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The introduction and release of *Chiasmia inconspicua* and *C. assimilis* (Lepidoptera: Geometridae) for the biological control of *Acacia nilotica* in Australia

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Abstract

Two geometrid moths *Chiasmia inconspicua* and *Chiasmia assimilis*, identified as potential biological control agents for prickly acacia *Acacia nilotica* subsp. *indica*, were collected in Kenya and imported into quarantine facilities in Australia where laboratory cultures were established. Aspects of the biologies of both insects were studied and CLIMEX® models indicating the climatically favourable areas of Australia were developed. Host range tests were conducted using an approved test list of 74 plant species and no-choice tests of neonate larvae placed on both cut foliage and potted plants. *C. inconspicua* developed through to adult on prickly acacia and, in small numbers, *Acacia pulchella. C. assimilis* developed through to adult on prickly acacia and also in very small numbers on *A. pulchella, A. deanei, A. decurrens*, and *A. mearnsii.* In all experiments, the response on prickly acacia could be clearly differentiated from the responses on the non-target species. Both insects were approved for release in Australia. Over a three-year period releases were made at multiple sites in north Queensland, almost all in inland areas. There was no evidence of either insect's establishment and both colonies were terminated. A new colony of *C. assimilis* was subsequently established from insects collected in South Africa and releases of *C. assimilis* from this new colony were made into coastal and inland infestations of prickly acacia. Establishment was rapid at one coastal site and the insect quickly spread to other infestations. Establishment at one inland area was also confirmed in early 2006. The establishment in coastal areas supported a CLIMEX model that indicated that the climate of coastal areas was more suitable than inland areas.

Keywords: Biological control; Prickly acacia; *Acacia nilotica*; *Chiasmia inconspicua*; *Chiasmia assimilis*

1. Introduction

Prickly acacia, *Acacia nilotica* subsp. *indica* (Benth.) Brenan, has become one of the worst woody weeds of northern Australia. It was introduced from the Indian sub-continent as a shade and fodder tree but now infests over 7 million hectares of the Mitchell grass downs (Fig. 1) of western Queensland (Mackey, 1997), an area being rapidly converted from a natural grassland into a thorny scrubland similar to the African thornveldt. Small infestations of the weed also exist in coastal areas of Queensland between Bowen and Townsville (Fig. 1). These infestations are significant because coastal areas are climatically more favourable for the plant than the more arid western areas (Kriticos et al., 1999, 2003).

Prickly acacia was recognised as a serious weed after a dramatic increase in its abundance in the mid-1970s and, subsequently, investigations commenced to find biological control agents. As part of the biological control project, studies of the insects associated with *A. nilotica* have been undertaken in Pakistan (Mohyuddin, 1981), Kenya (Marohasy, 1995) and South Africa (R. Stals, Plant Protection Research Institute, unpublished). The significance of these and other studies in relation to biological control of prickly

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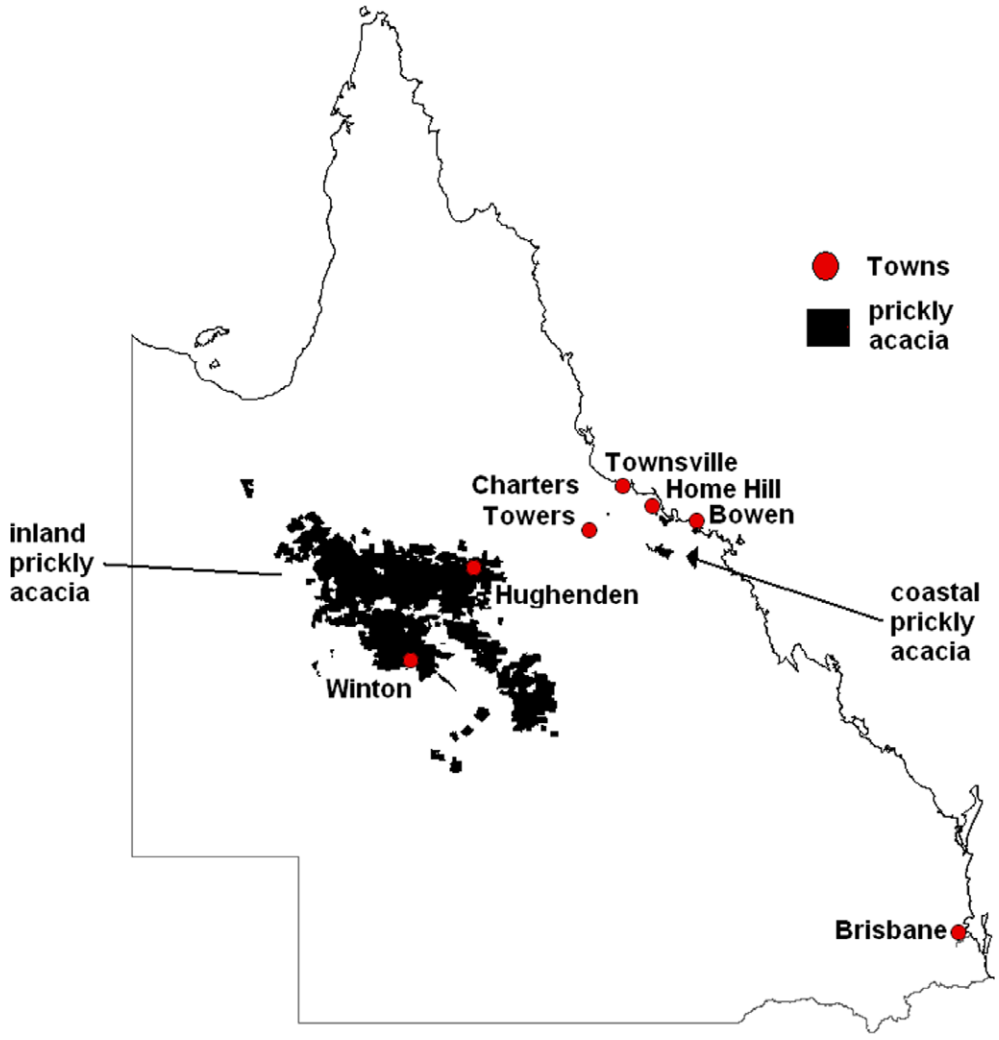

Fig. 1. The distribution of prickly acacia in Queensland.

acacia and collecting technique has been discussed elsewhere (Marohasy, 1995; Palmer, 1996).

