Application for the field-release of *Cecidochares connexa* (Macquart) (Diptera: Tephritidae) for the biological control of *Chromolaena odorata* (L.) King & Robinson (Asteraceae) in Australia

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1.0 Summary

Chromolaena odorata (Asteraceae) is a woody shrub native to tropical America. It flowers prolifically and the lightweight seeds are dispersed by wind, water, wildlife, and people and on their possessions, including machinery. It is now found in many countries in Africa, Asia and the western Pacific, where it invades grasslands, plantations and forests, reducing productivity and biodiversity. It is also present in Australia in north Queensland where it was the target of a national cost-share eradication program until 2012. While current infestations are confined to several river catchments in north Queensland, there is potential for the weed to spread along the Queensland coast south to Sandy Cape, concomitant impacts to agriculture and the environment.

In most countries, control is by slashing as the use of herbicides is beyond the means of most farmers. However, it is labour intensive and regrowth occurs and needs to be treated. Biological control of *C. odorata* first began in 1966, with agents introduced into West Africa. However, they failed to either establish or control the weed. In a new project funded by ACIAR and managed by the Queensland Government, the gall fly *Cecidochares connexa* (Diptera: Tephritidae) was introduced into Indonesia following host specificity testing. The gall fly has since been further tested in three more countries and galls failed to develop on any of the 80 non-target plant species tested. *Cecidochares connexa* has been released in nine more countries and has spread from Cote d'Ivoire to Ghana in West Africa.

In Papua New Guinea where detailed monitoring has occurred, the gall fly has controlled *C. odorata* in most areas in which it has established. *Chromolaena odorata* was declared a target for biological control in Australia when it was thought that eradication was no longer feasible. *Cecidochares connexa* was thought to be useful in trying to control and limit the spread of *C. odorata* in Queensland. Host specificity testing using multispecies choice-minus *C. odorata* and no-choice trials on 18 species, all belonging to the tribe Eupatorieae, was conducted in quarantine at the Ecosciences Precinct, Brisbane. Galls formed on, and adults emerged from *C. odorata* and *Praxelis clematidea*, a minor weed in Queensland. Additional tests, involving paired choice, paired no-choice, multiple generation and time dependency trials all showed that the number of galls formed on, and adults emerged from *P. clematidea* was significantly fewer than that on *C. odorata* and populations of the gall fly could not be maintained.

We believe the damage to populations of *P. clematidea* would be negligible and that *C. connexa* poses minimal risk to other species. This document presents information supporting an application seeking the field release of *C. connexa* for the control of *C. odorata* in Australia.

2.0 Information on target species

2.1 Taxonomy

Order: Asterales
Family: Asteraceae
Tribe: Eupatorieae
Genus: *Chromolaena* DC
Species: *odorata* (L.) King and Robinson

2.1.1 Synonyms

There are numerous synonyms:

Chrysocoma maculata Vell.
Chrysocoma volubilis Vell. Conc.
Eupatorium affine Hook. & Arn.
Eupatorium atriplicifolium Vahl
Eupatorium brachiatum Sw. ex Wikstr.
Eupatorium clematitis DC.
Eupatorium conyzoides Mill.
Eupatorium dichotomum Sch.Bip.
Eupatorium divergens Less.
Eupatorium floribundum Kunth
Eupatorium graciliflorum DC.
Eupatorium incisum Rich.
Eupatorium klattii Millsp.
Eupatorium margaritense var. *glabrescens* Steetz
Eupatorium odoratum L.
Eupatorium sabeanum Buckley
Eupatorium stigmatosum Meyen & Walp.
Osmia atriplicifolia (Vahl) Sch.Bip.
Osmia clematitis (DC.) Sch.Bip.
Osmia conyzoides (Vahl) Small
Osmia divergens (Less.) Sch.Bip.
Osmia floribunda (Kunth) Sch.Bip.
Osmia graciliflora (DC.) Sch.Bip.
Osmia graciliflorum (DC.) Sch.Bip.
Osmia odorata (L.) Sch.Bip.

2.1.2 Common name

Common names vary with country. Historically, it was known as Siam weed but chromolaena is now used globally to remove associations with Thailand. Other common names include bitter bush, Christmas rose and triffid weed. A more detailed list is found in Holm *et al*. (1991).

2.1.3 Close relatives in the Australian region

The genus *Chromolaena* contains 165 species, all of which are tropical American in origin. *Chromolaena* belongs to the tribe Eupatorieae, within the family Asteraceae (King and Robinson 1987). This large family contains many ornamentals but very few crops (Toelken 1983). Within the tribe Eupatorieae, *Stevia rebaudiana* (Bertoni) Bertoni is grown in some countries as a source of sweeteners.

There are 22 species (*Eupatorium serotinum* was eradicated) in the Eupatorieae reported in Australia (ICON 2010; APC CHAH 2011) (Table 1) but only *Adenostemma lavenia* (L.) Kuntze and *Adenostemma macrophyllum* (Blume) DC. are native (Orchard 2011). Three species *Chromolaena squalida* (DC.) King and Robinson, *Gymnocoronis spilanthoides* (D.Don ex Hook. & Arn.) DC. and *Mikania micrantha* Kunth (mile-a-minute) are targets for eradication, although *C. squalida* does not appear to be invasive (DAFF 2012). *Ageratina adenophora* (Spreng.) R.M. King & H. Rob. (crofton weed), *Ageratina riparia* (Regel) R.M. King & H. Rob. (mistflower) and *M. micrantha* are targets for biocontrol in Australia or elsewhere (Winston *et al*. 2014). *Ageratum conyzoides* L. subsp. *conyzoides*, *Ageratum houstonianum* Mill. and *Praxelis clematidea* King and Robinson are exotic and deemed minor weeds. The remaining species are all exotic and none are of economic importance (ICON 2010; APC CHAH 2011).

Table 1. Plant species of the tribe Eupatorieae found in Australia and their status.

Species	**Status**
Adenostemma lavenia (L.) Kuntze	native
Adenostemma macrophyllum (Blume) DC.	native
Ageratina adenophora (Spreng.) R.M. King & H. Rob.	introduced & biocontrol target
Ageratina altissima (L.) R.M. King & H. Rob.	introduced & horticultural
Ageratina ligustrina (DC.) R.M. King & H. Rob.	introduced & naturalised in Victoria
Ageratina riparia (Regel) R.M. King & H. Rob.	introduced & biocontrol target
Ageratum conyzoides L. subsp. *conyzoides*	introduced & minor weed
Ageratum houstonianum Mill.	introduced
*Bartlettina sordida (*syn *Eupatorium megalophyllum)* (Less.) R.M. King & H. Rob.	introduced & horticultural
Chromolaena odorata (L.) R.M. King & H. Rob.	introduced & ex eradication target
Chromolaena squalida (DC.) R.M. King & H. Rob.	introduced & eradication target
Conoclinium coelestinum (L.) DC.	introduced & horticultural
Eupatorium lindleyanum DC.	introduced & horticultural
Eupatorium purpureum (L.)	introduced & herbal
Eupatorium serotinum Michx.	introduced but eradicated
Gymnocoronis spilanthoides (D. Don ex Hook. & Arn.) DC.	introduced & eradication target
Liatris spicata (L.) Willd.	introduced & horticultural
Mikania micrantha Kunth	introduced & eradication target
Praxelis clematidea (Griseb.) R.M. King & H. Rob.	introduced & minor weed
Stevia eupatoria (Spreng.) Willd.	introduced
Stevia ovata Willd.	introduced& minor weed
Stevia rebaudiana (Bertoni) Bertoni	introduced & horticultural

Reference: (ICON 2010; APC CHAH 2011; Catalogue of Life 2015)

2.2 Description

Chromolaena odorata is a perennial shrub, growing to 2-7 m high. It has straight stems which branch readily. The leaves are three-veined and ovate-deltoid, acuminate, about 6-12 cm long and are arranged opposite on the stems. *Chromolaena odorata* has a deep taproot, with a massive fibrous root system. Flowers are usually terminal, in clusters, with the corollas varying from white to mauve. Achenes are black with a pale pappus (Holm *et al*. 1991).

2.3 Biology and ecology

Chromolaena odorata prefers open, sunny areas, receiving over 1000 mm rainfall pa, but can tolerate areas with lower rainfall (~600-800 mm pa), as well as partial shade as an understorey species (Waterhouse 1994; Day & Bofeng 2007). It flowers in the dry season. In the northern hemisphere, flowering usually occurs in December-January, while for the southern hemisphere, flowering usually occurs in June-July. Flowering is prolific, and fertile seed is produced without pollination, as the species is apomictic (Coleman 1989; Rambuda & Johnson 2004). Seeds are lightweight and are dispersed by wind, as well as on machinery, clothing and animal fur (Holm *et al*. 1991; Day & Bofeng 2007). In north Queensland, there has been substantial dispersal of seeds by rivers. *Chromolaena odorata* dies back after flowering and can become a fire hazard. Fire does not usually kill mature plants which can reshoot from the crown (Waterhouse 1994).

2.4 Native range and centre of origin

Chromolaena odorata is endemic to much of tropical America where it is found in southern USA, Mexico, Central and South America and the Caribbean (Zachariades *et al*. 2009).

2.5 Australian and overseas distribution

Two forms of *C. odorata* are thought to exist. The Asia-West African form is found in many Asian countries, most countries in sub-Saharan Africa, Papua New Guinea, Micronesia and northern Queensland. The other form is found only in South Africa (Zachariades *et al*. 2009).

Chromolaena odorata was first reported in Australia around Bingil Bay, in northern Queensland in 1994, where it was probably introduced in contaminated pasture seed. Other infestations were later found in 2003 at Mossman and Townsville (Fig. 1), probably as a result of seed again being accidently introduced. It is also present on the Cocos Islands and Christmas Island, Australian Territories in the Indian Ocean (DAFF 2012). A climate-matching model predicts that coastal lands in the Northern Territory and areas along the Queensland coast south to Sandy Cape (24.7ºS 153.2ºE) would be climatically suitable for *C. odorata* (Kriticos *et al*. 2005) (Fig. 1).

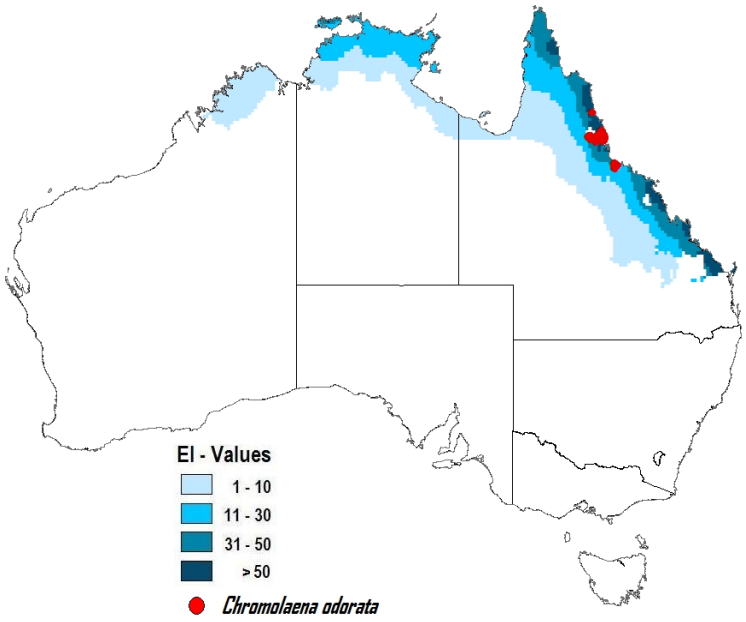

Figure 1. The current distribution of *Chromolaena odorata* in Australia (red dots) and the predicted distribution using CLIMEX (Kriticos *et al*. 2005).

2.6 Importance of the plant

2.6.1 Detrimental aspects

Chromolaena odorata is a fast-growing multi-branched shrub, reaching heights of about 7 m. Overseas where it has been introduced, *C. odorata* is a major weed of agricultural areas and can form dense monocultures. In grazing lands, *C. odorata* can outcompete preferred pasture species, is toxic to cattle and can impede mustering. *Chromolaena odorata* can become the dominant understorey species in plantations, reducing yield and interfering with the harvesting of coconuts and oil palm (Day & Bofeng 2007; Day *et al*. 2013).

Chromolaena odorata is also a major weed of subsistence farms, where it can quickly establish in areas where land has been cleared for the planting of crops. It can smother and kill taro, cassava, papaya and bananas, reducing productivity and income (Day *et al*. 2013). *Chromolaena odorata* can increase fire frequency and intensity, potentially damaging rainforest margins. It has the ability to cause skin problems and asthma in some people. In most countries where *C. odorata* has been introduced, it invades roadsides and disturbed forests and can climb up and smother other vegetation (Zachariades *et al*. 2009).

In Queensland, *C. odorata* is found in a range of habitats and land uses. As occurs overseas, it invades grazing lands, where it again reduces productivity. *Chromolaena odorata* is also a problem in timber plantations, where it affects the growth of saplings and interferes with harvesting. It is not a problem of sugarcane and other intensively managed crops. In national parks, *C. odorata* can outcompete other species, blocking successional processes and reducing biodiversity. It is also found on Australian Defence Force land where seeds may become attached to vehicles and thus be spread further in Australia and overseas (DAFF 2012).

