Abstract

Populations of the leaf-feeding moth *Pareuchaetes insulata* from Florida (USA), Jamaica and Cuba were mass-reared by the South African Sugarcane Research Institute (SASRI) and released extensively in KwaZulu-Natal (KZN) province of South Africa from 2001-2009 for biological control of the invasive shrub *Chromolaena odorata*. Establishment was first confirmed in 2004, at only a single site, near Umkomaas just south of Durban, at which the Florida population had been last released nearly two years earlier. A population outbreak and range expansion occurred during 2006, followed by a decline to a much lower level. Annual monitoring from 2006-2013, along a 100 km coastal stretch and slightly inland from the original established site, indicated gradual further spread within this range with time, although populations were generally low. In 2014 and 2015, additional populations and outbreaks were recorded further north in KZN, well beyond the area that had been regularly monitored, in a region that had been previously considered to be climatically unsuitable for the moth. In late 2015 and early 2016, surveys located the moth even further afield, in Swaziland and south-eastern Mpumalanga, having probably spread from KZN. To better understand the highly variable establishment and performance of *P. insulata*, various aspects of the moth’s biology have been examined. The cross-breeding and molecular analysis of the Cuban, Jamaican and Floridian populations indicated the lack of a mating barrier between them. Studies on the moth’s performance on *C. odorata* from South Africa and Florida showed that it is not affected by variation in host plant genotype. However, studies on the nutritional ecology and thermal physiology of *P. insulata* demonstrated that multiple factors, including low temperatures and spatio-temporal variations in the physical and phytochemical characteristics of *C. odorata* leaves, may cause variable performance of the moth. This paper discusses the results of monitoring and research activities on the moth, which has been established on *C. odorata* in
South Africa for more than a decade, and the implications of these for the biocontrol of the weed in southern Africa.

**Keywords:** agent dispersal, biocontrol, mass-rearing, South Africa, Swaziland, trifid weed

**Introduction**

*Chromolaena odorata* (L) RM King and H Rob (Asteraceae) has a large native distribution, from southern USA to northern Argentina, and first became invasive in Asia in the 19th century. From there it was taken to West Africa in the 1930s (Zachariades *et al.*, 2009). It became one of the most problematic invasive alien plants in the subtropical eastern areas of South Africa during the final decades of the 20th century. The plant was first recorded as naturalised near Durban in 1947, and by the turn of the century was present throughout the warmer parts of KwaZulu-Natal (KZN), and invading the Lowveld of Mpumalanga and Limpopo provinces as well as Swaziland and southern Mozambique (Goodall and Erasmus, 1996; Zachariades *et al.*, 2011). Although its worst impacts were on native biodiversity, *C. odorata* also affected grazing lands, silviculture and to some extent cropping agriculture. Given its rapid growth rate and the production of large numbers of easily-dispersed seeds, *C. odorata* is difficult to control. Although individual plants are easily killed by herbicide and fire, and their shallow root systems facilitate mechanical removal, the logistics and costs involved in large-scale mechanical and chemical control operations were prohibitive. Biological control involving the deployment of introduced natural enemies was thus considered as a more sustainable solution. The aim of this contribution is to review biocontrol activities against *C. odorata*, notably those involving species of *Pareuchaetes* (Lepidoptera: Erebidae).

**Biological control**

The scale of the problem in Asia and West Africa prompted a survey of the weed’s natural enemies in countries within its native range, mainly Trinidad, in the 1960s and 1970s, with a view to introducing suitable insects as biological control agents. This survey was conducted by the Commonwealth Institute of Biological Control (now called CABI) with funding from the Nigerian Institute for Oil Palm Research (see Zachariades *et al.*, 2009 and references therein). One of these, the leaf-feeding moth *Pareuchaetes pseudoinsulata* Rego Barros (Lepidoptera: Erebidae), was finally established in Sri Lanka in the 1970s, and thereafter in several other countries, although with variable impact (Zachariades *et al.*, 2009). A number of other suitable candidate agents were also identified during field surveys and subsequent assessments.

