Journal of Biological Control, 28(2): 81–86, 2014

Research Article

The leaf-feeding geometrid *Isturgia disputaria* (Guenée) - a potential biological control agent for prickly acacia, *Vachellia nilotica* subsp. *indica* (Benth.) Kyal. & Boatwr. (Mimosaceae) in Australia

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ABSTRACT: Prickly acacia (*Vachellia nilotica* subsp. *indica*), a native multipurpose tree in India, is a weed of National significance, and a target for biological control in Australia. Based on plant genetic and climatic similarities, native range surveys for identifying potential biological control agents for prickly acacia were conducted in India during 2008-2011. In the survey leaf-feeding geometrid, *Isturgia disputaria* Guenee (syn. *Tephrina pulinda*), widespread in Tamil Nadu and Karnataka States, was prioritized as a potential biological control agent based on field host range, damage potential and no choice test on non target plant species. Though the field host range study exhibited that *V. nilotica* ssp. *indica* and *V. nilotica* ssp. *tomentosa* were the primary hosts for successful development of the insect, *I. disputaria*, replicated no - choice larval feeding and development tests conducted on cut foliage and live plants of nine non-target acacia test plant species in India revealed the larval feeding and development on three of the nine non-target acacia species, *V. tortilis, V. planiferons* and *V. leucophloea* in addition to the *V. nilotica* ssp. *indica* and *V. nilotica* ssp. *tomentosa*. However, the proportion of larvae developing into adults was higher on *V. nilotica* subsp. *indica* and *V. nilotica* subsp. *tomentosa*, with 90% and 80% of the larvae completing development, respectively. In contrast, the larval mortality was higher on *V. tortilis* (70%), *V. leucophloea* (90%) and *V. planiferons* (70%). The no-choice test results support the earlier host specificity test results of *I. disputaria* from Pakistan, Kenya and under quarantine in Australia. Contrasting results between field host range and host use pattern under no-choice conditions are discussed.

KEY WORDS: Prickly acacia, *Acacia nilotica*, native range survey, biological control, India.

(Article chronicle: Received: 20-03-2014; Revised: 15-4-2014; Accepted: 20-04-2014)

INTRODUCTION

Vachellia nilotica subsp, *indica* (Benth.) Kyal. & Boatwr. (Previously known as *Acacia nilotica* subsp. *indica*) (Mimosaceae), commonly known as prickly acacia in Australia, is a multi-purpose tree that occurs naturally and cultivated throughout the country. It is widely used in agroforestry, social forestry, reclamation of wastelands and rehabilitation of degraded forests in India. Prickly acacia is a woody, leguminous tree which was introduced to Australia in the 1890s (Parsons and Cuthbertson, 2001). In Australia, it was planted extensively in western Queensland for shade and fodder, but spread widely after wet years during the 1950s and 1970s, to form dense impenetrable thickets (Mackey, 1997). Prickly acacia was recognised as one of the most serious weeds in Australia because of its invasiveness, potential for spread, and economic and environmental impacts, when it was placed on the list of weeds of National significance (Thorp and Lynch, 2000). Prickly acacia is also present in the coastal regions of Queensland, the Northern Territory and Western Australia (Spies and March, 2004), and has the potential to infest vast areas of Australia's native grassland ecosystems (Kriticos *et al*., 2003a, 2003b). Mechanical and herbicide treatments are available to manage this weed (Jeffrey, 1995; Spies and March, 2004), but their use is not always economical. Classical biological control, a low-cost and permanent alternative, is considered as a viable option for the long term sustainable control of this weed.