Elsewhere in Queensland, *C. odorata* is found in open shrublands, along rainforest margins, roadsides and creek banks. On the Cocos Islands, *C. odorata* has impeded the movement of coconut crabs to and from the water (DAFF 2012).

2.6.2 Legislative status

Chromolaena odorata is listed on the Australian ICON restricted import list. It is a declared a Class 1 weed under the Queensland *Land Protection (Pest and Stock Route Management) Act 2002* and the New South Wales *Noxious Weeds Act 1993* (NSW), a Prohibited Species in Western Australia under the *Biosecurity and Agriculture Management Act 2007*, and has a Class C rating (not to be introduced) in the Northern Territory.

2.6.3 Potential threats

In Australia, *C. odorata* is found in only north Queensland as well as the Australian territories of the Cocos Islands and Christmas Island. It has considerable potential to spread (Fig. 1) and thus have far greater impacts than currently experienced.

The Wet Tropics area in Queensland supports over 4,600 plant species, of which 25% are endemic. The localised distribution of many of these species makes them particularly vulnerable to extinction due to threatening processes such as weed invasion (Werren *et al.* 1995). A report to the Wet Tropics Management Authority listed *C. odorata* as the third highest ranked species for potential environmental risk to the Wet Tropics (Werren 2001) and is a threat to natural reserves outside the Wet Tropics.

Chromolaena odorata has the potential to spread to Kakadu National Park and other significant ecosystems, where its presence may impact on biodiversity and alter fire regimes. Studies in Africa reported that dense thickets of *C. odorata* along riverbanks increased shading which reduced egg incubation temperatures resulting in an altered sex ratio and failed development of Nile crocodiles (Leslie & Spotila 2001). There is potential for similar impacts on freshwater and estuarine crocodiles in Australia if *C. odorata* populations continue to increase and spread.

2.6.4 Beneficial aspects

Chromolaena odorata is not known to be of any benefit, although some landholders overseas believe that soil under the plant is improved.

2.7 Control methods

Chromolaena odorata can be controlled by several methods. Overseas on subsistence farms, the preferred method is by slashing as resource-poor farmers cannot afford herbicides. However, this method is time-consuming and plants often re-shoot from the broken fragments or from the base of plants (Day & Bofeng 2007). Single plants can be grubbed out. Fire is thought to offer only short-term control as plants are not necessarily killed and infestations can quickly re-occur. Effective chemical control methods are available but are used only by commercial enterprises or various government organisations. In Queensland, large infestations are controlled by herbicide, particularly Starane Advanced, with a 99% mortality rate, or Grazon extra with a 93% mortality rate. These can be applied using a QuikSpray unit or by ATV-mounted spray rigs (DAFF 2012).

2.8 Stakeholders

Stakeholders include the agricultural and fisheries sectors (including landscaping and earthmoving companies), national parks, Wet Tropics Management Authority, Department of Main Roads, Queensland Rail, Local government authorities, utilities (such as Ergon Energy, PowerLink) and the Australian Defence Force.

2.9 Approval as target species for biological control

The Australian Weeds Committee approved *C. odorata* as a target for biological control in Australia in August 2010**.**

2.10 History of biological control

Biological control of *C. odorata* was initiated in 1966 when the weed was first surveyed in Trinidad for potential control agents (Cruttwell 1974). From those surveys, two agents were released into Nigeria, Malaysia, India, Sri Lanka and Ghana in the 1970s. However, only one agent, the moth *Pareuchaetes pseudoinsulata* Rego Barros (Lepidoptera: Arctiidae) established and it was later introduced into numerous other countries, including Guam, Federated States of Micronesia (FSM) and Northern Mariana Islands (NMI) in the 1980s. South Africa initiated a project in 1988 and two agents were released on their *C. odorata* biotype. In 1993, an ACIAR-funded project commenced in Indonesia and the Philippines, managed by the Queensland Government. The gall fly *Cecidochares connexa* (Macquart) (Diptera: Tephritidae) was collected from Colombia and introduced into Indonesia and the Philippines following host specificity testing (Aterrado & Bachiller 2002; McFadyen *et al*. 2003; Appendix 1). The gall fly was subsequently released into Guam, FSM, NMI, Palau, Papua New Guinea and Timor Leste following further host specificity testing (Esguerra 2002). Of the agents that have established globally on *C. odorata*, the gall fly is by far the most effective (Zachariades *et al*. 2009; Day & McFadyen 2012; Day *et al*. 2013).

3.0 Information on biological control agent

3.1 Taxonomy

Class: Insecta
Order: Diptera
Family: Tephritidae
Genus: *Cecidochares*
Species: *connexa* (Macquart)

3.1.1 Related species and a summary of their host range

Sixteen other species in the Tephritidae have been utilised for weed biological control against 17 weed species, of which all but one are members of the Asteraceae (Table 2). No other members of the genus *Cecidochares* have been utilised as biological control agents. However, two closely related species *Procecidochares alani* Steyskal and *P. utilis* Stone have been released to control *Ageratina riparia* (mistflower) and *A. adenophora* (Crofton weed) respectively, both of which are also members of the tribe Eupatorieae (Winston *et al.* 2014).

Table 2. Species of Tephritidae successfully used as biocontrol agents. All weed species except for *Lantana camara* (Verbenaceae) belong to the family Asteraceae. (Ref: Winston *et al.* 2014).

Biocontrol agent	Target weed	Country
Procecidochares utilis Stone	*Ageratina adenophora* (Spreng.) R. M. King & H. Rob.	Australia, India, NZ, RSA, Thailand, USA (Hawaii)
Procecidochares alani Steyskal	*Ageratina riparia* (Regel) R. M. King & H. Rob.	Australia, NZ, USA (Hawaii)
Urophora solstitialis (L.)	*Carduus acanthoides* L.	Canada
	Carduus nutans L.	Australia, Canada, NZ
Chaetorellia australis Héring	*Centaurea cyanus* L.	USA
	Centaurea solstitialis L.	USA
Urophora affinis (Frauenfeld)	*Centaurea diffusa* Lam.	Canada, USA
	Centaurea stoebe L. sens. lat.	Canada, USA
	Centaurea virgata Lam. subsp. *squarrosa* (Boiss.) Gugler	USA
Urophora quadrifasciata (Meigen)	*Centaurea diffusa* Lam.	Canada
	Centaurea jacea L. nothosubsp. *pratensis* (W.D.J. Koch) Čelak.	Canada
	Centaurea stoebe L. sens. lat.	Canada
Urophora sirunaseva (Héring)	*Centaurea solstitialis* L.	USA
Chaetorellia acrolophi White & Marquardt	*Centaurea stoebe* L. sens. lat.	Canada, USA
Terellia virens (Loew)	*Centaurea stoebe* L. sens. lat.	USA
Mesoclanis polana Munro	*Chrysanthemoides monilifera* (L.) Norl. subsp. rotundata (DC.) Norl.	Australia
Urophora cardui (L.)	*Cirsium arvense* (L.) Scop.	Canada, NZ, USA
Urophora stylata (Fabricius)	*Cirsium vulgare* (Savi) Ten.	Australia, Canada, NZ, USA
Tetraeuaresta obscuriventris (Loew)	*Elephantopus mollis* Kunth	Fiji, USA (Hawaii)
Acinia picturata (Snow)	*Pluchea carolinensis* (Jacq.) G. Don	USA (Hawaii)
Euaresta aequalis Loew	*Xanthium strumarium* L.	Australia
Eutreta xanthochaeta Aldrich	*Lantana camara* L. sens. lat.	USA (Hawaii)

3.2 Brief biology of the agent

Adults ingest only water and live for between 5 and 11 days. Mating occurs on *C. odorata* and females deposit eggs in the axils of stems or tips. The eggs hatch in 4-7 days and the larvae burrow into the stem or tip. Feeding by larvae results in the formation of galls, first visible after about 15 days. Larvae feed for 30-50 days and construct a window in the side of the gall, prior to pupation, to facilitate adult emergence. The entire life cycle from egg to adult ranges from 47 to 75 days, with an average of 60 days (McFadyen *et al*. 2003).

3.3 Native range of the agent

Cecidochares connexa occurs naturally from Central America to northern Argentina (Crutwell 1974).

3.4 Proposed source(s) of the agent

Cecidochares connexa is now found in 11 countries (Zachariades *et al*. 2009; Winston *et al*. 2014). Galls will be field collected in PNG and held in the quarantine facility at the Ecosciences Precinct, Brisbane until adults emerge.

3.5 Agent's potential for control of the target

In all countries in which *C. connexa* has established, the gall fly appears to be having an impact on *C. odorata* (Zachariades *et al*. 2009). In PNG where the most intensive studies have occurred, *C. connexa* quickly established and spread to over 89% of sites where *C. odorata* was present. It is reported to control *C. odorata* at over 200 sites, covering nine provinces (Day *et al*. 2013). At three monitoring sites, *C. connexa* has completely controlled *C. odorata*, with all plants in all quadrats dying. At another three sites and along some transect lines, over 80% of *C. odorata* has been controlled, enabling preferred pasture species to return (Day *et al*. 2013).

3.6 Possible interactions, including conflict-of-interest with existing biological control programs

Cecidochares connexa is specific to *C. odorata* so no interactions or conflicts-of-interest are expected with existing biological control programs.

3.7 Details on the quarantine facility and methods of containment

Mature galls on *C. odorata* containing *C. connexa* will be imported into the quarantine facility at the Ecosciences Precinct, 41 Boggo Road, Dutton Park, Brisbane, Queensland. This is an AQIS approved facility, QAP No: Q2275, QC level: 5.3 and QIC level 7.3. The quarantine has double glazing of glasshouses, HEPA air filtering, negative air pressure between rooms, air-lock entrances, filtering and heat treatment of liquid waste and autoclaving of solid waste.

All staff involved in the project are experienced quarantine operators who strictly follow AQIS approved guidelines. All staff wear overalls, hairnets and booties when entering the laboratories and which they remove before leaving the facility. Insects are transported to the facility in double-sealed containers. Insects are held in cages in the glasshouses or controlled environment rooms. Method of disposal and treatment of refuse and packaging is by autoclaving.

3.8 Information on non-target organisms at risk from the agent

3.8.1 Host-specificity testing of *Cecidochares connexa*

Cecidochares connexa has been tested previously against 80 species, covering 18 families, including 22 species in Asteraceae and five species in tribe Eupatorieae, in four countries (Guam, Indonesia, Philippines and Thailand), prior to being tested in Australia (Appendix 1). Galls did not form on any test species conducted in these trials.

Host specificity testing in Australia was conducted against 18 plant species, using multiple species-minus-*C. odorata* choice tests, single species no-choice tests, paired choice tests, continuation tests and time dependent trials. In all tests, galls were formed on, and adults emerged from only *C. odorata* and *Praxelis clematidea*. However, adult emergence on *P. clematidea* was significantly less than on *C. odorata* and a population of *C. connexa* was not able to be maintained on *P. clematidea*.

A comparison of stem diameter of *C. odorata* and *P. clematidea* showed that stems of *C. odorata* (18.46±0.4 mm; n=20) were significantly larger than those of *P. clematidea* (15.64±0.4 mm; n=20) ($t=4.23$, $p<0.001$) which may help explain the poor performance of *C. connexa* on this species. In the field, plant condition is likely to be poorer than those plants grown in the glasshouse under optimal conditions.

Therefore, the only plant species at risk from *C. connexa* is *P. clematidea*, a minor weed in north Queensland. However, as populations of *C. connexa* cannot be sustained on *P. clematidea*, damage is likely to be restricted to spill-over effects of galls forming from adults emerging from stands of *C. odorata* near populations of *P. clematidea*.

3.8.2 Test list for determining the host-specificity of *C. connexa*

The test list comprised of 18 species, all belonging to the tribe Eupatorieae that could be sourced in Australia (Table 3). This list was shorter than would normally be used to confirm specificity of a biocontrol agent, as *C. connexa* had been tested previously against 80 species, covering 18 families, including 22 species in Asteraceae and five species in tribe Eupatorieae, in four countries (Guam, Indonesia, Philippines and Thailand). Galls did not form on any test species conducted in these trials. In addition, *C. connexa* has established in 11 countries and there have been no reports of galls forming on any other plant species (Winston *et al*. 2014).

Two related species, *Ageratum conyzoides* subsp. *conyzoides* and *Stevia eupatoria* were not tested as these species were not able to be obtained. However, *A. conyzoides* subsp. *conyzoides* had previously been tested in Indonesia, Guam and Thailand and galls did not form in any of the tests conducted.

Table 3. Plant species in the Eupatorieae used to test the host-specificity of *C. connexa* at the Ecosciences Precinct, Brisbane.

Adenostemma lavenia (L.) Kuntze
Adenostemma macrophyllum (Blume) DC.
Ageratina adenophora (Spreng.) R.M. King & H. Rob.
Ageratina altissima (L.) R.M. King & H. Rob.
Ageratina riparia (Regel) R.M. King & H. Rob.
Ageratum houstonianum Mill.
Bartlettina sordida (Less.) R.M. King & H. Rob.
Chromolaena odorata (L.) R.M. King & H. Rob.
Chromolaena squalida (DC.) R.M. King & H. Rob.
Conoclinium coelestinum (L.) DC.
Eupatorium lindleyanum DC.
Eupatorium purpureum (L.)
*Gymnocoronis spilanthoide*s (D. Don ex Hook. & Arn.) DC.
Liatris spicata (L.) Willd.
Mikania micrantha Kunth
Praxelis clematidea (Griseb.) R.M. King & H. Rob.
Stevia ovata Willd.
Stevia rebaudiana (Bertoni) Bertoni

3.8.3 Materials and Methods

For all tests, a weak honey solution was provided to the flies and extra moisture for flies was provided by finely spraying water into the cages each day. Plants were watered as required. All plants were monitored for gall development over the duration of the tests and test plant species that had no galls develop were discarded once all flies had emerged from the corresponding control cage. Once emergence windows became visible on the plants, cages were checked daily for adult emergence. The number of galls/stem, total number of galls on each plant, the gender and total number of flies to emerge and the time to emergence were recorded. Once all flies had emerged, the diameter of the galls on each plant was measured.

