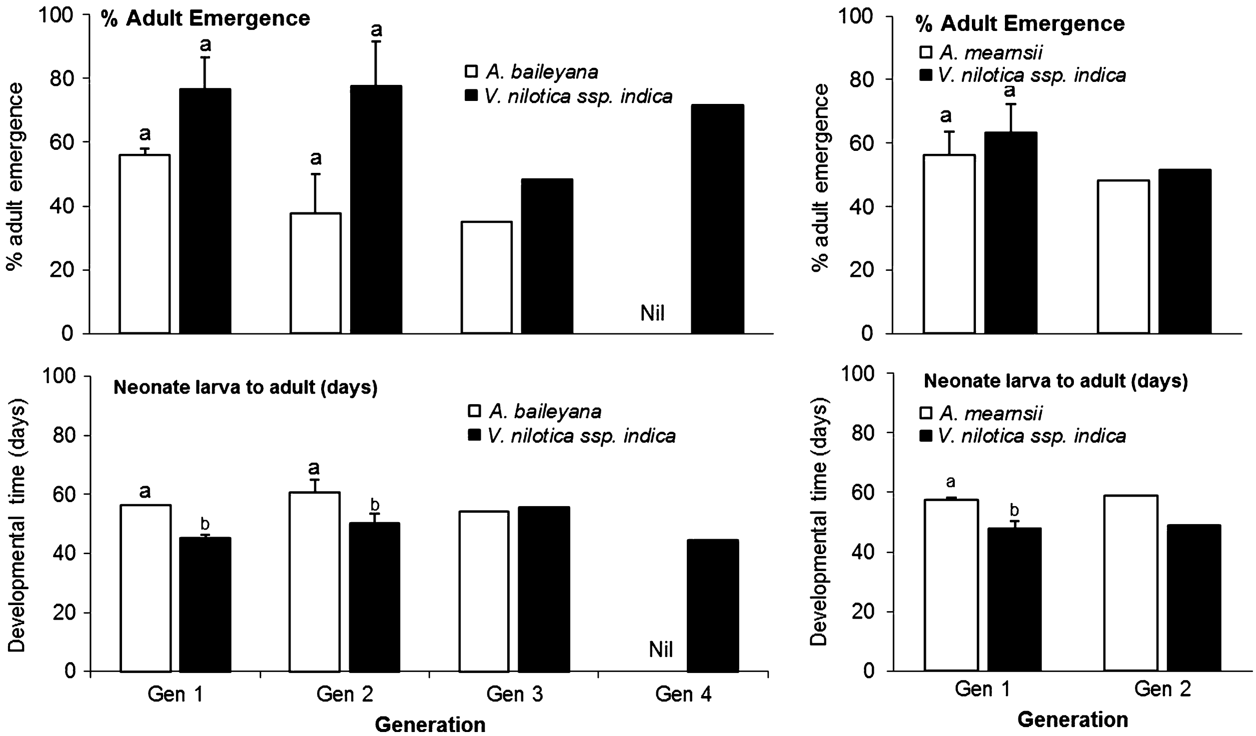

Fig. 4 Durations of larval development (mean + SE) and proportion of larvae that developed into adults (mean + SE) on two non-target test plant species, *Acacia mearnsii* and *A. baileyana* (empty bars) and the target weed (solid bars) over multiple generations under no-choice conditions in quarantine in Australia. Within each generation, treatment means with the same letter are not significantly different (Dunn's method, P > 0.05). In the columns where there are no letters, no analyses were performed due to lower number larvae developing on the non-target plant species.

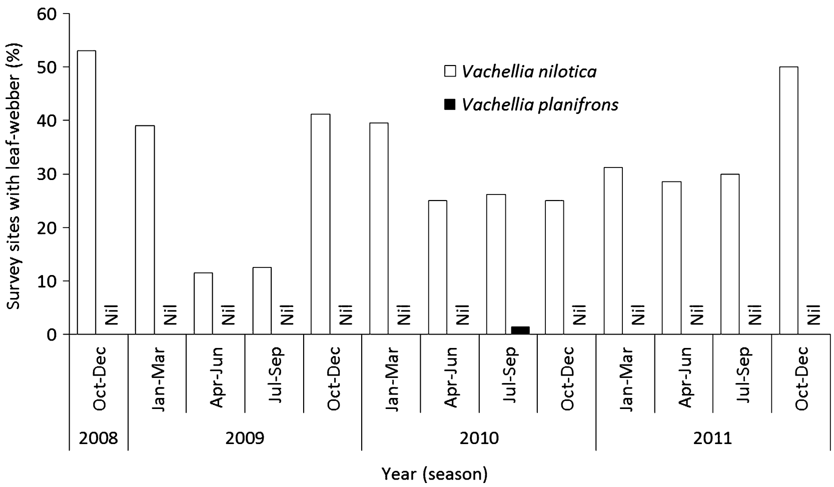

Fig. 5 Incidence (% of survey sites) of *Phycita* sp. larvae on *Vachellia nilotica* (target weed, empty bars) and *V. planifrons* (non-target tree species, solid bar) in relation to season over three years (2008–2011). *Phycita* sp. was not observed on other non-target tree species (*V. horrida*, *Acacia leucophloea* and *A. ferruginea*) that co-occurred at the survey sites.

