INVESTIGATION OF THE BIOLOGY AND CROSS-BREEDING OF POPULATIONS OF *PAREUCHAETES INSULATA* (LEPIDOPTERA: ARCTIIDAE) AND THE IMPLICATIONS FOR THE BIOLOGICAL CONTROL OF *CHROMOLAENA ODORATA* (ASTERACEAE) IN SOUTH AFRICA

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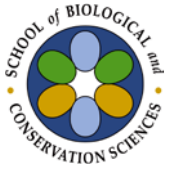

PREFACE

The experimental work described in this dissertation was carried out at the South African Sugarcane Research Institute and Agricultural Research Council Plant - Protection Research Institute with the School of Biological and Conservation Sciences, University of KwaZulu-Natal Pietermaritzburg, from April 2006-December 2008, under the supervision of Dr Terry Olckers and co-supervision of Dr Costas Zachariades.

This dissertation, submitted for the degree of Master of Science in the Faculty of Science and Agriculture, University of KwaZulu-Natal, Pietermaritzburg, represent original work by the author and have not otherwise been submitted in any form for any degree or diploma to any University. Where there is use of work done by others, text duly acknowledges that use.

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ABSTRACT

Larvae of *Pareuchaetes insulata* were released in South Africa for the biological control of the invasive weed *Chromolaena odorata*. *Pareuchaetes insulata* has proved to be a difficult agent to establish in the field in South Africa, for various possible reasons. Populations collected from Florida and Jamaica (their aboriginal home) were released separately at several sites each in South Africa, but only one population (Florida) was definitely established. It is possible that adults from this established population interbred with adults from the Jamaican population released at nearby sites.

The aims of this study were to determine whether there were any differences in biology between the two populations and whether hybridization affected the fitness of either. Trials involved: (i) pure-breeding of both Florida (F) and Jamaica (J) populations; (ii) cross-breeding of the two populations and; (iii) back-crossing of the hybrids with the parent populations. The fitness of these populations was determined by measuring adult longevity and fecundity, egg viability, and larval development and survival rates.

The F population was superior to the J population in most of parameters measured, including fecundity. Hybridization of these populations reduced the fitness of the F population. It is unknown whether these differences in fitness reflect differences in their native regions, laboratory cultures or response to South African *C. odorata*. It appears that different populations of *P. insulata* have different levels of fitness, and that hybridization negatively affects the fitness of stronger populations. The lower fitness of the J population may have reduced its likelihood of establishing successfully, and even reduced the fitness of the established F population where the populations came into contact. These results caution that the possible consequences of mixing different

genotypes of a biocontrol agent species should be properly investigated prior to their release in the same country.

Keywords: *Pareuchaetes insulata*, establishment, cross-breeding, back-crossing, biological control, *Chromolaena odorata*

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CONTENTS

ABBREVIATIONS/ACRONYMS

ARC-PPRI	Agricultural Research Council – Plant Protection Research Institute
SASRI	South African Sugarcane Research Institute
F	Florida
J	Jamaica
WfW	Working for Water
FJ	Cross of Florida male and Jamaica female
JF	Cross of Jamaica male and Florida female
FJ♂XF♀	FJ hybrid male mated with Florida female
FJ♀XF♂	FJ hybrid female mated with Florida male
FJ♂XJ♀	FJ hybrid male mated with Jamaica female
FJ♀X J♂	FJ hybrid female mated with Jamaica male
JF♂XF♀	JF hybrid male mated with Florida female
JF♀XF♂	JF hybrid female mated with Florida male
JF♂XJ♀	JF hybrid male mated with Jamaica female
JF♀XJ♂	JF hybrid female mated with Jamaica male

CHAPTER 1: GENERAL INTRODUCTION

1.1 Invasive alien plants in South Africa

Exotic plants have been introduced into South Africa since the mid 1600s when sailors to and from the Spice Islands used the Cape of Good Hope as a transit point (Wells et al., 1986; Zimmermann et al., 2004). Since then, South Africa has become a suitable arena for the establishment of alien plants from many countries, particularly Australia and the Americas, with some having been deliberately introduced as ornamentals and as crops, or accidentally as contaminants of agricultural produce (Mack, 1995; Zimmermann et al., 2004). Many of these alien species became invasive in South Africa, probably because they were introduced without any of their own natural enemies. Another reason is the absence of other regulatory factors such as unfavourable soil and climatic conditions that were present in their native region (Mack, 1995).