3.8.3.1 Multiple species-minus-*C. odorata* choice tests

Five pairs of randomly selected newly emerged *C. connexa* flies were added to cages holding between 4-6 test plants. Each plant species was tested five times in a semi-randomly designed experiment in such a way that no two plant species were tested together more than twice to limit potential masking of one plant by another if the females preferred a particular plant species on which to oviposit. A control cage was set up with five pairs of newly emerged flies from the same pool of adults with a single *C. odorata* plant for each batch of test plant trials. Adults were left in the cages until they had died.

Results

Oviposition by *C. connexa* was observed on both *C. odorata* and *P. clematidea*, with galls developing on both plants. Galls failed to develop on any other plant species. There were significantly more galls/plant formed on *C. odorata* (48.0±8.4; n=8) than on *P. clematidea* (2.4±1.5; n=5) ($t=5.38$, $p<0.001$). There was no significant difference in the size of galls on

C. odorata (8.6±0.2 mm; n=193) compared with those on *P. clematidea* (8.3±1.2 mm; n=10) (t=0.35, p=0.730).

Adults emerged from only *C. odorata* and *P. clematidea*. However, there were significantly more adults emerging from *C. odorata* (128.8±38.5; n=8) than from *P. clematidea* (1.0±0.77; n=5) (t=3.31, p=0.013). There was no significant difference in the average time (days) of individuals to complete development (from the time the cages were set up) for individuals reared on *C. odorata* (86.2±0.4; n=1030) compared to those individuals reared on *P. clematidea* (80.6±2.9; n=5) (t=0.98, p=0.328).

3.8.3.2 Single species no-choice tests

Each test plant species was placed singly in a cage with three pairs of randomly selected newly emerged *C. connexa* flies. A control cage containing three pairs of randomly selected newly emerged *C. connexa* flies from the same pool of adults and a single *C. odorata* plant were set up concurrently with each batch of test plants set up. Each plant species was tested at least once, depending on plant availability. As *P. clematidea* was the only test plant on which *C. connexa* galls developed in multiple choice trials, it was tested five times in single plant trials. Adults were left in the cage until they had died.

Results

Oviposition by *C. connexa* was observed on both *C. odorata* and *P. clematidea*, with galls developing on both plants. Galls failed to develop on any other plant species. There were significantly more galls/plant formed on *C. odorata* (40.7±8.4; n=6) than on *P. clematidea* (9.2±2.6; n=5) (t=3.57, p=0.012). The average diameter of galls on *C. odorata* (9.1±0.2 mm; n=244) was significantly greater than those on *P. clematidea* (7.4±0.5 mm; n=46) (t=2.93, p=0.004).

Adults emerged from *C. odorata* (168.8±70.4; n=6) and *P. clematidea* (7.6±3.1; n=5). While there were many more adults emerging from *C. odorata* than *P. clematidea*, the difference between the species was not significant (t=2.29, p=0.071), probably due to the high variation in the number of adults emerging from *C. odorata*. The average time (days) of individuals to complete development (from the time the cages were set up) was significantly greater for individuals reared on *C. odorata* (85.6±0.4; n=1013) compared to those individuals reared on *P. clematidea* (77.7±1.0; n=38) (t=7.59, p<0.001).

3.8.3.3 *Praxelis clematidea* choice tests

As galls developed and adults emerged from *P. clematidea* in multiple species-minus *C. odorata* choice tests and single species no-choice tests, this species was tested in choice tests. One *P. clematidea* plant and one *C. odorata* plant were placed into a cage with three pairs of randomly selected newly emerged *C. connexa* flies that had emerged from *C. odorata*. The test was replicated five times. Adults were left in the cage until they had died. Once galls had begun to develop, the plants were separated into individual cages for adult emergence.

Results

Oviposition by *C. connexa* was observed on both *C. odorata* and *P. clematidea*, with galls developing on both plants. While there were twice as many galls forming on *C. odorata* (29.6±8.7; n=5) compared with *P. clematidea* (13.0±4.1; n=5), the difference between the species was not significant (t=1.72, p=0.123), probably due to the high variation in the number of galls forming on *C. odorata*. However, the average diameter of galls on *C. odorata* (8.5±0.3 mm; n=148) was significantly greater than those on *P. clematidea* (6.5±0.3 mm; n=65) (t=4.59, p<0.001).

There were significantly more adults emerging from *C. odorata* (114.8±37.6; n=5) than from *P. clematidea* (5.2±3.0; n=5) (t=2.90, p=0.043). However, there was no significant difference in the average time (days) for individuals to complete development (from the time the cages were set up) for individuals reared on *C. odorata* (81.0±0.6; n=574) compared to those individuals reared on *P. clematidea* (78.4±1.7; n=26) (t=1.50, p=0.145).

3.8.3.4 *Praxelis clematidea* paired no-choice tests

For this test, there was a shortage of adequate and same-sized plants. As a result, in some instances, two plants were added to a cage. Two *P. clematidea* plants were placed into each of two cages and three pairs of randomly selected newly emerged *C. connexa* flies were added to each cage. A single cage containing one *P. clematidea* plant was also set up with three pairs of flies. Two cages each containing two *C. odorata* plants were set up concurrently. The first control cage had five pairs of randomly selected newly emerged *C. connexa* flies added while the second control cage had three pairs of randomly selected newly emerged *C. connexa* flies added. Adults were left in the cages until they had died. As galls developed, plants were placed into separate cages for adult emergence.

Results

As different numbers of pairs of adults were added to cages, with varying numbers of plants, all data was converted to galls/plant/female or adults emerged/female. Oviposition by *C. connexa* was observed on both *C. odorata* and *P. clematidea*, with galls developing on both plants. There were significantly more galls/plant/female formed on *C. odorata* (17.5±3.6; n=4) than on *P. clematidea* (4.2±1.2; n=5) (t=3.82, p=0.007). However, there was no significant difference in the size of galls on *C. odorata* (6.4±0.2 mm; n=139) and those on *P. clematidea* (5.7±0.3 mm; n=38) (t=1.93, p=0.057).

There was no significant difference in the number of adults/female emerging from *C. odorata* (38.7±13.6; n=4) than that from *P. clematidea* (1.5±0.4; n=5) (t=2.73, p=0.072). The high variation in the number of adults emerging from *C. odorata* again accounts for the lack of significance. There was also no significant difference in the average time (days) of individuals to complete development (from the time the cages were set up) for individuals reared on *C. odorata* (91.3±0.6; n=293) compared to those individuals reared on *P. clematidea* (91.7±3.2; n=14) (t=-0.15, p=0.879).

3.8.3.5 Continuation tests

Adults that emerged from multiple species-minus *C. odorata* choice tests and single species no-choice tests were used in continuation tests. Three pairs of newly emerged adults that had been reared on *P. clematidea* were placed in a cage with one or two *P. clematidea* plants, depending on the size of the plants and kept in the cage for five days. After five days, the surviving adults from each cage were collected and placed into separate new cages, each containing one *C. odorata* plant until all flies had died. Seven replicates were conducted.

Concurrently, three pairs of newly emerged adults that had been reared on *P. clematidea* were placed in a cage with one *C. odorata* plant until all flies had died. Due to the low numbers of flies that emerged from *P. clematidea*, only four replicates of this test could be conducted.

As a control, three pairs of newly emerged adults that had been reared on *C. odorata* were placed in a cage containing one *C. odorata* plant until all flies had died. Seven replicates were conducted.

Results

Oviposition was observed on *C. odorata* and *P. clematidea*, with galls developing on both species. However, there were significantly more galls formed on the control *C. odorata* plants, using flies that had emerged from *C. odorata* (43.7±6.3; n=7) than formed on *P. clematidea* (12.0±2.9; n=7) or on *C. odorata* (5.3± 3.4; n=4), each using flies that had been reared on *P. clematidea* ($F_{2,15}$=17.52, p<0.001) (Fig. 2).

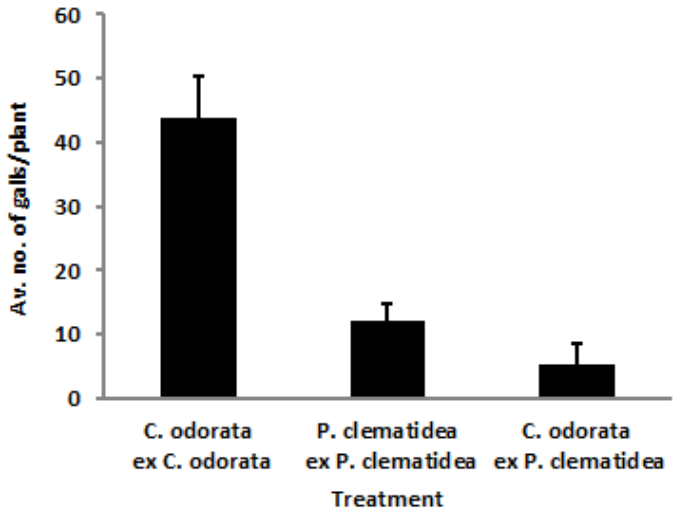

Figure 2. The mean number of galls formed on *C. odorata* when exposed to *C. connexa* reared on *C. odorata*, and *P. clematidea* and *C. odorata* when each exposed to *C. connexa* reared on *P. clematidea*, during continuation trials.

Only an average of 1.8±0.9 galls/plant (n=5) formed on *C. odorata* by flies that were reared on *P. clematidea* and had been placed on *P. clematidea* plants for five days initially. Only five trials were able to be set up and, of these, all adults had died within three days in two trials. In the remaining three trials, adults lived for up to eight days but females laid few eggs.

There was a significant difference in the size of the galls formed on *C. odorata* (control) (7.7±0.2 mm; n=306), *P. clematidea*, using flies that had been reared on *P. clematidea* (5.9±0.3 mm; n=73), and *C. odorata*, using flies that had been reared on *P. clematidea* (5.2±0.4 mm; n=21) ($F_{2,397}$=12.21, p<0.001) (Fig. 3).

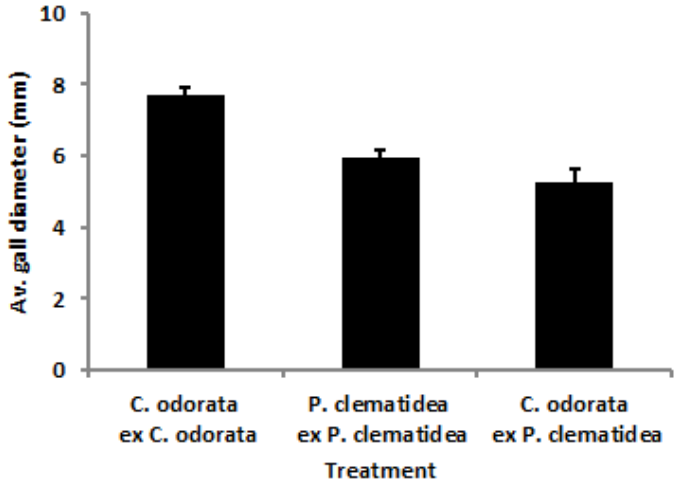

Figure 3. The mean diameter of galls formed on *C. odorata* when exposed to *C. connexa* reared on *C. odorata*, and *P. clematidea* and *C. odorata* when each exposed to *C. connexa* reared on *P. clematidea*, during Continuation trials.

There were significantly more adults emerging from *C. odorata* using flies reared on *C. odorata* (177.9±54.5; n=7) than from *P. clematidea* (2.9±1.3; n=7) or from *C. odorata* (1.3±0.8; n=4) using flies that had been reared on *P. clematidea* ($F_{2,15}$=7.93, p=0.004) (Fig. 4).

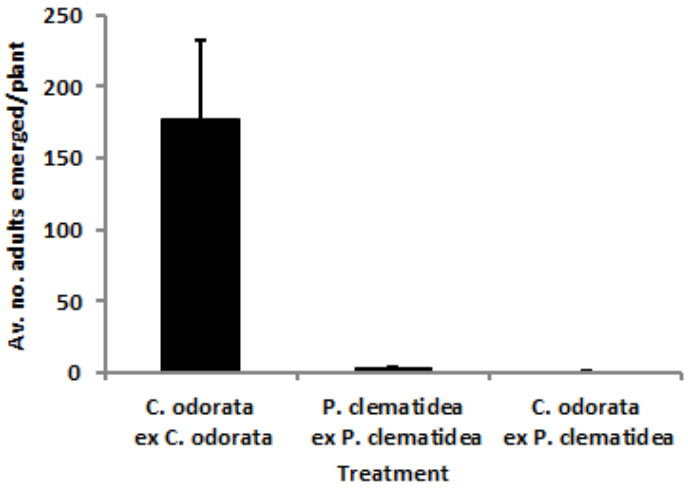

Figure 4. The mean number of adults that emerged from *C. odorata* when exposed to *C. connexa* reared on *C. odorata*, and *P. clematidea* and *C. odorata* when each exposed to *C. connexa* reared on *P. clematidea*, during continuation trials.

