## PRE-RELEASE RESEARCH ON BIOCONTROL AGENTS FOR CHROMOLAENA IN SOUTH AFRICA

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## ABSTRACT

With the establishment of two leaf-feeding agents on Chromolaena odorata in South Africa, some success in biocontrol of the weed has been achieved, but there is a need for additional agents. Current research is focused largely on stem-attacking insects. The two broad groups that have been targeted are (i) candidate agents which originate from the region of the Americas in which plants resembling the biotype of *C. odorata* invading southern Africa are present, and (ii) those which originate in regions which have a similar climate to the area in southern Africa invaded by C. odorata and which have a biology allowing them to withstand a prolonged dry season and/or fires. Promising agents in South African quarantine at present include a stem-tip galler, Dichrorampha n.sp. (Lepidoptera: Tortricidae), a stem-tip wilter Carmenta n.sp. (Lepidoptera: Sesiidae) and a stem borer Recchia parvula (Coleoptera: Cerambycidae). Field host-range surveys that have been carried out on three species, viz. the stem galler Conotrachelus reticulatus (Coleoptera: Curculionidae), Carmenta n.sp. and the root feeder Longitarsus sp.(Coleoptera: Chrysomelidae), and which are under way for a fourth, the stem-tip wilter Melanagromyza eupatoriella (Diptera: Agromyzidae), in the neotropics have proved valuable. Longitarsus sp. was rejected as a biocontrol agent due to an unacceptably wide host range, while the stem tip galler Mescinia n.sp (Lepidoptera: Pyralidae) and the stem galler Polymorphomyia basilica (Diptera: Tephritidae) were reduced in priority status due to rearing difficulties and the prioritization of more promising agents. Potential pathogens collected from C. odorata in

Jamaica did not perform well in quarantine, but trap plants to be planted in Jamaica may attract compatible fungi.

KEY WORDS: host biotype matching; climate matching; stem-attacking insects

## Original abstract:

Progress continues on several new species of insects and fungal pathogens for the biological control of Chromolaena odorata, both in quarantine in South Africa and in two countries in the Americas. Permission has been received by the South African Department of Agriculture to release the stem borer *Lixus aemulus* (Coleoptera: Curculionidae), but is still pending from the other relevant government authority. In quarantine, three species of Lepidoptera were successfully reared for the first time: the tip-gallers Mescinia n.sp. (Pyralidae) and Dichrorampha n.sp. (Tortricidae), both from Jamaica, and the tip-wilter Carmenta n.sp. (Sesiidae). A second generation of progeny of *Mescinia* n.sp. was not obtained. All three species are being currently described. An initial survey of the field host range of Carmenta n.sp. in Venezuela and preliminary host-range trials on *Dichrorampha* n.sp. in quarantine both indicate high specificity. Carmenta n.sp. is a promising agent because it originates from a region with a distinct dry season and diapauses in stems over winter. Dichrorampha n.sp. has a short lifecycle, and breeds easily and prolifically in cages. A culture of the damaging stem-borer Recchia parvula (Coleoptera: Cerambycidae), collected in northern Argentina in 2003, is slowly increasing in size in quarantine. A preliminary host-range trial was also promising, and because the insect is from a seasonally dry area and diapauses, it is suitable for the drier regions of southern Africa invaded by chromolaena. Several fungal isolates were collected on C. odorata in Jamaica during a field survey in 2005. Pathogenicity testing is currently being conducted and identification of any suitable fungal isolate will follow. The thirty isolates that have been tested did not cause any symptoms. There were several disappointments: the species previously identified as Longitarsus horni (Coleoptera: Alticinae) may be L. amazonas, and both field- and laboratory-based work has indicated that it is likely to have an unacceptably wide host range for use as a biocontrol agent. This is unfortunate as it remains the only root-feeding species identified for chromolaena. Secondly, the stem-galler Polymorphomyia basilica (Diptera: Tephritidae) could not be induced to breed in quarantine, even though a large number of adults were obtained and exposed to different conditions. Work on another stem-galler, Conotrachelus reticulatus (Coleoptera: Curculionidae) is being undertaken in Venezuela. This includes field host-range surveys, which so far indicate specificity to chromolaena; field biology and phenology; and host-range tests in the laboratory. This insect remains a high priority because it pupates in the soil and diapauses in the dry season, making it potentially fire-resistant. Research on the field host-range of the stem tip wilter Melanagromyza eupatoriella (Diptera: Agromyzidae) has recently started in Jamaica. Therefore within the next five years we expect to have substantially increased the number of agents released on chromolaena in South Africa, and expect to begin to see a significant measure of control of this invasive plant.