With this in mind, South Africa initiated a biocontrol programme against *C. odorata* in the late 1980s (Zachariades *et al.*, 1999, 2011). A culture of *P. pseudoinsulata* was obtained from Guam (USA), tested in quarantine at ARC-PPRI Cedara for host specificity, and released in fairly low numbers at several sites in the Durban area in 1989. The moth failed to establish, with ant predation on egg batches thought to be a possible barrier (Kluge, 1994). In 1989-1990, a second species of *Pareuchaetes*, *P. aurata aurata* (Butler) was collected from the closely related *Chromolaena hookeriana* (Griseb.) RM King and H Rob in Argentina, and imported into South African quarantine. This species was considered possibly superior to *P. pseudoinsulata* because it scatters its eggs singly on the ground, whereas *P. pseudoinsulata* lays them in batches on the underside of *C. odorata* leaves – the former behaviour was thought to be advantageous in reducing ant predation. Also, the climate of north-western Argentina was better matched to that of South Africa than Trinidad. Again host-specificity trials were
undertaken (Kluge and Caldwell, 1993a) and, following government permission for release, *P. aurata aurata* was transferred to the insectary at the South African Sugarcane Research Institute (SASRI) for mass-rearing. Between 1990 and 1993, some 150,000 insects were released at multiple sites in KZN and the Eastern Cape province, but again no establishment was recorded. This time, disease was implicated as a possible cause of the moth’s failure to establish (Zachariades et al., 1999).

A final attempt was made with a third *Pareuchaetes* species, *P. insulata* (Walker) (Figure 1), which also fed on *C. odorata*, but has a native range stretching around the Gulf of Mexico, from Florida through to Central America and on the Caribbean islands (Cock and Holloway, 1982). The use of a culture from Florida was considered desirable because it originated from continental America, and was thus thought to be less susceptible to ant predation than *P. pseudoinsulata* with an insular origin (islands often have lower ant diversity) (Zachariades et al., 2011). Also, the Florida climate was a closer match with South Africa than that of Trinidad (confirmed by Parasram, 2003). A culture was imported from Florida in the early 1990s and host-range testing indicated that *P. insulata* was safe for release (Kluge and Caldwell, 1993b). However, for several reasons, the moth was not released at the time and the culture was terminated. A release application was, however, applied for and obtained in 1997, using Kluge and Caldwell’s data.

In the early years of the South African biocontrol programme, it was recognised that the *C. odorata* invading southern Africa was substantially different in morphology (pubescence, odour and flower colour) from that invading other areas in the Old World. Furthermore, no plants identical to the southern African form could be found during exploratory trips in the Americas. Potential biocontrol agents therefore had to be collected from other forms of the plant. This was problematic because some natural enemies (both insects and fungal pathogens) were so specific that they did not develop well on the southern African *C. odorata* during attempts to culture them in quarantine in South Africa. Finding the origin of this local form of *C. odorata* thus became a major focus of the project, in order to locate natural enemies that were better matched to the host plant. Eventually, Jamaica or Cuba was shown, by several means, to be the most likely origin of the *C. odorata* invasion in southern Africa (Zachariades et al., 2011; Paterson and Zachariades, 2013).

Following major successes with *P. pseudoinsulata* in Ghana and Sumatra in the 1990s (Zachariades et al., 2009), a second attempt to establish *P. pseudoinsulata* was made in South Africa. One of the apparent keys to success of the Ghanaian programme was the repeated release of large numbers of insects over an extended period of time. It was believed that this overcame Allee effects caused by predation and dispersal (Stephens et al., 1999), and this methodology was therefore replicated in South Africa. Mass-rearing and releases were conducted in the Tzaneen area of Limpopo province, by the *Working for Water* (WfW) programme, then under the Department of Water Affairs. Despite initial promising signs of persistence, the population disappeared after the first winter, and no insects were found subsequently (Strathie and Zachariades, 2002).
Figure 1. Lifecycle of *Pareuchaetes insulata*, with typical damage caused by individual larvae. Clockwise from top left: (a) adult moth, (b) egg batch, (c) young larva with damage, (d) mature larva, (e) typical damage caused by older larva, (f) pupa. A description of the lifecycle can be found in Kluge and Caldwell (1993b) and Uyi *et al.* (2014c). Photographs (a), (b), (d), (e), (f): ARC-PPRI, (c): Sandile Gumedze, Swaziland National Trust Commission.
Releases of *Pareuchaetes insulata*