Prickly acacia is one of nine subspecies of *A. nilotica* (L.) Willd. Ex Del.; three being from the Indian sub-continent and six from Africa (Ross, 1979; Brenan, 1983). *Acacia*, with over 1100 species worldwide, is the largest genus (with over 950 endemic species) of flowering plants in Australia (Orchard and Wilson, 2001a) and the numerical and ecological importance of the genus presents particular issues for proposals to introduce biological control agents for prickly acacia. *Acacia* has been divided into three subgenera, namely subgenus *Acacia*, subgenus *Phyllodineae* and subgenus *Aculeiferum* (Maslin, 2001). *A. nilotica* belongs to the subgenus *Acacia*, which is represented in Australia by nine native species, and two introduced species (*A. nilotica* and *A.farnesiana*) (Maslin, 2001). All of these 11 species have bipinnate leaves and stipular spines, and are generally found in tropical areas. Except for two endemic species in the subgenus *Aculeiferum*, the remaining Australian *Acacia* spp. all belong to subgenus *Phyllodineae* (Maslin, 2001). The subgenus *Phyllodineae* comprises seven sections; the leaves of five of these sections being phyllodinous while the remaining two sections, *Botrycephalae* and *Pulchellae*, are bipinnate.

Because the genus *Acacia sens. lat*. is recognised as polyphyletic and its major groups warrant generic status (Maslin, 2001), the Nomenclature Session of the XVII International Botanical Congress in Vienna, Austria, voted in 2005 to conserve the name *Acacia* with a new type, as proposed by Orchard and Maslin (2003), to enable the splitting of *Acacia sens. lat.* into five genera. Under the proposed system the present subgenus *Acacia* will become the genus *Vachellia* Wight & Arn. and the Australian subgenus *Phyllodineae* will become the genus *Acacia*. New combinations for the New World (Seigler and Ebinger, 2006) and Australian (Kodela and Wilson, 2006) species of *Vachellia* have recently been proposed but African and Asian species have not yet been treated. However, because the transition is presently incomplete, and indeed may be contested, the nomenclature of the Flora of Australia (Orchard and Wilson, 2001a; Orchard and Wilson, 2001b) is adhered to in this paper.

One promising group of insects for biological control encountered in all three regions surveyed for biocontrol agents was the geometrid tribe Macariini (Lepidoptera: Geometridae: Ennominae), represented by its two largest genera *Chiasmia* Hübner and *Isturgia* Hübner. The Macariini are cosmopolitan though poorly represented in Australia (Scoble and Kruger, 2002). The moths are predominantly nocturnal. Legumes are the most frequent food plants and legume feeding appears to be the primitive condition within Macariini (Scoble and Kruger, 2002). The group appears to be originally from the Afrotropical region from whence it dispersed into the Palaearctic and later to the Nearctic, with dispersal accompanied by several host shifts to deciduous trees and conifers (Scoble and Kruger, 2002). Two *Isturgia* spp. were investigated as possible biological control agents for prickly acacia. *Isturgia deerraria* (Walker), previously reported as *Tephrina ? presbitaria* by Marohasy (1995), was found to develop on several Australian *Acacia* spp. and *Delonix regia* and was rejected as a biocontrol agent (Palmer and McLennan, 2006). Populations of *I. disputaria* were also found to be insufficiently host-specific (Palmer, unpublished report).

Chiasmia is an Old World genus and is thought to contain 265 species throughout the eastern Palaearctic, Afrotropical, and Indo-Australian regions (Scoble and Kruger, 2002). Hosts are known for only 26 of the 173 Afrotropical species and most of these are leguminous, particularly various *Acacia* spp. (Kruger, 2001). Non-leguminous hosts (for 1 species each) recorded are *Tamarix* (Tamaricaceae), *Sterculia* (Sterculiaceae), *Hippobromus* (Sapindaceae), and *Mallotus* (Euphorbiaceae), respectively. No congener is considered a pest of tropical leguminous food crops (Singh, 1990) but *C. clathrata* (L.) is considered a pest of *Medicago sativa* and *Trifolium* spp. in Europe (Zang, 1994).