Biological control of prickly acacia was initiated in the early 1980s, with surveys conducted in Pakistan (Mohyuddin, 1981, 1986), Kenya (Marohasy, 1992, 1995) and South Africa (Stals, 1997). So far six species of insects have been

released in Australia, but only two of these species have become established in the field. These include a seed-feeding bruchid, *Bruchidius sahlbergi* Schilsky from Pakistan (Wilson, 1985; Palmer, 1996) and a leaf-feeding geometrid *Chiasmia assimilis* (Warren) from Kenya and South Africa (Lockett and Palmer, 2005). Due to non-establishment of several of these agents in the targeted Mitchell Grass Downs areas, a climate matching analysis was carried out. The study indicated that several of these agents are more suited to coastal regions and they are less likely to establish in the hotter and drier weather conditions that is the norm in the Mitchell Grass Downs of western Queensland (Lockett and Palmer, 2003; Senaratne *et al*., 2006). The need for effective biological control agents continues to be a priority in the Mitchell Grass Downs, where the introduced agents have either not established or are ineffective (Dhileepan *et al*., 2010). Genetic studies have revealed that the invasive prickly acacia populations in Australia are the native to India and Pakistan (Wardill *et al*., 2005). India is the region climatically most similar to Queensland and Australia (Dhileeepan *et al*., 2006). Plant genotype and climatic similarities has accelerated a five year biological control project based in India since September 2008. Two years systematic surveys on prickly acacias at its natural host range in India revealed 94 different species of insects belonging to five families (Dhileepan *et al*., 2010; Kunjithapatham Dhileepan *et al*., 2013). Of which, 74 species are recorded as true pests causing damage on foliage, shoot, stem, flowers and seed pods. The leaf-feeding geometrid moth, *Isturgia disputaria* Guenee is one of the agents prioritized as a potential biological control agent for further studies based on its field host range, field abundance and damage levels (Kunjithapatham Dhileepan *et al*., 2013). *Isturgia disputaria,* available throughout the year was found more abundant in late spring to early summer (September-December and January – March) in Tamil Nadu, India. But for a little information on its pest status on *V. nilotica* ssp. *indica* in India currently no other details available on its field host range and host specificity. This paper describes the biology, host range and host specificity studies that were carried out in India. It also discusses the possibility of using this insect species as a potential biological control agent of prickly acacia.

MATERIALS AND METHODS

Mass rearing

Collections of *I. disputaria* were made in Tamil Nadu in 2008 and 2009 for mass rearing in the Institute of Forest Genetics and Tree Breeding (IFGTB) laboratory. The species was collected from both the subspecies *V. nilotica* ssp. *indica* and *Vachellia nilotica* ssp. *tomentosa* (Benth.) Kyal.& Boatwr. Most (75%) were collected at Coimbatore (10°59.298' N; 076° 54.908' E) to (11° 15.635' N; 077° 07.981'E) either as larvae or as adult moths. Groups of 50-60 larvae were kept in clean glass jars (30cm X 15cm) and fed with young cut foliage of prickly acacia held in glass vials containing water and placed in the jars covered with white cotton cloths. Another set of larvae was reared on group of potted 1 year old live plants. After two weeks, the pupae were collected and kept over moistened cotton placed on Petri dishes. The adults emerged within a week from the pupae. Group of 70-80 newly emerged moths were allowed in glass jars for oviposition supplied with diluted honey solution together with prickly acacia foliage. In mass rearing cages cotton strips wet with honey solution were hung for adult feeding. The oviposition jars with cut foliage and cages with live plants in pots were maintained at 26 – 32°C and 12h light/ 12h dark photoperiod. Day fourth onwards eggs were seen on cloth used for covering the culture jars and wall of insect cages. It was difficult to locate the eggs on leaves or on cut foliage. The oviposition period was observed to last for 10 days and the eggs were hatched in a week time. Newly laid eggs collected from the culture were used in lifecycle studies. Similarly, the newly emerged larvae were used in no-choice host specificity tests.

Life Cycle

A colony of *I. disputaria* was established at the IFGTB insectary. Adults emerged out of the colony were fed with diluted honey solution. Pairs of newly emerged adults (n=10) were introduced into rearing cages (60cm X 60cm X 100cm) made of insect proof net, and the duration of oviposition and fecundity were recorded. The females did not oviposit on potted prickly acacia plants or on cut foliage. The eggs were usually laid on the wall of the cages. Newly emerged larvae from the eggs were collected and introduced on potted prickly acacia plants kept inside the insect proof cages. The duration of larval development was studied. Measurements of the head capsule width of 50 larvae in various instars stages were also calculated. Fifty newly formed pupae were transferred individually to containers and the pupal duration was recorded.