However, there was no significant difference in the average time (days) of individuals to complete development (from the time the cages were set up) for individuals reared on *C. odorata* using flies reared on *C. odorata* (88.0±0.4; n=1245) compared to those individuals reared on *P. clematidea* (86.8±2.4; n=20) or *C. odorata* (83.2± 0.8; n=5), each using flies that had been reared on *P. clematidea* ($F_{2,1268}$=0.46, p=0.633).

Due to the very low numbers, and lack of synchrony of flies emerging on *P. clematidea*, no further trials could be set up and populations of *C. connexa* could not be sustained on *P. clematidea*. Even when flies that had been reared on *P. clematidea* were placed on *C. odorata*, very few galls formed and very few adults emerged, suggesting that adults developing on *P. clematidea* were inferior in health to those developing on *C. odorata*.

3.8.3.6 Time-dependent tests

To determine how readily *C. connexa* females oviposit on *C. odorata* and *P. clematidea*, three pairs of randomly selected newly emerged adults that had been reared on *C. odorata* were placed in a cage containing one *P. clematidea* plant. Three pairs of randomly selected newly emerged adults that had been reared on *C. odorata* were placed in a cage containing one *C. odorata* plant, as a control. All flies from each cage were removed after five days and placed in separate cages, each containing one *C. odorata* plant and left until all adults had died. The test was replicated three times.

The number of galls on each plant was counted, and the gall diameter for galls that developed on each plant measured. The number of adults that emerged from each plant and the time taken to complete development were also recorded.

Results

There was a significant difference in the number of galls that developed on *C. odorata* (44.0±1.0; n=3), *P. clematidea* (11.0±7.0; n=3), *C. odorata* following adults previously exposed to *C. odorata* for five days (35.3±4.1; n=3) and *C. odorata* following adults previously exposed to *P. clematidea* for five days (24.5±0.5; n=2) ($F_{3,7}$=10.51, p=0.006) (Fig. 5).

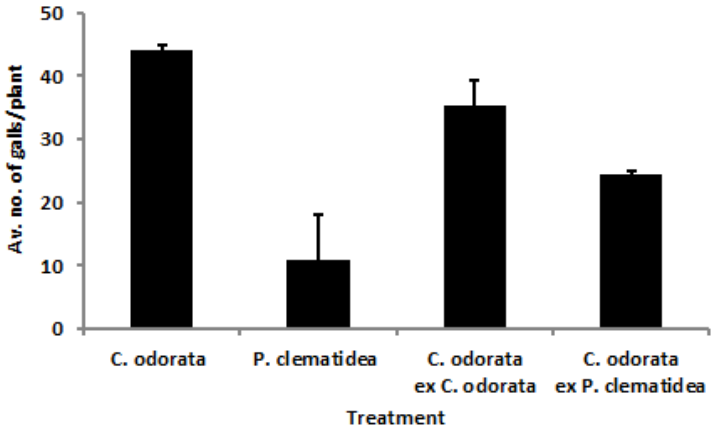

Figure 5. The mean number of galls formed on *C. odorata*, *P. clematidea*, *C. odorata* following adults previously exposed to *C. odorata* for five days and *C. odorata* following adults previously exposed to *P. clematidea* for five days, during time dependency trials.

There was a significant difference in the size of galls formed on *C. odorata* (7.2±0.2 mm; n=132), *P. clematidea* (6.2±0.4 mm; n=33), *C. odorata*, following adults previously exposed to *C. odorata* for five days (7.1±0.3 mm; n=106) and *C. odorata*, following adults previously exposed to *P. clematidea* for five days (8.9±0.4 mm; n=49) ($F_{3,316}$=7.96, p<0.001) (Fig. 6).

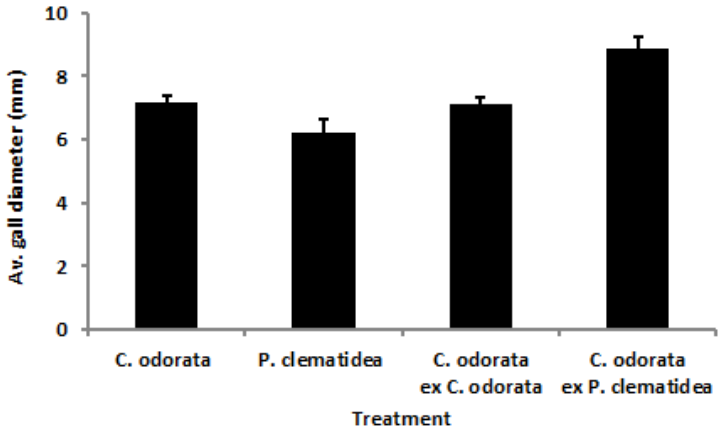

Figure 6. The mean diameter of galls formed on *C. odorata*, *P. clematidea*, *C. odorata* following adults previously exposed to *C. odorata* for five days and *C. odorata* following adults previously exposed to *P. clematidea* for five days, during time dependency trials.

There was a significant difference in the number of adults that emerged from *C. odorata* (109.0±25.7; n=3), *P. clematidea* (2.3±0.9; n=3), *C. odorata* following adults previously exposed to *C. odorata* for five days (41.7±11.9; n=3) and *C. odorata* following adults previously exposed to *P. clematidea* for five days (39.5±9.5; n=2) ($F_{3,7}$=8.26, p=0.011) (Fig. 7).

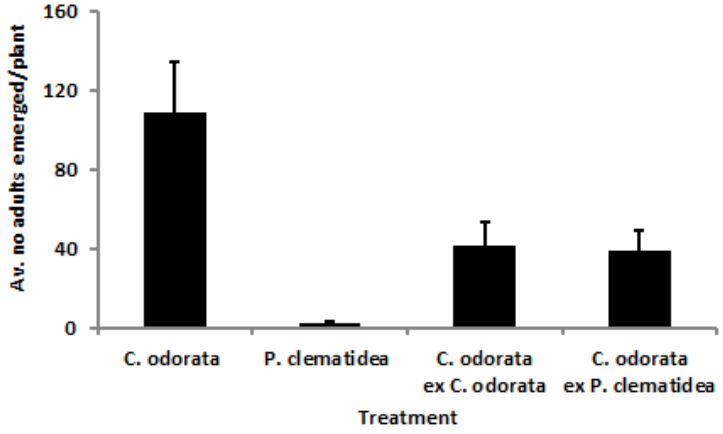

Figure 7. The mean number of adults that emerged from *C. odorata*, *P. clematidea*, *C. odorata* following adults previously exposed to *C. odorata* for five days and *C. odorata* following adults previously exposed to *P. clematidea* for five days, during time dependency trials.

There was no significant difference in the time to development of adults emerging from *C. odorata* (79.7±0.6 days; n=327), *P. clematidea* (71.7±3.9; n=7), *C. odorata* following adults previously exposed to *C. odorata* for five days (81.6±1.3; n=125) and *C. odorata* following

adults previously exposed to *P. clematidea* for five days (78.6±1.8; n=79) ($F_{3,527}$=2.01, p=0.112).

These trials suggest that while *C. connexa* will lay on *P. clematidea*, they prefer to lay on *C. odorata*. When flies were exposed to *P. clematidea* for five days before being placed on *C. odorata*, more galls were formed on *C. odorata* compared to *P. clematidea*, indicating that females were holding onto eggs rather than laying on a less-preferred host.

3.9 Additional information on *Praxelis clematidea*

Following oviposition and adult development on *P. clematidea*, additional information was sought on the species.

3.9.1 Phylogeny

Phylogenetically, the genus *Praxelis* is the most closely related genus to *Chromolaena*, with both belonging to the subtribe Praxelinae within the tribe Eupatorieae (Robinson *et al*. 2009) (Fig. 8).

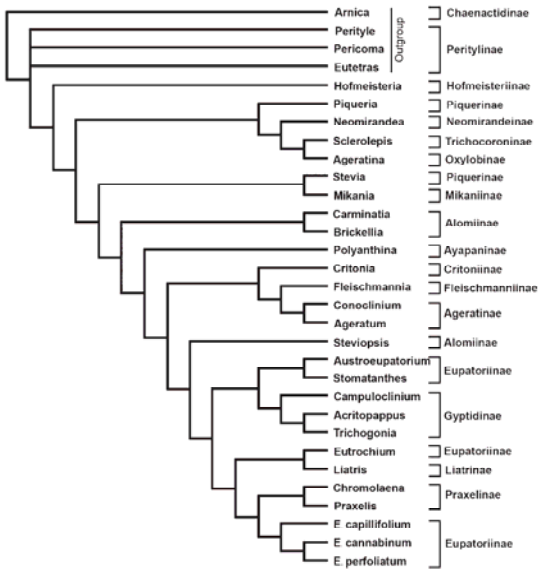

Fig. 43.5. Phyletic tree of the tribe Eupatorieae with selected outgroups based on ITS data from GenBank with additional genera intercolated on the basis of their positions in the cpDNA RFLP results of Ito et al. (2000b). Tree prepared by V.A. Funk and R. Chan. Excluded here, but included in the text, members of the subtribes Adenostemmatinae, Disynaphiinae, Hebecliniinae, and Oaxacaniinae, for which no DNA data are available. A biogeographic tree of Compositae can be found in Chapter 44.

Figure 8. A phylogenetic tree showing the relationships of the genera within the Eupatorieae (from Robinson *et al*. 2009).

3.9.2 Distribution and pest status in Australia

Praxelis clematidea has a mainly coastal distribution, being found from Cape York down to the Sunshine Coast, in southern Queensland. However, the heaviest infestations appear to be in far north Queensland, along the coast and on the Atherton Tablelands (Fig. 9). Consequently, its northern distribution would overlap that of *C. odorata* which has a far more restricted distribution, being found around Mossman, Innisfail, Tully, Mission Beach, Mount Garnet and Townsville (Fig. 10).

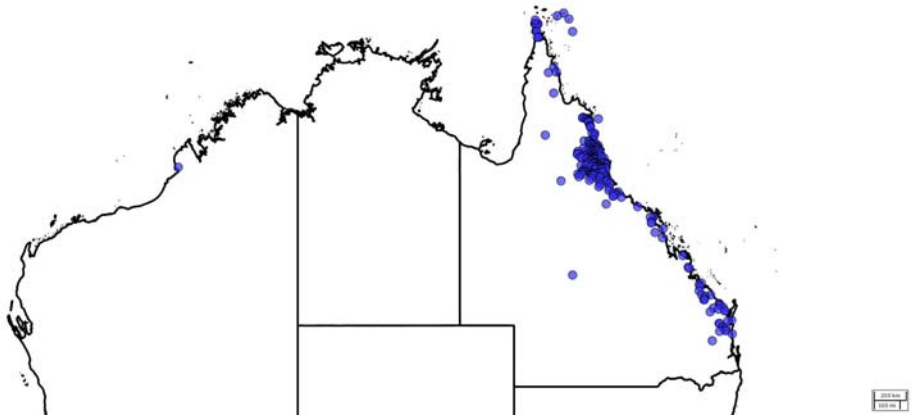

Figure 9. A map showing the distribution of *P. clematidea* in Australia. (Source: Atlas of Living Australia 2015).

Figure 10. The current distribution of *C. odorata* in Australia (S. Brooks, DAF, unpublished data).

Praxelis clematidea is viewed as a minor weed in Queensland, but is not a declared species under the Queensland *Land Protection (Pest and Stock Route Management) Act 2002*. It is however, on the *Alert List for Environmental Weeds* (Department of Environment 2015).

3.9.3 Plant structure

Praxelis clematidea is an annual shrubby herb with hairy and brittle stems that grows to about 1 m. It has lilac-bluish flowers and flowers prolifically, producing vast quantities of seeds that can be spread by wind (Department of Environment 2015).

Stem diameters of *P. clematidea* (15.6±0.4 mm, n=20) grown in the glasshouse were significantly smaller than that of *C. odorata* (18.5±0.4, n=20) ($t=4.23$, $p<0.001$) grown under the same conditions.

3.9.4 Field observations in Palau

In Palau where *C. connexa* is widely established, no galls have ever been reported on *P. clematidea* despite the plant and *C. odorata* growing in the same vicinity at numerous sites (J. Miles, Ministry of Natural Resources, pers. comm. 2014).

3.10 Information and results on any other similar assessments undertaken on the species

Cecidochares connexa had previously been tested in four countries, namely Guam (Esguerra 2002; Appendix 2), Indonesia (McFadyen *et al*. 2003: Appendix 3), Philippines (Aterrado & Bachiller 2002; Appendix 4) and Thailand (Kernasa *et al*. 2013; Appendix 5), prior to being tested in Australia. A total of 80 species, covering 18 families, including 22 species in Asteraceae were tested by at least one of the four countries. Species within the Asteraceae represented 10 tribes, including five species from the tribe Eupatorieae (Appendix 1). Galls did not form on any test species, including *P. clematidea* which was tested in Thailand, in these trials.

