Chromolaena in the Asia-Pacific region

Proceedings of the 6th International Workshop on biological control and management of chromolaena held in Cairns, Australia, May 6–9, 2003

Editors: M.D. Day and R.E. McFadyen

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COVER
Clockwise from top left: *Cecidochares connexa* adult (R. Desmier de Chenon); *Pareuchaetes pseudoinsulata* adult (W. Orapa); *Actinote anteas* adult (R. McFadyen); *Actinote anteas* larva (C. Zachariades); *Chromolaena odorata* (unknown); *Calycomyza eupatorivora* adult (C. Zachariades).

Contents

Preface

Chromolaena is a serious weed of tropical pastures and a threat to national parks and other biodiversity conservation areas.

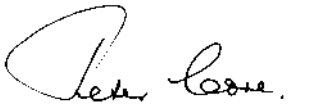
It has quickly spread from its original home in the West Indies to large areas of the wet tropics of Africa and Asia. It is now considered the major weed threat to Australia.

Chromolaena has many natural enemies and biological control has long been touted as a control option. Through ACIAR-supported projects a leaf-feeding moth has been released into oil palm and pastoral areas in Indonesia, where it has successfully established in several areas. Another ACIAR project introduced a new control agent, the stem-galling fly from South America, which had not been tried or host-tested anywhere else in the world. It was first released in Indonesia in 1995 and later in the Philippines. These controls have significantly reduced weed populations in the two countries.

Current ACIAR projects aim to enhance the biological control of chromolaena in Indonesia, Papua New Guinea and the Philippines and to introduce bio-control to East Timor, where chromolaena has invaded pastures, crops, gardens and other areas, causing significant livestock losses.

Successful biological control of chromolaena will remove the need for costly manual removal that is currently necessary in most areas. In Australia, each new infestation costs about $800,000 to eradicate.

ACIAR is pleased to be able to publish this series of papers, which were presented at the 6th International Workshop on Biological Control and Management of Chromolaena. It is hoped this publication will facilitate the uptake of research and allow greater collaboration between countries where chromolaena is a problem.



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Experience with chromolaena in different countries

An infestation of chromolaena in the Erap Valley, Morobe Province of PNG. M. Day

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Chromolaena in East Timor: history, extent and control

Rachel Cruttwell McFadyen[1]

Abstract

Due to its relative isolation, East Timor was free of chromolaena until after the Indonesian invasion in 1974. The weed is now widespread and has invaded most of the grasslands and savannah woodlands, both natural and secondary. The presence of chromolaena is severely impacting on native biodiversity and reducing pasture availability for livestock, including the Timor ponies, the only transport for most hill villages. Biological control is the only feasible management method, and there is a need for the immediate importation and release of agents already tested and utilised by other countries.

History

CHROMOLAENA first arrived in southeast Asia about 1930 (McFadyen 1989), and its subsequent spread through the region was largely the result of human movement, especially the movement of vehicles and machinery. Up until 1974, East Timor was a small isolated Portuguese colony, with little trade and no significant contacts with the Dutch East Indies or the previously British countries Malaysia, India and Singapore. East Timor was conquered by the Japanese in 1942–43 after fierce fighting, but there seems to have been no direct importation of chromolaena to any part of Timor, presumably because the Japanese troops and their equipment came from areas still free of the weed at that time.

A detailed survey of the vegetation of East Timor undertaken in 1969 and 1970 (Metzner 1977) lists several weed species but not chromolaena, and Dr Metzner is sure that the weed was not present in East Timor when he made his survey (J.K. Metzner pers comm., Oct 2002). However, Stephen Simpson (AusAid project, pers comm., 1988) reported that the weed was already present and increasing in Flores, where it was first seen in West Flores in 1970. Chromolaena was first recorded in Timor in the 1980s, when it was reported along the north coast of West Timor (Ing. Wayan Mudita, pers comm., 1995), probably brought in from Flores through the internal immigration (Immigrasi) program which started about then. By 1988, there were several small infestations in West Timor and it was increasing rapidly in the Besi Pae and Soe area east of Kupang (Alan Smith, Simon Field, AusAid project, pers comm., 1988). By 1995, it was common and widespread from Kupang to Soe (Rachel McFadyen, unpublished report to Weed Management Workshop, Kupang, May 1995).

Indonesia invaded East Timor in 1974 and incorporated it as a province of Eastern Indonesia (NTT). Subsequently, there was a great deal of movement of people and machinery into East Timor from various parts of Indonesia, including Java and Sumatra and probably Flores, where the weed was increasingly widespread. Troop movements in East Timor, plus village resettlement schemes and the construction of new roads and infrastructure, would have aided the general spread of the weed. However, because foreign travel to East Timor was restricted until 1989, there is no documented information on the first occurrences or subsequent spread of chromolaena until 1995, by which time there were already dense infestations in some places (R. Desmier de Chenon, pers comm., 1995). When Australian troops arrived in East Timor after the referendum in September 1999, the hillsides were covered with dense infestations and the need to clean seed from vehicles and machinery returning to Darwin was raised at the Australian Weeds Conference that year (Barbara Waterhouse, pers comm., 1999).

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Current situation

No detailed survey of the distribution of the weed has been undertaken. On my visit in October 2002, which is the end of the dry season, I found dense thickets of chromolaena in all climatically suitable areas visited; west from Dili to Ermera, and south from Manatuto to Laklubar and to Natar Bora on the south coast. Along the north coast west and east of Dili, conditions are too dry for the weed, but it was present as little as 5 km inland where the rainfall is slightly higher. Chromolaena was also absent in dense rainforest and in the coffee plantations, as it is not shade-tolerant. Above 900 m altitude, it became increasingly scarce, being replaced by lantana, melastoma and *Tithonia diversifolia*.

In the *Eucalyptus alba*-acacia grassland savannahs which cover much of the non-arable lands of East Timor, the grasses have now been largely replaced by dense stands of chromolaena. Bushes are between 2 m and 3 m tall, except in gullies where they may reach 5 m or more when they climb up trees. The plants produce new growth immediately after the first storms and maintain dense cover during the wet season, so that no grass or other herbaceous plants survive in their shade. Plants recover very rapidly after fire, with new stems appearing within a week of the first storm rains.

The consequences of this replacement of natural and secondary grasslands by dense chromolaena stands are disastrous both for animal production and for the natural ecosystem and biodiversity. Chromolaena burns readily in the dry season, and a fire in the dry stalks is hotter and goes much higher into the trees than does a pure grass fire. As a result, although the acacia, eucalypt and other trees survive the fires, their seedlings do not, and recruitment ceases. The grass and herb layer has been totally replaced, with the loss of all the native species represented there, and the consequent loss of insects, birds and other animals that depend on these plants. Chromolaena produces a dense crop of flowers in June and seed in July, but no seeds are available to feed birds or animals for the rest of the year. In Timor, chromolaena has a very reduced insect fauna compared with the native plants, which limits the food supply for insectivorous birds and animals. The leaves and stems are unpalatable or poisonous to most herbivorous animals, therefore native herbivores cannot survive on it.

For the same reason, the weed has a very serious impact on animal production. East Timor does not have a significant cattle herd at present, but cattle are kept as an income source, being sold when money is needed (da Cruz 2003). Goats are also kept for meat and to a lesser extent milk, and Timor ponies are an important method of transport for produce and people in the hill villages. All these animals graze on an open-range system on the grasslands that are held in common, and there is little attempt to gather forage or supplement the animals' diet. Consequently, as the grasslands are replaced by chromolaena, the animals starve and the pressure on the arable and cultivated land is increased. Goats have also died from eating the flowers of chromolaena, presumably because the nectar makes them sweet or because no other fodder was available at the time. The flowers contain high levels of pyrrolizidine alkaloids, which cause severe liver damage leading to death (Biller et al. 1994).

Control

Hand clearing, first cutting the stems then digging out the roots with a hoe, is used to clear arable land for cultivation, but is only feasible for small areas. In these, clearing chromolaena may be easier than removing weedy grasses such as *Imperata cylindrica*, and for this reason chromolaena may be seen as a desirable fallow plant, even though it does not provide feed for livestock during the non-crop period. In the current economic situation of East Timor, chemical control is not economically feasible, and it is unlikely that it would ever be economic in the extensive grasslands. Biological control is therefore the only method available to manage the weed in the grasslands and fallow lands.

The present Minister of Agriculture is very supportive of biocontrol and is keen to start a program as quickly as possible. International donor support is being sought to import the gall fly *Cecidochares connexa* from West Timor, where it is now widespread in the western half. Suitable release sites have been identified along the Cribas River south of Manatuto. In accordance with the International Plant Protection Convention protocols for biocontrol, if host specificity has been established by testing undertaken in other countries (McFadyen et al. 2003) and there is no risk of the introduction of parasites or diseases, a biocontrol agent can be imported and released on the approval of the responsible government authority, once they are satisfied that this is in the best interests of the country as a whole (FAO 1996). This is the system used in Papua New Guinea, Micronesia, Fiji, and other Pacific island countries, all of which rely on host-testing carried out in other countries. No problems have been experienced by these countries, and East Timor is to be congratulated on adopting this sensible attitude.

If all goes well, the first releases will be made in 2003 during the wet season, using galls directly collected in West Timor and released into dense chromolaena at nursery sites. Minimal resources and

no infrastructure is required for this, as two or three people should be able to collect sufficient galls from West Timor sites in a day or so. Direct releases of the galls have worked well in both Indonesia and Papua New Guinea. Subsequent redistribution can occur once a sufficient population is present in the original nursery sites. It is anticipated that good control will be achieved in most of the island, more rapidly in sites where the rainy season is longer and more reliable, and more slowly where the dry season is longer and the growing season shorter, with fewer generations of the fly in each year. Control can be expected to be slower above 700 m where lower temperatures slow fly development.

Once the gall fly is established, the importation of other biocontrol agents already tested in South Africa should be considered, starting with the leaf mining fly *Calycomyza eupatorivora*. Rearing and releases of these insects may require greater expenditure, in particular the provision of facilities to rear the insects.

Acknowledgments

I would like to thank the Hon. Estanislaus da Silva, Minister of Agriculture, Forestry and Fisheries, East Timor, for inviting me to participate in the International Conference on: Agricultural Development in East Timor: New Directions for a New Nation, UNTL, Dili, 1–3 October 2002, and for facilitating my travel to see chromolaena infestations in East Timor. I am also grateful to Mr Lorenco Fontes Borgues for accompanying me on the trip to the south coast, and to the Australian Centre for International Agricultural Research (ACIAR) for funding my attendance at the Conference.

References

Biller, A., Boppre, M., Witte, L. and Hartmann, T. 1994. Pyrrolizidine alkaloids in *Chromolaena odorata.* Chemical and chemoecological aspects. Phytochemistry. 35: 615–619.

da Cruz, C.J. 2003. Livestock development in East Timor. In 'Agriculture: New directions for a new nation.' H. Da Costa, C. Piggin, C.J. da Cruz and J.J. Fox (eds), Proceedings 113. ACIAR: Canberra

FAO 1996. Code of Conduct for the Importation and Release of Exotic Biological Control Agents. FAO, Rome. 14 pp.

McFadyen R.E.C. 1989. Siam weed: a new threat to Australia's north. Plant Protection Quarterly 4, 3–7

McFadyen, R.E.C., Desmier de Chenon, R. and Sipayung, A. 2003. Biology and host specificity of the chromolaena stem gall fly, *Cecidochares connexa* (Macquart) (Diptera: Tephritidae). Aust. J. Ent. 42: 294–297

Metzner, J.K. 1977. Man and Environment in Eastern Timor. ANU Development Studies Centre Monograph 8, Canberra.

Biological control of chromolaena in Micronesia

R. Muniappan[1], K. Englberger[2], J. Bamba[1] and G.V.P. Reddy[1]

Abstract

Chromolaena odorata established in the Mariana Islands in the early 1960s and had spread to most of the Micronesian islands by the early 2000s. The natural enemy *Pareuchaetes pseudoinsulata* has established in the Mariana Islands and Pohnpei while the gall fly *Cecidochares connexa* has established in Palau and Guam. The gall fly is being released in Saipan and host specificity tested in Pohnpei. The eriophyid mite *Acalitus adoratus* has fortuitously established in the East Caroline and Mariana Islands. Attempts are being made to eradicate the chromolaena infestation in the Majuro Island of the Marshall Islands.

Introduction

THE island groups in Micronesia associated with the United States of America are the Marianas, Carolines and Marshall Islands located in the western Pacific. The humid tropical climatic conditions of these islands are very suitable for *C. odorata*. The first herbarium specimen of *C. odorata* was collected on Guam in 1963. In the early 1980s, it became a problem in the Mariana Islands. During the 1980s, chromolaena spread to Palau, Yap, Pohnpei and Kosrae in the Carolines. It established in Weno Island of Chuuk (East Carolines) in the late 1990s and in Majuro Island of the Marshalls in 2001.

Biological control

Mariana Islands: The menace of chromolaena in Rota was brought to the attention of (RM) in 1983 and a project proposal was submitted to the Tropical and Subtropical Agricultural Research Program of the USDA. Upon approval of this project, *Pareuchaetes pseudoinsulata* Rego Barros (Lepidoptera: Arctiidae) was introduced from India and Trinidad in 1985 and established on Guam (Seibert 1989). Subsequently it was introduced and established on Rota in 1985 and Tinian and Saipan in 1986. *P. pseudoinsulata* has effectively suppressed chromolaena thickets in all the four Mariana Islands.

In 1984, *Apion brunneonigrum* Beguin-Billecoq (Coleoptera: Apionidae) was introduced to Guam but it did not establish. The natural enemies *Mescinia parvula* (Zeller) (Lepidoptera: Pyralidae) and *Melanagromyza eupatoriella* Spencer (Diptera: Agromyzidae) were imported to Guam from Trinidad but no releases were made as *M. parvula* was difficult to culture in the laboratory and the shipment of *M. eupatoriella* was mostly parasitised. The eriophyid mite *Acalitus adoratus* Keifer (Acari: Eriophyidae) fortuitously established in the Marianas in the early 1990s. The introduction and establishment of *Cecidochares connexa* Macquart (Diptera: Tephritidae) in the Marianas has been reported elsewhere in this publication.

East Caroline Islands: the establishment of chromolaena in Yap and Palau were noted in 1987 and 1988 respectively (Muniappan and Marutani 1988). In 1988 three shipments of *P. pseudoinsulata* were sent to Yap from Guam. Even though releases were made at 14 different sites, *P. pseudoinsulata* was found established at only one site, Talaguw in a 10 m diameter area, in October 1988. It is not known whether establishment has sustained or died out as no follow-up studies have been made. Shipments of *P. pseudoinsulata* were also sent to Palau from Guam in March and April 1996 and October 1997. They were field released in Koror but no field establishment was observed.

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The eriophyid mite *A. adoratus* fortuitously established in Palau and Yap in 1988. A shipment of 26 females and 23 males of *C. connexa* was sent to Palau from Guam in February 1999. Host specificity tests were conducted on seven different species of plants. The insect was approved for release and then field released in October 1999 (Esguerra 2002). *C. connexa* has established well throughout the islands of Koror and Babelthuap in Palau.

West Caroline Islands: P. pseudoinsulata was imported from Guam in 1988. It was reared in the laboratory and field released from February 1989 to May 1990. In October 1990, field establishment was observed (Esguerra et al. 1991). From January to November 1992, shipments of *P. pseudoinsulata* were sent to Kosrae and field released at Tafunsak, Lelu and Utwe. Defoliation in the release sites were observed; however, it is not known whether it has permanently established in Kosrae (Esguerra et al. 1998).

Shipments of *P. pseudoinsulata* have been sent to Chuuk and field released since December 2002. A shipment of *C. connexa* was sent from Guam to Pohnpei and it is being reared in the quarantine laboratory for host specificity testing. It is planned to release *C. connexa* in the four FSM states (Chuuk, Kosrae, Pohnpei and Yap) in 2004.

Marshall Islands: Establishment of chromolaena in Laura, Majuro was observed in October 2001 (Muniappan and Nandwani 2002) and later in February 2003 near the International Airport (Van der Velde, pers. comm.). In both areas chromolaena has been cut and sprayed with herbicides in an attempt to eradicate it in the Marshall Islands.

Acknowledgment

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References

Esguerra, N.M. 1998. The Siam weed infestation in the Federated States of Micronesia — Seven years of attempting to control it. In: Proceedings of the Fourth International Workshop on Biological Control and Management of *Chromolaena odorata*, Bangalore. 80–81.

Esguerra, N.M. 2002. Introduction and establishment of the tephritid gall fly *Cecidochares connexa* on Siam weed, *Chromolaena odorata*, in the Republic of Palau. In: Proceedings of the Fifth International Workshop on Biological Control and Management of *Chromolaena odorata*, Durban, S.A., 2000. 148–151.

Esguerra, N.M., William, W.S. and Smith, J.R. 1991. Status of biological control of Siam weed, *Chromolaena odorata* (L.) R.M. King and H. Robinson on Pohnpei, Federated States of Micronesia. Ecology and Management of *Chromolaena odorata*. BIOTROP Special Publication, No. 44: 99–104.

Muniappan, R. and Marutani, M. 1988. Ecology and distribution of *Chromolaena odorata* in Asia and the Pacific. In: Proceedings of the First International Workshop on Biological Control of *Chromolaena odorata*, Ag. Exp. Sta., University of Guam, Mangilao, U.S.A., Bangkok, Thailand. 21–24.

Muniappan, R. and Nandawani, D. 2002 Survey of arthropod pests and invasive weeds in the Republic of Marshall Islands. Marshall Islands Community College Publication No. 1.

Seibert, T.F. 1989. Biological control of the weed, *Chromolaena odorata* (Asteraceae), by *Pareuchaetes pseudoinsulata* (Lep.: Arctiidae) on Guam and the Northern Mariana Islands. Entomophaga 34, 531–539.

Chromolaena and other weed problems in the Pacific Islands

Warea Orapa[1], Konrad Englberger[2] and Sada Nand Lal[1]

Abstract

The spread of chromolaena (*Chromolaena odorata*) into the Pacific is of serious concern for many small Pacific island countries and territories (PICTs). Many islands are already faced with dealing with other serious weed problems and threats. Chromolaena has spread as far as the Federated States of Micronesia, Palau, Guam and Majuro in the Marshall Islands. In Melanesia, chromolaena is increasingly becoming a problem in Papua New Guinea (PNG) where it occurs as far east as Bougainville Island close to the international sea border with the Solomon Islands. Chromolaena is absent but is a threat to PICTs east and south of PNG, including the Solomon Islands, Vanuatu, New Caledonia and Fiji, and the Polynesian countries and territories. Efforts to combat chromolaena are underway in Micronesia and PNG, with emphases placed on public awareness to prevent its spread, eradication of small outbreaks on Majuro Island in the Marshall Islands and management of large infestations using classical biological control. The leaf-feeding arctiid moth *Pareuchaetes pseudoinsulata* and the stem-galling tephritid fly *Cecidochares connexa* have been introduced and released in PNG, Palau and Guam. In the absence of chromolaena (at least for the time being) in many of the remaining 16 PICTs, management of other invasive weeds are the focus of national and regional weed control programmes, with focus on biological control where it is possible. Other weeds such as *Cyperus rotundus*, *Merremia peltata*, *Mikania micrantha* and *Panicum maximum* are already very serious weeds regionally. A whole range of others, including *Miconia calvescens*, *Coccinia grandis*, and *Piper aduncum*, are increasingly becoming more troublesome in agriculture as well as in natural areas.