Host-specificity tests

No-choice host testing of *I. disputaria* was carried out at the insectary of IFGTB by exposing unfed neonates on the plant species raised in poly bags (20X30 cm). The insect was tested on 9 plant species under laboratory conditions with *V. nilotica* subsp. *indica* and *V. nilotica* ssp. *tomentosa* as control. The test plants used in the study were *Vachellia tortilis* (Forssk.) Galasso and Banfi, *Vachellia planifrons* Wright and Arn., *Vachellia leucophloea* (Roxb.)

Maslin, Seigler and Ebinger *Senegalia mellifera* (M. Vahl) Seigler and Ebinger, *Senegalia catechu* (L.f.) P.J.H.Hurter and Mabb., *Senegalia ferruginea* (DC.) Pedley, *Vachellia auriculiformis* A.Cunn. exBenth., *Vachellia farnesiana* (L.) Willd., *Vachellia deanei* (R.T. Baker) (all Fabaceae) and *Delonix regia* (Bojer ex. Hook.) (Caesalpiniaceae).

The test on each plant species was replicated five times. In each test, 10 unfed neonate larvae were placed on cut foliage of individual test plants having good amount of young foliage. Care was taken to use only young foliage in the test. Bunch of young cut foliages were held in glass vials containing water so as to maintain the freshness of the leaves. Once in two days, the foliage was changed and observations on the numbers of larvae survived and feeding damage (if any) were noted. The plants were monitored daily. Because no larvae survived beyond 7 days on many plants other than the two *V. nilotica* subspecies and three *Acacia* species, the tests were terminated at this stage.

The experiment was also replicated with potted plants to confirm that the larvae did not respond differently in terms of survival and damage caused on cut foliage and growing, whole plants. In this experiment, all the 10 species were tested. The test plants used were raised and maintained in 20 x 30 cm poly pots in nursery. Unfed 10 neonate larvae were placed on a potted plant of each plant species including the two subspecies of *A. nilotica*. Five replications were maintained for each test. The plants were monitored daily and observation on the duration of larva, pupal survival and proportion of larvae and pupae developing into pupae and adults, respectively, were recorded.

RESULTS AND DISCUSSION

Biology

A female moth lay (60- 70) 64 ± 2.01 (n=10) eggs in its lifetime of 10 days. More than 90% of the eggs laid were fertile, and the eggs hatched in 3-4 days (4.01± 0.08). Newly hatched neonates fed on tender foliage and it appeared to be important that they had access to thorns on which to rest. There were five larval instars which last for about 15-17 days (16.42 ± 0.12) and the developing larvae fed on the leaves causing complete defoliation of potted prickly acacia plants in the insectary. As the larvae matured they dropped in to the floor of the cage ground (cage floor) and pupated. A male and a female pupae weighed to be 0.05g and 0.07 g, (n=50) respectively. The pupal duration lasted 5- 7 days (6.10 ± 0.11). Newly emerged moth had a wing span of about 22 mm was dirty whitish in colour with tree transverse brownish bands on the forewings.