### INTRODUCTION

The South African research programme on the biological control of *Chromolaena odorata* (L.) King & Robinson was active between 1988 and 1991, and was reinstated in 1996 (Kluge, 1990, 1991; Zachariades et al., 1999). Since Strathie and Zachariades (2004) reported on progress at the Sixth International Workshop on Biological Control and Management of Chromolaena held in 2003, the project has advanced both in the establishment of agents in the field (Strathie et al., this proceedings) and in pre-release work on several potential agents. The latter work is continuing because the two agents established in the field are both leaf-feeders which have no obvious diapause period. These characteristics make it unlikely that they will be effective inland from the moist coastal belt. Among the developments in research on potential agents, one potential insect agent has been rejected, two have decreased in priority because of rearing difficulties in the laboratory, and two insects have been cultured in guarantine for the first time. This period of research was also marked by an increase in the amount of data on the host range of several insect species that was collected in the field in countries of origin. Potential biocontrol agents are still being researched both from areas in the neotropics (i) in which plants identical to the southern African C. odorata biotype have been found (Zachariades et al., 2004), viz. the islands of the northern Caribbean, particularly Jamaica, and (ii) that are predicted to be climatically similar to the region of South Africa invaded by C. odorata (northern Venezuela and north-west Argentina). No insect species with a biology that infers resistance to a seasonally dry climate and/or fire (e.g. having a diapause period or soil dwelling stages) have been discovered on C. odorata in Jamaica or Cuba (Strathie & Zachariades, 2004), and these islands are climatically quite dissimilar to South Africa (Robertson et al., in prep.).

In this paper we discuss progress on each agent that has been studied since our report (Strathie & Zachariades, 2004) at the previous workshop.

#### **SPECIES UNDER STUDY**

### Lixus aemulus Petri (Coleoptera: Curculionidae)

The larvae of this weevil are stem-borers in *C. odorata*. The insect was collected in western Brazil in 1995 and imported into quarantine in South Africa. In subsequent laboratory tests, it was shown to be both host specific (Zachariades & Strathie, 2002) and damaging (Kluge & Zachariades, 2006). The South African Department of Agriculture granted permission for its release as a biocontrol agent on *C. odorata* in South Africa in May 2006. However, before any releases of *L. aemulus* can occur, a similar permit must be obtained from the Department of Environmental Affairs and Tourism. This department has been delaying the issue of permits for other host-specific weed biocontrol agents for up to four years; therefore it is uncertain when *L. aemulus* will finally be released in South Africa. The insect is expected to do well in seasonally drier areas, but its effectiveness may be reduced by parasitism and incomplete compatibility with the southern African biotype of *C. odorata*.

#### Mescinia n.sp. (Lepidoptera: Pyralidae: Phycitinae)

#### Biology, origin, taxonomy

In Trinidad, *C. odorata* is attacked by a moth identified in Cruttwell (1977) as *Mescinia* sp. nr *parvula* (Zeller), whose larvae gall both the terminal and axillary shoots, causing cessation of growth. The moth was shown to be host specific by Cruttwell (1977), but never mated in captivity, and could thus never be used as a biocontrol agent (although a small number of field-collected adults were unsuccessfully released in Guam in the 1980s, Julien & Griffiths, 1998). A similar insect is widespread on *C. odorata* through much of the plant's native range (PPRI, unpubl. data; Cruttwell, 1977). Specimens collected in Jamaica in 1999 were identified first as *Phestinia costella* Hampson and later as *Mescinia* n.sp. nr *indecora* Dyar by Dr M.A. Solis (USDA-SEL) but appear to be the same species as that studied by Cruttwell (1977) in Trinidad (M.A. Solis, pers. comm.). The species is currently being described.