The stem-galling fly Cecidochares connexa *has been released on several Pacific Islands.*
R. Desmier de Chenon

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Biological control of *Chromolaena odorata* in Papua New Guinea

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Abstract

The status of the biological control of *Chromolaena odorata* in Papua New Guinea is reviewed. An ACIAR-funded project on the biological control of chromolaena began in 1997 in collaboration with the Papua New Guinea National Agricultural Research Institute and Queensland Department of Natural Resources and Mines. Two agents have so far been introduced and both have established. The moth *Pareuchaetes pseudoinsulata*, which was introduced from Guam in 1999, has established only in the Markham Valley, Morobe Province, despite being released in eight provinces. The gall fly *Cecidochares connexa* was introduced from the Philippines in 2001 and has established in six of the ten provinces in which it has been released. At some sites where it established, up to 20 galls per plant have been recorded and the insect spread up to 7 km from the release site within 18 months. Rearing of *P. pseudoinsulata* is continuing with the view to achieving establishment in other provinces of PNG, while the fly is currently being field collected for re-distribution by provincial officers. Two other agents, the leaf-mining fly *Calycomyza eupatorivora* and the stem-boring weevil *Lixus aemulus*, both of which were tested in South Africa, will be imported into PNG in the future. The project concludes in 2005 and it is hoped that these two new biocontrol agents can be released and will establish in all chromolaena-infested provinces.

Introduction

CHROMOLAENA ODORATA (L.) King and Robinson (Asteraceae), was first reported officially in Papua New Guinea (PNG) in 1970 on the Gazelle Peninsula of New Britain Island (Henty and Pritchard 1973). However, its presence in the area was known as early as the 1960s (Samson Laup pers comm.). Chromolaena affects a number of different land uses, namely: oil palm, cocoa and coconut plantations affecting harvesting and production; pastures of cattle grazing areas; subsistence food gardens; disturbed forests, roadsides and fringes of settlements and villages (McFadyen 2002; Orapa et al. 2002). Chromolaena now occurs in mainly lowland areas in 12 provinces with varying levels of infestations and has the potential to spread to other parts of the country.

Biological control of chromolaena in PNG began in 1997, with the introduction of the moth *Pareuchaetes pseudoinsulata* Rego Barros (Arctiidae) (McFadyen 2002). The moth had previously been used successfully in Indonesia, the Philippines and Guam. A second agent, the gall fly *Cecidochares connexa* (Macquart) (Tephritidae) was introduced in 2001. The project, funded by the Australian Centre for International Agricultural Research (ACIAR), is implemented by the PNG National Agricultural Research Institute (NARI) and administered by the Queensland Department of Natural Resources and Mines (NRM) (McFadyen 2002).

The project is based at the NARI's Research Centre at Labu, west of Lae, proximal to major chromolaena infestations of the Markham Valley. It has several main objectives, namely: to locate all chromolaena infestations in PNG; to release, monitor and re-distribute biocontrol agents to known infesta-

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tions throughout the country; and to train provincial officers in the above aspects of the project. The geography of PNG constrains the project substantially. Developed areas of Morobe Province, Madang Province and the Highland provinces are accessible by vehicle from Lae. Other provinces are accessible only by air, and flights to and from Lae are irregular. Most of the project operations are conducted in Morobe Province, but staff travel to other provinces to make releases of agents and monitor as required.

Since the project commenced, the location and range of known chromolaena infestations in PNG has increased greatly (Orapa 1998; Orapa et al. 2002). Previously known infestations have spread considerably and new infestations have been discovered. This paper outlines the current activities: documenting the distribution of the weed, the release of both *P. pseudoinsulata* and *C. connexa*, and extension and awareness.

Current distribution of chromolaena

Chromolaena is currently found in 12 provinces. In the Morobe Province, the main infestations occur in the Markham Valley, where chromolaena is invading cattle grazing areas, reducing productivity. Infestations have also been found scattered along the foothills of the Sarawaget Range, adjacent to the Markham Valley, and in several of its major tributary valleys. Chromolaena has spread from the upper Markham Valley into the Ramu Valley of Madang Province near Gusap, affecting pastures and sugar plantations, and it has also been found on Siassi Island in the east of Morobe Province.

Two isolated chromolaena infestations have been found along the Highlands Highway at Kassam Pass, near Watarais, at altitudes over 900 m in the Eastern Highlands Province. This is the highest recorded altitude of chromolaena in PNG. Chromolaena has been recorded around Wutung, Bewani and Vanimo in Sandaun Province, near the Indonesian border and around Lorengau and Lombrum on the island province of Manus to the north. In East New Britain (ENB) Province, chromolaena occurs across the Gazelle Peninsula, and in West New Britain (WNB) Province it has been found around Kimbe, Hoskins and Bialla. Chromolaena occurs along most of the east coast and some of the west coast of the mainland of New Ireland Province, where the worst infestations in PNG occur. The weed spread widely after El Niño-associated forest fires in 1997 and it is particularly conspicuous near Namatanai. At Huris, southeast of Namatanai, some areas of grasslands used as cattle pastures have become severely infested.

Smaller infestations occur on Misima Island in the Milne Bay Province, between Popondetta and Kokoda in Oro Province, and at Arawa (Aropa Airport) on Bougainville Island in the North Solomons Province.

Biological control

P. pseudoinsulata was successfully introduced from Guam in March 1999 after an unsuccessful attempt in December 1998. Mass rearing of *P. pseudoinsulata* was carried out in an insectary at Labu, until the colony died out in October 2001 and again from mid-2002. Pairs of newly emerged moths are placed in cages containing several vigorously growing potted chromolaena plants. Females are allowed to oviposit on the plants and larvae emerge about eight days later. Larvae feed on the leaves and new plants are added to the cage when required. Pupation occurs on the surface of the pot or on the floor of the cage. Pupae are collected, sexed and placed in a emergence container. Emerging adults are placed in a clean cage containing fresh actively growing plants (Orapa et al. 2002).

Over 200 000 larvae were released at 35 sites in ENB, Madang, Manus, Milne Bay, Morobe, Oro, Sandaun and WNB Provinces. However, establishment has been achieved at only ten sites, all in the Markham Valley, Morobe Province, which is considerably drier than the other release areas. Larvae at several sites have caused severe but sporadic defoliation of stands of chromolaena. Further releases will continue in other provinces in the future.

In January 2001, a colony of laboratory-reared *C. connexa* was imported from the Philippine Coconut Authority laboratories in Davao, Philippines. The colony was cleared through one generation in the Bubia quarantine insectary before mass rearing commenced at Labu. At Labu, potted chromolaena plants are exposed to gall flies in oviposition cages in the insectary. After three days, the plants are removed from the cages and placed in the open for larval development. Releases are made by taking potted plants or stems with mature galls to various chromolaena infestations and allowing adult flies to emerge. Mass rearing and release techniques are described in more detail by Orapa and Bofeng (this proceedings).

Over 23 000 galls have been released at 46 sites in the provinces of ENB, East Sepik, Eastern Highlands, Madang, Manus, Milne Bay, Morobe, New Ireland, Oro, Sandaun and WNB. Field inspections indicate that the gall fly has established at a total of 26 sites: ENB (3 sites), Madang (1), Morobe (15), New Ireland (2), Sandaun (4) and WNB (1). Recent inspections of sites near Erap in Morobe Province and Namatanai in New Ireland have shown that the fly has spread up to 7 km since being released in 2001. There has been no evidence of parasitism on the larvae, but occasional chewing (probably by

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grasshoppers) of the tender developing galls (including larvae) has been observed.

The project plans to introduce an additional two agents, the leaf-mining fly *Calycomyza eupatorivora* Spencer (Agromyzidae) and the stem-boring beetle *Lixus aemulus* Petri (Curculionidae). Applications for import permits for *C. eupatorivora* have been made to the National Agricultural Quarantine Inspection Authority (NAQIA) and the Department of Environment and Conservation in PNG and permits are expected to be issued in the near future. Both insects have been tested in South Africa by the Plant Protection Research Institute and are host specific to chromolaena. The fly causes mines on the leaves, initiating premature leaf drop, reducing plant vigour and flowering. *L. aemulus* larvae tunnel into the stems, causing the plants to become stunted with reduced flowering and seed production. (C. Zachariades, pers comm.).

Collaboration and extension

Apart from the strong collaborative links between PNG and Australia, there is also substantial collaboration with various organisations within PNG. Staff of NARI and NAQIA and provincial Departments of Primary Industries (DPI) have assisted project staff visiting provincial infestations. Provincial staff are being alerted to the problems of chromolaena and its impact to the regions. They have been involved in identifying new infestations and the release and monitoring of biocontrol agents. The Oil Palm Research Association (OPRA) based at Dami, West New Britain Province and Popondetta, Oro Province have agreed to mass-rear biocontrol agents for release in their local areas. Cages have been provided to OPRA for this purpose. Ramu Sugar Ltd, based at Gusap in Madang Province, has also been involved in the field release and monitoring of the gall fly on chromolaena in the Ramu Valley. Information leaflets are currently being produced and will be distributed to all potentially suitable regions where chromolaena can grow, in order to increase awareness on the impacts of the weed.

As part of the training component of the project, a three-day workshop is planned in 2003 to train key provincial staff in weed (particularly chromolaena) awareness and in handling, releasing and monitoring biocontrol agents.

Funding by ACIAR for the project ends in March 2005 and it is planned that four agents will have been introduced and established in at least the major infestations. Through the training and awareness program, it is expected that provincial officers will be able to continue to collect and re-distribute agents to infestations without agents after the project has finished. With concerted effort, it is hoped that biocontrol of chromolaena in PNG can be achieved, resulting in a significantly reduced impact of the weed in agricultural areas and village gardens.

Acknowledgments

The authors wish to acknowledge support from Dr Rachel McFadyen, Co-operative Research Centre for Australian Weed Management (formerly of NRM) for initiating and administering the project until 2002; Dr Wendy Forno for reviewing the first phase of the project; Dr Lastus Kuniata, Ramu Sugar Ltd and OPRA for rearing and distributing biocontrol agents, and various staff from NARI, NAQIA and provincial DPI offices for assisting in the release, monitoring and re-distribution of biocontrol agents and other project activities. We thank Dr Muniappan for supplying the founder colony of *P. pseudoinsulata* and Mr Emmanuel Atterrado for supplying *C. connexa*. The authors gratefully acknowledge the support of ACIAR for continuing to fund the project in PNG and to the Secretariat of the Pacific Community for funding the attendance of participants to the workshop.

References

Henty, E.E. and Pritchard, P.H. 1973. Weeds of New Guinea and Their Control. Botany Bulletin No. 7, Department of Forests, Lae, Papua New Guinea.

McFadyen, R.E.C. 2002. Chromolaena in Asia and the Pacific: spread continues but control prospects improve. In: C. Zachariades and and L. Strathie (eds), Proceedings of the Fifth International Workshop on Biological Control and Management of *Chromolaena odorata*. Durban, South Africa, October 2000. 13–18.

Orapa, W. 1998. The status of *Chromolaena odorata* in Papua New Guinea. In: Paul Ferrar, R. Muniappan and K.P. Jayanath (eds), Proceedings of the Fourth International Workshop on Biological Control and Management of *Chromolaena odorata.* Bangalore, India, October 1996, Agricultural Experiment Station, Mangilao, Guam. 82–85.

Orapa W., Bofeng, I. and Donnelly, G.P. 2003. Mass production, establishment and impact of *Cecidochares connexa* on chromolaena in Papua New Guinea (these proceedings).

Orapa, W., Donnelly, G.P. and Bofeng, I. 2002. The distribution of Siam weed, *Chromolaena odorata*, in Papua New Guinea. In: C. Zachariades and L. Strathie (eds), Proceedings of the Fifth International Workshop on Biological Control and Management of *Chromolaena odorata*. Durban, South Africa, October 2000. 19–25.

Chromolaena — the weed

Chromolaena odorata *infestation*.

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Determining optimal growth conditions for the South African biotype of *Chromolaena odorata*

M. Gareeb[1,2], N.W. Pammenter[2], J.P. Bower[3] and C. Zachariades[1]

Abstract

The rapidly growing, perennial shrub, *Chromolaena odorata* (L.) King and Robinson (Asteraceae) continues to invade the tropical and subtropical parts of southern Africa at an alarming rate. Its distribution is apparently limited by its intolerance to frost and, to a lesser extent, low rainfall. The South African chromolaena biocontrol program is based at Cedara, in the KwaZulu-Natal midlands, a subtropical region above 1000 m altitude, lying outside the natural range of *C. odorata*. During the past few years, there have been significant problems with maintaining healthy potted chromolaena plants at the laboratory. This has had serious consequences, especially for endophagous insect cultures which cannot be transferred between plants.

In order to maintain insect cultures, rooted saplings of *C. odorata* are regularly collected from the field around Durban, where the plant is abundant, and potted into 18 cm or 26 cm pots at Cedara. Initial growth is generally very good, but once the plants are larger, some begin yellowing, wilting and dying back. This often occurs in epidemics, several times a year. Installation of a heated tunnel and fertigation system has not significantly alleviated the problem. Therefore, it is likely that the primary problem is a physiological and not a pathological one. In order to investigate further, pot trials have been set up in Durban and Cedara to compare growth and physiological characteristics. Included amongst these will be responses of photosynthetic rate to light intensity and leaf and soil temperatures as these may influence carbohydrate production for root regeneration. To complement these studies total non-structural carbohydrate of different plant structures will be measured. Results from the study will assist in improving techniques used at the Cedara laboratory for growing and maintaining healthy *C. odorata* plants.

MSc thesis at University of Natal-Durban, December 2005

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Allelochemicals from *Chromolaena odorata* (L.) King and Robinson for increasing crop productivity

S.R. Ambika[1] and S. Poornima[1]

Abstract

Wild varieties of many present day crop plants are known to possess allelopathic chemicals that make them resistant to pests and weeds. During the course of selection for high yielding varieties, such genes are either rendered weak or eliminated, resulting in loss of allelopathic attributes in crop plants. However, this quality still exists in most of the weeds, where such selection does not occur.

Chromolaena odorata is reported to be highly allelopathic compared with some of the other weeds that are among the world's worst. Allelochemicals are natural products and do not accumulate in soil as persistent pollutants. In its crude form at a particular concentration, *C. odorata* allelochemicals increased the vegetative growth, metabolite content and yield in pulses, grain crops and vegetables.

In this study, the crude allelochemicals were used as seed invigorants, liquid fertilisers and as foliar spray. The treated plants were even resistant to pests and diseases. Details of the preparation of allelochemicals, appropriate concentration, the crop growth pattern, yield assessment and biochemical analyses are discussed.

Introduction

CHROMOLAENA ODORATA is a perennial weed of plantations, agricultural fields, pasturelands, wastelands and roadsides. The leaves, seeds and stem contain growth inhibitors, which were found to be allelopathic. Laboratory and field studies have very clearly demonstrated that the leachates and the extracts of the weed inhibited crop growth (Ambika and Jayachandra 1980).

It was also shown that the root exudates and the rhizosphere soil of chromolaena had allelochemicals that inhibited crop growth (Ambika and Jayachandra 1992). The aerial part of the weed released volatile inhibitors that caused crop growth inhibition (Ambika and Jayachandra 1992). The weed residues that were allowed to decompose in the soil remained a toxic medium for crop growth for up to six months. After six months, crop growth was promoted by the decomposing medium (Ambika and Jayachandra 1984).

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Studies established that phenolics, alkaloids and aminoacids were the main allelochemicals in this species. In the soil medium, where the plant was allowed to decompose, the concentration of allelochemicals increased in the first 100 days and declined thereafter. Further, the level of micro and macro nutrients increased and reached its maximum after 180 days (Ambika and Jayachandra 1984).

These studies clearly establish that the weed is toxic and caused inhibition of crop growth. The main growth effects induced by the allelochemicals were reduction in linear growth of root and shoot, reduction in leaf number, leaf expansion, decrease in cell wall elongation, cell elongation and reduction in cell division. As a result, the plants remained stunted, small and less vigorous. This decrease in growth of the crop also affected the yield of the crop (Ambika and Jayachandra 1984 and 1992). Although these studies revealed the interference of allelochemicals with cell division and elongation, no conclusive evidence could be documented to show the relative contribution of these processes to allelochemical-mediated growth inhibition. However, in one of the field studies, ragi plants grown using *Chromolaena* allelochemicals as liquid fertiliser (prepared in a ratio of

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1:16), remained stunted and small. However, ragi plants were resistant to artificially created water stress and, unlike the control plants, could recover fast. This highlighted the possibility of using allelochemicals from *C. odorata* at very low concentrations as liquid fertiliser for increasing the crop growth and yield.

Material and methods

The crops — fingermillet, clusterbean, soybean, palak and radish — were grown following the normal package of practices in the quadrangle of the Department of Botany, Bangalore University, in completely Randomised Block Design. The leachate was prepared by soaking one gram of air-dried powdered leaves of chromolaena in 100 ml of glass-distilled sterile water for 24 hours at the laboratory temperature of 22 ± 1°C and normal pressure. The solution was further filtered through glass wool and stored under refrigeration. Crop plants were grown until they flowered and fruited.

Harvested seeds were broadcast sown throughout the plots. After emergence, plants were thinned to maintain 25 plants in each of 10 rows. The crops were tested in the following manner: ragi seeds were soaked in leachate and distilled water separately for three hours before sowing; soybean plants were given five aerial sprays from the twentieth day on alternate days with leachate and water; and clusterbean, palak and radish plants were each fertilised with tap water and crude allelochemicals from the twentieth day onwards.

At the end of the vegetative phase, 20 plants of each crop were harvested, when linear growth and fresh and dry matter accumulation were recorded. As each crop is normally grown for a specific purpose, the data collected from each reflected the crop's use. For palak, which is grown mainly for the edible leaves, the yield of leaves was recorded. The yield of leaves and roots were recorded in radish, while the number of pods/grain produced in cluster bean, soybean and ragi were recorded. Data were statistically analysed for significance following Sokal and Rohlf (1973).

Results

Plant height and root depth in cluster bean increased by 50% and 67% respectively. The fresh and dry weight of these increased from 130%–138% and the yield increased by 25% (Figure 1).

Shoot and root linear growth of soybean increased by 15% and 40% respectively, while fresh weight increased by 79% and 68%, and dry weight increased by 110% and 72% respectively. The increase in pod yield was 162% by dry weight (Figure 2).

The root length of radish decreased by 24% but the fresh and dry weight increased by 42% and 15% respectively. The length of the leaf, and the fresh and dry weight of the leaf increased by 86%, 200% and 113% respectively (Figure 3).

The root length of palak decreased by 11% but the number of leaves and the fresh and dry weight of leaves increased by 74%, 182% and 220% respectively (Figure 4).

Ragi plants were slightly stunted. The plant height decreased by 6% while the root length decreased by 11%. The numbers of leaves and the fresh and dry weight in shoot and root increased by 24% to 78% (Figure 5). The yield increased by 144% and the 1000 grain weight was 17% more than the control

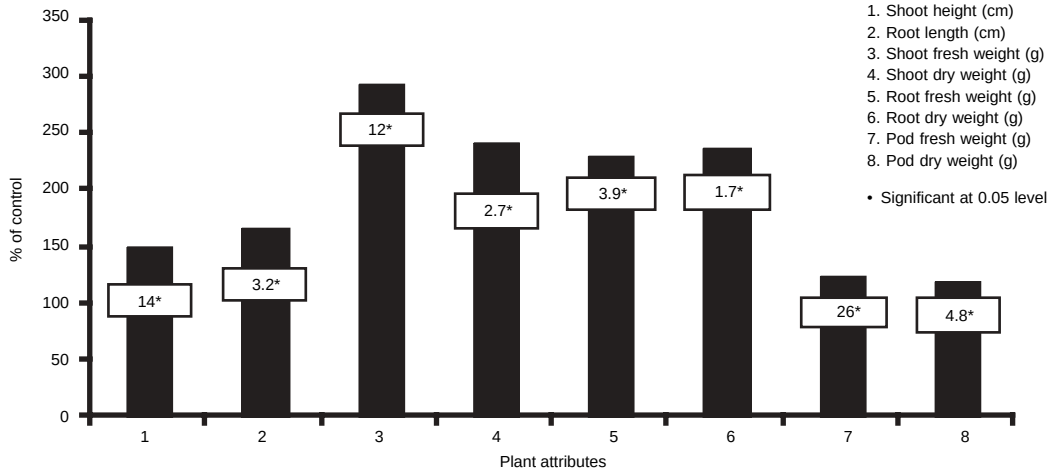

Figure 1. Effect of allelochemicals from *C. odorata* on the growth of cluster bean.