In the absence of other immediately promising agents (Zachariades et al., 1999, 2011) and as the origin of the southern African biotype was still unknown at the time, it was again considered worthwhile attempting to establish *P. insulata*, but using large releases over a longer time period. The only organisation that was able to undertake such large-scale mass rearing of healthy insects was SASRI, due to its considerable entomological experience with intensive rearing of natural enemies for the biocontrol of sugarcane pests. A culture of *P. insulata* was thus imported from Florida in late 2000, transferred to SASRI, and mass-rearing was initiated with funding from WfW (Walton and Conlong, 2003; Muir and Conlong, 2003). Releases began early in 2001 (Figure 2), and were restricted to KZN; they were scheduled initially for two years, i.e. until early 2003. Around 781,000 insects (mainly larvae) were released on *C. odorata* infestations at 17 sites throughout KZN province. Release numbers per site varied from about 5,000 to 335,000, and the duration of releases from once-off to 21.5 months (Zachariades et al., 2011). The site that received the largest number of larvae, released over the longest period of time, was in the Sappi Cannonbrae plantation near Umkomaas, south of Durban (30.22060°S, 30.78132°E) (hereafter referred to as Cannonbrae). Regular monitoring of release sites (every three months for two years after the last release) suggested that initial persistence of *P. insulata* was poor at most sites. Although there was initial feeding damage to the leaves, and at some sites adults were caught using light traps, it appeared that the insect, once again, was unable to establish.

The main reasons for the failure of weed biocontrol agents to establish are incompatibility between the climates of the regions of origin and introduction; incompatibility between the agent and its host plant; and factors such as predation, parasitism, disease and dispersal of agents. All these, apart from a mismatch between agent and host plant, should have been overcome by using a population of *P. insulata* from Florida, and releasing large numbers over a long period at a particular site. By 2002, there was already reliable anecdotal evidence that the southern African *C. odorata* originated from Cuba or Jamaica. Therefore, following the perceived failure of the Floridian *P. insulata* to establish, additional cultures of this species were collected in both Cuba and Jamaica in late 2002. Releases were initiated in early 2003, and the Florida culture was soon terminated to enable greater focus on the Cuban and Jamaican cultures. Some 590,000 individuals, mostly larvae, from Jamaica and 327,000 from Cuba were released at seven and five sites respectively, which were mostly different to those at which the Florida culture had been released (Zachariades et al., 2011). Initial persistence seemed better at some sites than achieved with the Floridian culture, with greater damage recorded. However, yet again, the populations appeared to become extinct soon after releases were stopped at a given site, and especially after the winter. Releases of the Jamaican culture continued until 2008 and the Cuban culture until 2006. Climatic matching between Cuba and Jamaica on the one hand and South Africa on the other was low (Robertson et al., 2008), which may have played a role in this apparent failure. Once an established population of *P. insulata* was located at the Cannonbrae site (see below), it was field-collected and mass-reared at SASRI. About 220,000 insects were released at four additional sites between 2006 and 2009 (Zachariades et al., 2011). This was done because it was thought that field-established material would be better adapted to South African conditions. However, as before, these releases did not result in any obvious establishment.
Figure 2. Sites in South Africa at which *Pareuchaetes insulata* from Florida, Jamaica and Cuba (not differentiated in map) was released between 2001 and 2009 (N=31) (blue dots), and sites at which it has been recovered through intensive monitoring efforts or *ad hoc* surveys (N=45) (yellow dots). Cannonbrae plantation, the only original release site where establishment is known to have occurred, is indicated by the red arrow. The distribution of *Chromolaena odorata* in South Africa and Swaziland is shown in the inset map (from L Henderson, ARC-PPRI, SAPIA database, 2016).
Initial establishment and spread of *Pareuchaetes insulata*

In December 2004, nearly two years after the final release, a small outbreak of *P. insulata* larvae was unexpectedly found about 500 m from the Cannonbrae release site. Such an outbreak had never before been observed in South Africa, and was strong evidence that establishment had occurred at this site. Cannonbrae was characterised by a diverse habitat with non-sandy soils, and dense, continuous stands of *C. odorata* which remained in good condition year-round, some growing in ‘conservation units’ either in forested riverine areas or in the open, and others under eucalyptus plantation. This *P. insulata* population was observed throughout 2005, and it became increasingly abundant throughout the plantation and surrounds up to about a 1 km radius from the release site. By the end of summer in April 2006, it constituted a large outbreak, with extensive areas of defoliated *C. odorata* plants (some of which died), hundreds of adult moths around light sources, and larvae starving on plants that had been entirely defoliated due to the excessive numbers. Any remaining foliage on *C. odorata* plants typically turned yellow, due to a chemical defence stimulated by the saliva of the larvae (Figure 3), as is the case with *P. pseudoinsulata* (Marutani and Munaiappan, 1991; Raman et al., 2006). Visible die-back of *C. odorata* stands surrounding trees in unsampled areas of Cannonbrae occurred during this outbreak (Figure 3). The population then declined rapidly (‘crashed’) during the winter of 2006 (Zachariades et al., 2011 and references therein). This population pattern had been recorded in several other countries for *P. pseudoinsulata* (Zachariades et al., 2009).