Four *Chiasmia* spp. have an association with *A. nilotica*. *Chiasmia streniata* (Guenée) is known from *A. nilotica* (presumably *A. nilotica* subsp. *indica*) in India (Browne, 1968) and is also known to occur in Africa on *Acacia, Hippobromus* and *Albizia* (Kruger, 2001). *Chiasmia furcata* (Warren) is a southern African species found on *A. nilotica* and *A. karroo* Hayne (Kruger, 2001). *Chiasmia assimilis* (Warren) [=*Semiothisa assimilis* (Warren)] is found in southern Africa from Durban to northern Zimbabwe, East Africa, Zaire and Cameroon. It has only been taken from *A. nilotica* but from all three subspecies common in these areas: *A. nilotica* subsp. *kraussiana* (Benth.) Brenan, *A. nilotica* subsp. *subalata* (Vatke) Brenan and *A. nilotica* subsp. *leiocarpa* Brenan. *Chiasmia inconspicua* (Warren) [=*Semiothisa inconspicua* (Warren)] was treated as two subspecies with disjunct distributions by Kruger (2001); *C. inconspicua inconspicua* (Warren) which was found in southern Africa (South Africa, Botswana, northern Namibia, Zimbabwe and Mozambique) and *C. inconspicua pertaesa* (Prout) which was found in East Africa. However Dr. Kruger (personal communication) now regards the distributions as disjunct only because of paucity of collecting and has recommended not recognising the subspecies. *C. inconspicua* has the same observed host range as *C. assimilis*.

This paper describes the studies undertaken and procedures employed to introduce *C. assimilis* and *C. inconspicua* into Australia for the biological control of prickly acacia. The insects had first to be introduced into quarantine facilities in Australia where comprehensive host specificity testing was undertaken and their biologies studied. Models were also developed to determine climatic suitability. After the insects were approved for release in Australia they were then mass reared and released throughout the range of the weed.

2. Materials and methods

2.1. Insect source and laboratory culture

Both *C. inconspicua* and *C. assimilis* were collected from both *A. nilotica* subsp. *leiocarpa* and *A. nilotica* subsp. *subalata* growing near Voi, Coast State, Kenya (3°24′S; 38°35′E), in May 1997. The collected larvae were airfreighted to Brisbane where they were housed in the quarantine facility of the Alan Fletcher Research Station. The identities of both species were confirmed from extensive series of pinned specimens by Dr. M. Kruger. Voucher specimens were lodged with the Australian National Insect Collection, Canberra. Both species were reared through approximately 20 generations during the course of these studies by either feeding larvae cut foliage of prickly acacia (earlier generations) or maintaining on potted plants (later generations). These colonies were used for all host specificity studies reported in this paper and were subsequently released from the Tropical Weed Research Centre. A second importation of *C. assimilis* was made from several locations in South Africa in 2002 (Wardill et al., 2004), with insects collected from *A. nilotica* subsp. *kraussiana* (Benth.) Brenan. A new colony was established at the Tropical Weeds Research Centre from this importation.

2.2. Biology studies

During the course of the host specificity studies, various observations and measurements were made about the biology and life cycle of the two insects. Eggs were measured using a microscope and a calibrated eye piece graticule. Freshly sclerotised pupae were weighed and stored in individual vials and followed through to emergence to determine sex and time taken to emerge. Two cohorts of 19 and 13 pairs of male and female moths of *C. assimilis* and *C. inconspicua*, respectively, were kept in individual plastic containers and oviposition recorded at intervals until the death of the females. Females were then dissected and the remaining mature eggs present in the ovarioles were counted.

2.3. Host range testing

The insects were tested against a list of species closely related to prickly acacia and selected according to the

centrifugal phylogenetic method (Wapshere, 1974). Because *Acacia* spp. comprise a very significant segment of Australia's natural flora and because many other leguminous species are agriculturally significant, the test list of 67 species approved by the regulatory authorities was relatively long. Extra species were also tested for various reasons so that 74 species (Table 1) were eventually tested.

Most tests were conducted using cut foliage. In each test, 10 unfed neonates were exposed to a sprig of cut foliage of one test plant placed on a moistened filter paper in a Petri dish. The foliage was changed every 3 days and the number of surviving larvae recorded. The tests were conducted in batches of 10–15 plant species, with each batch including one test on *A. nilotica* subsp. *indica* as a control. Three replications of each plant species on the host test list were conducted. In each test, the number of larvae surviving at 6 days, the number of larvae successfully pupating, the number of moths ultimately emerging, the mean development time from neonate to pupation and the pupal weights (*C. assimilis* only) were recorded or calculated.

The ability of larvae to develop on potted plants was also tested. Thirteen plant species were selected from the cut foliage list, with most coming from groups thought more likely to be susceptible to these insects (i.e. subgenus *Acacia* and section *Botrycephalae*). Twenty neonates were placed on the foliage of each plant. A fine mesh bag was placed over them so that they could be more easily observed and recovered. Surviving larvae were counted on the sixth day. Any resultant pupae were counted, weighed and the mean larval development time calculated. Two replications of all 13 plants were conducted.

2.4. Climate matching for australian release

The climate matching model CLIMEX® Version 2 (Skarratt et al., 1995; Sutherst et al., 2004) was used to assess where each insect was likely to be effective in Australia and as an aid to the selection of release sites. This model uses an integrated climate index to describe the climate suitability of an area for each species. The climate profile of each insect was first determined by recursively trying various sets of parameter values until the model's distribution matched the known distribution of the insect in Africa. The set of parameters was then used to predict the potential distribution of each insect in Australia.

2.5. Mass rearing and release

Application was made to the Australian Quarantine Inspection Service (AQIS) and the Department of Environment and Heritage (DEH) to release both insects in Australia. As soon as approval to release was obtained from these authorities, the insects were shipped to the Tropical Weeds Research Centre in Charters Towers for mass rearing.

The mass rearing procedure involved exposing 4–8, 40–60 cm tall, potted plants in 90 cm × 55 cm × 87 cm gauze-covered cages to 80–100 unsexed moths for 6–7 days to allow oviposition. Plants with eggs were transferred to new cages where resultant larvae developed to pupation some 15 days later. Pupae were then collected from cage floors.