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(Figure 6). Amino acids, proteins, carbohydrates and sugars were all found to be greater in the treated palak plants when compared with the control plants on a weight basis (Figure 7).

Discussion

Allelochemicals are secondary metabolites produced by many higher plants. A wide array of these compounds is released into the environment in appreciable quantities via volatilisation and exudation as leachates through the rain-wash of leaves and during their decomposition. These are known to play a major role in the inhibition of growth of several crops (Liu and Lovette 1993). Allelopathic interactions between plants have been implicated in the patterning of vegetation and weed growth in agricultural systems (Aldrich 1987; Rice 1987). These are revealed to interact with plant growth regulators, either synergistically or additively while exerting their action (Tomaszewski and Thimann 1966; Tayal and Sharma 1985; Kathiresan et al. 1990).

The results of this study demonstrate that the allelochemicals from *C. odorata* can be successfully exploited for enhancing crop productivity. These

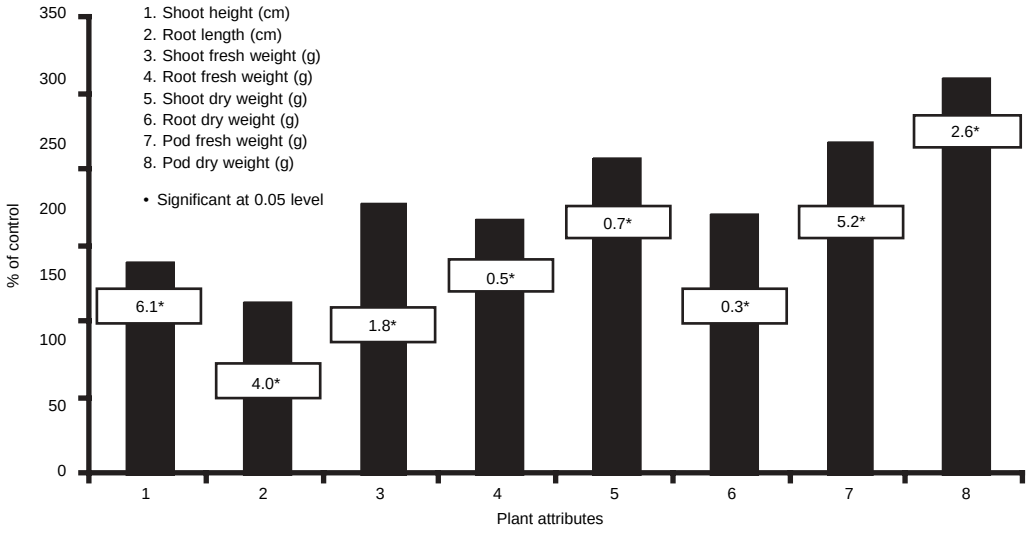

Figure 2. Effect of allelochemicals from *C. odorata* on the growth of soybean.

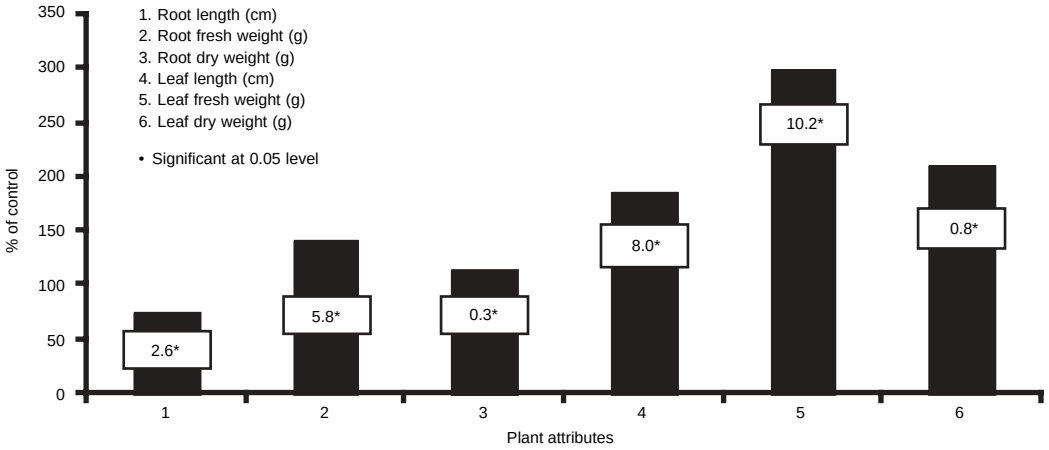

Figure 3. Effect of allelochemicals from *C. odorata* on the growth of radish.

allelochemicals also rendered the plants resistant to pathogens and water stress. However, in the present study, in the 1:50 dilution, the hormonal level would be too low to cause any growth difference and it may be the very low concentrations of the inhibitors (allelochemicals) that brought about the increase in growth and productivity. This effect of allelopathy is a biotechnological aspect where one can use allelochemicals as growth regulators. A number of sesquiterpene lactones (secondary metabolites) are known to possess this property (Fischer et al. 1989; Chen and Leather 1990). Their activity is comparable to known plant growth regulators which are otherwise expensive (Batish et al.1996).

In addition, the treated plants were found to be resistant to pathogen attack and water stress and remained healthy compared with the control plants. Allelochemicals controlling plant diseases in crude and purified form have been reported by using neem (Ghewande 1989), eucalyptus (Singh and Dwivedi 1990), tobacco (Menetrez et al. 1990), ginger (Endo et al. 1990), tagetes (Kishore and Dwivedi 1991) and *Salvinia* species (Qureshi et al. 1989).

Therefore, allelochemicals may provide a cheap source of growth regulators. They can be successfully exploited for enhancing crop productivity, providing excellent alternatives for integrated crop protection programs thus restricting the use of synthetic and

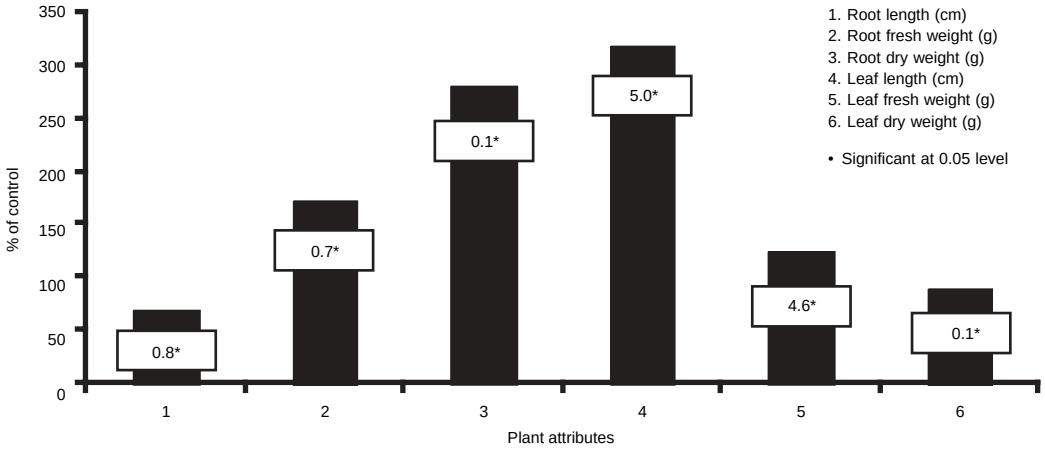

Figure 4. Effect of allelochemicals from *C. odorata* on the growth of palak.

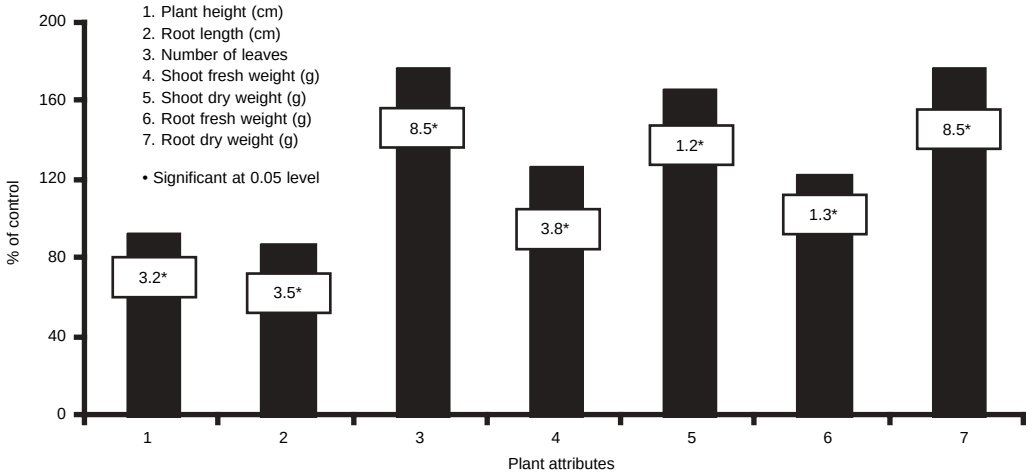

Figure 5. Effect of allelochemicals from *C. odorata* on the growth of ragi.

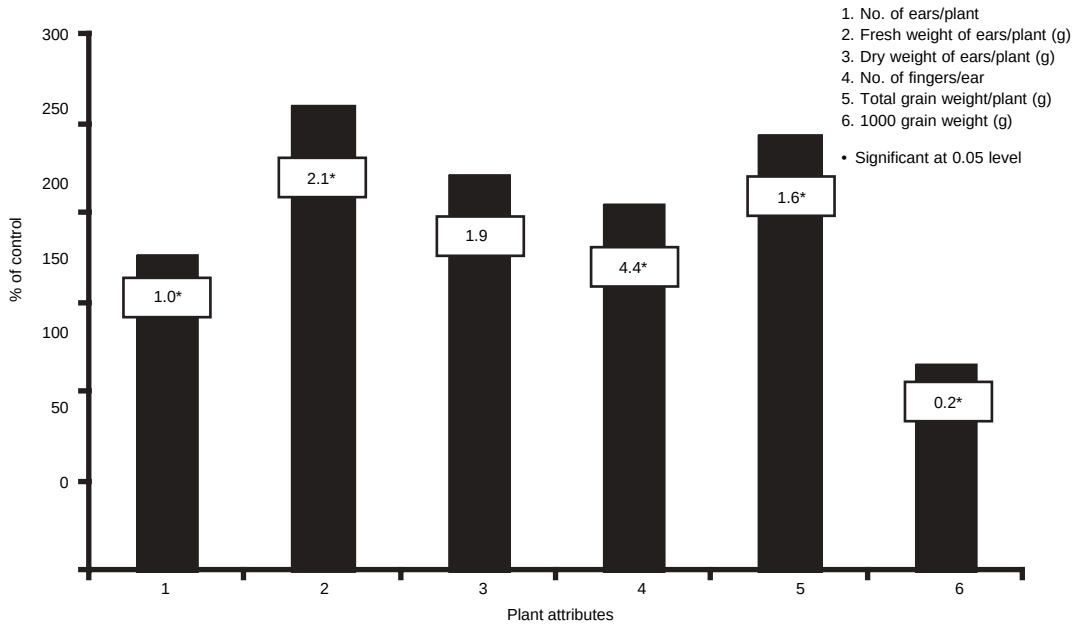

Figure 6. Yield of ragi plants treated with allelochemicals from *C. odorata.*

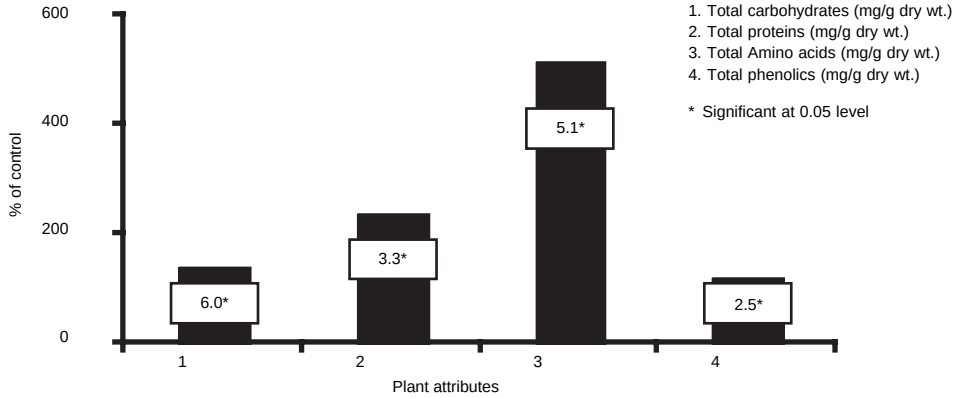

Figure 7. The level of metabolites in the leaves of palak plants fertilised with liquid allelochemicals of *C. odorata*.

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References

Aldrich, R.J. 1987. In: Allelochemicals: Role in agriculture and Forestry (G.R. Walter, ed.), Vol. 330, American Chemical Society, Washington, DC, 300–312.

Ambika, S.R. and Jayachandra 1980. Suppression of plantation crops by *Eupatorium* weed. Curr. Sci., 49(22): 874–875.

Ambika, S.R. and Jayachandra 1984. *Eupatorium odoratum* L. in plantations — an allelopath or a growth promoter? *Placrosym-V.* Proceedings of the fifth annual Symposium on plantation crops: 247–259.

Ambika, S.R. and Jayachandra 1992. Allelopathic effects of *Chromolaena odorata* (L.) King and Robinson. Proceedings of First National Symposium — allelopathy in Agroecosystems, P. Tauro and S.S. Narwal (eds.), Indian Society of Allelopathy, Haryana Agricultural University, Hisar: 139–141.

Batish, D.R., Kohli, R.K., Saxena, D.B. and Singh, H.P. 1996. Growth regulatory response of parthenin and its derivatives, Journal of Crop Production, 1(1), 1998.

Chen, P.K. and Leather, G. 1990. Plant growth regulatory activities of artemisinin and its relatives compounds, Journal of Chemical Ecology, 16: 1867–1876.

Endo, K., Kanno, E. and Oshima, Y. 1990. Structures of antifungal diarytheprenones, gingerenones A,B,C and isogingerenone B, isolated from the rhizomes of *Zinger officinalis*, Phytochemistry, 29: 797–799.

Fischer, N.H., Weidenhamer, J.D. and Bradow, J.M. 1989. Inhibition and promotion of germination by several sesquiterpenes, Journal of Chemical Ecology, 15: 1785–1793.

Ghewande, M.P. 1989. Management of foliar diseases of groundnut (*Arachis hypogaea*) using plant extracts. Indian Journal of Agricultural Sciences, 59: 133–134.

Kathiresan, K., Ravishankar, G.A. and Venkataraman. 1990. Curr. Sci., 59: 430–431.

Kishore, N. and Dwivedi, R.S. 1991. Fungitoxicity of the essential oil of *Tagetes erecta* L. against *Pythium aphanidermatum* Fitz. The damping-off pathogen. Flavour and fragrance Journal, 6: 291–294.

Liu, D.L. and Lovette, J.V.E. 1993. J. Chem. Ecol, 19: 2231–2244.

Menetrez, M.L., Spurr, H.W. Jr., Danchower, D.A. and Lawson, D.R. 1990. Influence of tobacco leaf surface chemicals on *Peronospora tabacina* Adam sporangia. Journal of Chemical Ecology, 16: 1565–1576.

Qureshi, I.H., Ahmed, S. and Kapadia, Z. 1989. Antimicrobial activity of *Salvia splendens*. Pakistan Journal of Scientific and Industrial Research, 32: 597–599.

Rice, E.L. 1987. In: Allelochemical-Role in agriculture and Forestry. G.R. Walter (ed.), 330, American chemical Society, Washington, DC, 8–22.

Singh, R.K. and Dwivedi, R.S. 1990. Fungicidal properties of neem and blue gum against *Sclerotium rolfsii* Sacc., a foot-rot pathogen of barley. Acta Botanica Indica, 18: 260–262.

Sokal, R.R. and Rohlf, F.J. 1973. Introduction to Biostatistics. W.H. Freeman and Co. San Francisco., Tappan Co. Ltd., Tokyo, Japan, 368.

Tayal, M.S. and Sharma, S.M. 1985. Indian journal of Plant Physiology, Curr.Sci., 59: 430–431.

Tomaszeuski, M. and Thimann, K.V. 1966. Plant Physiol., 41: 1443–1454.

Evidence for a northern Caribbean origin for the southern African biotype of *Chromolaena odorata*

Costas Zachariades[1], Inge von Senger[2, 3] and Nigel P. Barker[2]

Abstract

The biotype of *Chromolaena odorata* invading southern Africa differs markedly from that invading other parts of the paleotropics. Until 1997 no plants identical to the southern African biotype had been found by ARC-PPRI researchers in the neotropics, so that of necessity, candidate biocontrol agents were collected from *C. odorata* plants which differed in morphology to it. For some candidates, this resulted in suspected or obvious agent-host incompatibility in the quarantine laboratory in South Africa. Between 1988 and 1994, several techniques were used to determine the origin of the southern African *C. odorata*, but these gave conflicting and unclear results. In 1997, plants identical to the southern African biotype were collected in Jamaica. Since then, diverse strands of evidence have accumulated, confirming that this biotype is present in, and apparently restricted to, the Greater Antillean islands of Jamaica, Cuba, Puerto Rico and possibly nearby islands in the northern Caribbean. DNA matching indicated some trends and excluded some areas as origins of the southern African biotype, but was inconclusive with respect to exact origin. Comparison of the inflorescence morphology using herbarium specimens resulted in the exclusion of the South American continent as an origin, and showed that plants with identical inflorescences were present in Jamaica, Cuba and Central America. Populations of *C. odorata* plants apparently identical to the southern African biotype with respect to leaf, stem and flower morphology, colouration, odour and growth form were found in Jamaica, Cuba and Puerto Rico, and reported from Hispaniola and the Bahamas. Leaf pathogens collected from Jamaica and Cuba were among the only ones to form lesions on the southern African biotype. A literature search indicates that *C. odorata* plants from Jamaica were growing in the Cape Town Botanic Garden by 1858.

Introduction

In-field matching

POPULATIONS of *C. odorata* plants morphologically identical to the southern African biotype with respect to leaf, stem and flower anatomy, colouration, odour and growth habit (hereafter referred to as 'southern African-morphology' plants) were found in Jamaica, Cuba and Puerto Rico (C. Zachariades, unpubl. data), which together with Hispaniola, form the Greater Antilles. On all three islands these were interspersed with *C. odorata* plants with different morphological features. Because no information is currently available on whether variation in appearance between *C. odorata* plants within a region in the Americas is correlated with any biological, ecological or genetic differences, we refer to these simply as 'morphological forms'. Southern African-morphology plants were also reported from Hispaniola (S. Neser, pers comm.) and the Bahamas (I.A.W. Macdonald, pers comm.).

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Genetic studies

A study was conducted using DNA sequence data from the nuclear Internal Transcribed Spacer (ITS) region (von Senger 2002; Barker et al. 2003). ITS sequencing indicated some trends and excluded some areas as origins of the southern African biotype, but was inconclusive with respect to its exact origin. All *C. odorata* specimens resolved as a group, separately from several other Eupatorieae, which had been used

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as an outgroup. The *C. odorata* specimens included several whose species identity was questionable, but which was later confirmed using morphological features (H. Robinson, pers comm.). The taxonomy is thus supported by the DNA data at a species level.