Cecidochares connexa has been released and has established in 10 countries. It has also spread from Cote d'Ivoire to Ghana in West Africa (Paterson & Akpabey 2014) (Table 4). In Indonesia, Timor Leste and the western Pacific, where most studies have been conducted, *C. connexa* is reported to be aiding the control of *C. odorata* (e.g. Day *et al*. 2013). It has not been reported on any other plant species where it has been introduced. In Palau where *C. connexa* has established, it has not been reported on *P. clematidea* even though this plant and *C. odorata* are found growing in the same area (J. Miles, Ministry of Natural Resources, pers. comm. 2014).

Table 4. List of countries where *Cecidochares connexa* has been released and/or established (Winston *et al*. 2014**).**

Cote d'Ivoire
Federated States of Micronesia
Ghana
Guam
India
Indonesia
Northern Mariana Islands
Palau
Papua New Guinea
Philippines
Timor Leste

3.11 Information on where, when and how initial releases will be made

Chromolaena odorata is currently found in northern Queensland, around Mossman, Innisfail, Tully, Mission Beach, Mount Garnet and Townsville. It is also present on the Cocos Islands and Christmas Island, Australian Territories in the Indian Ocean. A culture is no longer being maintained in quarantine at the Ecosciences Precinct. Upon approval of release, a fresh colony will be obtained from PNG and the agent will be reared through one full generation before being transferred to plants outside quarantine.

Cultures of the agent will be maintained at the Ecosciences Precinct, Brisbane and South Johnstone in north Queensland. Initially batches of 100-200 galls will be placed at all key locations, in early summer when plants are actively growing. Once gall numbers reach suitable levels in the field, field collection and re-distribution will occur. Records of where galls are placed in the field and where establishment occurs will be maintained.

3.12 Establishment and evaluation

All release sites will be monitored for gall fly establishment and impact on *C. odorata*. Other species including *P. clematidea* in the vicinity of the field sites will also be monitored for the presence of the gall fly.

3.13 Discussion

During multiple species-minus-*C. odorata* choice tests, galls developed on and adults emerged from only *C. odorata* and *P. clematidea*. However, the numbers on *P. clematidea* were significantly fewer than on *C. odorata*. In further trials to clarify to what extent *C. connexa* can develop on *P. clematidea*, numbers of galls formed on and the resulting adults emerged were also significantly fewer for *P. clematidea* than for *C. odorata* in most trails. In continuation trials, populations could not be sustained on *P. clematidea* as the numbers of adults emerging were very low and there was a lack of synchrony in those that did emerge. Galls did not form on any other plants species tested. The results are consistent with results of previous host specificity work conducted overseas where 80 species, including 22 in the Asteraceae were tested in four countries.

The relatively low numbers of galls formed and adults emerging from *P. clematidea* in our trials are not necessarily inconsistent with results in Thailand where gall formation did not occur on *P. clematidea*. Populations of *C. connexa* could not be maintained on *P. clematidea* in our studies and observations in Palau have failed to find gall formation on *P. clematidea*. Differences could be attributed to the environmental conditions under which the trials were performed and the condition of the plants.

Although galls formed on *P. clematidea*, the size of galls was generally smaller, as was the number of adults emerging compared to those from *C. odorata*. The diameter of stems of *P. clematidea* was also smaller on average compared to *C. odorata* and this is true for plants in the field as *P. clematidea* is a small herbaceous plant. While gall formation may occur on *P. clematidea* in the field, our tests repeatedly show it is unlikely that populations will persist on this species and any damage will be minor.

While *Praxelis* is the most closely related genus to *Chromolaena*, it is interesting to note that galls did not form on *Chromolaena squalida* in any trials. Galls also did not form on either

of the two native *Adenostemma* species. No DNA sequences were available to determine the relatedness of the genus to *Chromolaena*. However, there are no structural features that show particularly close resemblance to other members of the tribe (Robinson *et al*. 2009). They believe the placement of *Adenostemma* in the tribe Eupatorieae is based on the chromosome number, $x = 10$, which is essentially consistent for the remainder of the tribe. If this is true and the two genera are not closely related, then it is not surprising that gall formation did not occur on either of the two species and they would not be at risk from *C. connexa* if it was released.

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Appendices

Appendix 1. List of plant species previously tested using *C. connexa*. No galls formed on any test plant.

Family	Genus/species	Where tested
Amaranthaceae	*Amaranthus tricolor* L.	Indonesia
Asteraceae		
Eupatorieae	*Ageratum conyzoides* L.	Indonesia, Guam, Thailand
	Austroeupatorium inulaefolium (L.)	Indonesia
	Eupatorium adenophorum (Spreng.) R.M. King & H. Rob.	Thailand
	Mikania scandens (L.) Willd.	Guam
	Praxelis clematidea (Griseb.) R.M. King & H. Rob.	Thailand
Anthemideae	*Artemisia vulgaris* L.	Philippines
	Chrysanthemum indicum L.	Philippines
	Chrysanthemum morifolium Ramat	Indonesia
Astereae	*Aster* sp.	Indonesia
Coreopsideae	*Bidens pilosa* L.	Guam
	Cosmos caudatus H.B.K	Indonesia
	Cosmos sulfureus Cav.	Guam
Heliantheae	*Clibadium surinamense* L.	Indonesia
	Dahlia pinnata Cav.	Indonesia
	Helianthus annuus L.	Indonesia, Guam, Philippines, Thailand
	Tithonia diversifolia Gray.	Indonesia
	Zinnia elegans Jacq.	Indonesia
Inuleae	*Blumea aurita* L.	Thailand
	Blumea balsamifera (L.) DC	Philippines
Mutiseae	*Gerbera jamesonii* Bolus.	Indonesia
Plucheae	*Pluchea indica* (L.) Less.	Indonesia
Senecioneae	*Gynura aurantica* DC	Indonesia
Tageteae	*Tagetes erecta* L.	Thailand
Amaryllidaceae	*Allium sativum* L.	Indonesia
Brassicaceae	*Brassica oleracea* L.	Guam
Convolvulaceae	*Ipomoea aquatica* Forsk.	Indonesia
	Ipomoea batatas (L.) Lamk.	Indonesia
Cucurbitaceae	*Citrullus lanatus* (Thunb.)	Indonesia, Guam
	Cucumis melo L.	Indonesia
	Cucumis sativus L.	Indonesia
	Curcubita moschata Duch. ex Poir	Indonesia
Euphorbiaceae	*Hevea brasiliensis* (HBK)	Indonesia
	Jatropha curcas L.	Thailand
	Manihot esculenta Crantz	Indonesia, Thailand
	Ricinus communis L.	Indonesia
Fabaceae	*Albizia falcataria* (L.) Fosberg	Indonesia
	Arachis hypogaea L.	Indonesia
	Caesalpinia pulcherrima (L.) Swartz	Indonesia
	Calliandra haematocephala Benth.	Indonesia
	Crotalaria juncea L.	Indonesia
	Desmodium heterocarpon (L.) DC	Indonesia
	Dolichos lablab L.	Indonesia
	Flemingia strobilifera R.Br.	Indonesia
	Gliricidia sepium Walp.	Indonesia
	Glycine max (L.) Merr.	Indonesia, Thailand
	Leucaena glauca Merr	Indonesia

Appendix 1 (continued). List of plant species previously tested using *C. connexa*. No galls formed on any test plant.

Fabaceae (cont)	*Leucaena leucocephala* (Lam.) de Wit	Philippines
	Pachyrhizus erosus (L.) Urb.	Indonesia
	Phaseolus sp.	Guam
	Psophocarpus tetragonolobus DC	Indonesia
	Pterocarpus indicus Willd.	Philippines
	Sesbania grandiflora Pers	Indonesia
	Vigna radiata (L.) Wilczek	Thailand
	Vigna unguiculata (L.) Walp.	Indonesia
Lamiaceae	*Vitex negundo* L.	Philippines
Malvaceae	*Abelmoschus esculentus* (L.) Moench	Guam
	Gossypium obtusifolium Roxb.	Indonesia
	Hibiscus rosa-sinensis L.	Indonesia
Meliaceae	*Swietenia macrophylla* King	Philippines
Myrtaceae	*Eugenia aquea* Burm.	Indonesia
	Eugenia caryophyllus Bull & Harris	Indonesia
	Psidium guajava L.	Indonesia
Poaceae	*Oryza sativa* L.	Indonesia, Thailand
	Saccharum officinarum	Thailand
	Sorghum vulgare Persoon	Thailand
	Zea mays L.	Indonesia, Guam, Thailand
Rubiaceae	*Coffea arabica* L.	Thailand
	Coffea robusta Linden ex De Wild	Indonesia
Rutaceae	*Citrus aurantifolia* (Christm.) Swingle	Guam & Thailand
	Citrus nobilis Lour	Indonesia
	Citrus reticulata Blanco	Thailand
Solanaceae	*Capsicum annuum* L.	Indonesia, Guam, Thailand
	Capsicum frutescens	Thailand
	Lycopersicum esculentum Mill.	Indonesia, Thailand
	Nicotiana tabacum L.	Indonesia
	Solanum melongena L.	Indonesia
	Solanum tuberosum L.	Indonesia
Sterculiaceae	*Theobroma cacao* L.	Indonesia
Verbenaceae	*Lantana camara* L.	Indonesia

Summary

Indonesia	56 species in 14 families
Guam	12 species in 8 families
Philippines	8 species in 4 families
Thailand	19 species in 7 species

Total: 80 species in 18 families, including 22 in Asteraceae and 5 in Eupatorieae

Source: Guam (Esguerra 2002; Appendix 2), Indonesia (McFadyen *et al*. 2003; Appendix 3), Philippines (Aterrado & Bachiller 2002; Appendix 4) and Thailand (Kernasa *et al*. 2013; Appendix 5)

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PROCEEDINGS OF THE FIFTH INTERNATIONAL WORKSHOP ON
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ZACHARIADES, C., R. MUNIAPPAN AND L.W. STRATHIE (EDS). ARC-PPRI (2002) PP. 148-151

INTRODUCTION AND ESTABLISHMENT OF THE TEPHRITID GALL FLY *CECIDOCHARES CONNEXA* ON SIAM WEED, *CHROMOLAENA ODORATA*, IN THE REPUBLIC OF PALAU

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Since its introduction into Palau in the early 1980s, *Chromolaena odorata* (Siam weed) has invaded agricultural lands. As a result, thickets of the weed have reduced the amount of land in Palau available for cultivation, particularly in Airai, Ngatpang, Aimeliik and Ngaremlengui States. This study was conducted to introduce, determine host specificity of, and establish the tephritid gall fly, *Cecidochares connexa*, to control *C. odorata* in the Republic of Palau.

A culture of *C. connexa* was imported from Guam and reared for seven generations on potted Siam weed enclosed with muslin cloth sleeves in a rearing shed. Host-specificity tests, conducted on three root crops and four medicinal plants, revealed that the gall flies did not attack root crops such as taro, cassava and sweet potato. The flies also did not attack *Coleus blumei, Phyllanthus* sp., *Physalis* sp. and *Mimosa* sp. Adult gall flies were subsequently released in an area infested with Siam weed in Nizimatz, Ngaremlengui. Shoots of the weed were enclosed with muslin cloth sleeve and the adult flies were released inside. The sleeves were removed after three days. The fly was released on four occasions at the same site, and had established 8 months after the first release. By this stage numerous galls were present on the shoots and stems of *C. odorata*, even 4km from the release site. Adult gall flies will be collected from the release site and released in other areas of Palau where Siam weed is abundant.

KEY WORDS: agent establishment and spread, biological weed control, field releases, host specificity

INTRODUCTION

Siam weed, *Chromolaena odorata* (L.) R.M. King and H. Robinson (Asteraceae), a native of South and Central America, was introduced into Palau in the early 1980s. Since then it has invaded many areas and has become a dominant weed in Babeldaob, particularly in Airai, Aimeliik, Ngatpang, Ngaremlengui, and Koror States (Muniappan *et al.*, 1999). The weed has occupied roadsides, vacant lands, pasture areas, and cultivated lands. Because *C. odorata* is an aggressive, fast-growing, scrambling perennial shrub, it is likely that it will continue to spread throughout Palau if left uncontrolled.

The weed has a rapid growth rate, profuse branching and prolific seed production, enabling it to impede access to croplands. Besides, the weed can withstand slashing and burning, as regeneration from the deep roots is rapid. During a dry spell, it becomes a fire hazard. Furthermore, the weed has allelopathic chemicals that suppress the growth of surrounding vegetation, so that some economic plants do not grow in areas infested with Siam weed (Muniappan, 1996).

Chromolaena odorata can be controlled by spraying herbicides such as picloram and triclopyr, but because of its rapid recolonization, this method of control is expensive and uneconomical. Also, herbicides can harm the fragile ecosystems of Palau. Hence, the use of effective, host-specific biocontrol agents is an ideal approach to controlling Siam weed.

In Indonesia, with the assistance of Australian and French entomologists, a biological control agent, *Cecidochares connexa* Macquart (Diptera: Tephritidae), was introduced and established on Siam weed (ACIAR, 1993). The gall fly produced galls on stems and shoots of *C. odorata*, thereby reducing the formation of flowerheads and seeds (Desmier de Chenon *et al.*, this Proceedings). Thus, *C. odorata* is prevented from spreading to noninfested areas.

In 1998, Dr. R. Muniappan of the University of Guam received shipments of the gall fly from Indonesia. The gall fly has since been reared for several generations at this university. Palau Community College Cooperative Research and Extension received a shipment of a pure culture of the gall fly from Guam, and since then it has been successfully reared on *C. odorata* in a rearing shed in Ngaremlengui State.