Regular annual monitoring of the population was then undertaken, both at Cannonbrae (which is still ongoing) and at 5 km intervals from Cannonbrae, along the coast in both directions and inland for 15 km (which continued until 2013).

**Methodology**

*Monitoring at Cannonbrae*

In 2002 and 2003, monitoring was conducted 2-3 times per annum, and from 2004 until the present time, on an annual basis at the end of the growing season, during April, May or June. The following were recorded: at the two original release points (one in the shade of the plantation, the other in full sun about 25 m across the road) in Cannonbrae, and then at 5 m intervals from them up to 30 m, along plantation roads, the *C. odorata* plants were classified into categories (by obtaining the consensus of three people) for height, density and the percentage of feeding damage caused to the leaves by *P. insulata* larvae, visually quantified. Plants were then beaten over two canvas beating trays with a total surface area of 0.7 m², and any larvae collected in them were counted. Beyond 30 m from the release points, the same procedure was followed at 20 m intervals up to 90 m, and then at 50 m intervals up to a maximum distance of 700 m, along plantation roads, with two additional short (15-25 m) cross-transects into the vegetation away from the roads. Initially 22 points were sampled, and from 2005 on, once the outbreak had occurred, 54 points. This was supplemented by the use of four light traps (Figure 4) placed at regular intervals along the transects and set from dusk until about 22h00. The number of *P. insulata* adults caught in each trap was recorded before dismantling the traps, and the moths were released after capture.
Figure 3. Damage caused to Chromolaena odorata by an outbreak of Pareuchaetes insulata in April 2006 in the Cannonbrae plantation, Umkomaas. (a) C. odorata plants which have been defoliated and died as a result, visible as grey patches around forest patches. (b) A C. odorata bush heavily fed on by P. insulata larvae, where the leaves have turned yellow as a result.

Photographs: ARC-PPRI.
Figure 4. (a) Purpose-built light trap used to non-destructively sample *Pareuchaetes insulata* adults. Moths are attracted to the light at night and land on the outside of the gauze netting material of the trap (b), as well as crawling inside through the gap at the base. Lights use two standard fluorescent tubes and are connected to a deep-cycle battery. Multiple traps were erected before dusk and moths counted between 22h00 and 23h00, before release, on each sampling occasion. Photographs: ARC-PPRI.
Spread from Cannonbrae

From Cannonbrae, which was denoted as point zero, a GPS was used to determine about 5 km intervals between sampling points along a transect travelled by vehicle from Cannonbrae, along secondary roads, in a north-easterly (up the coast), south-westerly (down the coast) and westerly (inland) direction, in as straight a line as possible. A substantial infestation of *C. odorata*, as close to the 5 km point as possible, was selected. At each point sampled, the infestation was examined by three people for 15 minutes, amounting to a 45 minute survey duration, up to a distance of at most 1 km, for signs of *P. insulata* damage. The amount of damage was then scored from 0-4, with 0=no damage and 4=complete defoliation. Visual searches for insects were also made, particularly where damage was present. The number of larvae and adult moths, if encountered, were recorded. This procedure was repeated at the same sites on an annual basis from 2006-2013. Initially 18 sites were examined, up to a maximum of 30 km up the coast towards Durban, 25 km down the coast towards Port Shepstone, and 15 km inland towards Dududu. Where two consecutive sites in the same direction lacked any signs of *P. insulata*, the assumption was made that the moth had not spread that far from Cannonbrae, and the transect was terminated. By 2013, surveying was done at 34 sites up to a maximum of 66 km up the coast through Durban, 59 km down the coast to Port Shepstone, and 15 km inland towards Dududu.