Within *C. odorata*, specimens of the West African/Asian biotype resolved into a distinct group. Specimens from Guatemala and Mexico also formed a separate group, but reasons for this are unclear, and these samples possess a range of morphologies. The results of Scott et al. (1998) were supported in that one Australian *C. odorata* specimen resolved with the West African/Asian biotype, and the other with a plant from southern Brazil. Within *C. odorata*, South African specimens resolved in a scattered fashion, as did Jamaican and Venezuelan specimens. The reasons for these results are unclear. No correlation between the ITS sequence and morphology emerged for the Jamaican specimens. Specimens from Cuba and Puerto Rico were not available for genetic comparisons at the time.

Involucral morphology

Two comparisons of the involucral morphology were made. The first was a qualitative assessment using herbarium specimens at the Smithsonian Institution, Washington DC. It indicated that plants with an involucre similar to the southern African *C. odorata* were most common in the West Indies, less common in Central America, and absent from the South American mainland (H. Robinson, pers comm.). The second was a quantitative study using herbarium specimens at the Royal Botanic Gardens, Kew. This indicated that plants from the Amazonian and Brazilian Floristic Regions *sensu* Takhtajan (1986) had much shorter, broader involucres. Only four specimens were the same as the southern African biotype in all parameters measured, one each from Nicaragua, Costa Rica, Jamaica and Cuba. The two DNA and morphological studies are thus in broad agreement.

Pathogen-host plant matching

Three isolates of *Pseudocercospora eupatorii-formosani* (Sawada) J.M. Yen (Deuteromycotina: Hyphomycetes) collected in 1997 from Jamaica developed better on southern African-biotype *C. odorata* than any previously collected pathogens from 12 other countries in the Americas (den Breeÿen 2002). Further collections of leaf pathogens from Cuba and Jamaica have supported this trend (den Breeÿen 2003).

Introduction into South Africa

The large distance between the three sites at which *C. odorata* was first collected in the field in KwaZulu-Natal province (KZN) in the late 1940s, and the abundance with which it was present at these sites (C. Zachariades, unpubl. data), suggests that *C. odorata* may have been present in KZN earlier than previously supposed (e.g. Liggitt 1983). A record of *C. odorata* plants from Jamaica growing in the Cape Town Botanic Garden in the mid-nineteenth century (McGibbon 1858) places the introduction of the species into South Africa almost a century before it was first recorded as naturalised. No records of it being grown in the Natal Botanical Garden in Durban have yet been found, but seed packets were received from the British West Indies several times around the turn of the twentieth century (Wood 1886–1910). Alternatively, imports of agricultural or other products during the decades around 1900 may have brought *C. odorata* in as a contaminant from the West Indies (e.g. Anon. 1939; Schrire 1983), or it could have been brought as an ornamental by an individual.

Conclusions

No single method has provided sufficient evidence to draw a conclusion on the origin of the southern African *C. odorata* biotype, but taken together they are adequately convincing for the purposes of biocontrol research. The evidence indicates the islands of the northern Caribbean as the most likely origin of the southern African biotype of *C. odorata*. Unfortunately, indications are that the suite of phytophagous insects on *C. odorata* in the Greater Antilles is depauperate, and that important candidate agents such as *Conotrachelus*, *Lixus* and *Longitarsus* spp. are absent (Strathie and Zachariades, this Proceedings). Thus other regions in the Americas will still be used to source agents, if it can be shown that they are not biotype-specific. Several insect species that occur on *C. odorata* in the Greater Antilles, such as *Polymorphomyia basilica* Snow (Diptera: Tephritidae), *Melanagromyza eupatoriella* Spencer (Diptera: Agromyzidae), *Calycomyza eupatorivora* Spencer (Diptera: Agromyzidae) and *Pareuchaetes insulata* (Walker) (Lepidoptera: Arctiidae) can usefully be employed as biocontrol agents (Strathie and Zachariades, this Proceedings). Furthermore, the position of these islands at the edge of the tropics, and the presence of high-altitude, drier areas there, should allow for good climatic matching with the area of southern Africa invaded by *C. odorata* (R.E. McFadyen pers comm.).

A contract between ARC-PPRI and the University of the West Indies, Jamaica is currently in the final stages of negotiation. It is likely that under this contract, South African *C. odorata* plants will be grown in field-plots in Jamaica, in order to determine preferences of natural enemies for this biotype versus those in Jamaica. Insects and pathogens developing on South African plants will be harvested for import to South Africa.

Acknowledgments

The first author thanks the Working-for-Water Program of the Department of Water Affairs and Forestry, South Africa, for providing funding to attend the Sixth Workshop. We are grateful to Noma Sishuba and Lorraine Strathie for commenting on earlier drafts of the manuscript, and to Steve Compton, Steve Johnson and Andrew Mitchell for clarifying terms.

References

Anonymous 1939. The origin and history of the Natal and Zululand sugar industry. South African Sugar Journal 23: 473–486, 671–677.

Barker, N.P., von Senger, I., Howis, S., Zachariades, C. and Ripley, B.S. 2003. Plant phylogeography based on rDNA ITS sequence data: two examples from the Asteraceae. In: Bakker, F.T., Chatrou, L.W., Gravendeel, B. and Pelser, P.B. (eds), Plant Species-Level Systematics: Patterns, Processes and New Applications. Koeltz, Königstein. Regnum Vegetabile. 142. In press.

Den Breeÿen, A. 2002. *Chromolaena odorata*: biological control using plant pathogens — a South African perspective. In: Zachariades, C., Muniappan, R. and Strathie, L.W. (eds), Proceedings of the Fifth International Workshop on Biological Control and Management of Chromolaena odorata. Durban, October 2000. ARC-PPRI, Pretoria, South Africa, 167–169.

Den Breeÿen, A. 2003. Biological control of the southern African *Chromolaena odorata* biotype using pathogens — the search continues. Poster presented at the Eleventh International Symposium on Biological Control of Weeds, Canberra, Australia, April–May 2003.

Goodall, J.M. 2000. Monitoring serial changes in coastal grasslands invaded by *Chromolaena odorata* (L.) R.M. King and Robinson. MSc Thesis, University of Natal, Pietermaritzburg, South Africa.

Liggitt, B. 1983. The Invasive Alien Plant *Chromolaena odorata*, with Regard to its Status and Control in Natal. Monograph 2. Institute of Natural Resources, University of Natal, Pietermaritzburg, South Africa. 41 pp.

McGibbon, J. 1858. Catalogue of Plants in the Botanic Garden, Cape Town, Cape of Good Hope. Saul Solomon and Co, Cape Town, South Africa.

Schrire, B.D. 1983. Centenary of the Natal Herbarium, Durban, 1882–1982. Bothalia 14: 223–236.

Scott, L.J., Lange, C.L., Graham, G.C. and Yeates, D.K. 1998. Genetic diversity and origin of siam weed (*Chromolaena odorata*) in Australia. Weed Technology 12: 27–31.

Strathie, L.W. and Zachariades, C. (this proceedings). Insects for the biological control of *Chromolaena odorata* in South Africa.

Takhtajan, A.L. 1986. Floristic Regions of the World. University of California Press.

Von Senger, I. 2002. An assessment of the genetic diversity and origin of the invasive weed *Chromolaena odorata* (L.) King and Robinson in South Africa. MSc Thesis, Rhodes University, Grahamstown, South Africa.

Von Senger, I., Barker, N.P. and Zachariades, C. 2002. Preliminary phylogeography of *Chromolaena odorata*: finding the origin of a South African weed. In: Zachariades, C., Muniappan, R. and Strathie, L.W. (eds), Proceedings of the Fifth International Workshop on Biological Control and Management of Chromolaena odorata. Durban, October 2000. ARC-PPRI, Pretoria, South Africa, 90–99.

Wood, J.M. 1888–1910. Annual Reports on the Natal Botanic Gardens. Durban Botanic Society.

Zachariades, C., Strathie-Korrûbel, L.W. and Kluge, R.L. 1999. The South African programme on the biological control of *Chromolaena odorata* (L.) King and Robinson (Asteraceae) using insects. African Entomology Memoir 1: 89–102.

Chromolaena biocontrol agents

M. Day

Collecting C. connexa galls in PNG.

M. Day

Releasing C. connexa *galls in New Ireland Province, PNG.*

W. Orapa

P. pseudoinsulata *adult.*

M. Day

P. pseudoinsulata *damage in Morobe Province, PNG.*

Chromolaena in the Asia-Pacific region
Edited by M.D. Day and R.E. McFadyen
ACIAR Technical Reports No. 55
(printed version published in 2004)

Mass production, establishment and impact of *Cecidochares connexa* on chromolaena in Papua New Guinea

Warea Orapa[1] and Ingu Bofeng[2]

Abstract

Chromolaena (*Chromolaena odorata*) is localised but spreading in 12 of the 20 provinces of Papua New Guinea (PNG) and the weed is becoming invasive and is expanding its range. Effort to combat and prevent its impacts on agriculture, the environment and people began in 1997 with the commencement of an ACIAR-supported biological control project. The moth *Pareuchaetes pseudoinsulata* has been released in eight provinces but has established only in the Markham Valley of Morobe Province where chromolaena has invaded open dry grassland environment. A second biological control agent, the stem-galling fly *Cecidochares connexa*, was introduced in 2001 and is being reared for field release. *C. connexa* has been released in eight provinces so far and is established at most release sites in these provinces. With the extension of the biological control project for a further three years, two additional agents are being considered for introduction to PNG. In this paper, we describe the techniques for mass rearing and releasing *C. connexa* used in PNG.

Introduction

CHROMOLAENA was introduced into Papua New Guinea (PNG) prior to 1970 (Henty and Pritchard, 1973) and several workers reported the threats and national distribution of the weed during the last decade (Waterhouse 1992; Orapa 1998; and Orapa et al. 2002a). Chromolaena occurs in mostly localised areas of 12 lowland and island provinces with varying levels of infestations.

The main threats of the weed are to subsistence food gardens based on shifting cultivation, semi-subsistence cultivation areas, young and poorly maintained cash crop areas under oil palm, cocoa, coconut, sugarcane, and vanilla, and natural pastures under cattle production. Disturbed forests, wastelands, roadsides, hillsides, fringes of settlements and villages in the lowland provinces face invasion by chromolaena (Orapa et al. 2002a). Chromolaena has the potential to spread to many parts of PNG and further east into the Solomon Islands and other small South Pacific island countries. Chromolaena mainly grows at low altitude areas and has been found growing at an altitude of about 1000 metres above sea level along the important Highlands Highway in Eastern Highlands Province.

Biological control efforts against chromolaena in PNG began in 1998 as part of an ACIAR-supported southeast Asian regional program (Orapa 1998). The PNG National Agricultural Research Institute (NARI), in collaboration with the Queensland Department of Natural Resources and Mines (NRM), is implementing the project in PNG with biological control agent rearing facilities established at Labu near Lae in the Morobe Province. The biocontrol agent *Pareuchaetes pseudoinsulata*, which has been widely released on chromolaena elsewhere, was introduced to PNG in March 1999 (Orapa et al. 2002b). Releases of the moth were made at 27 locations in eight provinces but field populations have only established on chromolaena growing in open areas of the Markham Valley of Morobe Province. The Markham Valley is characterised by seasonal wet-dry rainfall and shrub and grassland. The agent's impact on chromolaena has been minimal, with leaf defoliation localised and brief only during seasonal population build-up, but not significant enough to suppress chromolaena. Most chromolaena stands in the Markham Valley burn during the dry season from

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fires ignited by local villagers, but regrowth is rapid during the onset of rains, which occur from December to March. Moth numbers were found to be consistently low and unevenly distributed. At all the other release areas in PNG high levels of predation are suspected to be responsible for this agent's failure to establish. Many of these locations were in areas where chromolaena occurred in forested or high rainfall areas (Orapa et al. 2002b).

A second agent, the stem galling fly *Cecidochares connexa*, was introduced and released in 2001. Here, we report the rearing, field release and monitoring regimes for *C. connexa* (Diptera; Tephritidae), which was introduced and released in 2001 and established successfully at most release sites. We also report on a short study conducted to compare the impact of gall densities on the growth, flowering and seed production of chromolaena during the rearing program as a simulation of field conditions.

Importation of *C. connexa*

C. connexa was considered to be host-specific to chromolaena in tests conducted in Indonesia (Sipayung and de Chenon 1999) and in additional tests in Guam (Muniappan 1999). As a result, the fly was introduced and released without further testing in PNG in January 2001. Approximately 300 pupae and newly emerged adults were imported from The Philippine Coconut Authority in Davao.

After post-entry quarantine rearing at the NARI facilities at Bubia near Lae, the agent was initially released at two nearby chromolaena infestations (Pususuatu and Erap) in the Markham Valley. Pususuatu is located in the high rainfall (2000–3000 mm per annum) tropical rainforest area near Lae while Erap (30 kilometres west) is in a rain shadow area with annual rainfall of about 1000 mm. The chromolaena at Erap grows under semi-arid conditions and mostly under shade provided by stands of introduced rain trees (*Samanea saman*) and leucaena (*Leucaena leucocephala*).

Mass rearing of *C. connexa*

Mass rearing of gall flies on chromolaena grown in pots (200–250 mm size plant pots) and cut-stems was carried out at the facility at NARI Labu. Equal numbers of female and male gall flies, easily identified by the presence or absence of an ovipositor, were collected from the emergence cages (90 cm × 56 cm × 88 cm) in clear screw-capped tubes. Ten pairs of flies were placed in each cage containing potted chromolaena plants. Potted chromolaena plants with vigorously growing multiple branches from single rootstock to about 50–60 cm in height were selected for oviposition. The plants were thoroughly cleaned of any predators, dirt and weeds before placing them into the oviposition cages (90 cm × 56 cm × 88 cm). We found the flies easy to handle individually before 9 am in the mornings.

The flies were allowed to mate and oviposit on the growing tips of chromolaena for three days. Every day the pots were moved in the cages to allow maximum oviposition on all available tips, as the adults were found to favour the walls receiving the most sunlight. After three days, the plants were removed from the cages, ensuring the flies remained behind in the cages. New potted chromolaena plants were then placed in the cages for the flies to continue oviposition. Plants were changed every three days until all adults had died, allowing females to oviposit on as many plants as possible.

Plants with eggs were kept out in the sun and allowed to grow on black Visqueen® plastic sheets laid on the ground. Galls took up to seven (7) weeks to develop and mature, indicated by a window at the side of the gall. When the windowed galls darken, signifying adult emergence, the galls were harvested by cutting the stems below the second internode below the gall. The cut-stems were cleaned of most leaves and any branches without galls. The cleaned stem cuttings and branches with galls were placed into 500 ml food cups containing water through holes in nylon gauze taped over the top of the cups. The containers of "planted" galled cuttings were placed into emergence cages. The water kept the stems or galls alive until all flies completed development and emerged. The fine gauze was used to prevent newly emerged flies from drowning.

Field release and establishment of *C. connexa*

Releases of *C. connexa* were conducted by transplanting potted plants with galls containing larvae at sites close to Lae, or releasing mature galls containing pupae in stem sections at more distant sites. Potted plants with developing or mature galls were taken by vehicle and planted in holes dug among chromolaena bushes accessible by road from Lae. The flies emerged from the potted plants and move onto naturally growing chromolaena plants.

To ensure the fly's survival and establishment following air transportation to field release sites in the other provinces, a modified version of the method described by Wilson and Widyanto (1998) was employed. Cut-stems with galls and some small leaves, harvested in the manner described above, were wrapped in moist newspaper and sealed in clear plastic bags before hand carrying them in aircraft.

Sites with actively growing or healthy chromolaena plants were selected as release points. Instead

of following the technique used in Indonesia (Wilson and Widayanto 1998), we 'planted' the stem cuttings in vases made of 500 mm plastic food cups to ensure the survival and emergence of adult flies in the galls. The cuttings were 'planted' through several holes made in the gauze covering the cups and filled with water, as described in the rearing program.

Impact of *C. connexa* on chromolaena

During the rearing of *C. connexa* for field releases, a trial was conducted to assess the impact of gall formation on plant growth and seed production. Plants were exposed to gall flies for three days to allow flies to oviposit. A control where plants did not possess galls was also set up. The number of leaves and branches were counted, and the plant height above the lowest gall on the central stem was measured over a 12-week period. On control plants, counts and measurements were made from a reference point marked at the growing stem's tip, at the same time that gall plants were taken out from oviposition cages. All plants used were potted in similar sized pots using potting soil from the same source and watered and fertilised at the same rates and kept in full sunlight conditions. Only fully developed flower heads and seeds were considered. Data on flowering and seeds were recorded in July, which was near the end of the flowering period but before plant senescence.

Results and discussions

Mass rearing of *C. connexa*

The 'planting' technique kept the galls alive while flies emerged, as the stem sections often developed roots at the internodes when submerged in water. Stem sections were kept alive for up to five weeks. The adult flies emerged over a month, with most emerging during weeks 2–4 after harvesting the galls and placing them in the emergence cages (Figure 1).

An average of 1.7 flies emerged from each gall. The newly emerged flies were collected 2–5 hours after emergence and placed in oviposition cages containing potted plants to continue the rearing. Most of the flies emerged over a period of three weeks from all the stems. There were sex differences in emergence, with more male gall flies emerging than females.

Field release and establishment of *C. connexa*

Keeping cut stems with galls in moist paper and placing the stems in water at field sites reduced the mortality of late *C. connexa* larvae and pupae due to drying and hardening of the chromolaena stem cuttings. Field releases were conducted at 25 locations in the provinces of Sandaun, Morobe, Eastern Highlands, Oro, Milne Bay (Misima Island), East New Britain, New Ireland and Madang. The first confirmation of field establishment of *C. connexa* was at

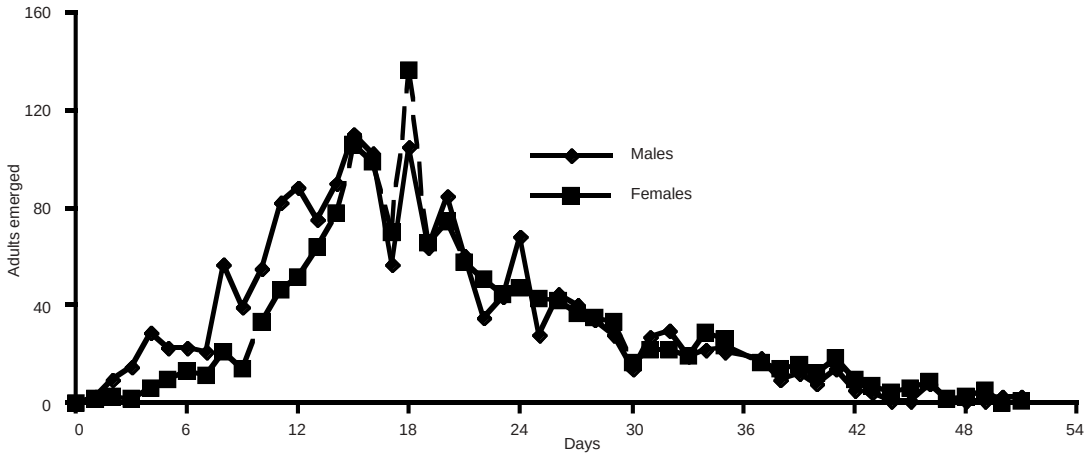

Figure 1. Average daily adult emergence of *C. connexa* over three generations between February and September 2001 after galls were collected and placed in cages at Labu, PNG.