This paper reports on the rearing, release and establishment of the gall fly, *C. connexa*, on *C. odorata* infestations in several areas in Palau.

Table 1. Number of galls formed on each plant species 1.5 months after release of adult gall flies onto them.

Plant species (crop name)	Family	No. of galls
Chromolaena odorata	Asteraceae	5
Colocasia esculenta (taro)	Araceae	0
Ipomoea batatas (sweet potato)	Convolvulaceae	0
Manihot esculenta (cassava)	Euphorbiaceae	0
Phyllanthus sp.	Euphorbiaceae	0
Coleus blumei	Lamiaceae	0
Mimosa sp.	Mimosaceae	0
Physalis sp.	Solanaceae	0

MATERIALS AND METHODS

Importation and Rearing

A shipment (26 females and 23 males) of adult *C. connexa*, packed in test tubes, was received from Guam in February 1999. Flies were provided with honey to serve as food while in transit. Three male gall flies were dead upon arrival of the shipment. Live adults were released from test tubes onto potted *C. odorata* plants which had been individually enclosed with a frame of mesh wire covered with a muslin cloth sleeve. Gall flies *in copula* could be seen on each plant. The plants were kept in the rearing shed and watered twice a week.

Host-Specificity Testing

Cecidochares connexa has been shown to develop on only *C. odorata*. Despite its confirmed safety to economically important crops, it was decided to test it further on three commonly grown root crops (taro, cassava and sweet potato) and four medicinal plants (*Coleus blumei* Benth., *Phyllanthus* sp., *Physalis* sp., and *Mimosa* sp.) occurring in Palau (Table 1).

One plant of each of the three root crops and medicinal plants was grown in pots. The plants were individually enclosed with a mesh wire frame covered with muslin cloth sleeve. Five newly-emerged gall flies (one male and four females), collected from the existing culture, were released onto each plant. A *C. odorata* plant, treated in the same way, was used as a control. The plants were watered twice a week. After 1.5 months, the frame and muslin sleeve were removed from each plant, and the number of galls was counted.

Field Releases of the Gall Flies

Four field releases of adult gall flies were made from August 4 to October 5, 1999, with a total of 26 flies (7 males and 19 females). The flies were released in an area in Nizimatz, Ngaremlengui where *C. odorata* was growing abundantly. For each release, young shoots of *C. odorata* were enclosed with a muslin cloth sleeve and adult flies were released into the sleeve. The end of the cloth sleeve was tied to the stem with twine to prevent the flies from escaping. The cloth was removed after three days, allowing time for mating and oviposition.

RESULTS AND DISCUSSION

Rearing of Gall Flies

The gall flies, originally from Colombia and received via Indonesia and Guam, were reared successfully for more than one year on potted *C. odorata* in the rearing shed. The flies produced large, prominent galls on the shoots and shoot buds of the weed.

Host-Specificity Tests

Sweet potato, cassava, taro and the medicinal plants tested had produced no galls 1.5 months after exposure to adults. On one *C. odorata* plant, however, five prominent galls were evident (Table 1). This indicates that the test plants were not suitable as alternative hosts for the flies. Consequently it would not be possible for *C. connexa* to maintain a population on other plant species.

Field Releases of Gall Flies

When released as adults in areas infested with *C. odorata* in Nizimatz, Ngaremelengui, the flies established readily (Tables 2, 3), especially where young *C. odorata* was growing vigorously. Despite the fact that much of the release site was burnt a few days after the release, some *C. odorata* shoots that regrew had galls. This indicates that the adult female flies that escaped the fire were readily attracted to young succulent shoots and laid their eggs on them. By December 1999, less than 3 – 5 months after releases, an average of 23% of the plants in the area was infested with a mean of 1.7 galls per plant (Table 2). In April 2000, the percentage of galled *C. odorata* plants increased to 46%, with an average of 1.34 galls per plant. *Chromolaena odorata* plants with galls could be observed as far as 4km from the release site (Table 3).

Table 2. Number of *Chromolaena odorata* plants, average plant height and number of galls in a 2 x 3m quadrat at different distances from the release site in Nizimatz, Ngaremlengui, on December 20, 1999.

Distance from site (m)	Average plant height (m)	Total no. of *C. odorata* plants	No. of plants with galls	Total no. of galls
0 (release site)	0.4	23	5	10
10	0.8	16	4	10
20	0.6	40	11	19
30	0.7	34	5	7
40	0.8	21	4	6
50	0.8	20	5	10
60	1.2	18	6	7
Total		172	40	69

Table 3. Number of *Chromolaena odorata* plants, average plant height and number of galls in a 2 x 3m quadrat at different distances from the release site in Nizimatz, Ngaremlengui, on April 26, 2000.

Distance from site (m)	Average plant height (m)	Total no. of *C. odorata* plants	No. of plants with galls	Total no. of galls
0 (release site)	1.2	17	7	8
100	1.4	16	5	7
250	1.3	17	9	13
500	1.4	14	8	11
750	1.5	17	10	15
1 000	1.5	19	9	13
2 000	1.6	19	12	18
3 000	1.4	22	7	9
4 000	1.5	20	8	7
Total		161	75	101

Therefore, in a short period of 8 months, the flies dispersed rapidly and attacked the weed within a 4km radius of the release site, despite the presence of some predatory arthropods. Both non-web- and web-forming spiders were observed preying on adult gall flies in the field. Black ants also broke the 'windows' (a paper-thin layer of epidermis created by larval tunneling before pupation in order to facilitate the adult's escape) on galls and fed on larvae and pupae of the flies.

Since the gall fly aggressively attacks *C. odorata* and causes stunting of young plants, it can be used together with other biological control agents to reduce the rate of establishment of the weed in other areas of Palau.

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Biology and host specificity of the chromolaena stem gall fly, *Cecidochares connexa* (Macquart) (Diptera: Tephritidae)

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Abstract The stem-galling tephritid fly *Cecidochares connexa* (Macquart) was investigated as a potential biocontrol agent against the weed *Chromolaena odorata* (L) King & Robinson in Indonesia. Adults were tested in choice and no-choice tests, on 55 non-target plant species in 17 families. No oviposition was recorded on 53 of the species, while oviposition but no larval development was recorded on two. Field releases commenced in 1995 and establishment was immediate. The gall fly is now widely established in most Indonesian provinces, where gall parasitism and predation is generally low.

Key words Asteraceae, biological control, *Chromolaena odorata*, weed, parasitism.

INTRODUCTION

Chromolaena (Eupatorium) odorata (L) King & Robinson is a herbaceous shrub native to the tropical Americas which has become a serious invasive weed in the wet/dry tropics of Africa and Asia (McFadyen 1989). It is one of the world's worst invasive alien weeds (International Union for the Conservation of Nature list in Baskin 2002) and is recognised as the worst weed threat to northern Australia (McFadyen & Skarratt 1996). A project to find and introduce agents for biological control of this weed into Indonesia and the Philippines commenced in 1993. The only infestation of chromolaena in Australia is currently being eradicated (Waterhouse 1998).

The stem gall fly was originally collected from *C. odorata* in Mexico, Brazil and Bolivia (Cruttwell 1974), and identified as *Cecidochares connexa* (Macquart) by Dr AL Norrbom, USDA, Washington, in 1992. Because of the successful control in Hawaii of *Ageratina (Eupatorium) adenophora* (Sprengel) and *A. riparia* (Regel) by the similar gall flies *Procecidochares utilis* Stone and *P. alani* Steyskal, respectively (Julien & Griffiths 1998), *C. connexa* was proposed as a suitable agent for the control of *C. odorata* (Cock 1984). Stem galls act as nutrient sinks, reducing stem growth, seed production and carbohydrate storage (Erasmus *et al*. 1992; Fay *et al*. 1996). If present in large numbers, galls severely reduce growth of the host plant and may result in plant death (Dodd 1961; Ehler *et al*. 1984). Population size in gall flies is frequently restricted by parasitism, both in the country of origin (Ehler *et al*. 1984; Hawkins & Goeden 1984) and in the introduced range (Dodd 1961; Harris & Shorthouse 1996). This has limited their use as biocontrol agents. Nevertheless, as *C. connexa* was believed to be easy to rear and host-specific, the decision was made to trial the insect as a biocontrol agent in Indonesia. This paper reports on the biology of the gall fly and the results of the host-specificity testing undertaken in Indonesia.

TAXONOMY AND DISTRIBUTION

The tephritid genera *Cecidochares* Bezzi and *Procecidochares* Hendel are native to the Americas, from the USA to central South America. All are stem gallers or, less commonly, flower gallers or flower feeders, with host plants in the Asteraceae. Most of the gall-forming species are highly host specific, sometimes attacking only a single plant species (Foote *et al*. 1993). Many species are difficult to separate morphologically, yet do not interbreed (A Norrbom pers. comm. 2001). *Cecidochares connexa* (Macquart) is the type species for the genus.

Adult flies identified as *C. connexa* have been reared from larvae in stem galls in *Eupatorium* and *Chromolaena* species from Central America to northern Argentina (Aczel 1953; d'Araujo Silva *et al*. 1968; Cruttwell 1974; A Norrbom unpubl. data 1992). Adults of *C. connexa* have also been reared from larvae feeding without gall formation in the flowers of *Chromolaena* spp. in Brazil and Trinidad (A Norrbom unpubl. data 1992; de Prado 1999). It is likely that cryptic species or host-races are involved, which will require DNA analysis to clarify (A Norrbom pers. comm. 2001).

In some earlier publications, the species was incorrectly referred to as *Procecidochares connexa* (Julien & Griffiths 1998; McFadyen 1999), due to the close similarity of the morphology and biology of this species with *P. utilis* and *P. alani*.

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MATERIALS AND METHODS

Parasitism studies

Opportunistic sampling for gall parasitism was undertaken in the Neotropics and in Indonesia. Adult parasitoids reared from galls in the Neotropics were sent for identification to the United States National Museum, Beltsville, USA. The parasitoids reared in Indonesia were identified by RDC. Parasitism rates in Indonesia were estimated by dissecting galls and recording the number of parasites encountered.

Gall-fly culturing

A culture of the gall fly was established in early 1993 in the quarantine facility at Marihat, North Sumatra, Indonesia, from adults reared from stem galls on *C. odorata* on the Caribbean coast of Colombia. This colony was used in the experiments described in this paper and all insectary and field colonies in Indonesia and elsewhere in SE Asia were derived from this colony. The colony was held and all testing undertaken in a naturally lit quarantine insectary and shade-house without temperature controls at Marihat (altitude 300 m; latitude 3°N) with 12 h L: 12 h D photoperiod and a temperature range of 26°C at night to 32°C in the day. Voucher specimens of flies from this culture are lodged at the Australian National Insect Collection, Canberra.

Host testing

Plants tested were chosen by the Indonesian Department of Agriculture and are listed in Table 1. Simple paired choice tests were used, with the test plant and a plant of *C. odorata* put together in a 0.5 by 0.5 by 1.5 m cage, made from a wooden frame covered with metal gauze. Four plants of each test species were tested and all plants used were growing in pots and were healthy with young shoots. Five female and five male flies were put into each cage and left for 3 d before being removed. Removal after 3 d ensured that the plants were not overloaded with eggs that might have reduced survival of larvae. Flies were observed and their activity (resting, mating, probing the plant, ovipositing) was noted each 30 min from 0830 h to 1330 h. After exposure to the adults, one test plant of each species was examined microscopically for eggs or oviposition scars, while the remaining three plants were kept to check if galls developed.

No-choice tests, using the same number of adults and size of cage but without potted *C. odorata* in the cage, were carried out with the closely related plants *Austroeupatorium inulaefolium* and *Ageratum conyzoides* and with sunflower, *Helianthus annuus*. Two replicates were run for each species. After exposure to the adults, plants were kept to check for gall development.