Currently, there are some 200 invasive alien plants that are subject to legislation in South Africa, including trees and shrubs, grasses and reeds, climbers, terrestrial herbs and aquatics (Henderson, 2001). These alien plants displace indigenous vegetation or planted crops and pose threats to the biodiversity of natural environments, agriculture, human health, water supplies and the economy of South Africa (Olckers *et al.*, 1998; Moran *et al.*, 2005) (Henderson and Wells, 1986; Geldenhuys *et al.*, 1986; Klein 2002a). Among the invasive alien plants present in South Africa, *Chromolaena odorata* (L.) King and Robinson (Asteraceae: Eupatorieae) (previously *Eupatorium odoratum* L.) is rated as one of the most problematic and was hence targeted for a number of control measures, including chemical, mechanical and biological control (Holm *et al.*, 1977; Goodall and Erasmus, 1996; Zachariades *et al.*, 1999). In this chapter, I provide the background to this

weed, focusing on its ecology and impact in South Africa, the methods of control and aspects relating to the biological control programme that was launched. The aims of this study are highlighted at the end.

1.2 *Chromolaena odorata* in South Africa

Chromolaena odorata (chromolaena), also known as Siam weed, triffid weed, paraffienbos or isandanezwa originates from the Americas, occurring from the southern USA to northern Argentina, and on the Caribbean islands (Erasmus, 1988; Kluge, 1990). *Chromolaena odorata* is also one of the most invasive alien species in the humid tropics and subtropics of the Old World and is highly invasive in several parts of Africa, Southeast Asia and Oceania (Holm *et al.*, 1977; Kluge and Caldwell, 1993a; Zachariades and Strathie, 2006). The morphologically distinct form of *C. odorata* invading southern Africa is a biotype different from other forms of *C. odorata* and is probably from one of the Caribbean islands (Timbilla *et al.*, 2003; Zachariades *et al.*, 2004; Kriticos *et al.*, 2005). Initially, it was thought that *C. odorata* was introduced into South Africa, in KwaZulu-Natal province (KZN), during the 1940s (Zachariades *et al.*, 1999). However, Zachariades *et al.* (2004) argued that its abundance throughout KZN at that time suggested that *C. odorata* might have been introduced earlier than was assumed. In addition, the plant was recorded growing in the Cape Town Botanical Garden in the mid 1800s, indicating that it was introduced into South Africa at least a century before it was recorded as being naturalised (Zachariades *et al.*, 2004). Because of its copious seed production and high growth rate, the weed has spread within South Africa along the KZN coastal belt and occurs from the Transkei region of the Eastern Cape to as far north as Kosi Bay in northern KZN and near Tzaneen in Limpopo Province. It is now considered to be one of the worst invasive alien plants in the subtropical eastern parts of southern

Africa including Swaziland, southern Mozambique and probably Zimbabwe (Liggitt, 1983; Kluge, 1990; Kluge and Caldwell, 1993a; Zachariades *et al.*, 1999). The presence of the other major invasive form of *C. odorata*, referred to as the Asian/West African biotype, has recently been confirmed for Tanzania and may be present in Northern Mozambique (Zachariades *et al*., 2009).

1.2.1 Description of *Chromolaena odorata*

Several published and electronic sources provide a description of the plant (Holm *et al.*, 1977; Liggitt, 1983; McFadyen, 1989; McFadyen and Skarratt, 1996; Zachariades *et al.*, 1999; ISSG, 2006). *Chromolaena odorata* belongs to the family Asteraceae and typically forms dense tangled bushes 1.5-2.0m in height (McFadyen, 1989, 1991; McFadyen and Skarratt, 1996; ISSG, 2006). However, under favourable conditions the weed can reach a height of up to 3m in one season and occasionally reaches a maximum height of 6-7m (Holm *et al.*, 1977; Liggitt, 1983; McFadyen and Skarratt, 1996; ISSG, 2006). The plant stems branch freely, with lateral branches developing in pairs from the axillary buds (McFadyen, 1989). The older stems are brown and woody near the base while the shoot tips and young shoots are green and succulent (McFadyen, 1988; McFadyen and Skarratt, 1996; ISSG, 2006). The root system is fibrous and does not penetrate beyond 20-30cm in most soils (McFadyen, 1988, 1989). Terminal corymbs of 20-30 heads bear the flowerheads on all stems and branches; the flowers are white or pale-bluish lilac (Holm *et al.*, 1977; McFadyen and Skarratt, 1996) and form masses covering the whole surface of the bush for a short period during the dry season (McFadyen, 1988; ISSG, 2006). The southern African biotype is characterized by white flowers, rather glabrous, bright green stems and leaves, and has a distinctive smell when the leaves are bruised (Zachariades *et al.*, 1999).