Three methods were used to release the Kenyan populations of both species. Shipments of approximately 500 pupae and a simple release kit (hanging container for the pupae and an information sheet) were sent to departmental officers and cooperating landholders for placement at field sites spread across the western areas. Multiple, free (uncaged) releases of moths were undertaken at 4 main sites on properties near Hughenden, Julia Creek and Winton and steel framed, gauze covered field cages, approximately 1.5 × 1.5 × 2 m, which covered a small tree were also trialled by staff and landholders at these sites. Progeny of the colony of *C. assimilis* from South Africa was released by larger, multiple, free releases at one coastal site near Bowen and two western sites near Hughenden.

Table 1
Attributes of *Chiasmia inconspicua* and *C. assimilis* (mean ± se)

Attribute	*Chiasmia inconspicua*	*Chiasmia assimilis*
Egg length (mm)	0.57 ± 0.004 ($n = 18$)	0.57 ± 0.005 ($n = 13$)
Egg width (mm)	0.35 ± 0.005 ($n = 18$)	0.38 ± 0.003 ($n = 13$)
Egg duration (days)	4	4
Larval duration (days)	14.56 ± 0.707 ($n = 104$)	13.75 ± 0.314 ($n = 103$)
Larval survival (percent)	65.0 ($n = 160$)	68.7 ($n = 150$)
Pupal weight—male (mg)	34.9 ± 1.09 ($n = 13$)	50.85 ± 1.42 ($n = 20$)
Pupal weight—female (mg)	43.8 ± 1.26 ($n = 13$)	51.92 ± 1.30 ($n = 20$)
Pupal duration—male (days)	6.9 ± 0.12 ($n = 15$)	9.0 ± 0
Pupal duration—female (days)	7.2 ± 0.11 ($n = 14$)	8.8 ± 0.09
Pupal survival	82.7 ($n = 104$)	87.4 ($n = 103$)
Male adult longevity (days)	—	9.17 ± 0.58
Female adult longevity (days)	—	9.95 ± 0.47
Preoviposition period (days)	1	1
Oviposition pattern	$\text{Eggs} = (1/0.005) * e^{(-0.048 * (\text{Day}-5.271)^2)}$	$\text{Eggs} = (1/0.008) * e^{(-0.053 * (\text{Day}-6.440)^2)}$
Fecundity (eggs per female)	533.3 ± 36.3 ($n = 13$)	436.9 ± 40.4 ($n = 19$)

Table 2
The survival and development of *Chiasmia inconspicua* and *C. assimilis* larvae fed on bouquets of cut foliage

Plant	*Chiasmia inconspicua*					*Chiasmia assimilis*				
	% Survival at 6 days	% Pupation	Larval development time (days)	Pupal weight (mg)	% Adult eclosion	% Survival at 6 days	% Pupation	Larval development time (days)	Pupal weight (mg)	% Adult eclosion
Family Mimosaceae										
Tribe Acacieae										
Genus *Acacia*										
Subgenus *Acacia*										
Section *Acacia*										
A. bidwillii Benth.	0	0	—	—	0	0	0	—	—	0
A. ditricha Pedley	0	0	—	—	0	0	0	—	—	0
A. farnesiana (L.) Willd.*	0	0	—	—	0	0	0	—	—	0
A. nilotica subsp. *indica**	73	59	15.9	36.8	49	84	69	14.9	55.0	61
A. pallidifolia Tindale	0	0	—	—	0	0	0	—	—	0
A. sutherlandii (F. Muell.) F. Muell	0	0	—	—	0	0	0	—	—	0
A. suberosa A. Cunn. ex Benth.	0	0	—	—	0	0	0	—	—	0
Subgenus *Phyllodineae*										
Section *Alatae*										
A. alata R. Br.	0	0	—	—	0	0	0	—	—	0
Section *Botrycephalae*										
A. cardiophylla A. Cunn. ex Benth.	0	0	—	—	0	0	0	—	—	0
A. deanei (R. T. Baker) Welch, Coombs & McGlynn	0	0	—	—	0	13	3	19.0		3
A. decurrens Willd.	8	0	—	—	0	17	7	21.5		3
A. filicifolia Cheel & Welch	0	0	—	—	0	0	0	—	—	0
A. glaucocarpa Maiden & Blakely	0	0	—	—	0	0	0	—	—	0
A. irrorata Seib. ex Streng.	0	0	—	—	0	0	0	—	—	0
A. mearnsii De Wild.	0	0	—	—	0	3	0	—	—	0
A. oshanesii F. Muell. & Maiden	0	0	—	—	0	0	0	—	—	0
A. parramattensis Tind.	0	0	—	—	0	0	0	—	—	0
A. spectabilis A. Cunn. ex Benth.	0	0	—	—	0	3	3	20		0
Section *Juliflorae*										
A. aneura F. Muell. ex Benth.	0	0	—	—	0	0	0	—	—	0
A. holosericea A. Cunn. ex G. Don	0	0	—	—	0	0	0	—	—	0
A. lysiphloia F. Muell.	0	0	—	—	0	0	0	—	—	0
A. mangium Willd.	0	0	—	—	0	0	0	—	—	0
A. plectocarpa A. Cunn. ex Benth.	0	0	—	—	0	0	0	—	—	0
Section *Lycopodiifoliae*										
A. spondylophylla F. Muell.	0	0	—	—	0	0	0	—	—	0
Section *Pulchellae*										
A. drummondii Lindley	0	0	—	—	0	0	0	—	—	0
A. lasiocarpa Benth.	0	0	—	—	0	0	0	—	—	0
A. lateriticola Maslin	0	0	—	—	0	0	0	—	—	0
A. pulchella R. Br.	67	40	22.3	22.3	23	33	10	25	34	7
Section *Phyllodineae*										
A. conferta A. Cunn. ex Benth.	0	0	—	—	0	0	0	—	—	0
A. macradenia Benth.	0	0	—	—	0	0	0	—	—	0