RESULTS

Biology

Adult flies live for 5–11 d and drink water but have not been seen to feed. They are active between 0800 and 1400 h (sunrise at 0600), usually in full sunlight. Mating takes place on the host plant between 0800 and 1100 h. Oviposition

Table 1 Plants species used in choice and no-choice tests for oviposition and larval survival in *Cecidochares connexa*

Amaranthaceae	*Amaranthus tricolor* L.
Asteraceae	
Eupatorieae	*Ageratum conyzoides* L.
	Austroeupatorium inulaefolium (L.)
Astereae	*Aster* sp.
Anthemideae	*Chrysanthemum morifolium* Ramat.
Heliantheae	*Clibadium surinamense* L.
	Cosmos caudatus H.B.K
	Dahlia pinnata Cav.
	Helianthus annuus L.
	Tithonia diversifolia Gray.
	Zinnia elegans Jacq.
Mutiseae	*Gerbera jamesonii* Bolus.
Plucheae	*Pluchea indica* (L.) Less.
Senecioneae	*Gynura aurantica* DC
Caesalpinaceae	*Caesalpinia pulcherrima* (L.) Swartz
	Sesbania grandiflora Pers
Convolvulaceae	*Ipomoea aquatica* Forsk.
	Ipomoea batatas (L.) Lamk.
Cucurbitaceae	*Citrullus lanatus* (Thunb.)
	Cucumis melo L.
	Cucumis sativus L.
	Curcubita moschata Duch. ex Poir.
Euphorbiaceae	*Hevea brasiliensis* (HBK)
	Manihot esculenta Crantz
	Ricinus communis L.
Papilionaceae	*Gliricidia sepium* Walp.
	Arachis hypogaea L.
	Crotalaria juncea L.
Papilionaceae (cont.)	*Desmodium heterocarpon* (L.) DC
	Dolichos lablab L.
	Flemingia strobilifera R.Br.
	Glycine max Merr.
	Pachyrhizus erosus (L.) Urb.
	Psophocarpus tetragonolbus DC.
	Vigna unguiculata (L.) Walp.
Mimosaceae	*Albizia falcataria* (L.) Fosberg
	Calliandra haematocephala Benth.
	Leucaena glauca Merr.
Liliaceae	*Allium sativum* L.
Malvaceae	*Gossypium obtusifolium* Roxb.
	Hibiscus rosa-sinensis L.
Myrtaceae	*Eugenia aquea* Burm.
	Eugenia caryophyllus Bull & Harris
	Psidium guajava L.
Poaceae	*Oryza sativa* L.
	Zea mays L.
Rubiaceae	*Coffea robusta* Linden ex De Wild
Rutaceae	*Citrus nobilis* Lour
Solanaceae	*Capsicum annuum* L.
	Lycopersicum esculentum Mill.
	Nicotiana tabacum L.
	Solanum melongena L.
	Solanum tuberosum L.
Sterculiaceae	*Theobroma cacao* L.
Verbenaceae	*Lantana camara* L.

usually occurs between 1000 and 1400 h. Females fly from plant to plant, walking over the stems and tips and then probing and ovipositing in the buds. Chromolaena has opposite leaves, with each pair orientated at 90° to the preceding pair. The female inserts her ovipositor through the vegetative tissue of terminal or axillary buds and lays eggs in packed masses of 2–16 in the bud tip. In field conditions, where oviposition sites are not restricted, females usually lay two eggs in each tip. Eggs are 0.8 by 0.2 mm, pale translucent white and elongated oval in shape. Each female lays 50–70 eggs over her lifetime.

The eggs hatch in 4–7 d and the larvae tunnel into the stem tissue. The gall swelling first becomes visible at about 15 d and the gall develops steadily until the larvae are fully grown 30–50 d after oviposition. The gall generally develops at a node with a single pair of leaves growing from the gall. Occasionally the gall is internodal or forms at an axillary bud and has no leaves. Mature galls are green but woody, 2–3 cm long and 0.8–1.5 cm wide. Gall size is determined by the size and vigour of the stem rather than the number of larvae in the gall.

In the field, there are usually 2–4 larvae per gall, each larva occupying a separate chamber within the gall. It is rare to encounter a single larva, except where a parasitoid has killed one. Up to 10 larvae may be found in a single gall in laboratory conditions. Mature larvae cut an emergence tunnel to the gall surface, leaving this closed by a 'window' of epidermal tissue that the adult breaks on emergence. Larvae usually construct separate emergence windows, but two larvae may occasionally use the same window. The prepupal and pupal period lasts 15–25 d, and the whole life cycle from egg to adult takes 47–75 d, averaging about 60 d.

In the field in Indonesia, breeding is continuous as long as leaf buds are available. Female flies only oviposit in leaf buds, never in flower buds, and larvae have never been encountered in the flowers. When the plants commence flowering with the start of the dry season (December in North Sumatra and June in Timor, Lombok and adjacent islands south of the Equator), pupation ceases and the mature larvae remain in the prepupal stage within the galls, without cutting emergence tunnels. This period is short in regions with a short or mild dry season, but may be up to 6 months in the drier parts of Timor and the other eastern islands. When plant growth recommences after the rains, the larvae cut emergence tunnels and pupate, to emerge as adults shortly afterwards.

Host testing

Eggs were laid in packed clumps of 4–16 in the terminal and axillary buds and between the bud leaves of *C. odorata* in all tests. These eggs developed normally to the pupal and adult stages. In choice tests, no eggs were laid in any other plants and no attempts at oviposition were observed in the other plants. In the no-choice tests, no eggs were laid in *H. annuus*, but oviposition was observed in *A. inulaefolium* and *A. conyzoides* (plant tips were not dissected so the number of eggs laid was not recorded). The larvae from these eggs did not develop and there was no gall formation.

Parasitism and predation

In the Neotropics, parasitism rates are high, although precise information was not collected. In Mexico, the hymenopteran parasitoids *Torymus umbilicatum* (Gahan) (Torymidae), *Eupelmus* sp. (Eupelmidae) and *Neocatolaccus* sp. (Pteromalidae) were reared from larvae in stem galls, as was an unidentified pteromalid from pupae. In northern Brazil, *Heterospilus pallidipes* Ashmead and *Heterospilus* sp. nr *humeralis* Ashmead (Braconidae) were reared from larvae, while in Bolivia *Heterospilus* sp., *Eupelmus* sp., *Dimeromicrus cecidomyidae* Ashmead (Torymidae) and *Syntomosphyrum* sp. (Eulophidae) were reared from larvae (Cruttwell 1974).

In Indonesia, only two parasites have been encountered. A solitary ectoparasitic eulophid was reared from small larvae in west Java, 5 years after the first releases at the sites. Parasitism was 50% of a sample of 18 galls at one site and 27% of a sample of 35 galls at a second. The solitary larval-pupal endoparasitic chalcid, *Ormyrus* sp., has been encountered at several sites in Sumatra and Java, but parasitism rates were always low, generally below 1% and never exceeding 15%. In Indonesia, predation can be locally significant. Adult flies are eaten by spiders, especially wolf spiders (species not identified) and by small lizards (not identified). Egg predation has not been observed. Larvae within the gall are predated by a large reduviid, *Sycanus* sp., which inserts its proboscis into galls containing large larvae where the wall is still not lignified. The larval contents are sucked out, leaving the shrivelled white skin. In Aceh, north Sumatra, an ant *Tetraponera* sp. (Pseudomyrmecinae) has been observed puncturing the emergence window and removing pupae and prepupal larvae. In some localities, galls have been found torn open with the larvae removed, apparently by small birds. Predation from these causes can exceed 50% but is local and patchy in distribution.

DISCUSSION

As was expected from the field host range and known specificity of *C. connexa*, the cage tests confirmed that *C. odorata* was the only acceptable host plant for this species. In no-choice cage conditions, eggs were laid on two closely related weedy species, but larvae did not develop in these. Based on these results, permission for field release was granted by the Indonesian Government in 1995 and the first releases were made soon after. The gall fly is now established in most of the larger Indonesian islands (Tjitrosemito 2002; Wilson & Widayanto 2002), and has since been released in Palau (Esguerra 2002), Papua New Guinea (Orapa *et al*. 2002), Guam (R Muniappan pers. comm. 2002) and Thailand (B Napompeth pers. comm. 2002).

The impact of the stem gall flies *P. utilis* and *P. alani*, released in several countries of South and SE Asia for the control of their host weeds *A. adenophora* and *A. riparia*, has been greatly reduced by parasitism by native Hymenoptera (Julien & Griffiths 1998). For *C. connexa* in Indonesia, parasitism rates have remained generally low, although in West Java, the solitary ectoparasitic eulophid has reached 50% at some sites. In the general absence of significant parasitism, large gall fly populations have developed in most areas, with >10 galls per meter of stem length. As previously recorded for *P. utilis* (Dodd 1961), this level of attack causes stem die-back and plant death within 12 months.

Die-back and death of plants over areas of 1–50 ha have now been recorded at many sites within 3–5 years of the first release, especially in low altitude sites (<300 m) with a short dry season (Tjitrosemito 2002; Wilson & Widayanto 2002). In these areas, successful control of chromolaena is being achieved (McFadyen 1999). At higher altitude sites (>600 m), or where frequent cloudy conditions reduce maximum daytime temperatures and restrict activity of adult flies, or where a long dry season limits the number of generations per year, fly populations have increased much more slowly and control may not be adequate. Overall, this gall fly has proved a very successful biocontrol agent and offers a real opportunity for control of this very serious weed.

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BIOLOGICAL CONTROL OF *CHROMOLAENA ODORATA*: PRELIMINARY STUDIES ON THE USE OF THE GALL-FORMING FLY *CECIDOCHARES CONNEXA* IN THE PHILIPPINES

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The gall fly, *Cecidochares connexa* has been imported to the Philippines from Indonesia for biological control of *Chromolaena odorata*. The fly oviposits into the tender shoots of the host plant. Galls start to appear within 12 – 15 days after oviposition. Windows appear on the gall one month after oviposition and attain a maximum width of 9.7mm and length of 13.7mm. Each gall contains from 2 – 10 pupae. Galls were harvested about a week after the appearance of windows. Adults start to emerge from the 51st day onwards. As many as 107 galls were recorded from a single *C. odorata* host plant. Host die-back was observed during heavy infestation. Host-specificity tests on selected plants showed no oviposition or gall formation.

KEY WORDS: biological weed control, lifecycle, host specificity, Tephritidae

INTRODUCTION

Chromolaena odorata (L.) R.M. King and H. Robinson (Asteraceae), known locally as 'hagonoy', has invaded agricultural fields, rangelands, forests, plantations and marginal areas of the Philippines. For rangelands and coconut and other plantations, the rapid invasion of the weed has had a severe impact in terms of decreased carrying capacity. It is unpalatable and, when ingested by cattle, causes diarrhoea. In extreme cases death has been reported (Sajise *et al.*, 1974).

This weed was reported to have been introduced into the Philippines in the early 1960s (Pancho and Plucknett, 1971). It spread throughout the country from the southern provinces towards the north. Since then, control of *C. odorata* has been an integral component of agricultural cultivation.

In the Philippines, a serious attempt towards biological control of *C. odorata* was initiated in 1993 when the moth *Pareuchaetes pseudoinsulata* Rego Barros (Lepidoptera: Arctiidae), previously discovered on the Philippine island of Palawan, to which it had possibly been accidentally introduced from Sabah, was found to heavily defoliate the weed. However, later mass rearing and field release of this insect did not result in high field populations or much defoliation.

Biological control of *C. odorata* in Indonesia has been gaining ground since *Cecidochares connexa* Macquart (Diptera: Tephritidae), a gall-forming fly, was imported from South America into quarantine in North Sumatra in 1993. It was found to be specific to *C. odorata* (Sipayung and Desmier de Chenon, 1994), and has been released around North Sumatra since 1995. Since then it has spread widely, with significant galling and growth suppression of *C. odorata* (Desmier de Chenon *et al.*, this Proceedings).

In this paper we report on the importation of *C. connexa* into the Philippines from Indonesia, and on host-specificity testing and other observations in the Philippines.

MATERIALS AND METHODS

Importation of the Gall Fly *Cecidochares connexa*

An application for a permit for the importation of *C. connexa* was filed with the Plant Quarantine Service of the Bureau of Plant Industry with copies of all the available literature. The quarantine containment facilities were checked to ensure that safety measures were in place and conformed to safety standards and quarantine regulations.

Mass Rearing of *Cecidochares connexa*

Emerging flies were immediately contained in small medicine vials for mating. Moistened cotton wool served as plugs to prevent escape. Mated flies were introduced in pairs into oviposition cages in the quarantine insectary. The cages contained from 2 – 4 host plants. Water, virtually the only substance the flies feed on, was sprayed regularly into the cage. Honey was occasionally offered as alternative food for adults. From six to 12 pairs of mated flies were introduced into each cage and kept in the cage for 2 – 3 days. After this time the potted host plants were taken out to the adjoining screen house for exposure to sunlight, to allow normal growth and development of the galls. As soon as the galls had enlarged, and 'windows' appeared (a

tunnel created by the mature larvae for the escape of adult flies during emergence, leaving only a parchment-thin layer on the gall surface), they were harvested and brought back to the insectary.

Host-Specificity Tests

Trials were conducted from August 1999 to March 2000 at the quarantine containment facilities of the PCA-Davao Research Center. No-choice and choice tests on the NCBP-prescribed host-plant species were conducted over a 6-month period. Tests were replicated 10 times, except for *Vitex negundo* L., with only six (Table 1).

No-choice Tests

Cages of 0.3 x 0.3 x 0.6m were used to contain, individually, the different host plant species. Three mated pairs of flies were introduced into each cage. They were kept in it for 3 days before being retrieved and either used for mass rearing or destroyed. *Chromolaena odorata* was placed in a separate cage as a control.

Choice Tests

An array of host plant seedlings of species prescribed for testing by the National Committee on Biosafety of the Philippines (NCBP), at most 0.51m high were placed all together in big cages measuring 0.6 x 0.6 x 0.76m. At least five pairs of flies were introduced into the cage each time. After the experiment was terminated, the flies were destroyed. *Chromolaena odorata* was always placed in the cage with the other plant species in this series of tests.

Longevity Test

Newly emerged flies were placed individually in medicine vials. Longevity was measured for (i) males only and (ii) females only, with twelve replicates.

Preliminary Test on Control of *Chromolaena odorata* by *Cecidochares connexa* under Confinement

To simulate field conditions, a preliminary test was set up using a 2.4 x 2.4 x 2.4m cage constructed inside a screen house. One hundred pairs of adult flies were introduced onto 15 healthy polybagged *C. odorata* plants. A similar set of untreated control plants was also set up inside the screen house.

Table 1. Results of host-specificity tests on *Cecidochares connexa*.