1.2.2 Ecology of *Chomolaena odorata*

Studies conducted on the ecology of *C. odorata* show that its ecology is similar in both native and introduced ranges (McFadyen, 1991). The morphology, physiology, biochemistry and seed production of *C. odorata* can differ between even close localities because of differences in soil moisture, relative humidity, temperature, sunlight (Ambika, 1998) and precipitation (Muniappan and Marutani, 1988). In its region of origin, *C. odorata* is not a serious weed, probably due to competition with many other former *Eupatorium* species and to attack by natural enemies, including insects and diseases absent in areas it invades (McFadyen, 1989, 1991). *Chromolaena odorata* flowers can be produced over a wide range of day-length conditions, but a shorter day length (about 10 hours) expedites flowering in both native and introduced range (Liggitt, 1983). In Trinidad (northern hemisphere), flowering occurs from late December until the end of March (McFadyen, 1991), whereas the main flowering period in KZN (southern hemisphere) takes place from June to December with a peak in July and August (Liggitt, 1983). *Chromolaena odorata* grows in many soil types but the plant has a short lifespan in poor soils with frequent waterlogging, hence it prefers well-drained soils (McFadyen, 1988, 1989; Goodall and Erasmus, 1996). It grows in a wide range of vegetation types such as forests, grassland and bushveld (Goodall and Erasmus, 1996). In arid areas the plant is restricted to riverbanks and will only become invasive in the frost-free areas of mesic to dry bushveld and woodland, which are not water-stressed in the growing season (Erasmus, 1988). *Chromolaena odorata* grows well at a relative humidity of 60-70% and at levels higher than 80% has poor growth performance (Kluge, 1990; ISSG, 2006). It does not tolerate shade (McFadyen, 1988) and thrives well in open areas (Kluge, 1990; McFadyen, 1991). The weed takes advantage of the flush of soil nitrogen that becomes available after disturbances like fire or land clearing for agriculture and exhibits relatively

high foliar nitrogen, phosphorus and potassium contents (ISSG, 2006). Chemicals with allelopathic properties produced by *C. odorata* have been shown to prevent the germination of adjacent plants (Holm *et al.*, 1977; Waterhouse, 1994).

In its invasive range, *C. odorata* often produces a phenomenal number of seeds that are dispersed easily and rapidly (McFadyen 1989; Goodall & Erasmus, 1996). These seeds have tiny barbs that cause them to adhere to clothes, fur, feathers and other objects, especially when these are wet (Waterhouse, 1994; ISSG, 2006). Seeds can also be transported with personal belongings or trade goods during movement of people through continents (ISSG, 2006). In South Africa, *C. odorata* may have been spread in the same way or along railways and roads, which may have provided reservoirs of seeds for infesting the surrounding countryside (von Senger *et al*., 2002). After flowering, *C. odorata* plants die back depending on the severity of the dry season. At the beginning of the rainy season, plants regenerate rapidly either from seed or from existing plants (Holm *et al*., 1977; McFadyen, 1991); vegetative growth continues throughout the rainy season.

1.2.3 Harmful impacts of *Chomolaena odorata*

In the Old World, *C. odorata* forms dense stands that damage and prevent the establishment of native species and have allelopathic effects (Sahid and Sugau, 1993) that further reduce biodiversity and the carrying capacity of native ecosystems (McFadyen, 1989; Kluge, 1990; Luwum, 2002). Leslie and Spotila (2000) showed that in KZN, Lake St. Lucia's nesting Nile crocodiles *Crocodylus niloticus* Laurenti (Reptilia: Crocodilidae) require open sunny, sandy areas in which to deposit their eggs. *Chromolaena odorata* plants overrunning the nesting sites created fibrous root mats unsuitable for egg chamber and nest construction. Shading by *C. odorata* led to a female-biased sex ratio and the

sites were abandoned (Leslie and Spotila, 2000). Whether dry or green, *C. odorata* provides a high fuel load because of the essential oils it contains (Liggitt, 1983). Wild bushfires in vegetation dominated by *C. odorata* kill native plant species (Goodall and Erasmus, 1996), particularly since fires fuelled by this weed are hotter than normal. *Chromolaena odorata* forms a higher fuel load than the vegetation it replaces (Cock and Holloway, 1982; Luwum, 2002; Klein, 2002b; ISSG, 2006). Fires caused by *C. odorata* can also lead to soil erosion because hotter-than-normal fires caused by this weed sterilise the soil and kill the roots that keep the soil particles together (Klein, 2002b). In South Africa, *C. odorata* also displaces grassland which is used as pasture for domestic livestock of small scale and commercial farmers (Zachariades and Goodall, 2002). The weed has economic implications in South Africa in that it reduces tourism potential by obstructing game and bird viewing in recreation areas and also invades sugarcane cultivations (Goodall and Erasmus 1996; Luwum, 2002; Klein, 2002b). Due to the harmful impacts of *C. odorata* already discussed, it is one of the worst invasive alien plants and unwanted in subtropical regions of this country (Kluge and Caldwell, 1993a; Zachariades *et al.*, 1999). As such, *C. odorata* is legislated as a Category 1 plant or declared weed by the Conservation of Agricultural Resources Act in South Africa, which means it is no longer tolerated, neither in rural nor in urban areas (Klein, 2002c).