## Rearing in South African quarantine

This insect was collected in several countries during field trips between 1992 and 2003 (Zachariades et al., 1999; PPRI unpubl. data), but attempts to start a culture in the quarantine laboratory were unsuccessful. In December 2004, 29 adults were obtained from larvae collected in Jamaica. These were released into two walk-in cages (2x4x2m and 4x4x2m), one of which was

located in the glasshouse and the second, possessing a baffle, in the shadehouse. After 2 weeks, galls appeared, and thereafter about 70 F1 pupae were obtained (most from the shadehouse cage). The adults which eclosed from these pupae were released into the same cages, but no F2 galls appeared. At the time, possibilities for this failure included that either the release of too many adults into the cage together, or that pheromones from the previous generation, had interfered with mating. The presence of adverse weather conditions (e.g. low humidity) was also considered a possibility. In November 2005 the insect was again collected in Jamaica, and 22 adults were obtained and released into the walk-in cages which had previously been washed to remove any pheromones. However, no F1 galls appeared. It was thus decided not to re-import this species until its description has been completed; its conspecificity with the moth which Cruttwell (1977) showed to be host specific confirmed; and possibly more laboratory host range work conducted in Jamaica using more species of Eupatorieae. In addition, *Dichrorampha* n.sp., a tortricid moth from Jamaica whose larvae cause similar damage, is currently showing great promise and may prove a good substitute (see below).

#### Carmenta n.sp. (Lepidoptera: Sesiidae)

#### Biology, origin, taxonomy

The newly-hatched larva of this day-flying moth bores into a young *C. odorata* stem. Soon afterwards, the larva girdles the stem, thereby killing it in a similar way to the larva of the agromyzid *Melanagromyza eupatoriella* Spencer which has been long recognized as a potential biocontrol agent for *C. odorata*. The larva then bores further down the stem and pupates inside it. Before it pupates, it chews a 'window' covered only by a layer of epidermis. The insect enters diapause during the dry season, apparently often as a larva. *Carmenta* n.sp. was first collected in 1998 on *C. odorata* in northern Venezuela, where it is locally abundant; it may also occur in Panama (T. Eichlin, pers. comm.). The moth is currently being described by Dr T. Eichlin

## Potential importance for biocontrol in South Africa

Several *Carmenta* species have been used in other weed biocontrol projects to good effect (e.g. *C. ithacae* Beutenmüller on *Parthenium hysterophorus* on *Mimosa pigra*), which gives confidence in the usefulness of the genus for biocontrol. In addition, northern Venezuela has a

pronounced dry season, and climatic matching predicts that it is similar to the area in South Africa invaded by *C. odorata* (Robertson et al., in prep.). Also, the insect appears to prefer large plants in sunny, open areas for oviposition and diapauses in stems over the dry season. These characteristics should make it compatible with the drier parts of the range of *C. odorata* in southern Africa. However, because it was collected from a different form of *C. odorata* to that present in southern Africa, there could be biotype-matching problems.

#### Work in country of origin

The field host range of *Carmenta* n.sp. was investigated in November 2005 in northern Venezuela, at a site where damage levels on *C. odorata* were high. Numbers of shoot tips damaged by *Carmenta* n.sp. were counted on *C. odorata* and seven other Asteraceae species found growing at the site. If available, 30 plants per species were sampled. Shoot tips with *Carmenta* n.sp. damage were found only on *C. odorata*. No further data could be collected during a subsequent trip in August 2006 because no sites were located with both sufficient numbers of shoot tips damaged and sufficient Asteraceae species.