A. podalyriifolia A. Cunn. ex G. Don	0	0	—	—	0	0	0	—	—	0
A. tetragonophylla F. Muell.	0	0	—	—	0	0	0	—	—	0
Section Plurinerves										
A. harpophylla F. Muell. Ex Benth.	0	0	—	—	0	0	0	—	—	0
A. coriacea DC.	0	0	—	—	0	0	0	—	—	0
A. flavescens A. Cunn. ex Benth.	0	0	—	—	0	0	0	—	—	0
A. simsii A. Cunn. ex Benth.	0	0	—	—	0	0	0	—	—	0
A. stenophylla A. Cunn. ex Benth.	0	0	—	—	0	0	0	—	—	0
Subgenus Aculeiferum										
Section Filicinae										
*A. angustissima** (Mill.) Kuntze	0	0	—	—	0	0	0	—	—	0
Tribe Mimoseae										
Adenanthera pavonine L.	0	0	—	—	0	0	0	—	—	0
Albizia procera (Roxb.) Benth.	0	0	—	—	0	0	0	—	—	0
Cathormion umbellatum (Vahl) Kosterm.	0	0	—	—	0	0	0	—	—	0
Dichrostachys spicata (F.Muell.) Domin	0	0	—	—	0	0	0	—	—	0
Entada phaseoloides (L.) Merr.	0	0	—	—	0	0	0	—	—	0
*Leucaena leucocephala** (Lam.) de Wit	0	0	—	—	0	0	0	—	—	0
Neptunia gracilis Benth.	0	0	—	—	0	0	0	—	—	0
Prosopis pallida * (Humb. & Bonpl. ex Willd.) Kunth	0	0	—	—	0	0	0	—	—	0
Tribe Ingeae										
Pararchidendron pruinosum (Benth.) I.C.Nielsen	0	0	—	—	0	0	0	—	—	0
Paraserianthes lophantha (Willd.) I.C.Nielsen	0	0	—	—	0	0	0	—	—	0
Family Caesalpinaceae										
Tribe Caesalpinieae										
Caesalpinia ferrea Tulasne*	0	0	—	—	0	0	0	—	—	0
Delonix regia (Bojer ex Hook.) Raf.*	0	0	—	—	0	0	0	—	—	0
Tribe Cassieae										
Cassia brewsteri (F.Muell.) Benth.	0	0	—	—	0	0	0	—	—	0
Labichea lanceolata Benth.	0	0	—	—	0	0	0	—	—	0
Senna barclayana (Sweet) Randell	0	0	—	—	0	0	0	—	—	0
Tribe Cercideae										
Barklya syringifolia F.Muell.	0	0	—	—	0	0	0	—	—	0
Bauhinia hookeri F.Muell.	0	0	—	—	0	0	0	—	—	0
Tribe Detarieae										
Tamarindus indica L.*	0	0	—	—	0	0	0	—	—	0
Family Fabaceae										
Tribe Aeschynomene										
Arachis hypogaea L.*	0	0	—	—	0	0	0	—	—	0
Tribe Bossiaeeae										
Hovea acutifolia A.Cunn. ex G.Don	0	0	—	—	0	0	0	—	—	0
Tribe Cicereae										
Cicer arietinum L.	0	0	—	—	0	0	0	—	—	0
Tribe Desmodieae										
Desmodium heterophyllum (Willd.) DC.	0	0	—	—	0	0	0	—	—	0
Tribe Galegeae										
Swainsonia formosa	0	0	—	—	0	0	0	—	—	0
Tribe Mirbelieae										
Pultenaea villosa Andrews	0	0	—	—	0	0	0	—	—	0

(*continued on next page*)

Table 2 (*continued*)

Plant	*Chiasmia inconspicua*					*Chiasmia assimilis*				
	% Survival at 6 days	% Pupation	Larval development time (days)	Pupal weight (mg)	% Adult eclosion	% Survival at 6 days	% Pupation	Larval development time (days)	Pupal weight (mg)	% Adult eclosion
Tribe Phaseoleae										
Cajanus cajan (L.) Millsp.*	0	0	—	—	0	0	0	—	—	0
Erythrina vespertilio Benth.*	0	0	—	—	0	0	0	—	—	0
*Glycine max**	0	0	—	—	0	0	0	—	—	0
Lablab purpureus (L.) Sweet*	0	0	—	—	0	0	0	—	—	0
Tribe Sophoreae										
Castanospermum australe A.Cunn. & C.Fraser ex Hook.	0	0	—	—	0	0	0	—	—	0
Tribe Tephrosieae										
Tephrosia glomeruliflora Meisn.	0	0	—	—	0	0	0	—	—	0
Tribe Trifolieae										
Medicago sativa L.*	0	0	—	—	0	0	0	—	—	0
Tribe Vicieae										
*Pisum sativum**	0	0	—	—	0	0	0	—	—	0
Vicia faba L.*	0	0	—	—	0	0	0	—	—	0
Family Sterculiaceae										
Brachychiton acerifolius (A.Cunn. ex G.Don) Macarthur & C.Moore	0	0	—	—	0	0	0	—	—	0
Family Euphorbiaceae										
Mallotus sp.	0	0	—	—	0	0	0	—	—	0

3. Results

3.1. Biology

Both species have very similar biologies. Life history parameters are given in Table 1.