Results and Discussion

Monitoring at Cannonbrae

The population of insects at Cannonbrae has fluctuated over the years (2006-2015) but there has always been some damage present, and at least a few adults and/or larvae recorded, except in December 2003 when no insects were recovered (ARC-PPRI, unpublished data). In April 2006, 727 adults (N=4 light traps) and 207 larvae (N=54 sampling sites) were collected. Some parts of the plantation are clearly more favourable for the insect, with more insects and more damage consistently recorded from year to year. These areas may have been slightly warmer, as indicated by iButton data recorded in 2013-2014 (Uyi, 2014). Population levels have never again reached the high levels recorded in 2006. On some monitoring occasions, a larger number of larvae (24 larvae and only 18 adults in May 2015) have been recorded, with few adults; on others, the opposite (207 adults and no larvae in May 2013); and on other occasions, very few of either. However, once-off annual monitoring only reflects a short window-period of recent damage to plants and numbers of larvae (the past few weeks), and an even shorter one for adults: depending on factors such as night temperature, the phase of the moon, the amount of wind, in addition to the intrinsic adult population status. Many insect species have a seasonal peak in population size in late summer (e.g. Nzama *et al*., 2014), and the rationale for monitoring *P. insulata* at Cannonbrae in late summer each year was to obtain data from peak seasonal populations. However, this assumption was never tested by quantitative sampling at different times of the year.

Interestingly, the population of *C. odorata* has declined markedly since 2006, up to a point where it is no longer the dominant weed in the plantation (ARC-PPRI, unpublished data). Before the initial outbreak in 2006, many areas outside of the eucalyptus compartments (the ‘conservation units’) were covered in dense, monospecific *C. odorata* stands about 2 m in height. The 2006 outbreak of *P. insulata* lowered the height and density of many of these stands, and although to a large extent they recovered for a few years, they have in recent years decreased again (ARC-PPRI, unpublished data). On average, the *C. odorata* density has halved.
and the height has decreased by 30%, with disappearance of the plant from 20% of the sampling points (ARC-PPRI, unpublished data). The decrease in *C. odorata* during the initial *P. insulata* outbreak allowed other species of plants, both indigenous and weedy, to gain a foothold. This has been a continuing trend over the years, with each successive smaller outbreak of *P. insulata* contributing further to decreases in *C. odorata* stand density and height. Trees and shrubs which gained a foothold in previous years are now large and shading out *C. odorata*, which cannot tolerate shade. In its native range, *C. odorata* acts as a pioneer species, densely colonising disturbed land before being found and attacked by its natural enemies, declining and disappearing as a result (Zachariades et al., 2009). Although there was no control area to compare this decline to, in countries where *P. pseudoinsulata* has established, the density of *C. odorata* has, in general, declined similarly from around 80% to 30% cover (Braimah et al., 2013, REC McFadyen, personal communication).

**Spread from Cannonbrae**

Several patterns emerged from the surveys of spread conducted from 2006 to 2013 (Figure 2). Firstly, from early on, *P. insulata* was found quite far (30.40778°S, 30.63783°E and 30.03123°S, 30.90697°E) from Cannonbrae, indicating dispersal over a total of nearly 50 km. It was surmised that the population outbreak had prompted large-scale dispersal from the site, as is sometimes the case for other insect species (see Denno and Peterson, 1995). After this initial post-outbreak dispersal, the moth appeared to disperse more slowly. Secondly, the moth’s distribution was patchy; at some sites it was never found, and at others it was consistently found in relatively high numbers. This may indicate that some habitats were more suitable or that the moth simply did not reach other monitored sites during the initial dispersal event. The moth was only found on one occasion at the 15 km inland site (30.19487°S, 30.62522°E), probably due to the increasing altitude and seasonality of the sites inland – on the KZN South Coast, *C. odorata* itself is highly invasive only up to about 15 km inland from the coast. By 2013, the maximum distance along the coast that *P. insulata* or its damage had been found were 50 km down the coast (30.61580°S, 30.53808°E) and 56 km up the coast (29.76358°S, 31.03313°E) from Cannonbrae. It was noted that one of the active Jamaican release sites (Finningley Estates, 30.24747°S, 30.77748°E) was well within the range of this dispersal and that Florida material from Cannonbrae and Jamaican material from Finningley Estates may have interbred; there are no mating barriers between the two populations (Dube et al., 2014). Towards the end of the survey period, populations to the south of Cannonbrae appeared to decline and, at some sites, disappear, possibly due to a series of cooler winters and summers. A correlation between climate and population density will be explored in future.