Erap in Morobe Province. The gall flies were first released in April 2001 on chromolaena growing under rain trees (*Samanea saman*) along the Erap River flood plains on silty soils. Frequent ongoing visits to the site to monitor the gall fly population show that the numbers of galls on chromolaena stems have increased steadily and in November 2001, a total of 155 galls were counted within 10 minutes by three persons. By February 2003, 357 galls were counted on 20 stems. From the original release site at Erap, a total of 1100 galls were 'harvested' and flown to other provinces for releases.

Recently, *C. connexa* was found established at the edge of a rainforest at Pususuata near Lae. Gall flies were first released here in April 2001 and despite several subsequent releases, it had not been detected over the following two years. In March 2003, galls were found developing on chromolaena and the gall fly had spread to about one kilometre from the release site. A total of 23 galls were found on 20 stems after a brief search. The status of the biocontrol agent at the other release sites around PNG is listed in Table 1.

C. connexa was found established by June 2001, six months after release, in and around Vanimo (Sandaun Province), where chromolaena had spread from the neighbouring Papua Province of Indonesia (Orapa, 1998). By December 2002, the flies had spread, with galls found 0.5 kilometre from the release sites. Additional releases have been made at two new locations 10 kilometres away from the first release sites. At the time of reporting, there was no evidence of spread of the flies from Jayapura, where Indonesian workers had released it. In Jayapura, it was reported to have spread up to 10 kilometres towards PNG territory (Widayanto, pers comm.).

In East New Britain Province, subsistence farmers clearing chromolaena and other vegetation to grow food crops destroyed two *C. connexa* release sites at Kerevat and Vunadirdir. During February 2002, a NARI Entomologist returning to Rabaul from Lae released 100 galls at Kerevat. The site was revisited in February 2003 and the flies were found established with 27 galls found on 20 chromolaena stems.

Gall flies were released at Rasese and Rasirik in Namatanai District of New Ireland in October 2001 and establishment was confirmed in February 2003. At Rasese, gall flies have spread six kilometres east and four kilometres west from the release point. At Rasirik, gall flies were found to spread three kilometres east and four kilometres west from the release point. At Rasese 226 galls were counted on 20 stems and 66 galls on 20 stems at Rasirik.

The release site on Misima Island (Milne Bay Province) has not been revisited and in Oro Province the agent has failed to establish. Releases of *C. connexa* are yet to be conducted in West New Britain, Manus, and Bouganville and the follow-up releases need to be conducted in New Ireland, Oro Provinces Misima and East New Britain Provinces.

Impact of *C. connexa* on chromolaena

The results from the limited impact trial showed that there was an increase in the number of branches and

Table 1: *Cecidochares connexa* release sites and status in Papua New Guinea.

Province	Release location	Month of first release	Status
Sandaun	Vanimo Hill	December 2001	June 2002, established and spreading
	Mushu	December 2001	June 2002, established and spreading
	Lido-Bevani Junction	June 2002	To be checked
	Blackwater Refugee camp	June 2002	To be checked
Morobe	Erap Station	April 2001	Well established and spreading. Galls collected from this site have been redistributed elsewhere
	Munkip, Erap Valley	September 2002	Not established
	Kasuka, Erap Valley	October 2001	Not established
	Pususuatu	April 2001	March 2003, established and spreading
	Leron Valley	August 2002	Not established. Made 2nd release and yet be checked
Oro	Tanana	October 2002	To be checked
	Hohorita	October 2002	To be checked
Milne Bay	Misima Island	July 2001	To be checked
East New Britain	Kerevat	July 2001	Established and spreading
	Vunadidir	July 2001	Site destroyed
New Ireland	Rasese	October 2001	Established and spreading
	Rasarik	October 2001	Established and spreading
Madang	Gusap Plains	April 2002	Established and spreading
Eastern Highlands	Singsing Creek	December 2002	To be checked

leaves in galled plants (Figures 2 and 3). The presence of galls on main stems appeared to trigger the development of new axillary buds and leaves but the numbers of branches produced tapered after 12 weeks in both galled and gall-free plants. As expected, plant height was slightly more reduced in galled plants than in the control plants (Figure 4). Galled plants produced fewer flowers and seeds than the control plants, although the differences in the means were small (Table 2).

In this experiment, 10 female gallflies were allowed to oviposit for three days and there were an average of 3 galls/plant. The differences in plant height and seed production in the test and control plants were small. From these results, it is not possible to predict the true impact of *C. connexa* on chromolaena. However, at some field sites, there have been reported more than 10 galls/plant. Therefore, it is possible with higher densities, plant height and seed production could decrease as a result of gall fly activity.

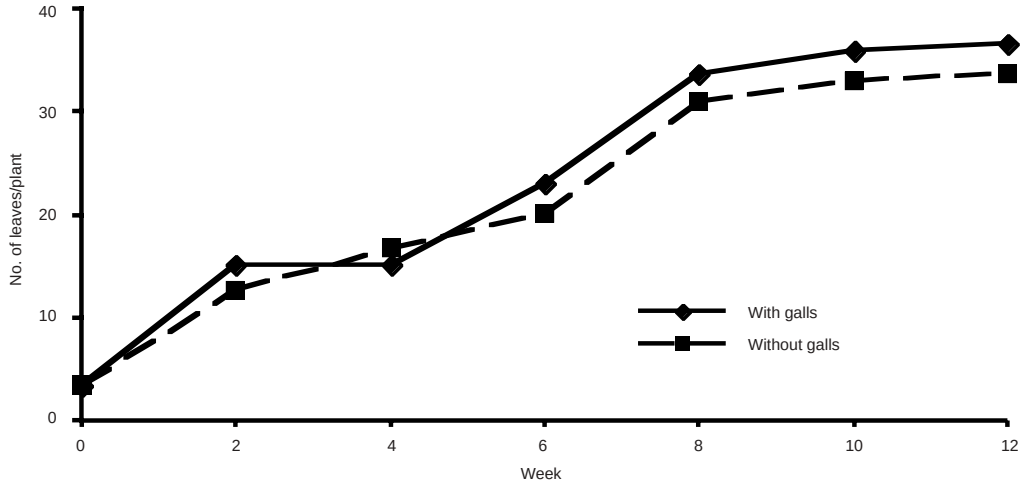

Figure 2. Average number of leaves above the lowest gall on galled plants and above a comparable reference point on plants without galls over a period of 12 weeks (n = 50).

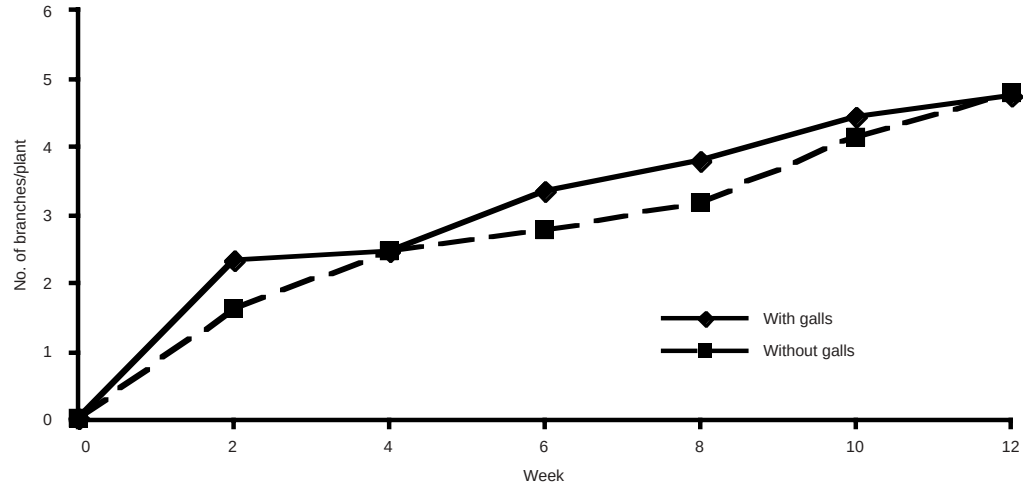

Figure 3. Average number of branches developed above the lowest gall on galled plants and above a comparable reference oint on plants without galls over a period of 12 weeks (n = 50).

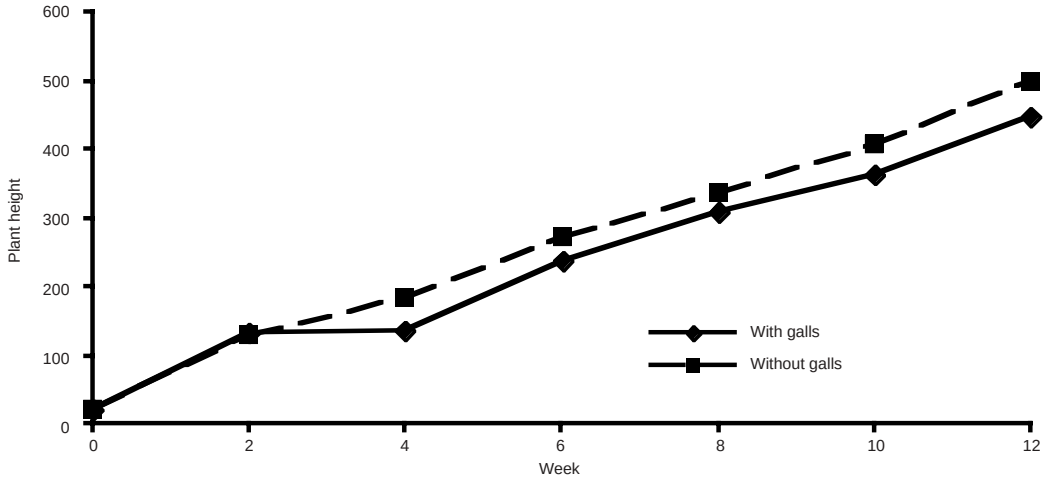

Figure 4. Average height above the lowest gall of galled plants and above a comparable reference point on plants without galls over a period of 12 weeks (n = 50).

Table 2. Summary of t-test on flower and viable seed counts on galled (n = 30) and gall-free (n = 30) chromolaena plants grown in pots at Labu, PNG. With $p_{0.05} > p_{obs}$, there were significant differences in both flower head and seeds counts. (WG = with galls; WOG = without galls).

	Flower heads per branch		Seeds per flower head	
	WG	WOG	WG	WOG
Total counts	642	643	770	790
Average	21.40	21.43	25.67	26.33
Stdev	11.722	7.147	4.366	2.721
T-test value (p_{obs})	0.989		0.481	

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References

Henty, E.E. and Pritchard, G.S. 1973. Weeds of New Guinea and their control. Botany Bulletin No. 7. Division of Forests, Lae, PNG. 195 pp.

Muniappan, R. 1999. (unpublished report). Environmental assessment of *Procecidochares connexa.* University of Guam. Guam (USA). 18 pp.

Orapa, W. 1998. The Status of *Chromolaena odorata* in Papua New Guinea. Proceedings of the Fourth International Workshop on Biological Control and Management of *Chromolaena odorata*. In Ferrar, P., Muniappan, R. and Jayanth, K.P. (eds), Oct. 1996, Bangalore, India. 83–85.

Orapa, W., Donnelly, G.P. and Bofeng, I. 2002a. The distribution of Siam weed, *Chromolaena odorata,* in Papua New Guinea. In: Zachariades, C., Muniappan, R. and Strathie, L.W. (eds.), Proceedings of the Fifth International Workshop on Biological Control and Management of *Chromolaena odorata*. Durban, South Africa, 23–25 October 2000. 19–25.

Orapa, W., Bofeng, I. and Donnelly, G.P. 2002b. Management of *Chromolaena odorata* in Papua New Guinea: status of a biological control programme. In: Zachariades, C., Muniappan, R. and Strathie, L.W. (eds.), Proceedings of the Fifth International Workshop on Biological Control and Management of *Chromolaena odorata*. Durban, South Africa, 23–25 October 2000. 40–45.

Sipayung, A. and Chenon, D.D. 1999. Biology and Host-Specificity of the *Chromolaena* stem gall fly, *Procecidochares connexa*. Unpublished report on results of investigations at Marihat Research Station, Sumatra, Indonesia.

Waterhouse, B.M. 1992. Survey of the Papua New Guinea border with Indonesia for the presence of exotic pests and diseases. Unpublished report to the Australian Quarantine and Inspection Service.

Wilson, C.G. and Widayanto, E.B. 1998. A technique for spreading the Chromolaena gall fly *Procecidochares connexa*, to remote locations. In: Ferrar, P., Muniappan, R. and Jayanth, K.P. (eds), Proceedings of the Fourth International Workshop on Biological Control and Management of *Chromolaena odorata*. Publication No. 216. Banglore, India, October 1996. 63–65.

Rearing *Actinote thalia pyrrha* (Fabricius) and *Actinote anteas* (Doubleday and Hewitson) with Cutting and Potted *Mikania micrantha* Kunth

Li Zhigang[1,2], Han Shichou[1], Guo Mingfang[1], Luo Lifeng[1], Li Liying[1] and Roch Desmier de Chenon[3]

Abstract

Two *Actinote* spp. have been utilised for the biological control of the exotic weeds *Chromolaena odorata* and *Mikania micrantha*. Since the end of the 1990s, *M. micrantha* has been spreading rapidly in the south of Guangdong Province, China, and has become a serious weed, especially in nature reserves. We introduced two *Actinote* spp. from the Indonesian Oil Palm Research Institute in 2001, and reared them in the quarantine laboratory of the Guangdong Entomological Institute. A method of rearing *Actinote thalia pyrrha* and *Actinote anteas* using cut and potted *Mikania micrantha* was developed. When reared on *Mikania micrantha* in the laboratory, with controlled temperature, humidity and light, *A. t. pyrrha* and *A. anteas* completed their life cycle very well. Emergence and oviposition of adult *Actinote* spp. occurred successfully in a screen cage in a plastic screen house even during the cold season (6–9°C night and 12–18°C day). This method can be used to rear *A. t. pyrrha* and *A. anteas* for experiments.

Introduction

MIKANIA MICRANTHA Kunth is a perennial vine, native to central and south America. Because it grows very fast and is hard to control, it has become one of the world's worst weeds, invading southeast Asia and the Pacific region (Waterhouse 1994). In recent years, it has spread to the Hong Kong and Guangdong coastal area in the People's Republic of China, especially Zhujiang River delta. In Neilingding Island National Nature Reserve (Shenzhen), about 6–7 ha of forest have been degraded by this weed. The weed spreads rapidly and is an enormous threat to the environment and to agricultural production.

Chemical control of *M. micrantha* is difficult and expensive. Biological control is considered the most practical method to reduce the weed's impact. *Actinote thalia pyrrha* (Fabricius) and *Actinote anteas* (Doubleday and Hewitson) are two potential biological control agents for *M. micrantha* as well as for *Chromolaena odorata*. *A. anteas* has been successfully reared for the control of *Chromolaena odorata* in Indonesia (Desmier de Chenon et al. 2002). In December 2001, the Guangdong Entomological Institute introduced both species and initiated the research work with the approval of the relevant organizations. The technology used and method for rearing *Actinote* spp. is reported in this paper.

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Materials and methods

Rearing condition and containers

The rearing room in the quarantine laboratory was an area of 50 m^2. The temperature in the room was 20–30°C, with a photoperiod of 13/11 L/D and the relative humidity was 70–90%. The doors and windows were screened with mesh. The room was kept clean, and, prior to use, surfaces sterilised with 2% lysol solution and an ultraviolet lamp.

Plastic screen houses were positioned on the roof of the quarantine laboratory. A screen cage (2 × 1.5 × 2 m) was placed inside one. Temperature: 18–35°C;

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RH: 50–80%; natural light. 20 pots (30 cm high and 28–30 cm diameter) of cultivated *M. micrantha* were placed in the cage. Plastic soft drink bottles were used to rear caterpillars.

Culture of *M. micrantha*

M. micrantha plant material was obtained from the suburb of Dongguan City, Guangdong Province. Stems of strong one-year-old plants were taken from the field into the laboratory. The stems were cut into sections of about 25 cm with nodes. These stems were planted in pots with farmland soil, with 4–5 pieces of stem in each pot because *M. micrantha* grows rapidly from cut stems (Waterhouse 1994).

Newly planted *M. micrantha* plants were put in a shady, cool place and watered morning and evening. New stems grew from the nodes after about 20 days. The survival rate of the stems was greater than 85% and the surviving plants were then moved to a more open sunny area. When the stems reached 20 cm, bamboo sticks (about 1.5 m length) were placed in the pots to support the vines and allow them to climb freely. When the branches reached the top of the bamboo stick, the terminal bud was removed to promote the growth of new stems. Daily watering was continued.

Rearing method for *Actinote* spp.

The first eggs of *Actinote* spp. were sent from the Indonesian Oil Palm Research Institute by Dr. Roch Desmier de Chenon. The eggs were laid on leaves. Small branches of *M. micrantha* with fresh leaves were placed in bottles with water, 3–4 branches in each bottle. The leaves with eggs were placed in contact with the fresh leaves. The water in the bottle was refilled regularly. Room temperature was 20–28°C; RH about 85%.

Young caterpillars in the first three instars feed little, living in groups, and spin silk to form a protective net. They eat the upper epidermis and mesophyll of the leaves and the lower epidermis dries up and changes colour. Several dozen caterpillars form a colony on a single leaf that is difficult to disperse, so the use of leaves in bottles saves time, space and labour.

New leaves were placed in the water bottle each day, close to the old leaves. After the caterpillars moved to the new leaves, the old leaves were removed. Any caterpillars remaining on the old leaves were moved using a fine paintbrush. The water in the bottles was constantly replaced to keep the leaves fresh.

After the fourth instar, the larvae eat more and feed singly, so they were moved to potted *M. micrantha.* The leaves with caterpillars were placed evenly on the potted *M. micrantha*, 20 to 30 caterpillars at 5–6 places on each pot. New potted *M. micrantha* plants were constantly supplied, placing these in contact with the old pot. The caterpillars readily moved to the new leaves of *M. micrantha.* Any caterpillars remaining on old plants were transferred with a fine brush.

During the cold weather (6–9°C), caterpillars are still alive but not active and feed slowly. At temperatures above 34°C, the caterpillars are also inactive.

The caterpillars generally pupate in the sixth instar, but sometimes in the fifth instar if nutrition is inadequate. The pupae usually hang from stems and for pupation it is preferable to use potted *M. micrantha* with several stems. Pupae must not be handled immediately after pupation or they risk being injured. The stems with pupae were cut two days later and kept in the hanging position for the emerging butterflies to spread their wings. Unattached pupae were fixed on the stems or bamboo sticks with a small amount of glue. The pupae could be stored in a refrigerator (at about 10°C) for a week and the butterflies emerged normally, to obtain simultaneous emergence of females and males.

The female butterflies prefer to lay on fully open fresh leaves, so the best quality potted plants of *M. micrantha* were used in the screen cage for emergence and oviposition. Just before emergence, the pupae were moved to the screen cage. The outside temperature of about 25–30°C was preferable; however, during the cooler season when the temperature was about 18–20°C, *Actinote spp*. still emerged and laid eggs. Fluorescent lamps were used if the weather was cloudy and rainy because the natural light was insufficient. Temperature and sunshine are important factors that influence adults' mating and laying eggs, although on cloudy days with high temperatures they will still mate and lay eggs. The males emerged earlier than the females. For normal copulation and oviposition, a large population of butterflies (about 100 pairs of females and males in the screen cage) is necessary. Mating usually occurred in the afternoon (peak time 4 pm–6 pm) and oviposition in the morning (2–3 days after emergence). Trays with 20% honey solution were hung within the cage for the adults' supplemental nutrition. Copulation lasts for several minutes to more than 20 hours, and the longer times resulted in more fertile eggs. Oviposition generally lasted 1–4 hours and a larger batch of eggs (>200 eggs) is preferable, as smaller batches were usually infertile. Eight to nine days later the plants with eggs were moved to the rearing room for hatching and the rearing of the new generation.