Host range testing

Under laboratory conditions adults indiscriminately laid eggs all over the surfaces, including insect-proof cage walls. Hence, oviposition tests could not be conducted in the no- choice method using cut foliage and live plant. In the cut foliage test, a very few larvae (<10%) survived for 5 days on 6 of the 11 test plants. Feeding damage was not easy to detect in first instar stage, particularly on the older pinnate of plants. The presence or absence of frass in the base of the culture cages appeared to be the best indicator of feeding. There appeared to be no larval feeding and development on *S. mellifera*, *S. catechu*, *S. ferruginea*, *V. auriculiformis*, *V. farnesiana* and *D. regia* and all larvae died within 4 days on cut foliage and live plants (Fig. 2). Larval survival at 17 days on the control plants, *V. nilotica* ssp. *indica* and *V. nilotica* ssp. *tomentosa*, was 99% and 94%, respectively, and this difference was not significant (One-way Anova; $P > 0.05$). The larvae also fed and developed on the *V. tortilis*, *V. planiferons* and *V. leucophloea* (Fig. 1). On these plants, frass was evident in the base of the culture cages. However, there was also high larval mortality of 90%, 70% and 70% on *V. leucophloea*, *V. tortilis* and *V. planiferons*, respectively (Fig. 3). About 10% of larval mortality was also evident in *V. nilotica* ssp. *tomentosa* whereas all larvae developed normally on *V. nilotica* subsp. *indica*. Larval duration of *I. disputaria* varied significantly among the test plant species used in the study. The duration of larval survival of *I. disputaria* was significantly longer on *V. nilotica* ssp. *indica* (17 ±0.02 days) and *V. nilotica* ssp. *tomentosa* (16.8± 0.13 days) than on the non-target plants: *V. leucophloea* (5.2±0.54 days), *V. deanei* (2.7 ± 0.29 days), *V. auriculiformis* (1.7 ± 0.11 days), *S. mellifera*(3.1± 0.34 days), *S. catechu* (1.9± 0.16 days), *S. ferruginea* (2.8± 0.08 days), *V. farnesiana* (3.3± 0.08 days) and *D. regia* (1.9± 0.19 days) (One-Way ANOVA, $F = 156.2$, $P < 0.01$).

Host range of this agent under natural conditions in the native range is very host specific and gave no indication that it would be able to utilize any hosts other than *Acacia nilotica* sub species. Subsequent no-choice host specificity test revealed that larvae of *I. disputaria* completed development on five species, including control all in the genus acacia: *V. nilotica* ssp. *indica*, *V. nilotica* ssp. *tomentosa*, *V. planiferons*, *V. leucophloea* and *V. tortilis*. However the life span of *I. disputaria* varied on these species in respect of larval duration, larval mortality, pupal duration, pupal mortality and reproductive efficiency. Similar host specificity study with the species *V. tortilis* by Marohasy, 1992 and Palmer, 2003 complement the results obtained in the present study.

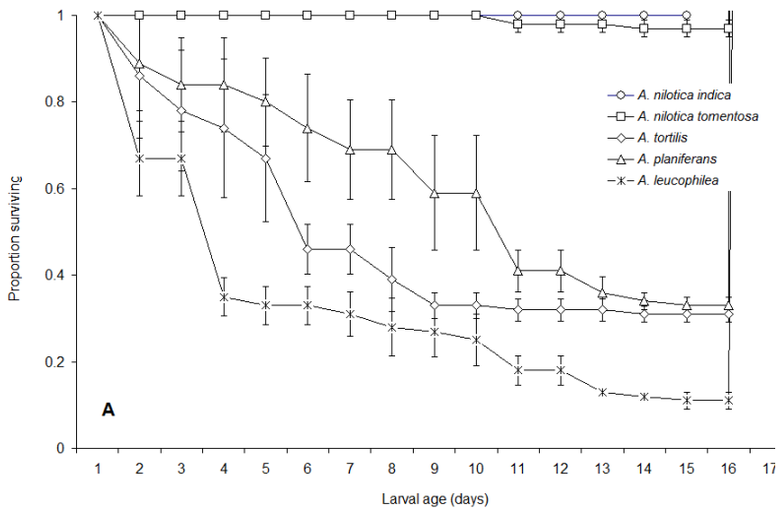

Fig. 1. Proportion of *Isturgia disputaria* larvae surviving on various test plants by no-choice host specificity tests over time. Mean of five replicates (± standard error)