Eggs are green with a slightly flattened ovoid shape and sculptured exochorion. In nature, the eggs of *C. assimilis* have been observed on leaflets. In the laboratory, eggs of both species are collected from gauze where they are found singly or in groups of 2–3, rarely in larger clusters.

Neonates emerge four days after oviposition at 25 °C. They readily climb the sides of vials and gather around the lips of the vial from where they hang from silken strands if disturbed. Movement on these strands may well be a dispersal mechanism. Larvae fed on excised foliage develop through to pupation in 14–15 days in a laboratory kept at 25 °C. Early instars are green and the last instar is brown. The last instar wanders from the foliage or plant to pupate in soil or litter.

Pupae are initially greenish but darken with age. Although male and female pupae were of similar weight (50.9 and 51.9 mg, respectively) in *C. assimilis*, with *C. inconspicua* the male pupae (34.9 mg) were significantly lighter than the females (43.8 mg).

Adults of *C. inconspicua* and *C. assimilis* emerged 7 and 9 days, respectively, after pupation at 25 °C and the *C. assimilis* moths lived for 10 days. Oviposition commenced 1 day after eclosion of the moth and continued for a further 4–7 days. In the laboratory, female *C. inconspicua* and *C. assimilis* oviposited 533 ± 36 and 436 ± 40 eggs, respectively, with maximum oviposition occurring on day 3. Mathematical functions were developed to describe the oviposition pattern with respect to time (Table 1). Both species oviposited indiscriminately on the sides of cages, gauze strips and any plant matter.

3.2. Host range testing

Both species proved to have very narrow host ranges.

In the cut foliage tests (Table 2), *C. inconspicua* developed only on *A. nilotica* and *A. pulchella*, with 49% and 23%, respectively, of neonates surviving to emerge as moths. Those developing on *A. pulchella* also had a longer development time of 22.3 days compared to that of 15.9 days on *A. nilotica.* A few larvae (8%) also survived for 6 days on *A. decurrens* but there was complete mortality by the 6th day on all other test plants. When potted plants were used (Table 3), 50% survived to adult on *A. nilotica*, only 2% on *A. pulchella* and there was no survival on any of the other 11 plant species. Again the development time on *A. pulchella* (26.5 days) was longer than that on *A. nilotica* (19.1 days).

In cut foliage tests (Table 2), 61% of *C. assimilis* neonates developed to emerge as moths on *A. nilotica* whereas only 7%, 3% and 3% survived to adult eclosion on *A. pulchella, A. decurrens* and *A. deanei* and in these cases there were

Table 3
The survival and development of *Chiasmia inconspicua* and *C. assimilis* larvae on potted plants

Plant species	*Chiasmia inconspicua*			*Chiasmia assimilis*			
	% Pupation	Larval development (days)	% Adult eclosion	% Pupation	Pupal weight (mg)	Larval development (days)	% Adult eclosion
Acacia nilotica	50	19.1	50	73	56.7	14.7	70
Acacia bidwillii	0	—	0	0	—	—	0
Acacia farnesiana	0	—	0	0	—	—	0
Acacia deanei	0	—	0	0	—	—	0
Acacia decurrens	0	—	0	5	—	23.0	5
Acacia mearnsii	0	—	0	3	43.0	22.0	3
Acacia pulchella	3	26.5	2	0	—	—	0
Acacia plectocarpa	0	—	0	0	—	—	0
Acacia conferta	0	—	0	0	—	—	0
Acacia flavescens	0	—	0	0	—	—	0
Acacia angustissima	0	—	0	0	—	—	0
Delonix regia	0	—	0	0	—	—	0
Arachis hypogaea	0	—	0	0	—	—	0

associated longer development times and lower pupal weights. There was no survival on the other 70 species. In the potted plant experiment there was 70% adult eclosion from neonates placed on *A. nilotica* and 3% and 5% eclosion on *A. mearnsii* and *A. decurrens*, respectively. There was no survival on the other 10 species tested, including *A. pulchella.*

3.3. Climate matching for australian release

The CLIMEX climate profiles of both *C. inconspicua* and *C. assimilis* were obtained using two growth indices (temperature and moisture) and four stress indices (wet, dry, heat and cold). These were used to identify the climatically suitable areas for the insects in Australia (Figs. 2 and 3). The two insects had similar climate profiles though *C. inconspicua* was more cold tolerant (because it is found in inland areas of South Africa, Botswana and Namibia). The models also suggested that both insects would be largely confined to northern areas of Australia and that coastal areas of Queensland would be more suitable than areas further west.

3.4. Mass rearing and release

Permission to release *C. inconspicua* and *C. assimilis* in Australia was obtained from the authorities in November 1998 and April 1999, respectively. Both species were mass reared at the Tropical Weeds Research Centre and released over a three-year period from 1999–2001. Approximately 72,000 *C. inconspicua* were distributed at 63 sites and over 74,000 *C. assimilis* were placed at 31 sites. With one or two exceptions, all releases were made on the Mitchell grass downs of western Queensland where the weed is such a problem. In 2002 a second population of *C. assimilis*, from South Africa, was introduced. Over the next two years over 75,000 adults were released at one site on the Mitchell grass downs and two sites on the coast.

3.5. Establishment

Chiasmia inconspicua and *C. assimilis* from Kenya failed to establish in Australia.