#### Rearing in South African quarantine

A permit was obtained for export of live insects from Venezuela for the first time in November 2005, and a large culture of larvae (n = 121) was brought into quarantine in South Africa. Although larvae transfer well into new (potted) plants, previous rearing attempts failed due to the small size of the starter culture in combination with asynchronous adult eclosion. In 2005, stems were harvested from the potted plants once eclosion windows had formed, the first of which were observed 5 weeks after field collection. More than 73 adults were obtained, most of which eclosed 7-12 weeks after field collection. A frequent problem for breeding other *Carmenta* species was the persistence of pheromones in cages. Adults of *Carmenta* n.sp. on *C. odorata* were placed in a variety of settings to induce mating and oviposition: small cages vs walk-in cages; glasshouse vs laboratory; a range of temperatures and humidities; heat lamps; confinement for mating; flowers for food. Only the later adults produced offspring (>105 F1 larvae and about 89 adults). There was no clear indication that adults placed into a walk-in cage preferred to oviposit on Venezuelan *C. odorata* plants over plants of the southern African biotype, but this was not conducted as a statistically valid trial. The generation time in the laboratory was about 2

months. An F2 generation was obtained, but diapaused as larvae over winter. Adults began eclosing from late August 2006.

The next steps are to (i) develop a host-range testing strategy and initiate tests in the quarantine laboratory. The eggs of *C. ithacae* on parthenium were easily found on leaves. A small leaf disc was cut out around the egg and glued with albumin to a leaf of control and test species. A similar method could be used here, and would constitute a larval no-choice test; (ii) determine if there are host biotype preferences for Venezuelan plants over South African plants.

## Dichrorampha n.sp. (Lepidoptera: Tortricidae)

#### Biology, origin, taxonomy

This small moth has yellow, mobile larvae which bore into vegetative and floral buds of *C*. *odorata* for up to 20mm. As a result, a small gall-like swelling is formed at the end of the stem, causing termination of growth. Little frass is exuded from the 'gall'. The mature larva exits the gall to pupate. It cuts and folds a section of leaf and creates a tight cocoon. When eclosing, the pupal skin is extruded from this leaf fold. The insect was first collected in Jamaica in 1999 in small numbers, and field damage was initially confused with *Mescinia*. It was also collected in Cuba in 2002 (Lepidoptera, unidentified sp.1, in Strathie & Zachariades, 2004). It is currently being described by Dr J. Brown (USDA-SEL). The genus is largely holarctic and feeds almost exclusively on Asteraceae.

#### Potential importance for biocontrol in South Africa

This insect is likely to complement *P. insulata* in wetter areas; its performance in drier areas will probably be limited, as there is no obvious diapause period. Because it originates in the northern Caribbean, incompatibility with the host-plant biotype in southern Africa is not expected. It is easy to rear in the laboratory and may thus prove an acceptable replacement for *Mescinia* n.sp.

#### Rearing in quarantine in South Africa

For the first time, a substantial number of larvae (n = 40) were collected in Jamaica in November 2005. Several attributes make it a seemingly excellent agent: (i) it breeds prolifically in standard,

small cages; (ii) it has a short lifecycle (6-7 weeks); (iii) larvae transfer easily between plants; (iv) preliminary host-range studies, conducted in the laboratory, are promising. Larval no-choice tests were used, in which five 5 mid-instar larvae were placed onto growing vegetative shoot tips of a control plant and five asteraceous test species at a time. Excluding *C. odorata*, a total of 10 Asteraceae, half of which were Eupatorieae, were exposed to *Dichrorampha* n.sp. The plants were kept aside until the pupation had occurred on the control, at which time the test species were examined. On only two species (*Ageratum conyzoides* L. and *Mikania capensis* DC) was any boring present, and here larval development was extremely slow; in contrast, all 10 larvae on *C. odorata* pupated. Larvae dissected out of the two species did not bore back in again. Given the biology of the insect, host-range testing should take only one to two more seasons.

## Recchia parvula (Lane) (Coleoptera: Cerambycidae)

## Biology and origin

This insect was briefly discussed in Strathie & Zachariades (2004) under 'unidentified sp. (Coleoptera: Cerambycidae)', and subsequently identified by Dr U. R. Martins (Universidade de São Paulo). Both the adults and larvae of this long-horn beetle are highly destructive. Adults feed on and girdle the shoot tips of *C. odorata*. They oviposit into the young stem, and the larva bores down the stem, expelling frass from holes in it. Stems that have been fed on by larger larvae often consist of only an epidermis filled with frass. The stem above the larva dies and usually collapses. The larva generally bores down to the root crown below the soil surface, leaving only a dead plant stump visible. The insect is apparently univoltine, as the adults eclose in spring and, in the laboratory, live for several months. The larva spends the summer feeding, and by the end of the season has killed the plant and diapauses in the stem at the soil surface until the following spring. The insect was collected in northern Argentina in 2002 and 2003, possibly from the closely related *C. jujuiensis*.