**Widespread presence of Pareuchaetes insulata**

In February 2013, damage caused by a late-instar *P. insulata* larva was found at the release site for another *C. odorata* biocontrol agent (the stem-boring weevil *Lixus aemulus* Petri (Coleoptera: Curculionidae)) near Empangeni (28.68217°S, 31.96528°E) on the KZN North Coast. This was about 150 km to the north of the most northerly established population known at this time. Hence, a decision was made to suspend the annual monitoring of spread described above as the moth had clearly expanded its range well beyond the monitored transect stretch. In May 2014, two major outbreaks of *P. insulata* were found near Phinda Private Game Reserve and outside Mkuze town, the first along an earthen farm-dam wall (27.91325°S, 32.29594°E), and the second along a tributary of the Mkuze river (27.60239°S, 31.99594°E). In both cases, hundreds of *P. insulata* larvae and adult moths were present and complete defoliation of plants occurred (Strathie and Zachariades, 2014). In October 2014, some damage was noted next to Pongolapoort dam (27.36848°S, 31.85543°E), and between November 2014 and June 2015,
widespread and frequent damage was recorded at several additional sites throughout northern KZN (e.g. at Maphelane, 28.52539°S, 32.35906°E; Monzi, 28.44042°S, 32.29161°E; Hluhluwe, 28.01347°S, 32.24822°E), while monitoring other biocontrol agents on C. odorata (Figure 2). In November 2015, damage and/or larvae were found at four sites in Swaziland (two in the north-east, at 26.17869°S, 32.02697°E and 26.17689°S, 32.04069°E; and two in Big Bend, at 26.79083°S, 31.94586°E and 26.78722°S, 31.93753°E), and in February 2016, at two sites in the Komatiport area of Mpumalanga (25.54053°S, 31.94836°E and 25.44608°S, 31.96214°E). These findings were surprising, because it had earlier been predicted that, being a leaf-feeding insect with no obvious diapause period, P. insulata would not establish inland in areas which become very dry in winter, as C. odorata loses condition and drops its leaves in such conditions (Zachariades et al., 2011). However, in most cases P. insulata was found only on C. odorata growing close to perennial water bodies; it appears that the moth is able to survive in these damper microhabitats where the plant remains in good condition year-round.

A similar situation was observed in Goiânia state, Brazil, in September 2013, before the start of the rainy season. The surrounding land was extremely dry, with most C. odorata plants having no leaves, but in a few places next to water, plants had fresh leaves and high levels of Pareuchaetes damage (probably P. aurata). Riverine habitats were also reported as preferential areas for P. pseudoinsulata (Cock and Holloway, 1982).

The question arises as to whether all the populations found from Empangeni northwards between 2013 and 2016 originate from a single established site at Cannonbrae, or whether establishment did indeed occur at one or more of the release sites in northern KZN. No releases were conducted in Swaziland, with only one small release in Mpumalanga, so the populations there spread from KZN. Systematic monitoring for P. insulata in northern KZN was terminated in 2006, and was never conducted in Swaziland or Mpumalanga. It is thus uncertain how long the insect has been present at these sites. It is also unknown as to how much further the insect may have spread already. It may well be present in Mozambique, and possibly further north and west in Mpumalanga and even in Limpopo province, although surveys at 10 sites around Thohoyandou and Tzaneen in late May 2016 located no larval damage. Further surveys will assist in determining this. Pareuchaetes pseudoinsulata has shown similar strong dispersal abilities. In West Africa it has spread from Ghana to Nigeria over, at most, 20 years (Uyi et al., 2011), while in South-East Asia it has spread from island to island (e.g. from Malaysia into the Philippines) (Waterhouse, 1994). It is thus quite possible that the only established site for P. insulata in South Africa was at Cannonbrae. If molecular techniques can be developed to determine whether the populations in the northern parts of the range of P. insulata contain markers unique to Cuban or Jamaican populations, this would indicate that Cannonbrae was not the only site that supported establishment. It would also eliminate the unlikely possibility that some of these insects are P. pseudoinsulata or P. aurata arising from earlier attempts at establishing these species.