South African populations of *C. assimilis* readily established at coastal sites between Home Hill and Bowen. Large numbers of adults and larvae were seen within six months of the first release at the coastal site near Guthalungra. Within a further two months, both adults and larvae had been collected from isolated prickly acacia infestations up to 30 km away. In early March 2005 infestations of prickly acacia between Home Hill and Bowen were heavily attacked by *C. assimilis.* Landholders then reported the presence of outbreak populations of moths and caterpillars, together with large areas of partial to total defoliation of the prickly acacia. Establishment at western sites was confirmed the following year. Small numbers of insects were first found in March 2006 at a release site located 20 km east of Hughenden. By May, numbers had increased greatly at this site and feeding damage was obvious on trees. However widespread defoliation such as occurred on the coast was not observed. During site inspections in June, insects were found at a further two prickly acacia infestations near Hughenden and on isolated plants along the roadside between sites.

4. Discussion

Both insects were shown to have a very restricted host range. Interestingly, neither insect showed any disposition towards any of the other species in the subgenus *Acacia.* On the other hand several species, from both the bipinnate

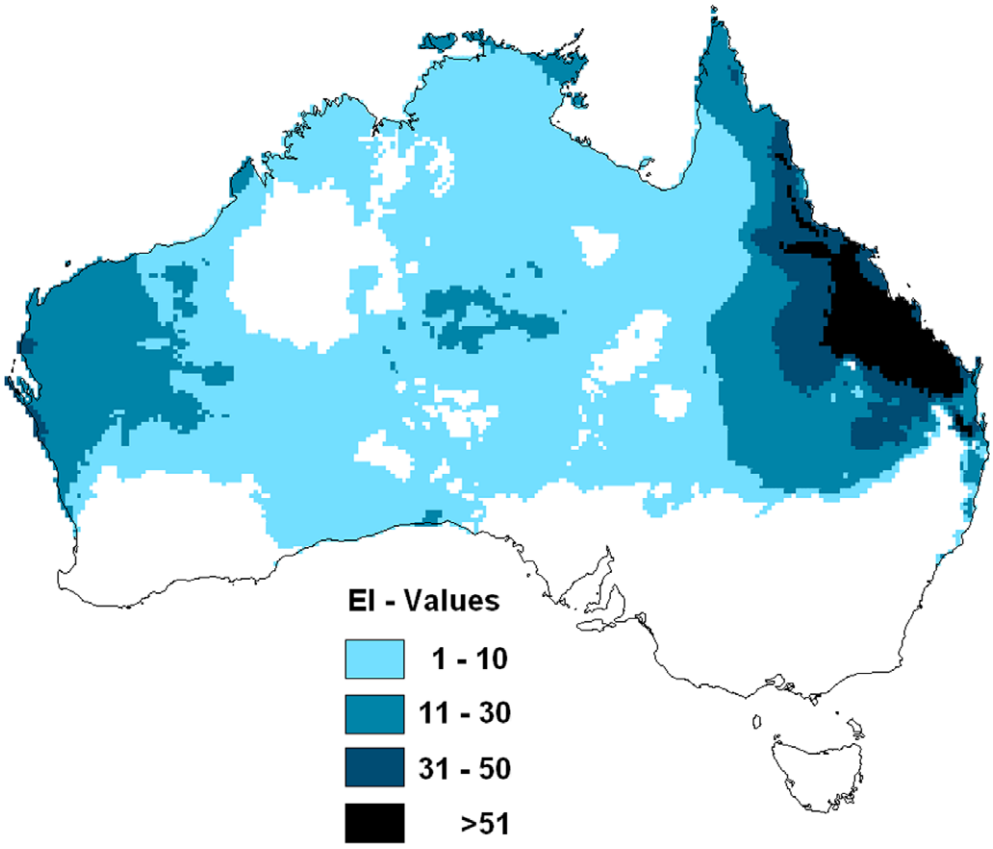

Fig. 2. The climatic suitability of Australia for *Chiasmia inconspicua* estimated by interpolation of Ecoclimatic Index (EI) values derived from a CLIMEX model (Temperature Growth Parameters: DV0 = 13, DV1 = 19, DV2 = 30, DV3 = 35. Moisture Growth: SM0 = 0.05, SM1 = 0.1, SM2 = 0.7, SM3 = 1.0. Cold Stress: TTCS = 8, THCS = −0.0002, DTCS = 20, DHCS = −0.001. Heat Stress: TTHS = 40, THHS = 0.002. Wet Stress: SMWS = 1.0, HWS = 0.001. Dry Stress: SMDS = 0.05, HDS = −0.005. Day Degrees: DVCS = 13). The darker colours and higher EI values indicate a more suitable climate. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

sections *Botrycephalae* and *Pulchellae*, were able to support neonate larvae through to adult eclosion. However when the much higher mortality, longer development times and lower pupal weights were considered, it was unlikely that these *Acacia* spp. would support populations of either species under field conditions. Further, the *Pulchellae* are confined to south-western Western Australia and the *Botrycephalae* are largely found in temperate eastern Australia, whereas the CLIMEX predictions were that both insects would be largely confined to tropical areas of Australia. It was therefore concluded that the insects were safe to release in Australia.

Several factors may have contributed to the failure of Kenyan populations of both insects to establish (Lockett and Palmer, 2004); the species may not have been able to adapt effectively to the Asian subspecies *A. nilotica* subsp. *indica*, there had been many generations between introduction to Australia and release so that the populations may have lost genetic diversity, they were released in climatically unsuitable areas, the predator complex may have been too effective, and the release strategy of single releases at many sites may have been ineffective.