## Potential importance for biocontrol in South Africa

Climatic matching indicates that northern Argentina is climatically similar to the areas of southern Africa invaded by *C. odorata* (Robertson et al., in prep.). For this reason and because of its biology, *R. parvula* should be a good agent for seasonally drier areas. Although the

morphology of *C. odorata* in Argentina differs to the southern African biotype, *R. parvula* appears to breed successfully on the latter. Nevertheless, performance and preference tests will be conducted in the laboratory using cuttings from the original host plant in comparison to South African plants.

## Rearing and host-range testing

The insect is fairly easy to breed, although which cues break the diapause period are still uncertain. The main constraints with using this insect as a biocontrol agent are its long lifecycle, slow rate of increase and the relatively short period of the year during which adults are available for host-range trials. A preliminary host-range test using field-collected adults in a multi-choice situation was promising. Four species of Asteraceae (three Eupatorieae) as well as control plants, with their foliage touching, were exposed to the *R. parvula* adults in a walk-in cage for one month. No feeding or oviposition was recorded on any of the test species.

#### Fungal pathogens ex Jamaica

Numerous isolates from throughout the native range of *C. odorata* have been inoculated onto southern African *C. odorata* plants in quarantine by PPRI since the start of the biocontrol programme (Morris et al., 1999; den Breeÿen, 2002). None produced symptoms except for 14 isolates of the leaf-spot fungi (Hyphomycetes) *Pseudocercospora eupatorii-formosani* (Sawada) Y.M. Yen from Jamaica, Mexico, Cuba, Florida and Costa Rica; and one *Mycovellosiella perfoliata* (Ellis & Everh.) Munt.-Cvetk from Florida. Of these, three isolates of *P. eupatorii-formosani*, all from Jamaica, were found to be the most pathogenic and host-specific, but were not virulent enough for release as a biocontrol agent. The low success of the pathogen project has been attributed to poor matching between the southern African *C. odorata* biotype and highly host-specific pathogens from other morphological forms of the plant. A trip was thus undertaken in 2005 to Jamaica to collect more pathogens, during which pathogens were sampled at 31 sites across island. However, by August 2006, most of these isolates had been inoculated without any success. It is now proposed that southern African chromolaena be grown in controlled field plots in Jamaica, inter-planted with Jamaican *C. odorata* plants naturally infested with pathogens and

transplanted from the field. The South African plants would act as trap plants for biotypecompatible pathogens.

#### Longitarsus sp. (Coleoptera: Chrysomelidae: Alticinae)

Several species of flea beetles in the genus Longitarsus have been used with great success in biocontrol programmes on other weeds. Three species whose larvae that feed in the roots of C. odorata have been found in the neotropics (Zachariades et al., 1999). Such insects have been given a high priority rating for the C. odorata biocontrol programme because their soil-dwelling stages infer resistance to seasonal dryness and fires. A species of *Longitarsus* collected on C. odorata in Trinidad and north-eastern Venezuela by Cruttwell (pers. comm.) and PPRI was identified as L. horni. The insect was first cultured in 1998 in South African quarantine, but was never a particularly prolific breeder. In 2002, during a field trip in Venezuela, adults were collected off a species of Asteraceae growing near C. odorata plants. Therefore on subsequent trips in 2003 and 2004, several species of Asteraceae growing both together with and separate from C. odorata were sampled in Venezuela and Trinidad. Longitarsus beetles were found on species in both the Eupatorieae & Heliantheae. In addition, beetles from one of the species, Wedelia calycina Rich. (Heliantheae), developed on C. odorata in quarantine. A morphological and molecular study was undertaken to determine if the beetles collected off the various asteraceous species in Trinidad and Venezuela all belonged to the same species of Longitarsus. This study indicated that the insects were very morphologically variable, with some being winged and others wingless (C. Duckett, unpubl. data). Molecular work showed two groups, one composed of both winged and wingless individuals from Venezuela and the other of wingless individuals from Venezuela and Trinidad, but there were no distinct morphological characteristics consistent with phylogeny. There was also no correlation between the plant species from which the insects were collected and their morphological or molecular characters. It thus appears that all the insects from the various Asteraceae in both countries belong to one species, and that this species is not L. horni and may be an undescribed species. These results confirm that this insect is unsuitable as a biocontrol agent.