Results

A. thalia pyrrha and *A. anteas* completed their life cycle very well using the above rearing method. Eggs are pale lemon-yellowish in colour, vase-shaped, becoming reddish before hatching. The neonates and first instar are yellowish and turn green-yellowish after feeding starts. Caterpillars have many spines on each segment. The colour of the two species is obviously different: young caterpillars of *A. anteas* are lighter and older caterpillars, after the fourth instar, darker than those of *A. thalia pyrrha.* Pupae are greenish in colour, becoming greyish later. Butterflies are brownish orange-yellow with black spots. *A. anteas* is paler coloured than *A. thalia pyrrha*.

The life cycle is 90–102 days, depending on the temperatures. At 25–32°C, the hatching of the eggs takes approximately 10–13 days (average 12 days); 11 days for the first instar; 22 days for the second instar; 11 days for the third instar; 10 days for the fourth instar; 11 days for the fifth instar and 9 days for the sixth instar. The pupal period lasts 11–12 days and adults live for 7–9 days. The two species vary slightly in the duration for each instar and stage.

Discussion

Environmental conditions are very important for hatching of eggs, especially the control of the humidity. The eggs cannot hatch when the humidity is too low, but high humidity may lead to mould on the eggs. Another key problem is handling pupae and butterflies. It is preferable to move the pupae into the screen cage in the screen house for emergence, and allow the female butterflies to lay eggs normally. If it is necessary to move them, to avoid injuring the butterflies, the butterflies can be manually moved first to a small plastic screen cage, then allowed to fly into the bigger screen cage in the screen house. Caterpillars must be adequately fed, otherwise they pupate early in the fifth instar, which causes abnormal emergence and reduced oviposition. High temperatures, above 35°C at midday in the summer, cause the death of pupae and affect the normal emergence and oviposition of females. As a result, emergence, mating and oviposition in laboratory in summer is still a problem.

References

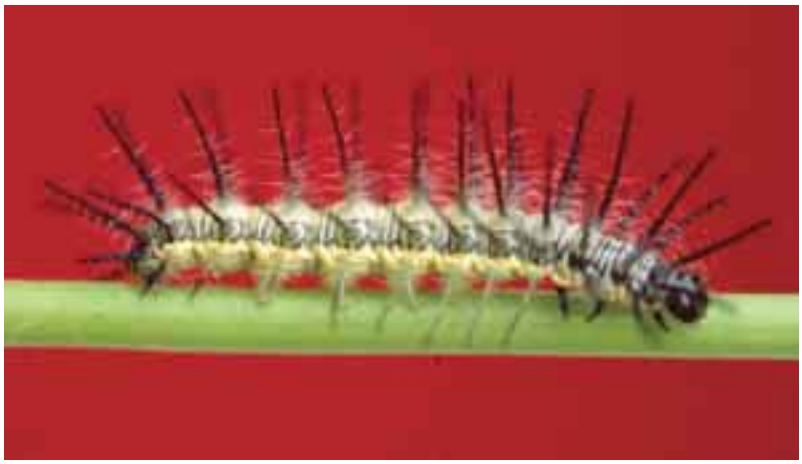
Caldwell, P.M. and Kluge, R.L. 1993. Failure of the introduction of *Actinote anteas* (Lep:Acraeidae) from Costa Rica as a biological control agent for *Chromlaena odorata* (Asteraceae) in South Africa. Entomophaga 38(4), 475–478.

Desmier de Chenon, R., Sipayung, A. and Sudharto, P. 2002. A new biological agent, *Actinote anteas*, introduced into Indonesia from South America for the control of *Chromolaena odorata*. Proceedings of the Fifth International Workshop on Biological Control and Management of *Chromolaena odorata*. Durban, South Africa, 23–25 October 2000. ARC-PPRI. 170–176.

Li Liying, Peng Tongxu, Liu Wenhui, Han Shichou, Li Kaihuang, Luo Lifen and Chen Qiaoxian. 2002. *Actinote anteas* (Doubleday and Hewitson), a new biological agent for controlling the weed *Mikania micrantha.* Natural Enemies of Insects, 24(2), 49–52.

Li Mingguang, Zhang Weiyin, Liao Weibo, Wang Bosun and Zan Qijie. 2002. The history and status of study on *Mikania micrantha.* Ecologic Science. 19(3), 41–45.

Waterhouse, D.F. 1994. Biological control of weeds: Southeast Asia Prospects. Canberra: 125–126.



Actinote caterpillars have many spines on each segment. C. Zachariades

Establishment and Spread of *Cecidochares connexa* in Eastern Indonesia

Colin G. Wilson[1] and Eko Budi Widayanto[2]

Abstract

The Australian Centre for International Agricultural Research (ACIAR) funded the biological control of *Chromolaena odorata* in eastern Indonesia between January 1993 and June 2002. This paper describes the introduction, release, establishment, spread and impact of the gall fly *Cecidochares connexa* initially in west Timor, and subsequently throughout the region between Bali in the west, Papua New Guinea in the east and to southern Sulawesi in the north. Much of this region relies on subsistence agriculture and lacks infrastructure and trained personnel to facilitate a biological control program. Transport is unreliable and political and religious turmoil frequently makes travel dangerous. The project has seen a plant- and insect-rearing facility established at Nusa Cendana University in Kupang, west Timor, and training provided to staff and students. *C. connexa* is now established across much of west Timor and is established and spreading rapidly on most other major islands in the region. The weed is already reducing in density in many areas, particularly where rainfall is more reliable. A proposal to extend the work to the new nation of East Timor is in preparation.

Introduction

SINCE the Second World War *Chromolaena odorata* (L.) King and Robinson has been spreading steadily down the Indonesian archipelago from the northwest towards the south and east (McFadyen 1989). This invasion has been extraordinarily successful and *C. odorata* is now the dominant plant species in rural landscapes across eastern Indonesia, surrounding many villages with dense, tangled thickets that smother other plant species and carry damaging fires (Mudita 2000; Wilson and Mudita 2000). It is known to occur on all of the larger islands (Wilson and Widayanto 2002) and almost certainly infests most of the smaller ones as well. It probably reached Timor, just 450 km off the north coast of Australia, in the late 1970s (McFadyen 1998) and has since become almost ubiquitous on the island.

C. odorata has a major impact on rural life in eastern Indonesia. It displaces grasses required for grazing animals, invades plantation crops, and restricts the area that subsistence farmers can maintain weed-free. Fire is a traditional management tool widely used to remove unwanted vegetation, but *C. odorata* is highly flammable and its recent dominance has created a serious fire hazard for villages, infrastructure and forests. The plant recovers quickly following fire by reshooting from the root crown or undamaged axillary buds (McFadyen 1989) and this helps it to maintain its ascendancy over other vegetation.

The Australian Centre for International Agricultural Research (ACIAR) funded a biological control program against *C. odorata* in Indonesia, the Philippines, and eventually Papua New Guinea, from January 1993 (McFadyen 1998). The segment of the program that covered the eastern Indonesian islands between Bali in the west, the Papua New Guinea border in the east and southern Sulawesi in the north, ultimately terminated in June 2002 following a successful mid-term review, an interruption in funding and delays caused by regional unrest.

Managing a scientific program in eastern Indonesia posed special challenges. Although physically close to Australia, the thousands of islands lack basic

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infrastructure, transport can be erratic and the region is beset by social, religious and political turmoil. In order to carry out the program effectively, a partnership was developed between the Northern Territory government in Australia and Nusa Cendana University (Undana) in Kupang, the administrative capital of Indonesia's Nusa Tenggara Timur province (NTT).

This paper updates one presented at the previous International Workshop in Durban, South Africa during October 2000 (Wilson and Widayanto 2002), and focuses specifically on the introduction, release, establishment and impact of the gall fly *Cecidochares connexa* Macquart (Diptera: Tephritidae) across the major islands of eastern Indonesia.

The biological control program

The base for the biological control program in eastern Indonesia was Undana in Kupang. This institution was chosen for its proximity to Darwin in Australia's Northern Territory, pre-existing links with institutions in Darwin, staff with some experience in biological control and the position of Kupang as a transport hub for the region (Wilson and Widayanto, 2002). Biological control agents to be released in eastern Indonesia were sourced from the International Oil Palm Research Institute (IOPRI) at Marihat, near Medan in northern Sumatra. Undana initially lacked suitable facilities for insect rearing, so ACIAR provided additional funding to refurbish a dilapidated shadehouse, provide a water supply, employ technical assistance and hire transport.

The first batch of larvae of the moth *Pareuchaetes pseudoinsulata* Rego Barros (Lepidoptera: Arctiidae) arrived at Undana in 1993 and rearing commenced. Despite releases of many thousands of larvae and adults in west Timor over a number of years, establishment has not been confirmed. The butterfly *Actinote anteas* Doubleday and Hewitson (Lepidoptera: Nymphalidae) was reared at Undana during 2000, but the colony died out before any field releases were made.

We detailed the releases, establishment and spread of *C. connexa* in eastern Indonesia to mid-2000 in our report to the previous International Workshop in Durban (Wilson and Widayanto, 2002). At that time, we had released the fly at sites on Lombok, Sumbawa, Flores, Alor, Sumba, Rote, west Timor, south Sulawesi and Irian Jaya, and had confirmed establishment at many of these sites. We made further surveys during March 2001 and March/April 2002.

The spread of a biological control agent away from a release site is difficult to assess in these remote areas. The islands of eastern Indonesia have few sealed roads that are navigable during the monsoon season when plants are growing and the gall flies are active. We invariably positioned release sites adjacent to a formed road and measured spread by roadside surveys for galls from a vehicle, using a GPS receiver to determine the straight-line distance from the point of release. There is typically only a single navigable road through an area in this region, and it is usually the focus for linear development of housing, small businesses and agricultural fields. There may be little or no *C. odorata* growing along extended stretches of road, and the spread of the agent may not be uniform in all directions. Nevertheless, roadside surveys give an overall impression of the establishment, abundance and rate of spread of the gall flies. Given the restrictions in available time and resources it was the only practicable technique for surveying the success of biological control agents in the extensive project area.

The only measure of impact of *C. connexa* on the host plant population was of necessity extremely subjective. Shifting slash-and-burn agriculture is the norm across the region, ownership of the land is difficult to ascertain and most areas could only be visited once every year or two. There was no easy way to establish, mark, maintain and monitor fixed plots or transects in an environment subject to such continuous anthropogenic perturbation. Hence, priority was given to distributing the gall flies as widely as possible across a vast archipelago, rather than attempting to set up an objective impact monitoring program.

West Timor

The first release of *C. connexa* in eastern Indonesia was in November 1995, near the village of Bipolo in west Timor, approximately 50 km northeast of Kupang (Table 1). Establishment was swift and we subsequently redistributed gall flies to a further 29 sites in west Timor (Wilson and Widayanto, 2002). Most of the release sites were concentrated in the lowlands near Kupang, but releases in the central highlands near the town of Soe, at an altitude of 820 m above sea level, ultimately led to successful establishment after several attempts. By March 2000, *C. connexa* was present in virtually all *C. odorata* infestations within 80 km of Kupang in the west of the island and at several sites beyond. One release was made near the town of Atambua in the far northeast of the Province near the border with East Timor, but the activities of armed militias have made it too dangerous to revisit the site to determine whether or not the flies have established. Gall flies were also released on the island of Rote, to the southwest of Timor, and have successfully established there (see Figure 1).

Travel away from the city of Kupang has been dangerous for westerners and locals alike for much

Table 1. Releases, establishment and spread of *Cecidochares connexa* at release sites in eastern Indonesia.

Release site location	No. of galls released	Release date	*C. connexa* established	Spread (km) by April 2002
West Timor				
Bipolo, 50 km northeast of Kupang	251	Nov 95	Yes	>40
Flores				
10 km north of Ende	363	Mar 98	Yes	19
Larantuka bus station	325	Dec 99	Yes	3.3
Boawae bus station, near Bajawa	250	Oct 00	Yes	>10
Village of Nitta (site 1), near Maumere	300	Mar 01	No	
Village of Nitta (site 2), near Maumere	300	Mar 01	Yes	<1
Ruteng airport	305	Nov 01	No	
Lombok				
North coast 15 km east of Gondang	200	Mar 98	Yes	30
South coast of Kuta Beach	480	Dec 98	Yes	35
Northwest coast opposite Gili Islands	423	Mar 01	Yes	<1
Sumba				
102 km west of Waingapu	283	Mar 98	Yes	11–31
16.5 km west of original site	269	Mar 00	?	?
21 km east of original site	480	Mar 00	Yes	<10
Sulawesi				
Bantimurung, 60 km northeast of Makassar	240	Mar 99		
Bantimurung, 60 km northeast of Makassar	300	Feb 00	Yes	13
Near Camba, 25 km northeast of Bantimurung	232	Feb 00	Yes	7
Irian Jaya				
Wasur National Park, 36 km east of Merauke	334	Mar 99	Yes	>1#
Senayu, ~30 km north of Merauke	533	Feb 00	?	?
Sumbawa				
Labu Ijuk, northeast of Sumbawa Besar	435	Mar 00	No	
24.5 km west of Sumbawa Besar	356	Mar 01	No	

#by February 2000.

of the time since the East Timorese voted for, and were granted, independence from Indonesia in 1999. In addition, the Australian government advised its citizens to avoid all travel to west Timor during the last two years of the project. Hence, our knowledge of the impact of gall flies on *C. odorata* thickets in areas where the flies have been established for the longest time is incomplete. There is some anecdotal evidence, however, that *C. odorata* is becoming less vigorous with the establishment and spread of *C. connexa*, especially in areas with higher rainfall or near permanent water.

Flores

We released *C. connexa* at six sites on the island of Flores between March 1998 and November 2001 (Table 1). Establishment may have failed at one of two sites near Maumere on the north coast and at Ruteng in the western highlands, as we found no galls during follow-up surveys less than one year after the release. Ruteng is high in the mountains and experience has shown that it can be difficult (although not impossible) to establish the flies above an altitude of 500 m. Additionally, it is not unusual for surveys to uncover no galls within one year of a release, but for galls to be plentiful in subsequent years. So it is possible that future surveys might find the galls to be established at these sites.

Nonetheless, gall flies have established at all other sites on the island of Flores, as well as on the island of Alor to the east of Flores where they were released in 1999 (Figure 1). Spread has been 19 km in four years from the original release site north of Ende on the south coast. We measured this along the single, winding road up into the central mountains, and spread may have been more extensive along the coastal lowlands away from formed roads. We found galls at least 10 km from the release site at Boawae

bus station near the town of Bajawa in the centre of the island, only 18 months after release.

Lombok

We released *C. connexa* galls at three sites on the island of Lombok (Table 1). After four years the flies had spread at least 35 km and now occur across more than half of the island. In the vicinity of the two earliest release sites, virtually all stems carry multiple galls. *C. odorata* is clearly becoming less abundant but is unfortunately being replaced, mainly by *Lantana camara* and other weeds. On the other hand, anecdotal evidence from local farmers is that these other weeds are easier to control than *C. odorata*.

Sumba

We set up three release sites on Sumba, all situated along the single sealed road that extends along the length of the island. The original site was established near the centre of the island in March 1998 and two years later, in March 2000, we established further sites 16.5 km to the west and 21 km to the east respectively. Our survey in April 2002 found galls continuously present in roadside *C. odorata* infestations from 10 km east of the eastern release site to the original central release site, a straight line distance of 31 km. Due to time constraints it was not possible to survey further west. We can conclude that *C. connexa* has spread a distance somewhere between 11–31 km in four years from the central site and up to 10 km in two years from the eastern release site (Table 1).

Sulawesi

C. connexa galls were released at two sites in southern Sulawesi (Table 1) in the limestone hills east of the city of Makassar (Ujung Pandang). We could find very few galls at the original site at

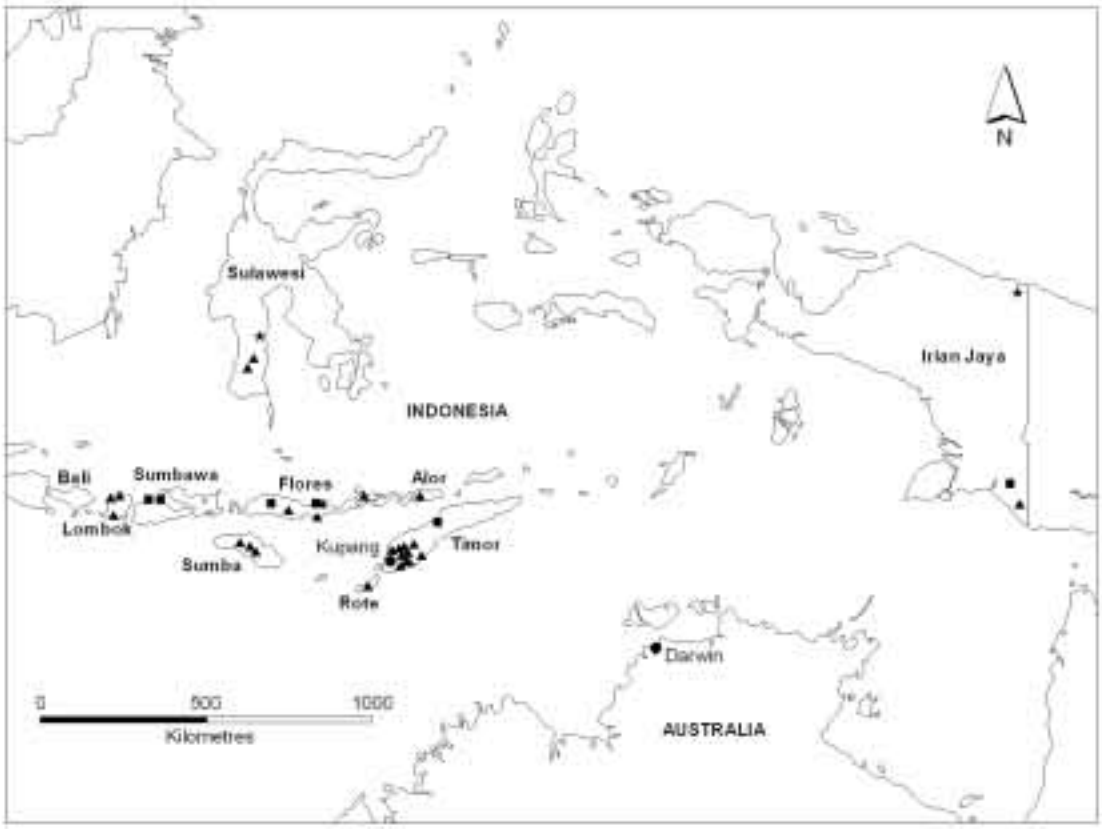

Figure 1. Release sites of C*ecidochares connexa* in eastern Indonesia to April 2002. (▲ = release sites created by the authors, establishment confirmed; ■ = release sites created by the authors, establishment not yet confirmed; ★ = release sites created by others, establishment confirmed). Note that triangle symbols in Timor (▲) represent a total of 29 separate release sites.

Bantimurung after one year and hence we made a second release of galls there in February 2000. By March 2002 galls were abundant around the release site and could be found up to 13 km away. Spread from the second release site near the town of Camba was approximately 7 km in two years. The road between the two sites along which the survey was conducted wound through precipitous hills cloaked in dense forest and *C. odorata* was restricted to occasional small clearings in the forest. Spread could have been more extensive in other directions.