At the time of the first releases there was some concern as to whether both species, which had been collected from *A.n.* subsp. *leiocarpa* and *A.n.* subsp. *subalata*, would successfully establish on *A.n.* subsp. *indica* despite the apparent ease with which they were reared over many generations on *A.n.* subsp. *indica.* The fact that the second importation of *C. assimilis*, collected from *A. n. subsp. kraussiana*, quickly established in Australia together with the fact that these three sub-species are similar to each other and more distant to prickly acacia (Wardill et al., 2005; Palmer and Witt, 2006) suggests that subspecies of the host was unlikely to be the critical factor. Indeed the defoliations caused by *C. assimilis* remain a good example where insects collected from plants other than their natural hosts can be effective biological control agents and support the strategy of searching more widely for suitable agents.

Genetic bottlenecks in laboratory cultures have long been recognised as a possible factor in the failure of biocontrol agents to establish successfully (Wardill et al., 2004). In the case of the Kenyan populations of *C. inconspicua* and *C. assimilis*, both had been cultured for more that 30 generations before the first releases though there were no obvious signs of population deterioration. Later, Wardill (2006) used the South African population of *C. assimilis* to demonstrate that a small loss of fecundity occurred after it was reared through 32 generations.

The CLIMEX models generated for both species clearly indicated that coastal areas would be more climatically

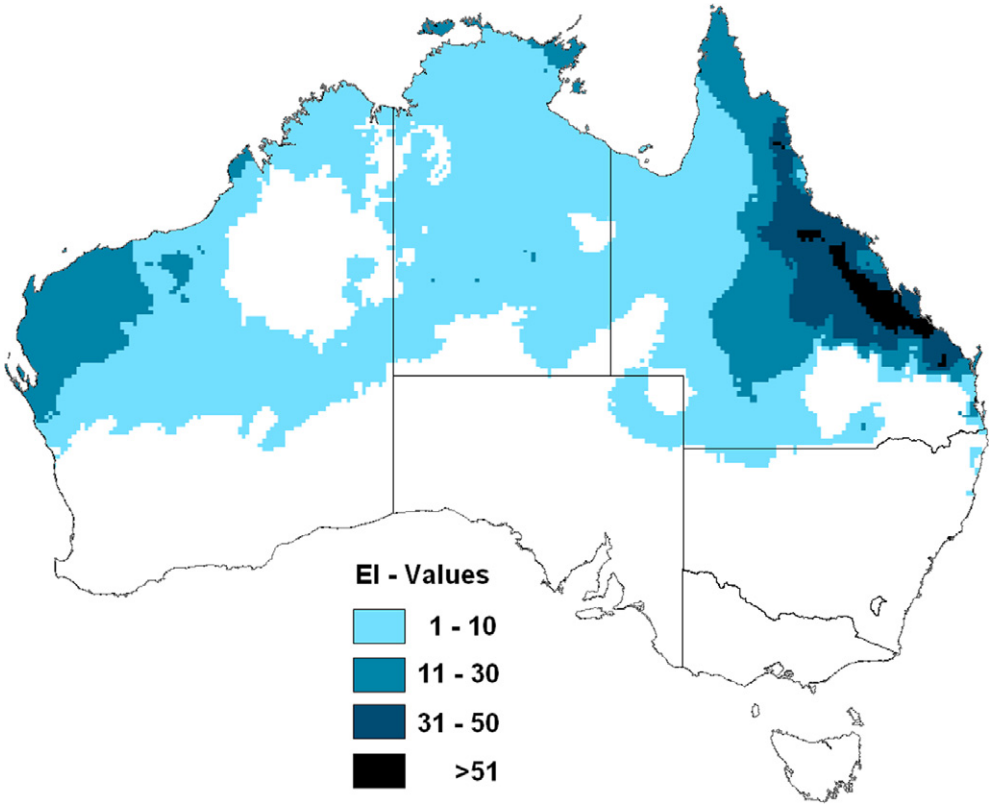

Fig. 3. The climatic suitability of Australia for *Chiasmia assimilis* estimated by interpolation of Ecoclimatic Index (EI) values derived from a CLIMEX model (Temperature Growth Parameters: DV0 = 13, DV1 = 19, DV2 = 30, DV3 = 35. Moisture Growth: SM0 = 0.05, SM1 = 0.1, SM2 = 0.69, SM3 = 0.75. Cold Stress: TTCS = 7, THCS = −0.0009, DTCS = 40, DHCS = −0.0002. Heat Stress: TTHS = 40, THHS = 0.002. Wet Stress: SMWS = 0.9, HWS = 0.001. Dry Stress: SMDS = 0.05, HDS = −0.005. Day Degree: DVCS = 13). The darker colours and higher EI values indicate a more suitable climate. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

favourable than inland areas. At the time of release of the Kenyan insects, efforts were being made to eradicate coastal populations of prickly acacia and releases in these areas were therefore discouraged. Establishment of the South African *C. assimilis* in coastal prickly acacia occurred rapidly and with relative ease and lack of climate suitability now appears the most likely explanation for the early failures to establish in western sites. From this experience it now seems important that releases of biocontrol agents should first be made into areas that are most climatically favourable rather than concentrating where the weed is the worse problem. In that respect reliable climate matching software, such as CLIMEX, is an invaluable tool for any biological control project.

Assessment of effectiveness of predator complexes is a major undertaking that was not pursued rigorously in this project. On occasion, ants were seen to carry off every prepupal larva as it dropped to the ground. However attempts to suppress ant populations around field cages by using Antex® granules (30 g/kg chlorpyrifos) applied directly into nests did not improve success.

There was also no evidence from the release of the *Chiasmia* spp. that the use of field cages improved or promoted the chances of field establishment of either species. Both free and cage releases of the Kenyan insects failed, while both free and cage releases of the South African *C. assimilis* were successful. Similarly there was no evidence to suggest that one release strategy was better than another.

Further studies on *C. assimilis* are presently being undertaken to assess their effectiveness as biocontrol agents.

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