#### Polymorphomyia basilica Snow (Diptera: Tephritidae)

This stem-galling tephritid is apparently restricted to islands of the Caribbean, and has been considered a possible substitute for the stem-galling tephritid Cecidochares connexa Macquart, which has been very successful as a biocontrol agent on C. odorata in South-East Asia but does not develop on the southern African biotype of the plant. The biology of *P. basilica* is somewhat different from that of C. connexa (Strathie & Zachariades, 2004), and it may be less damaging. Stem galls containing larvae and pupae collected in Cuba and Jamaica were imported into South African quarantine on several occasions but the insect could not be cultured because very few adults were obtained, due to high parasitism rates and because pupae cannot be dissected out of their galls without damaging them (their spiracles protrude through the epidermal window they create in the gall) and undissected galls deteriorate quickly. In 2004, 32 galls were imported from Jamaica, and 16 adults were obtained due to improved laboratory techniques which allowed the galls to be maintained in a healthy state (in some cases, when collecting galls in Jamaica, they were left attached to the entire rooted plant, which was potted in quarantine; others were left attached to a length of stem which was rooted in a heated mist-bed). Flies eclosed quite synchronously and lived for 2 weeks, but no F1 galls were obtained, even though the adults were exposed to a range of conditions with respect to cage size and climate. Work on this insect has thus been discontinued.

## Conotrachelus reticulatus Champion (Coleoptera: Curculionidae)

Weevils within this genus have been used in several biocontrol programmes for other weeds (e.g. *Conotrachelus albocinereus*?? on parthenium). The adults of *C. reticulatus* are nocturnal, and the female lays an egg in a fully expanded leaf often close to the stem tip. The larva bores down the petiole into the stem, where it forms a gall in the node, resulting in stunted stem growth. It exits the gall and burrows into the ground below the plant to pupate. Because this pupation habit confers resistance to seasonal dryness and fire, this insect has been given high priority as a biocontrol agent for South Africa. The species has a wide range in South African quarantine in 1998, where host-range tests on several Asteraceae indicated high specificity. However, possible poor compatibility with the southern African *C. odorata* biotype led to the eventual loss of the

culture. UCV-MIZA in Venezuela was subsequently contracted to conduct laboratory-based hostrange tests as well as field-based biology studies on *C. reticulatus*. Also, in 2005 and 2006, researchers from PPRI and MIZA conducted field-host range surveys in Venezuela. Three sites with high *C. reticulatus* densities on *C. odorata* were selected, and the number of eggs, galls and adults on each of 20 *C. odorata* plants were counted. A total of 18 Asteraceae species (??5-10 individuals) in the vicinity of *C. odorata* at the three sites were inspected for *C. reticulatus* in the same way. Only one species, possibly in the genus *Tagetes* (the plant is being identified), had similar damage (eggs, galls). Larvae from these galls will be reared out in due course, and if they prove to be a different species, these field surveys will have provided strong evidence that *C. reticulatus* is restricted to *C. odorata*. Once the work in Venezuela is completed, and assuming it shows that the weevil is acceptably host specific, it will be re-imported into South African quarantine, initially for preference and performance testing against the southern African plant biotype compared to Venezuelan plants.

#### Melanagromyza eupatoriella (Diptera: Agromyzidae)

This damaging tip-wilting fly is widespread over the native range of *C. odorata*, and in certain areas it is very abundant. It has proved difficult to culture in quarantine. Although PPRI first cultured it in 1996 and several times thereafter, cultures have never persisted for more than two generations. It is likely to be host specific, although two species of Heliantheae with similar damage were seen in Jamaica in 2006, and agromyzid adults were subsequently reared from one (*Bidens ?alba*). A field host-range study, using both roadside surveys and fixed plots, is currently being conducted by the University of the West Indies, Jamaica.