plant species (*V. leucophloea* and *V. planifrons*) under no-choice conditions, *Phycita* sp. larvae were never found on either of the non-target test plant species during field surveys in 72 sites at quarterly intervals over four years (2008–2011), except for a single collection of *Phycita* sp. larva on an *V. planifrons* tree at a single site (Ulakkudi kanmai) on one occasion (July 2010). *Phycita* sp. larva was never recovered from *V. planifrons* at this site on subsequent surveys, or in other sites, and hence, *V. planifrons* cannot be regarded as a natural host for the insect. Many herbivores develop very well in the laboratory on plants that they will not use in nature (e.g. Balciunas et al. 1996; Marohasy 1998; Frye et al. 2010), suggesting that field host range is influenced by numerous factors other than host suitability (e.g. Wapshere 1989; Janz et al. 1994; Fox et al. 1996; Sheppard et al. 2005).

In view of larval feeding and development on several non-target test plant species in no-choice tests, subsequent oviposition tests were conducted to predict the realized host range of *Phycita* sp. No-choice tests are prone to false-positive results, because the 'fundamental' host range is often wider than the field host range and hence may result in the rejection of safe agents (Heard 2000). In Australia, although *Phycita* sp. completed development on 16 of 27 non-target test plant species in no-choice tests, in paired-choice oviposition trials adults laid eggs only on prickly acacia. However, in paired-choice trials (which were conducted only for three test plant species), only a few eggs were laid, and in some trials, more eggs were laid on the cage walls than on test plants, rendering the results not reliable. In the field in India, *Phycita* sp. occurred only on prickly acacia, more often on mature prickly acacia trees than on young plants. Host selection mechanisms for *Phycita* sp. in the field are not known. It is possible that the female moths use the silhouette of prickly acacia trees as cue to locating host trees for oviposition (e.g. Cohen and Brower 1982; Wiklund 1984; Rabasa et al. 2005). Such preference for oviposition on mature trees over young plants has been shown in other lepidopterans (e.g. Thompson and Pellmyr 1991). Upon hatching, the neonate larvae will feed on the same host tree, as the mobility of early larval instars is very limited, with reduced chances of migration between host plants (Zalucki et al. 2002). This suggests that oviposition behaviour could be the key mechanism in host selection of *Phycita* sp., resulting in its occurrence on only prickly acacia in India.

If host discrimination takes place in different life stages (e.g. oviposition by female moths, feeding by larvae), no-choice discrimination (e.g. oviposition) tests may be the only tests required (e.g. Sheppard et al. 2005). In laboratory and quarantine conditions, the female moths laid more eggs on cage walls and other artificial surfaces than on prickly acacia plants. As a result, no-choice oviposition tests could not be conducted reliably in quarantine. In quarantine tests, the female moths showed a marked preference for oviposition on larger plants than smaller plants, but even when larger plants were offered, fewer eggs were laid than in oviposition containers (156 $\pm$ 63 eggs per female), suggesting that the plant sizes offered in oviposition trials were not suitable for oviposition. Due to limited space availability within quarantine, and logistic difficulties and time required (7 to 10 years) in growing test plants to require size, testing of very large plants/trees of all 16 test plant species that supported development of *Phycita* sp. was not feasible.

In many lepidopterans, oviposition behaviour, involving long-distance (visual and plant volatile) and short-distance (tactile, chemical stimulants and deterrents) cues are the principal mechanism for host selection in the field (e.g. Thompson and Pellmyr 1991; Keller 1999; Heard 2000; Singer 2004; Stefanescu et al. 2006). Under field conditions, a monophagous insect would search for several days for their preferred host species before accepting a second, less-preferred choice (Singer 2004). In contrast, within a restricted test arena in quarantine, where the test plants were offered directly for oviposition, some of the sequential steps in the natural oviposition behaviour (e.g. long-distance visual and chemical cues) would have been disrupted (e.g. Marohasy 1998; Withers and Barton-Browne 1998; Heard 2000; Singer 2004; Sheppard et al. 2005), making the results unreliable.

The restricted test arena and small size of test plants used in 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 (Balciunas et al. 1996; Briese 1999; Heard 2000; Frye et al. 2010). As the field observations suggest that the female moth laid eggs on mature trees, any choice trial in India should be conducted using fully grown Australian native test plant species on which the larvae completed development in no-choice tests under quarantine conditions in Australia. As this is not practical, further screening of other test plants was suspended, the insect was not considered further as a biocontrol agent for prickly acacia in Australia, and the colony in quarantine was destroyed.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. List of test plant species used in host specificity test with *Phycita* sp. in Australia and India.

Table S2. Survey sites in Tamil Nadu and Karnataka states in India.