Irian Jaya

Due to constraints of time, resources and the difficulty of reaching the main centres in Irian Jaya from Darwin, we have not re-surveyed the sites where *C. connexa* galls were released (Table 1) since our previous report on the project (Wilson and Widayanto 2002). At that time galls were found 15 km from the original release site at Cenderawasih University campus in Jayapura, and were established up to 1 km from the new release site in Wasur National Park near Merauke.

Sumbawa

We released galls at two sites near the main town of Sumbawa Besar in March 2000 and March 2001 respectively (Table 1), but by April 2002 had not found any sign that *C. connexa* had established. This apparent failure is difficult to understand, given the ease with which the flies established at most other sites in the region following releases of as few as 200 mature galls, but with such a small founder population, stochastic events can have a major affect on establishment. As discussed above, it is possible that future surveys will show that the gall flies have, in fact, established at these sites.

Discussion

The islands of Indonesia's Nusa Tenggara Timur province experience a monsoonal climate, heavily influenced by the proximity of the Australian mainland to the south and other Indonesian islands to the north and west which create a rain-shadow across the region. Rainfall is highly variable between seasons and between regions, but as a general rule winter dry seasons are severe, the western parts of the islands are wetter than the eastern, and the south coasts are wetter than those on the north (Monk et al. 1997).

With a generation time of approximately 7–8 weeks, the ability of *C. connexa* to reach high population densities is governed to a large extent by the length of the monsoonal wet season at a particular site. Once *C. odorata* begins to flower at the start of the dry season, final instar *C. connexa* larvae enter a diapause inside the gall, broken only by the resumption of plant growth at the start of the next rainy season (McFadyen 2002). Larvae undergoing diapause inside galls are highly susceptible to destruction during the widespread burning of *C. odorata*, which takes place each dry season. Those that survive and emerge as adults early in the following wet season must multiply rapidly if they are to cause significant damage to the host plant prior to flowering. In areas with a short rainy season they may have only two or three generations in which to increase to population densities damaging to the target weed, while in wetter areas there may be four or five generations in a season.

In most places across eastern Indonesia *C. connexa* has been established for no more than four years and populations are still actively spreading at a rate of approximately 5 km per year. We have not yet observed widespread impacts on the extent and density of *C. odorata* infestations caused by galling. However, early signs of significant reductions in the weed have been seen in areas with higher rainfall spread over a longer wet season, such as at Kuta Beach in southwest Lombok and Jayapura's Cenderawasih University campus in Irian Jaya. In these places gall densities have reached hundreds per plant, flowering and seeding has been suppressed and plant density has been reduced. We may begin to see a more widespread impact on plant vigour and density once the flies have occupied all suitable habitat on each of the islands and are no longer dispersing into new areas.

So far the gall flies appear to be free of parasites in eastern Indonesia as none have been found despite dissections of hundreds of galls from many sites. However, in some places predation is severe. Rats, birds and ants frequently take a high proportion of larvae from galls and salticid spiders have been seen to capture adult flies. Predation could become a factor in the effectiveness of biological control of *C. odorata* by *C. connexa* in the future.

This segment of the project on biological control of *C. odorata*, spanning the islands of eastern Indonesia, has now ended and there is no realistic opportunity for it to be resumed in the near future. However, there is a proposal in preparation for the biological control of *C. odorata* to be extended to the newly independent nation of East Timor. If funding is obtained for this project, it could see the agents already established in Indonesia (*Pareuchaetes pseudoinsulata*, *Cecidochares connexa* and *Actinote anteas*) being introduced into East Timor for rearing and release. It could additionally see the importation into East Timor of new agents that are being tested and released in South Africa.

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The Australian Centre for International Agricultural Research (ACIAR) funded the work reported here.

References

McFadyen, R.E. 1989. Siam weed: a new threat to Australia's north. Plant Protection Quarterly. 4: 3–7.

McFadyen, R.E. 1998. The ACIAR project for the biological control of *Chromolaena odorata*: future developments. In: Ferrar, P., Muniappan, R. and Jayanth, K.P. (eds), Proceedings of the Fourth International Workshop on Biological Control and Management of *Chromolaena odorata*, 14–18 October 1996. University of Guam, USA. 115–118.

McFadyen, R.E. 2002. Chromolaena in Asia and the Pacific: spread continues but control prospects improve. In: Zachariades, C., Muniappan, R. and Strathie, L.W. (eds), Proceedings of the Fifth International Workshop on Biological Control and Management of *Chromolaena odorata*, 23–25 October 2000. ARC-PPRI. 13–18.

Monk, K.A., de Fretes, Y. and Reksodiharjo-Lilley, G. 1997. The ecology of Nusa Tenggara and Maluku. Periplus, Hong Kong.

Mudita, W. 2000. Fire and the management of agricultural systems in East Nusa Tenggara. In: Russell-Smith, J., Hill, G., Djoeroemana, S. and Myers, B. (eds), Fire and sustainable agricultural and forestry development in astern Indonesia and Northern Australia. Proceedings of an International Workshop held at Northern Territory University, Darwin, Australia, 13–15 April 1999. ACIAR, Canberra, Proceedings No. 91, 56–61.

Wilson, C.G. and Mudita, W. 2000. Fire and weeds: interactions and management implications. In: Russell-Smith, J., Hill, G., Djoeroemana, S. and Myers, B. (eds), Fire and sustainable agricultural and forestry development in Eastern Indonesia and Northern Australia. Proceedings of an International Workshop held at Northern Territory University, Darwin, Australia, 13–15 April 1999. ACIAR, Canberra, Proceedings No. 91, 65–68.

Wilson, C.G. and Widayanto, E.B. 2002. The biological control program against *Chromolaena odorata* in eastern Indonesia. In: Zachariades, C., Muniappan, R. and Strathie, L.W. (eds), Proceedings of the Fifth International Workshop on Biological Control and Management of *Chromolaena odorata*, 23–25 October 2000. ARC-PPRI. 53–57.

J. Wright

C. connexa *larvae that emerge as adults early in the wet season must multiply rapidly if they are to cause significant damage to the host plant prior to flowering.*

Insects for the biological control of *Chromolaena odorata*: surveys in the northern Caribbean and efforts undertaken in South Africa

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Abstract

With recent evidence indicating that the southern African biotype of *Chromolaena odorata* has a northern Caribbean origin, surveys of insects on chromolaena were conducted in Cuba and Jamaica. These revealed a depauperate entomofauna with no 'new' agents. Compatible biotypes of insects from other countries (e.g. Venezuela) are thus still needed to supplement promising agents from the northern Caribbean. Diptera, Lepidoptera and eriophyid mites were the main groups of arthropods collected on *C. odorata* in Cuba and Jamaica. After a prolonged delay, releases of the host-specific, leaf-mining fly *Calycomyza eupatorivora* from Jamaica commenced in South Africa in 2003. Studies completed on the stem-boring weevil *Lixus aemulus*, that originates from Brazil, have shown it to be host-specific and permission for release should be imminent. The root-feeding flea beetle *Longitarsus horni* from Venezuela and an unidentified stem-boring cerambycid recently collected from a *Chromolaena* species in Argentina are also being cultured in quarantine. The stem-tip mining fly, *Melanagromyza eupatoriella*, from Florida, USA was successfully cultured for a few generations, but died out and a new culture will be imported from Jamaica to enable host-specificity testing. Other candidate agents that have been imported but unsuccessfully cultured include the stem-galling fly *Polymorphomyia basilica* and two stem-tip boring moths, *Mescinia* sp. nr. *parvula* and an unidentified species. During the past two years, the defoliating moth *Pareuchaetes insulata* from Florida, USA has been released in large numbers, for extended periods, at several sites in KwaZulu-Natal province in South Africa, with little establishment success. Populations of this insect from Jamaica and Cuba, which may be more compatible with the southern African *C. odorata* biotype, will be released in a new attempt to achieve establishment in South Africa. Despite successful culturing for several generations, the stem-galling weevil, *Conotrachelus reticulatus* from Venezuela, died out in the laboratory, with biotype incompatibility a possible contributing factor. This agent will not be re-imported until further biology and host range studies have been conducted in the country of origin. Studies on the biology of the stem-tip mining sesiid moth, *Carmenta* sp. nov., will also be conducted in Venezuela. Also, studies on the biology and host-specificity of the stem-galling fly *P. basilica*, and other insect species on *C. odorata*, will commence in Jamaica once biotype compatibility has been determined. In this regard, cooperation with overseas research organisations is of considerable importance in sustaining progress with the biological control of *C. odorata*.

Introduction

CHROMOLAENA ODORATA (L.) King and Robinson (Asteraceae) continues to spread and increase in density in the subtropical areas of southern Africa, ranking it as one of the major invasive plants of this region. Biological control is still considered to be the only viable option to reduce the weed to manageable levels and a suite of insects and pathogens is currently under evaluation (Zachariades et al. 1999).

Since 2000, further evidence was collected to confirm the northern Caribbean region as the origin of the southern African biotype of *C. odorata* (see Zachariades et al., this Proceedings, for an explanation of the usage of the term 'biotype' in this context). This involved completion of the comparative genetic

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study by von Senger (2002), observations of the plants in their countries of origin, and measurements of floral morphology (Zachariades et al., this Proceedings). The lines of evidence all indicate the northern Caribbean, that is the Greater Antilles group of islands, as the centre of origin for the southern African chromolaena biotype. Populations of plants resembling this biotype (referred to from here on as the 'southern African form') occur in Jamaica, Cuba and Puerto Rico (Zachariades et al., this Proceedings).

In this paper, we discuss the outcome of recent surveys for 'new' insect agents, or biotypes of known agents, on *C. odorata* in the northern Caribbean region. Also, we review the status of agents that have been introduced into quarantine from both this region and South America. Studies on some of the most promising species will be completed in quarantine in South Africa while others will be initiated in the country of origin in collaboration with overseas organizations.

Surveys in the northern Caribbean

Surveys of the phytophagous insects and pathogens associated with chromolaena were conducted in Cuba and Jamaica (Table 1). Present in both countries were the southern African form of *C. odorata*, a hairier form resembling the common invasive *C. odorata* biotype, and a range of intermediates.

Field surveys in Jamaica during this and previous surveys indicated a depauperate entomofauna, with few 'new'/unknown insect agents associated with chromolaena. Work in Cuba was focused predominantly on the eastern side of the island (Las Tunas, Holguin, Granma, Santiago de Cuba and Guantanamo states), and constituted the first survey of insects and pathogens associated with *C. odorata* here (Zachariades 2003). Cuba's larger landmass with accompanying range of habitats was expected to yield a more diverse insect fauna on chromolaena than Jamaica. However, the entomofauna on the two islands was found to be similar (Zachariades and Strathie 1999, Strathie 2003). No 'new' agents were discovered but a more extensive survey of the wetter, hilly parts of Cuba is required.

The insects and pathogens associated with *C. odorata* in Jamaica were surveyed briefly in 1997 and 1999. On the latter visit, the southern African *C. odorata* form was found mostly in the Blue Mountains region in the eastern part of the island, so this and the drier northern-central region were surveyed more intensively in September and October 2002.

A booster culture of *Calycomyza eupatorivora* Spencer (Diptera: Agromyzidae), and starter cultures of *Pareuchaetes insulata* (Walker) (Lepidoptera: Arctiidae) and *Polymorphomyia basilica* Snow (Diptera: Tephritidae) were collected.

Surveys were conducted first to determine whether species of *Conotrachelus*, *Lixus* (both Coleoptera: Curculionidae) or *Longitarsus* (Coleoptera: Chrysomelidae) with similar modes of action to *C. reticulatus* Champion, *L. aemulus* Petri and *L. horni* Jacoby respectively, were present on the two islands, and second to collect any other potentially promising agents. *Conotrachelus reticulatus*, *L. aemulus* and *L. horni* are high priority agents, but they were collected from hairier forms of *C. odorata* on the South American continent and may not be fully compatible with the southern African chromolaena biotype. However, because none of these genera were found in either Jamaica or Cuba, insects from other areas such as Venezuela and Brazil will continue to be used, once they have been assessed for compatibility with the plant biotype. No previous records exist of

Table 1: Insects and mites collected on *Chromolaena odorata* in Jamaica and Cuba.

Species	Feeding guild	Jamaica	Cuba
DIPTERA			
Calycomyza eupatorivora	Leaf-miner	✓	✓
Melanagromyza eupatoriella	Stem-tip miner	✓	✓
'*Ophiomyia*' sp.	Herringbone leaf-miner	✓	✓
Polymorphomyia basilica	Stem-galler	✓	✓
LEPIDOPTERA			
Pareuchaetes insulata	Defoliator	✓	✓
Mescinia sp. nr. *parvula*	Stem-tip galler	✓	✓
Unidentified sp. 1	Stem-tip galler	✓	✓
Dysschema sacrifica	Defoliator	✓	✓
Unidentified sp. 2	Shoot/leaf binder	✓	✓
ACARI			
Eriophyidae (several unidentified species)	'Furry' leaves, leaf distortion, 'witches broom'	✓	✓

these genera being collected on *C. odorata* from the northern Caribbean islands (Cruttwell 1974). Although few 'new' insect species were discovered in Jamaica or Cuba, it is desirable to use biotypes of widespread species from these islands, as they should be more compatible with both the southern African biotype of *C. odorata* and, if collected from drier areas, with the climate in the parts of South Africa in which chromolaena is invasive.

Promising agents collected in the northern Caribbean

The agents collected on *C. odorata* in Cuba and Jamaica fell into three main taxonomic categories, namely dipterans, lepidopterans, and eriophyid mites (Table 1). Pathogens were also extensively collected (den Breeÿen, 2003) but are not discussed here. Information on the most important species collected is summarised below.

Calycomyza eupatorivora (Agromyzidae)

The leaf blotch-mining fly *Calycomyza eupatorivora* was collected on the southern African chromolaena form in Jamaica in 1999. It was shown during a series of choice and no-choice tests to be host specific, with no feeding, oviposition, or development on 24 non-target species (Zachariades et al. 2002). There was a two-year delay in obtaining approval from the regulatory authorities to release this agent due to changed legislation, but this was granted in April 2003. The fly is being mass-reared on potted plants in a shade-house and the first experimental release took place in July 2003. Additional releases will commence at trial sites around KwaZulu-Natal province (KZN) during the spring of 2003. *C. eupatorivora* will probably be best suited to more humid areas, as sensitivity to low humidity has been noticed in the quarantine culture. Due to the short development period (about six weeks per generation), it is expected that this agent will establish readily in the field. The potential effects of parasitism in the field are unknown but hymenopteran parasitoids have attacked developing larvae on plants in the shadehouse. Also, *Calycomyza lantanae* (Frick), which causes similar damage to *Lantana camara* L. (Verbenaceae), is heavily parasitised in South Africa (Baars and Neser 1999), so the same may be true for *C. eupatorivora*.

Melanagromyza eupatoriella Spencer (Agromyzidae)

The stem-tip mining fly *M. eupatoriella* is damaging and widespread, occurring on *C. odorata* throughout Central and South America. During numerous attempts to rear this candidate agent in quarantine, there were problems in inducing mating and oviposition, and keeping adults alive for any length of time. Following on from the successful rearing of *C. eupatorivora* in a large walk-in cage in the glasshouse, there was a recent breakthrough with the successful breeding of *M. eupatoriella* from Florida using similar techniques. However, for reasons unknown, the *M. eupatoriella* culture declined after two generations and died out in the following generation. A new culture will be imported in late 2003 for host-specificity testing, but it will be imported from Jamaica to eliminate potential host incompatibility problems. Complementary work may also be conducted simultaneously in Jamaica.

Herringbone leaf-miner (Agromyzidae)

Little is known about this leaf-mining fly which seems to be fairly widespread on *C. odorata*, having been observed in Jamaica, Cuba, the Dominican Republic, Puerto Rico and Venezuela. The damage caused is similar to that of *Ophiomyia camarae* Spencer (Agromyzidae) on *L. camara* (Baars and Neser 1999) and this species may be a congener. The larvae form a conspicuous mine along the chromolaena leaf midrib, from the petiole towards the leaf tip, with successive diagonal mines each about 3 mm in length on both sides of the midrib, giving the mine a 'herringbone' appearance. Unlike *O. camarae*, the larvae do not mine the lateral veins of the leaf, and they move between opposite leaves and sometimes to pairs of leaves adjacent along the same stem. Larvae pupate within the main vein of the leaf. Larval mining kills the midrib but does not cause leaf death or abscission, so it may have limited effect on the plant.

Polymorphomyia basilica (Tephritidae)

This stem-galling fly occupies the same niche on *C. odorata* in the northern Caribbean that *Cecidochares connexa* Macquart (Diptera: Tephritidae) occupies in South America. Due to the widespread success of *C. connexa* (McFadyen et al. 2003) as a biocontrol agent of *C. odorata* in south-east Asia, and the incompatibility of *C. connexa* with the southern African chromolaena biotype, *P. basilica* is currently of great interest to South Africa. Eggs are deposited into the internodes of young, actively growing stems and spiral-shaped galls are formed. Galls are smaller than those of *C. connexa*, with a single larva per gall. The pupal case attaches to the epidermal 'window' formed by the larva prior to pupation. Larval mortality may be experienced when galls with developing larvae are collected. However, survival is increased by collecting galls at a later stage, when pupae have formed. *P. basilica* was imported into quarantine from Jamaica in 1999 and 2002, but could not be cultured due to insufficient numbers collected.

Pareuchaetes insulata (Arctiidae)

Despite renewed efforts from 1998–2000 to release *P. pseudoinsulata* on a large scale in Limpopo province, this agent did not establish in South Africa (Strathie and Zachariades 2002). *Pareuchaetes insulata*, which had not been released as a biocontrol agent for chromolaena anywhere in the world, was collected from Fort Lauderdale, Florida, USA in late 2000. This area is climatically fairly similar to the coastal region of KZN (Parasram et al., this Proceedings). Mass-rearing of *P. insulata* was conducted at the South African Sugar Experiment Station near Durban because of their technical expertise and laboratory facilities, and the culture was reared under rigorous hygiene standards. About 8000 first to third instar larvae were produced weekly for release. More than 700,000 larvae and about 10,000 pupae and adults were released at 17 sites in KZN (Table 2) between January 2001 and April 2003, as part of the national government's alien plant clearing and poverty relief operation, a multi-departmental initiative headed by the Working for Water Program (D. Muir, unpubl. data). Figure 1 shows the distribution of release sites that were selected along the coastal belt of KZN to the north and south of Durban. Many sites were selected within conservation areas to protect the sites from clearing, spraying, or burning.

The initial release strategy was to release 20,000 *P. insulata* larvae over a period of two to three months at each of about 10 sites (Table 2). Sites were monitored for the presence or absence of *P. insulata* by beating bushes along transect lines and counting larvae that were collected in beating trays. Following this release strategy, partial defoliation occurred on plants at the release points but no sites showed signs of persistence or establishment of *P. insulata*. The strategy was thus modified to conduct larger, long-term (8–22 months) releases at only a few sites. Two sites were selected on each of the coasts to the north and south of Durban and larvae were released at each site at two-weekly intervals. *Pareuchaetes pseudoinsulata* was established in Ghana and Sumatra by releasing large numbers of larvae and/or adults over a long period at few sites (Braimah and Timbilla 2002; Timbilla and Braimah 2002; and Desmier de Chenon et al. 2002).

Higher levels of damage by *P. insulata* were observed at the four long-term release sites, with some persistence at two sites. Releases at two of the long-term release sites were only terminated in March 2003, so it is still too early to confirm establishment. Unusually dry winter conditions have hampered the establishment of *P. insulata* in South Africa, with chromolaena plants at several sites losing all their foliage and plants dying back more than usual. As a result, releases at several sites had to be terminated earlier than planned, and new sites selected.