#### Ophiomyia sp. (Diptera: Agromyzidae)

This insect was discussed as a 'herringbone leaf-miner' in Strathie & Zachariades (2004). It was subsequently identified as *Ophiomyia* sp. by M. von Tschirnhaus (University of Bielefeld) in 2005. It is similar to, but is unlikely to be, *O. chondrillae* Spencer because the latter was collected (Washington State, USA) far from the edge of *C. odorata*'s range, from a stem of the asteraceous *Chondrilla juncea* L. (Spencer & Steyskal, 1986). It is thus probably a new species,

and was discussed in Martinez et al. (1993). It is not being considered as a biocontrol agent because it appears to cause little damage and because there are already two leaf-feeding insects established as biocontrol agents of *C. odorata* in South Africa (Strathie et al., this proceedings).

### CONCLUSIONS

Several insect species, most of which attack different parts of the stem, remain promising as biocontrol agents for *C. odorata. Lixus aemulus* has been cleared for release by the Department of Agriculture but remains in quarantine pending clearance from the Department of Environmental Affairs and Tourism. *Dichrorampha* n.sp., *Carmenta* n.sp. and *R. parvula* are currently being cultured in quarantine in South Africa, and in some cases preliminary host-range trials have been conducted. Research conducted by contracted universities and/or PPRI in Jamaica, Venezuela and Trinidad on field host ranges of *C. reticulatus, Carmenta* n.sp., *Longitarsus* sp. and *M. eupatoriella* have proved valuable. They have indicated that *Longitarsus* sp. has too broad a host range to be suitable as a biocontrol agent, and that *C. reticulatus* and *Carmenta* n.sp. are likely to be restricted to *C. odorata*. Host-range work on *M. eupatoriella* has begun too recently to draw conclusions on its likely host range.

There remains a dichotomy between (i) insects from the islands of the northern Caribbean, from where the biotype of *C. odorata* invading southern Africa appears to originate, in that the climate of these islands is not particularly similar to that of southern Africa, and none of the agents found so far has a biology with a distinct diapause period or soil-dwelling stage which make it better adapted to a seasonally dry, fire-prone area; (ii) insects from areas of the neotropics which are climatically similar to southern Africa, including north-west Argentina and northern Venezuela, where insects with a distinct diapause period are present, but where the morphology of *C. odorata* differs substantially from that of the southern African biotype. However, this problem may have been overstated, in that some insect species from the Caribbean islands may be quite adaptable climatically, and some biocontrol candidates from the South American mainland may have a host-range broad enough to perform equally on the southern African biotype in the field. In any case, the insects from South America that are being studied are all useful to the biocontrol programme against *C. odorata* in the drier parts of South East Asia, where *Pareuchaetes* 

*pseudoinsulata* Rego Barros (Arctiidae) and *C. connexa* have been less effective. Because the single biotype of *C. odorata* that has invaded Asia and West Africa is much more similar to the common morphological form that is present in South America, it is unlikely that there will be host-plant/agent compatibility problems for these agents on this biotype.

Apart from suspected biotype problems, some agents have simply proved very difficult to rear in the laboratory for logistical reasons, e.g. they have complex mating behaviour that is reliant on cues not present in the laboratory; they are choosy in terms of the quality of oviposition sites; they require actively growing tissue which is not always available in the laboratory; they require high humidity, which is not always available, etc. A new quarantine facility, planned for the PPRI laboratory at which research on insects for the biocontrol of *C. odorata*, and due to be completed in 2007, will provide better environmental conditions and thus alleviate some of these problems. Up till now, the contracts with institutions in Jamaica and Venezuela have been of most value in terms of facilitating the logistics of research in countries of origin, in terms of obtaining permission and assistance in collecting and exporting agents, collecting field data, and allowing the importation of southern African biotype plants.

Unlike in South-East Asia, where *C. odorata* has been brought under a good measure of control fairly quickly and cheaply though the use of *C. connexa* and to a lesser extent, *P. pseudoinsulata*, the South African programme has proved lengthy and expensive. However, with the currently established agents and some of those described in this paper, it is likely that adequate control will be achieved in South Africa as well.

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