Studies on Pareuchaetes insulata in South Africa

Several complementary studies have been conducted on P. insulata in South Africa, which add to our understanding of the insect. Concern about how the Floridian, Jamaican and Cuban populations would interact in the field, should more than one become established, led to molecular characterisation of the three populations (using mitochondrial DNA) and to interbreeding trials in the laboratory between Floridian and Jamaican insects. No genetic structure and no interbreeding depression was found, indicating that there should be no breeding barriers in the field, and that moths from different populations encountering one another would form a single population (Dube et al., 2014), for better or worse.
Given the initial concern of possible incompatibility between *P. insulata* from Florida and the *C. odorata* invading southern Africa, laboratory trials comparing the performance of the established *P. insulata* (largely or all Floridian in origin) on South African and Floridian *C. odorata* found no differences (Uyi et al., 2014a). A preliminary trial did imply that the larvae may still prefer *C. odorata* from Florida. Laboratory and field trials (Uyi et al., 2015, 2016) on the nutritional ecology of *P. insulata* indicated that the insect performs better on plants growing in the shade and with leaf nitrogen content of around 4%. Larvae also performed better on plants in late summer than in winter after they had flowered (Uyi, 2014). Such studies go some way to explaining distribution patterns in field populations. Finally, thermal physiology studies looking at developmental rates of the insect at different temperatures, as well as lower and upper lethal temperatures and locomotory studies (Uyi, 2014; Uyi et al., 2014b) have given an indication that low temperatures in the cooler parts of the southern African range of *C. odorata* may prevent the insect from establishing there, or render it marginal habitat for the insect. Conversely, this may explain why the insect initially dispersed slowly, in the relatively cool area around Umkomaas, but may now be dispersing more rapidly in warmer areas further north.

**Implications for biological control of *Chromolaena odorata***

In comparison with other biocontrol agents and target weeds, it has proven atypically difficult and expensive to establish a *Pareuchaetes* species as a biocontrol agent of *C. odorata* in South Africa. Certainly, without the considerable support from government funding and extensive mass-rearing by SASRI to produce large numbers of larvae for releases, this agent would probably not have established at all. The initial outbreak in 2006 was seen as a promising start to the biocontrol of *C. odorata*, but the subsequent performance of the insect in the field seemed disappointing. Since at least 2014, however, *P. insulata* appears to have been performing well again, with an increasing distribution in the invaded range of South Africa and neighbouring countries, and some major outbreaks. Although these outbreaks have been mainly in moister microhabitats (in drier areas), this may have a disproportionately positive effect in controlling the weed. This is because during periods of drought, *C. odorata* only persists in these moist microhabitats, and the plants here act as a seed source when rainfall returns to normal. By targeting plants in these moist areas, biocontrol agents can prevent or reduce the re-infestation of the areas further from water bodies.

Anecdotally, *C. odorata* has become less prevalent and invasive in coastal areas of KZN in recent years. While chemical and manual clearing programmes have certainly played an important role here, it is quite possible that biocontrol, particularly by *P. insulata*, may have contributed in some areas (REC McFadyen, personal communication), as many invaded areas were not intensively monitored for establishment or impact of the moth. In most countries in which a *Pareuchaetes* species has been established, anecdotal records indicate a reduction in *C. odorata* from 80% to 30% cover. Showing this experimentally is very difficult, however, because the unpredictable population dynamics of these species makes it difficult to select long-term monitoring sites.

It is unlikely that *P. insulata* will provide adequate control of *C. odorata* in all areas. A number of other insect species have been studied for their potential as biocontrol agents in South Africa (Zachariades et al., 2011). Releases of four agents have been carried out, of which one (the leaf-mining fly *Calycomyza eupatorivora* Spencer (Diptera: Agromyzidae)) has established, one (*L. aemulus*) has probably established, and current attempts are under way to establish another two (the shoot tip-boring moth *Dichrorampha odorata* Brown and Zachariades
(Lepidoptera: Tortricidae) and the stem- and crown-boring longhorn beetle *Recchia parvula* (Lane) (Coleoptera: Cerambycidae)). Another agent, *Polymorphomyia basilica* Snow (Diptera: Tephritidae), a fly which galls the stems of *C. odorata*, is currently being tested for host specificity in the quarantine laboratories of ARC-PPRI Cedara. Current and future prospects for the significant contribution of biological control, by *P. insulata* and additional agents, to the management of *C. odorata* in southern Africa are therefore considerable.

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