Mass-rearing and release of *P. insulata* from Florida was terminated at the end of March 2003, after two years of releases, to ascertain whether or not the agent has established. Four adults were found at one release site (Mkuze 3) six months after the last release of larvae was conducted (Table 2). At the

Table 2: Numbers of *Pareuchaetes insulata* (Florida material) released at sites in KwaZulu-Natal province, South Africa during 2001 to 2003 (D. Muir, unpubl.). Shaded blocks indicate long-term releases (see text for details). See Figure 1 for the location of the release sites.

Site no.	Site	No. of larvae released	No. of adults released	No. of releases
1	Mkuze Reserve 1	22,450	—	4
2	Mkuze Reserve 2	—	1,013	2
4	Fanies Island	14,741	—	3
5	Hluhluwe Reserve	18,368	—	6
6	Eastern Shores, St Lucia	6,187	—	1
7	Honeymoon Bend Island, St Lucia	25,870	—	6
8	Monzi	16,059	—	3
9	Thula Thula Reserve 1	26,253	—	3
10	Thula Thula Reserve 2	—	937	3
12	Umhlanga	19,991	113	8
15	Cannonbrae 2	—	709	2
16	Vernon Crookes Reserve	18,835	—	3
*3	Mkuze Reserve 3	101,043	—	15
#11	Richards Bay	93,658	—	11
13	New Germany Reserve	—	6,291	75
#14	Cannonbrae 1	311,892	—	43
17	Mbambazi Reserve	45,910	—	13

* insects present 6 months after the last release; # releases terminated in March 2003.

time, the plants were almost depleted of foliage due to the dry conditions.

Although larger, long-term releases appear preferable, it may be too early to confirm establishment. Indications suggest that *P. insulata* from Florida will not persist; sites will continue to be monitored for several years. The reasons for these disappointing results are still uncertain. A study on the relationship between temperature and development rates, in conjunction with climatic modeling, indicates that the *P. insulata* from Florida produces a sub-optimal number of generations per year in KZN (Parasram et al., this Proceedings).

With the large numbers of insects released at some sites, it seems unlikely that predation or premature dispersal account for their non-establishment. Parasitism is also unlikely to be a significant factor. Although biotype incompatibility was initially discounted as a constraining factor, it now seems more likely as a possible explanation. *P. insulata* was consequently collected from Jamaica and Cuba in late 2002. Large numbers will be released at a few newly-selected sites for at least the next year, pending initial results. No further attempts will be made to establish *Pareuchaetes* species in South Africa should these efforts prove unsuccessful.

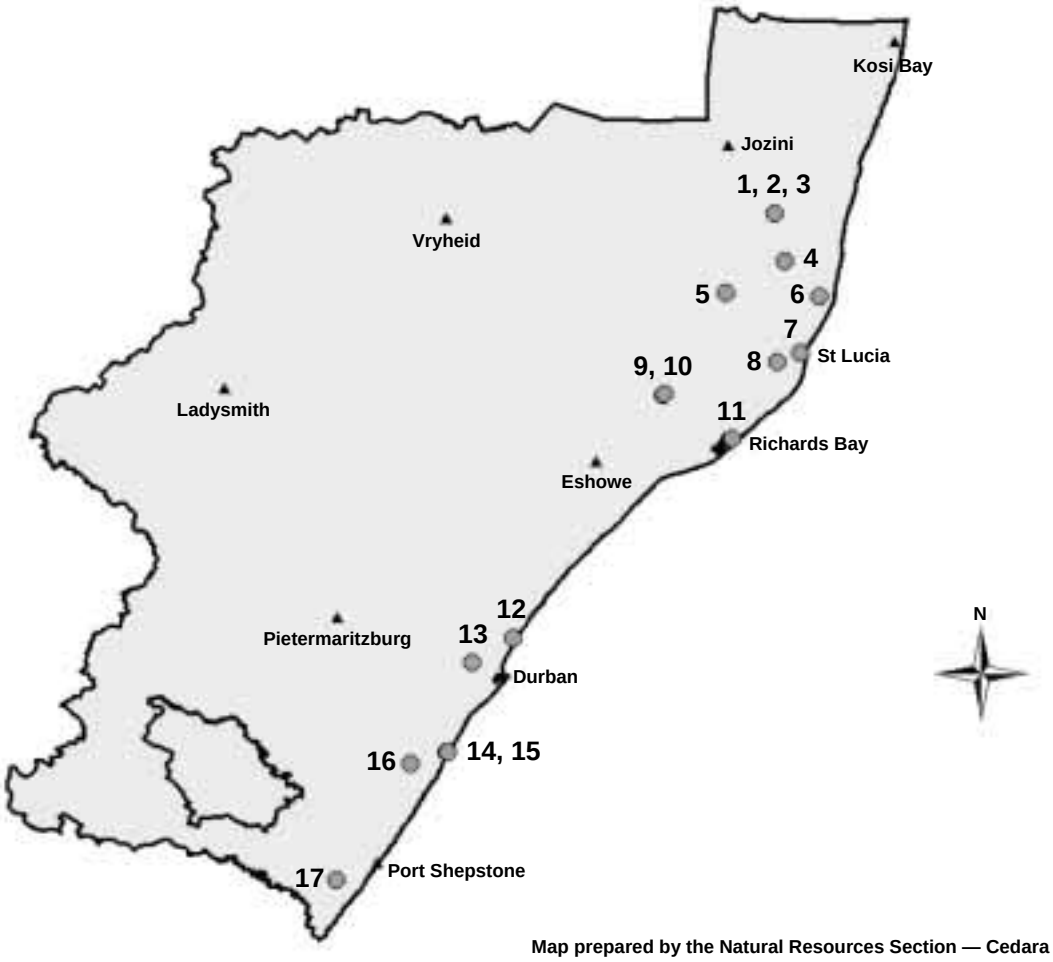

Figure 1: Distribution of sites (numbered dots) in KwaZulu-Natal province, South Africa where *Pareuchaetes insulata* (Florida material) was released during 2001 to 2003. Refer to Table 2 for further details of sites.

Mescinia sp. nr. *parvula* (Zeller) (Pyralidae)

This widespread and damaging agent (Cruttwell 1977a) has previously been imported into South African quarantine from Florida and Venezuela, but could not be induced to mate and/or oviposit (Strathie and Zachariade 2002). Numerous galled stem-tips with developing larvae were collected in Jamaica in 2002. Frass is pushed out of a hole on galls that form near shoot-tips, making the damage easily observable in the field. However, some material was lost due to a lengthy time in transit, and a culture could not be established in quarantine.

Another lepidopteran found on *C. odorata* in Jamaica and Cuba causes similar damage to that of *M.* sp. nr *parvula*. The larvae are yellow in colour, whereas those of *M.* sp. nr *parvula* are dark green. Specimens have been submitted for identification. Stem galling lepidopterans other than *M.* sp. nr *parvula* have been recorded previously (Cruttwell 1974).

Dysschema sacrifica Hübner (Arctiidae)

The defoliating moth *D. sacrifica* was imported into South Africa from Brazil in 1988 but preliminary host-specificity tests indicated that its host range was too broad for it to be considered as a potential bio-control agent for *C. odorata* (Kluge and Caldwell 1994). Larvae of this species were found in Cuba and similar larval feeding damage was observed in Jamaica in 2002.

Unidentified mites (Acari: Eriophyidae)

A variety of damage caused by unidentified eriophyid mites was observed on *C. odorata* in the field in the northern Caribbean. One species caused increased hairiness of the leaves and deformities such as leaf curling. Cruttwell (1977b) reported that heavy attacks of the eriophyid mite *Acalitus adoratus* Keifer on *C. odorata* in Trinidad causes erineum patches, stunts and distorts leaves and slows or arrests plant growth. Damage in the form of 'witches brooms' was also observed in Jamaica and is probably the result of eriophyid mites. 'Witches brooms' are compact clusters of small shoots that grow densely from a common point on a major branch; the brooms have a clumped or bush-like appearance. No further research has been conducted on these eriophyids.

Promising agents collected in South America

During earlier surveys in South America, several promising agents were introduced into quarantine in South Africa (Zachariades et al. 1999) and information on the most important of these is summarised below.

Lixus aemulus *(Coleoptera: Curculionidae)*

Lixus aemulus, a stem-boring weevil, was collected on *C. odorata* in Brazil in 1995. It was tested on 29 non-target species in choice and no-choice tests and shown to have a sufficiently narrow host range (Zachariades et al. 2002). Larvae are damaging and kill individual stems (Kluge and Zachariades, unpubl.). An application for permission to release this agent will be submitted to the regulatory authorities in 2003. *L. aemulus* was collected from a hairy form of *C. odorata* in Brazil so its compatibility with the southern African chromolaena biotype in the field is unknown, although it has been sustained on this biotype in quarantine for several years. Ichneumonid parasitoids may be a problem in the field as they have been recovered from the quarantine culture. It is likely that *L. aemulus* will have an impact on chromolaena, but it may take some time to establish due to its relatively slow development period (four months) and winter diapause within stems.

Longitarsus horni (Coleoptera*:* Chrysomelidae)

The flea beetle *Longitarsus horni* is of high priority as it is suited to areas with distinct wet and dry seasons, such as occurs in South Africa. It is also likely to be suitable for areas that are periodically burnt, as the larvae are soil dwelling. Adult feeding on leaves creates a 'shot hole' appearance, but larval feeding in the roots causes the most damage. The laboratory culture that was imported from the seasonally dry, coastal north-eastern region of Venezuela in 1999 declined and died out, but another founder culture was collected in late 2002. Because neither *L. horni* nor any equivalent root-feeding species has so far been found in Jamaica or Cuba, work will continue on the Venezuelan material.

A culture was established and biology studies are being conducted. Adults are maintained on sleeved, potted plants in the quarantine laboratory, where they feed and lay eggs, and larvae develop within the roots. Recently, adults were successfully reared by collecting eggs, hatching them on moist filter paper in Petri dishes and transferring newly-hatched larvae to the soil surface of potted plants to enable root penetration and further development. Host-specificity testing, using choice and no-choice tests, is under way.

Unidentified sp. (Coleoptera*:* Cerambycidae)

A cerambycid species was opportunistically collected on a *Chromolaena* species near Tucuman, Argentina in December 2002. Adult feeding severely

damages the stem tips. Eggs are deposited in stems, probably near the shoot-tips, or possibly in petioles. Larvae are very damaging, tunnelling down lateral branches, leaving only a paper-thin epidermal layer, into the main stem. The larvae bore down to the root crown and later return to the main stem, where they pupate. This species is probably univoltine, with larvae or pupae diapausing inside the plant over winter (D. Gandolfo, S. Neser, N. Sishuba, all pers comm.). This species is being cultured in quarantine and, pending identification, will later be subjected to biology and host-specificity studies.

Cooperative research in countries of origin

Further research on candidate agents that have been difficult to rear in quarantine, or about which little is known, is being conducted in their countries of origin. The Museo de Instituto de Zoología Agrícola Francisco Fernández Yépez (MIZA) at the Universidad Central de Venezuela, and the University of the West Indies, Jamaica have been contracted to conduct research on *C. odorata* biocontrol agents.

The stem-galling weevil *C. reticulatus* died out after two years in quarantine in South Africa. The reasons for this are unknown but it was possibly due to biotype incompatibility, as it was collected on a hairier type of *C. odorata* in Venezuela. This insect is favoured as a biocontrol agent for drier, fire-prone areas because it pupates and diapauses in the soil during the dry season. Tests conducted so far in quarantine have suggested that *C. reticulatus* is host-specific. However, fresh stocks will not be re-imported until further biology, host-range and biotype compatibility studies have been conducted in Venezuela.

The damaging stem-tip mining moth *Carmenta* sp. nov. (Lepidoptera: Sesiidae), will also be studied further in Venezuela. The species will be described and its biology elucidated. *Carmenta* sp. nov. has previously been imported into South African quarantine but was not successfully cultured, possibly due to insufficient numbers obtained. This agent is of particular interest due to the damage caused to shoot-tips, thereby stunting the growth of chromolaena. Congeneric species have been used to good effect elsewhere in weed biocontrol programs, for example *Carmenta mimosa* Eichlin and Passoa on *Mimosa pigra* L. (Mimosaceae) in Australia (Julien and Griffiths 1998).

Contract work in Jamaica will initially involve the planting of South African chromolaena plants in the field, to enable surveys and thereby determine the associated entomofauna that will be compatible with this biotype. Of the insects that have previously been found on chromolaena in Jamaica, those that are currently of particular interest are *M. eupatoriella* and *P. basilica*. Biology and host-specificity studies are to be conducted on *P. basilica* and other insect species in Jamaica once biotype compatibility has been determined.

Conclusions

It appears that the insect guilds on *C. odorata* in Jamaica and Cuba are very similar, and have distinctly different as well as overlapping species with the mainland Americas. Additional surveys of insects on *C. odorata* in Cuba are required. Species such as *M. eupatoriella* and *M.* sp. nr *parvula* are known from mainland America where they occur on the hairier form of *C. odorata*, but it is preferable to import material from the northern Caribbean that is more compatible with the southern African chromolaena biotype. The absence of certain priority groups such as *Conotrachelus*, *Lixus* and *Longitarsus* species from the northern Caribbean islands make it necessary to continue research on those species from South America. These agents are also of interest to south-east Asia and probably West Africa, as they were originally collected from the hairier biotype of *C. odorata*.

Although *P. insulata* from Florida does not seem to have established, *P. insulata* larvae from Jamaica and Cuba are being mass-reared and released as a last attempt to establish this agent in South Africa. It is hoped that these genetic stocks will be better suited to the southern African chromolaena biotype.

Contract research in some countries of origin of *C. odorata* will provide valuable information on agents that have been difficult to rear in quarantine. This is a new facet of the South African chromolaena biocontrol program. A further development is the release of *C. eupatorivora*, to be followed by the release of *L. aemulus* once permission for release has been approved. The host range of *L. horni* and *M. eupatoriella* will be investigated during the next few seasons and a combination of leaf-, stem- and root-attacking agents in the field within the next few years should provide a measure of control against *C. odorata* in South Africa.

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References

Baars, J-R. and Neser, S. 1999. Past and present initiatives on the biological control of *Lantana camara* (Verbenaceae) in South Africa. African Entomology Memoir 1: 21–33.

Braimah, H. and Timbilla, J.A. 2002. A decade of successful biological control of Siam weed, *Chromolaena odorata*, in Ghana: lessons and future plans. In: Zachariades, C., Muniappan, R. and Strathie, L.W. (eds) Proceedings of the Fifth International Workshop on Biological Control and Management of *Chromolaena odorata*, 23–25 October 2000, ARC-PPRI, Pietermaritzburg, South Africa. 58–65.

Cruttwell, R.E. 1974. Insects and mites attacking *Eupatorium odoratum* in the neotropics. 4. An annotated list of the insects and mites recorded from *Eupatorium odoratum* L., with a key to the types of damage found in Trinidad. Tech. Bull. Commonw. Inst. Biol. Control 17: 87–125.

Cruttwell, R.E. 1977a. Insects attacking *Eupatorium odoratum* L. in the Neotropics. 5. *Mescinia* sp. nr. *parvula* (Zeller). Technical Bulletin of the Commonwealth Institute of Biological Control 18: 49–58.

Cruttwell, R.E. 1977b. Insects and mites attacking *Eupatorium odoratum* L. in the Neotropics. 6. Two eriophyid mites, *Acalitus adoratus* Keifer and *Phyllocoptes cruttwellae* Keifer. Technical Bulletin of the Commonwealth Institute of Biological Control 18: 59–63.

Den Breeÿen, A. 2003. Biological control of the southern African *Chromolaena odorata* biotype using pathogens — the search continues. Poster presented at the Eleventh International Symposium on Biological Control of Weeds, Canberra, Australia, April–May 2003.

Desmier de Chenon, R., Sipayung, A. and Sudharto, P. 2002. A decade of biological control against *Chromolaena odorata* at the Indonesian Oil Palm Research Institute in Marihat. In: Zachariades, C., Muniappan, R. and Strathie, L.W. (eds) Proceedings of the Fifth International Workshop on Biological Control and Management of *Chromolaena odorata*, 23–25 October 2000, ARC-PPRI, Pietermaritzburg, South Africa. 46–52.

Julien, M.H. and Griffiths, M.W. 1998. Biological Control of Weeds: A world catalogue of agents and their target weeds. Fourth edition. CABI Publishing.

Kluge, R.L. and Caldwell, P.M. 1994. *Dysschema sacrifica* Hübner (Lep.: Arctiidae) from Brazil: a biological control candidate for *Chromolaena odorata* (L.) King R.M. and Robinson H. (Asteraceae) in South Africa. *Chromolaena odorata* Newsletter 8: 1–2.

McFadyen, R.E.C. Desmier de Chenon, R. and Sipayung, A. 2003. Biology and host specificity of the chromolaena stem gall fly *Cecidochares connexa* (Macquart) (Diptera: Tephritidae). Australian Journal of Entomology 42: 294–297.

Parasram, W.A., Byrne, M. and Zachariades, C. 2003. Climatic compatibility and selection of biocontrol agents: are *Pareuchaetes* spp. suitable for release on *Chromolaena odorata* in South Africa? Proceedings of the Sixth International Workshop on Biological Control and Management of *Chromolaena odorata*, this issue.

Strathie, L.W. 2003. Report on a field trip to Jamaica and Venezuela 30/09/2002–26/10/2002, towards the biological control of *Chromolaena odorata*. ARC-PPRI Unpublished Report, 20 pp.

Strathie, L.W. and Zachariades, C. 2002. Biological control of *Chromolaena odorata* in South Africa: developments in research and implementation. In: Zachariades, C., Muniappan, R. and Strathie, L.W. (eds) Proceedings of the Fifth International Workshop on Biological Control and Management of *Chromolaena odorata*, 23–25 October 2000, ARC-PPRI, Pietermaritzburg, South Africa. 74–79.

Timbilla, J.A. and Braimah, H. 2002. Successful biological control of *Chromolaena odorata* in Ghana: the potential for a regional programme in Africa. In: C. Zachariades, R. Muniappan and L.W. Strathie (eds) Proceedings of the Fifth International Workshop on Biological Control and Management of *Chromolaena odorata*, 23–25 October 2000, ARC-PPRI, Pietermaritzburg, South Africa. 66–70.

Von Senger, I. 2002. An assessment of the genetic diversity and origin of the invasive weed *Chromolaena odorata* (L.) King and Robinson in South Africa. MSc Thesis, Rhodes University, Grahamstown, South Africa.

Zachariades, C. 2003. Report on a field trip to the U.K., Cuba and Florida (U.S.A.) 08/09/2002–31/10/2002, towards the biocontrol of *Chromolaena odorata.* ARC-PPRI Unpublished Report, 24 pp.

Zachariades, C. and Strathie, L.W. 1999. Survey and collecting trip to Venezuela and Jamaica towards the bio-control of *Chromolaena odorata*. ARC-PPRI Unpublished Report. 23 pp.

Zachariades, C., Strathie-Korrûbel, L.W. and Kluge, R.L. 1999. The South African programme on the biological control of *Chromolaena odorata* (L.) King and Robinson using insects. African Entomology Memoir 1: 89–102.

Zachariades, C., Strathie, L.W. and Kluge, R.L. 2002. Biology, host specificity and effectiveness of insects for the biocontrol of *Chromolaena odorata* in South Africa. In: C. Zachariades, R. Muniappan and L.W. Strathie (eds) Proceedings of the Fifth International Workshop on Biological Control and Management of *Chromolaena odorata*, 23–25 October 2000, ARC-PPRI, Pietermaritzburg, South Africa. 160–166.

Zachariades, C., von Senger, I. and Barker, N.P. 2003. Evidence for a northern Caribbean origin for the southern African biotype of *Chromolaena odorata*. Proceedings of the Sixth International Workshop on Biological Control and Management of *Chromolaena odorata*, this issue.