Common name	Scientific name	Family	n	Galling observed	
				No-choice test	Choice test
Hagonoy	*Chromolaena odorata*	Asteraceae	10	+	+
Sambong	*Blumea balsamifera*	Asteraceae	10	-	-
Damong maria	*Artemisia vulgaris*	Asteraceae	10	-	-
Manzanilla	*Chrysanthemum indicum*	Asteraceae	10	-	-
Sunflower	*Helianthus annuus*	Asteraceae	10	-	-
Ipil-ipil	*Leucaena leucocephala*	Asteraceae	10	-	-
Narra	*Pterocarpus indicus*	Fabaceae	10	-	-
Mahogany	*Sweitenia macrophylla*	Meliaceae	10	-	-
Lagundi	*Vitex negundo*	Verbenaceae	6	-	-

Table 2. Results of preliminary trials on control of *Chromolaena odorata* by *Cecidochares connexa* under confinement.

Treatment	Mean no. of branches per plant (n = 15 plants)	% branches galled	% die-back of total branches
Exposed to flies	8.46	73	43
Flies excluded	6.80	0	4.4

RESULTS AND DISCUSSION

Importation of *Cecidochares connexa*

Having complied with all quarantine regulations, the gall fly was imported in May 1999. Four hundred and forty four galls containing pupae were collected on the outskirts of Marihat Research Station, North Sumatra. Only galls with windows were collected. The cargo was securely packed to prevent possible escape of flies *en route* to the containment facility. The cargo was properly documented at the quarantine office upon entry in to the Philippines. In the quarantine room, the cargo was unpacked inside an emergence chamber. The flies were provided with sprayed water. A total of 17 chalcid parasitoids was collected and preserved.

Mass Rearing

As early as 12 days after exposure to adults, galls started to form on the host plant. The growth of the shoot above the gall was considerably reduced. Rosetting of the terminal growth, an indication of slowed growth of the infested part, was apparent. Galls reached a maximum width of 9.7mm and length of 13.4mm, and each contained 2 – 10 pupae. It took about a month for a gall to develop windows. Harvesting of the galls was done a week after the appearance of windows, to anticipate early emergence. The galls were dissected and pupae kept in plates inside an emergence box.

First-generation flies appeared to have acclimatized easily, since nothing unusual about the population was noted, in terms of health, abnormalities, or death upon emergence. The flies were found to be so prolific that the population had to be regulated to limit oviposition, to allow for easier management under quarantine.

Host-Specificity Tests

In all trials of both no-choice and choice tests, gall formation occurred only on *C. odorata* (Table 1), with a range of 12 – 15 days before gall appearance on this species. The results of these tests confirm that *C. connexa* is highly host-specific on *C. odorata*, and are in conformity with the tests conducted by Sipayung and Desmier de Chenon (1994).

Longevity Study

Initial trials on the longevity of *C. connexa* showed that females outlived the males. Males lived from 4 – 9 days, with an average longevity of 6.41 days, while females lived from 6 – 14 days, with an average of 11.6. This study provides an indication of the number of egg-laying days available to female flies.

Control of *Chromolaena odorata* by *Cecidochares connexa* under Confinement

After a single release of 100 pairs of adult flies, results show that over 6 months, 73% of the branches developed galls and 59% of these branches died. On the other hand, the untreated plants had zero infestation and a die-back of 4.4%, which was due to natural causes (Table 2).

Conclusion

Colonization of *C. odorata* by *C. connexa* in the field and its subsequent suppression should eventually allow the regrowth of beneficial plants in coconut and other plantations and the grass to grow in rangeland used for livestock rearing. It will also reduce the fire hazard caused by *C. odorata* thickets during the dry season in these rangelands.

The costs of agricultural production would decrease correspondingly, since *C. odorata* would be relegated to a lower significance level and may not require priority action for control.

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Biological control of *Chromolaena odorata* in Thailand

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Research into the biological control of chromolaena, *Chromolaena odorata* (L.) R.M. King and H. Robinson (Asteraceae), in Thailand has been conducted by the National Biological Control Research Center since 1986. Two species of natural enemies were introduced. The defoliating moth *Pareuchaetes pseudoinsulata* Rego Barros (Lepidoptera: Arctiidae) was twice introduced from Guam, in 1986 and again in 2006, and the gall fly *Cecidochares connexa* (Macquart) (Diptera: Tephritidae) was introduced from Indonesia in 2001. These species were investigated under quarantine, reared in the laboratory and released in areas which were invaded by *C. odorata*. However, neither species established. In renewed efforts to control chromolaena, *C. connexa* was reintroduced from Papua New Guinea in 2009. A detailed study on the biology of *C. connexa* found that the female adult fly preferred to lay eggs on tender shoots of *C. odorata*. Eggs hatched in 6.00 ± 0.90 days. Larvae developed in 38.10 ± 3.80 days. Each gall contained one to four larvae. Pupae developed in 22.60 ± 1.80 days. The longevity of male and female adults was 8.00 ± 0.89 and 14.00 ± 1.00 days, respectively. The total length of the life cycle was 67.60 ± 5.60 and 73.20 ± 6.10 days respectively. Host-specificity trials indicated that *C. connexa* adults did not lay eggs on 20 plant species in both choice and no-choice tests. Inoculative field releases of the gall fly are planned in 2011.

KEYWORDS: biocontrol; biology; *Cecidochares connexa*; host-specificity

INTRODUCTION

Chromolaena odorata (L.) R.M. King and H. Robinson (Asteraceae), also known as chromolaena, is a serious weed of many economic field crops in Thailand. It has spread throughout the country, even into the high hill plantations situated at 600-800m above sea level. *Chromolaena odorata* is a shrub native to the tropical Americas and continues to spread through south-east Asia into the South Pacific, and into central and eastern Africa from the infestations in western and southern Africa. It is regarded as a very serious threat to agriculture and the environment in most of these countries. (McFadyen and Skarratt 1996; McFadyen et al. 2003).

In Thailand, biological control of *C. odorata* is being conducted by the National Biological Control Research Center (NBCRC). Earlier, attempts at biological control of *C. odorata* conducted in Thailand from 1975 to 1988 were described by Napompeth (1982) and Napompeth et al. (1988). No native insect species that showed adequate potential as biological control agents were found in Thailand (Napompeth and Winotai 1991). Consequently, two agents, the defoliating moth *Pareuchaetes pseudoinsulata* Rego Barros (Lepidoptera: Arctiidae) and the gall fly *Cecidochares connexa* (Macquart) (Diptera: Tephritidae) were introduced. The former was twice introduced from Guam in 1986 and 2006 and the latter was first introduced from Indonesia in 2001. However, neither agent established. In 2009, NBCRC reintroduced *C. connexa* from Papua New Guinea (PNG). This

paper reports on research conducted on the biology and host specificity of *C. connexa* prior to its release in Thailand.

MATERIALS AND METHODS

Rearing of *Cecidochares connexa*

Approximately 200 mature galls were collected from the field in PNG in June 2009 to initiate a rearing colony in a quarantine insectary at NBCRC, CRC, Kasetsart University, Thailand. The galls that contained immature stages of *C. connexa* were kept in a round plastic box, 20cm in diameter and 10cm high, with soaked cotton wool for moisture. Emerging flies were moved to a screen cage (5.0m x 6.0m x 2.0m), with actively growing chromolaena plants to provide oviposition sites. Water was regularly sprayed by 1,000ml hand sprayer into the cage for the flies to drink. The flies were left in this cage for 14-16 days, until they died, and then the potted *C. odorata* plants were moved to natural conditions in the adjoining screen house, to allow normal growth and development of the galls. As soon as the galls had matured, as evidenced by the formation of a sealed window on the side of the gall, they were harvested and held in round plastic boxes 20 to 25 days for adult emergence. Using this method, gall flies could be continuously produced for biology and host-specificity studies.

Biology of *Cecidochares connexa*

A pair of flies was kept in a test tube (2.5cm x 15.0cm) for mating and then moved to an oviposition cage (50cm x 50cm x 60cm), in the quarantine insectary. Each cage contained four potted chromolaena plants and water was sprayed into the cage to provide free moisture for flies. The flies were left for 24 hours for oviposition to occur, after which they were removed. The plant shoots were examined daily. The developmental stages of the gall fly were evaluated by dissection of 4 galls every 5 days. The width and length of the galls were measured using vernier calipers.

Host-specificity testing of *Cecidochares connexa*

Twenty plant species were used to test host specificity. Paired-choice trials were conducted using one potted test plant and one *C. odorata* plant in a 50cm x 50cm x 60cm cage. Each species was tested six times. Vigorously growing potted plants with young shoots were used. Five pairs of flies were placed into each cage and left for three days before being removed. Survival of larvae and development of galls on each plant were observed. If galls developed, survival to the adult stage was monitored. No-choice tests were also conducted for each plant species using the same method as above but without potted *C. odorata* in the cage. Deposited eggs were studied under a microscope.

RESULTS

Biology of *Cecidochares connexa*

Eggs were laid in shoot tips and axillary buds and hatched within an average of 6.00 ± 0.94 days (Table 1). First instar larvae entered the top of the plant and fed, causing galls to appear within 14 days. The galls continually grew as

Table 1. Duration of developmental stages of *Cecidochares connexa* in Thailand.

Developmental stages		Mean ± SD (days)	Range (days)
Egg		6.00 ± 0.94	5-7
Larva		38.10 ± 3.84	33-44
Pupa		22.60 ± 1.84	20-25
Total development from egg to adult:	Male	57.88 ± 5.09	53-70
	Female	63.32 ± 5.31	54-74
Adult longevity:	Male	8.00 ± 0.89	7-9
	Female	14.00 ± 1.00	13-15
Total life cycle:	Male	67.60 ± 5.60	54-80
	Female	73.20 ± 6.10	63-85

Table 2. Plant species used, and adult progeny production, in choice and no-choice tests for oviposition and larval survival of *Cecidochares connexa* in Thailand.

Family	Test plant	Common name	No. adult *C. connexa* progeny obtained		
			No-choice test	Choice test	
			Test plant	Test plant	*C. odorata*
Asteraceae	*Ageratum conyzoides* L.*	Billy goat weed	0	0	17
	Blumea aurita L.*	Tropical white weed	0	0	6
	Praxelis clematidea (Griseb.) R.M.King & H.Rob*	Praxelis	0	0	9
	Ageratina adenophora (Spreng.) R.M.King & H.Rob.*	Crofton weed	0	0	11
	Helianthus annuus L.*	Sunflower	0	0	8
	Tagetes erecta L.*	Marigold	0	0	6
Euphorbiaceae	*Manihot esculenta* L.*	Cassava	0	0	10
	Jatropha curcas L.*	Physic nut	0	0	16
Leguminosae	*Glycine max* (L.) Merr.*	Soybean	0	0	12
	Vigna radiata (L.) Wilczek*	Mungbean	0	0	9
Poaceae	*Oryza sativa* L.	Rice	0	0	12
	Sorghum vulgare Person*	Sorghum	0	0	16
	Zea mays L.*	Corn	0	0	16
	Saccharum officinarum L.*	Sugarcane	0	0	11
Rubiaceae	*Coffea arabica* L.*	Arabica coffee	0	0	5
Rutaceae	*Citrus aurantifolia* (Christm.) Swingle	Lime	0	0	13
	Citrus reticulata Blanco*	Mandarin	0	0	18
Solanaceae	*Capsicum annuum* L.*	Chili pepper	0	0	15
	Capsicum frutescens L.*	Cayenne pepper	0	0	7
	Lycopersicon esculentum Miller*	Tomato	0	0	8

*Alien species.

the larvae fed. The larval development period was an average of 38.10 ± 3.84 days. Prior to pupation, mature larvae cut windows in the side of the galls, through which the adults emerged. The pupal stage was 22.60 ± 1.84 days. Male and female adults lived for an average of 8.00 ± 0.90 and 14.00 ± 1.00 days, respectively. The total life cycle for males and females was 57.88 ± 5.09 and 63.32 ± 5.31 days, respectively (Table 1).

The average number of pupae per gall was 1.74 ± 0.80. The mean gall dimensions were 1.01 ± 0.30cm in width and 1.04 ± 0.22cm in length.

Host-specificity testing of *Cecidochares connexa*

In both choice and no-choice tests, *C. connexa* did not lay eggs on any of the 20 plant species tested. Gall flies completed development only on chromolaena plants (Table 2).

DISCUSSION

The results from the biology studies show that *C. connexa* developed well and reproduced on chromolaena. The length of the life-cycle of the gall fly was 57.88 ± 5.09 and 63.32 ± 5.31 days for males and females respectively, which was slightly longer than that found by Muniappan and Bamba (2002), who reported that *C. connexa* took 55 days to complete its life-cycle. The present study found that there were 1-4 larvae per gall which is similar to that observed by Cruz et al. (2006) who reported three pupae per gall.

The results of the choice and no-choice host specificity tests indicated that the gall fly was highly specific to *C. odorata*. The results were similar to that by Aterrado and Bachiller (2002) and McFadyen et al. (2003). Based on these results, *C. connexa* should be safe to release in areas invaded by *C. odorata*. The gall fly has proved to be a useful agent in many countries e.g. PNG (Day and Bofeng 2007) and it is hoped that similar results will be achieved in Thailand. Further studies on the potential of *C. connexa* and its ability to establish in areas in Thailand and for it to control *C. odorata* in the field will be conducted in the future.

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