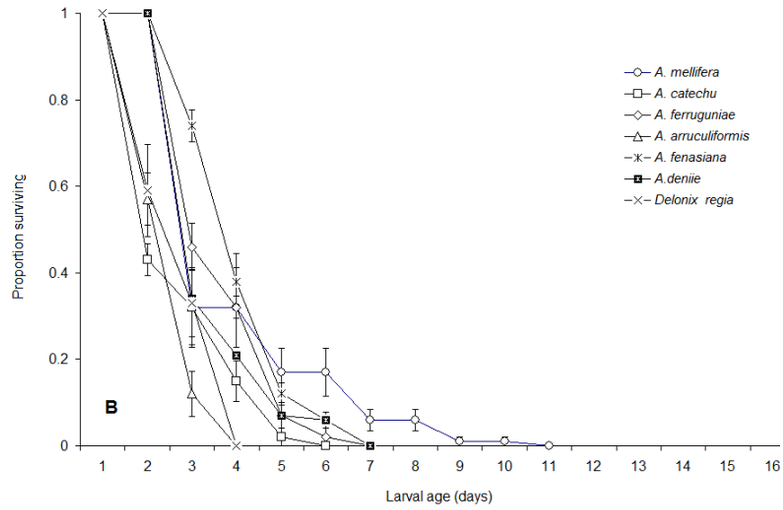

Fig. 2. Proportion of *Isturgia disputaria* larvae surviving on various non-target plants in no-choice host specificity tests over time. Mean of five replicates (± standard error).

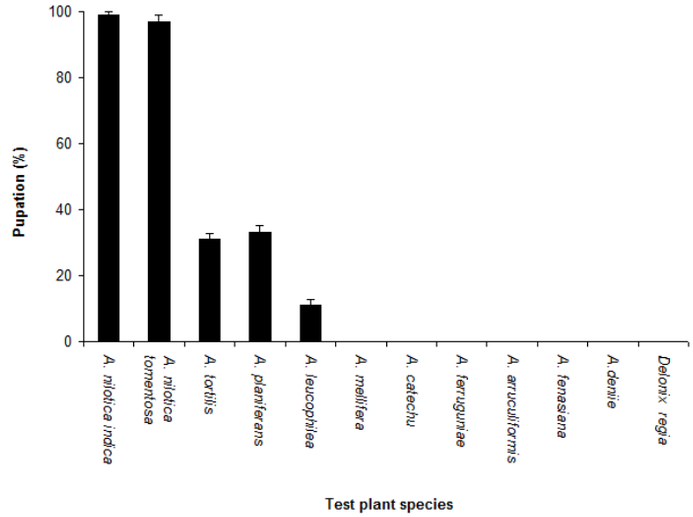

Fig. 3. Proportion of *Isturgia disputaria* larvae that developed into pupae on various non-target plants in no-choice host specificity tests. Mean of five replicates (± standard error).

The geometrid moth *I. disputaria* is a prospective agent for biocontrol of prickly acacia, *V. nilotica* ssp. *indica*. This insect is known from various subspecies of *V. nilotica* in both Africa and the Indian subcontinent. There are also a few specimens extant that were purportedly collected in Africa from the Australian species *V. mearnsii* De Wild. and *V. decurrens* Willd. but details of these collections are unknown (Palmer, 2003).The present study corroborate the view of the above authors that the insect species *I. disputaria* could feed on the species of *V. planiferons*, *V. leucophloea* and *V. tortilis* under forced condition in the no choice tests carried out in the lab. On no occasion occurrence of the insect was noticed on any of the acacia species including the test species which were available in adequate number in the field. Further the shorter larval duration, higher larval and pupal mortality, longer pupal period and no reproduction on the non target host plants strongly support that the insect species cannot successfully multiply and complete number of generations. As the species is able to develop and complete number of generations without any hindrance on *A. nilotica* ssp. *indica* and cause complete defoliation on seedlings and saplings, use of this species as a potential biocontrol agent at least at selective areas infested by *V. nilotica* ssp. *indica*, where the other non target acacia species are not co-occurring, can be thought of.

ACKNOWLEDGEMENTS

We thank Meat and Livestock Australia for funding the study and Dr N. Krishnakumar (Director, IFGTB) for the facilities and support.

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