1.2.4 Control measures for *Chromolaena odorata*

Different methods have been used to control *C. odorata* (Goodall and Erasmus, 1996; Luwum, 2002; Klein 2002b). Several foliar- and stump-treatment herbicides (Goodall and Erasmus, 1996) were tested for the control of *C. odorata* in South Africa and for some, application in summer resulted in 90% weed reduction. However, registration of herbicides for specific weeds is compulsory in South Africa, and many of these

herbicides were not registered for *C. odorata*. In addition, some of these herbicides were restricted internationally and were therefore not considered for use; others were not sufficiently effective or damaged plantation trees and crops (Goodall and Erasmus, 1996). Herbicides including tebuthiuron, glyphosate and triclopyr are registered for *C. odorata* and are effective at recommended concentrations (Goodall and Erasmus, 1996). Although chemical control of *C. odorata* is effective, the rapid growth rate and the spread of the plant make it very difficult to control chemically in the long term and over the large areas of often low-value or inaccessible land that the weed invades (Goodall and Erasmus, 1996; Zachariades *et al.*, 1999).

Mechanical control that involves manual slashing with brush cutters, mattocks, hoes or tractor-drawn implements was also applied to control *C. odorata* (Goodall and Erasmus, 1996). However, slashing causes regeneration and therefore needs to be followed by chemical control to be effective, manual weeding is labour intensive, and the use of tractor-drawn equipment is limited to accessible areas (Goodall and Erasmus, 1996; Field, 1991). Mechanical methods may also lead to soil disturbance and erosion, require repeated follow-up operations and may damage untargeted species that are mistakenly cleared in dense infestations of the weed (Luwum, 2002). Use of fire in grassland and savanna is an effective tool (Goodall, 2000).

Classical biological control restores some of the biotic constraints that exotic plant species experience in their home range, by deliberately importing and releasing natural enemies, notably plant-feeding insects and pathogens, which comprise biological control agents (Mack, 1995; Zimmermann *et al.*, 2004). In addition, biological control is the only viable method of control when large areas are invaded and repetitious chemical or

mechanical control becomes prohibitively expensive (Seibert, 1989; Mack, 1995). *Chromolaena odorata* was considered a good target for biocontrol in South Africa because there were plenty of potential agents available, it was morphologically homogenous throughout its southern African invasive range, no conflict of interest existed and it had susceptible stages in its biology (Kluge, 1990).

1.3 Biological control of *Chromolaena odorata* in South Africa

Research into the biological control of *C. odorata* was initiated by the Commonwealth Institute for Biological Control (CIBC) and Nigerian Institute for Oil Palm Research during the 1960s, with a survey of the phytophagous insects on *C. odorata* conducted largely in Trinidad (Timbilla *et al*., 2003). Host-range testing of selected insects was also undertaken at this time (Kluge, 1990; Zachariades *et al.*, 1999). In 1988, the Plant Protection Research Institute of the Agricultural Research Council (ARC-PPRI) initiated the biological control programme against *C. odorata* in South Africa (Zachariades *et al*., 1999; Timbilla *et al*., 2003). Since then, many candidate agents have been introduced into quarantine in South Africa, and some tested for host specificity. These include *Dysschema sacrifica* Hübner (Lepidoptera: Arctiidae) (Strathie and Zachariades, 2004), *Actinote anteas* (Doubleday and Hewitson) (Kluge, 1991; Kluge & Caldwell, 1996) and *Actinote thalia pyrrha* (Fabricius) (Lepidoptera: Nymphalidae) (Strathie and Zachariades, 2002; Li *et al*., 2004), all rejected due to lack of host specificity (Kluge and Caldwell, 1996; Zachariades *et al.*, 1999). Four species, the fly *Calycomyza eupatorivora* Spencer (Diptera: Agromyzidae) (Strathie and Zachariades, 2004) and three moths in the genus *Pareuchaetes*, namely *P. pseudoinsulata* Rego Barros, *P. aurata aurata* Butler and *P. insulata* (Walker) (Lepidoptera: Arctiidae) were all released in South Africa, but only *C.*

eupatorivora and *P. insulata* became established (Zachariades *et al.*, 1999; Zachariades and Strathie, 2006).

1.3.1 Release history of *Pareuchaetes pseudoinsulata* and *Pareuchaetes aurata aurata*

Pareuchaetes pseudoinsulata has been used in several countries as a biocontrol agent of *C. odorata*; it established in some countries but failed in others (Seibert, 1989; Waterhouse, 1994; Singh, 1998). *Pareuchaetes pseudoinsulata* established and controlled *C. odorata* to varying degrees in parts of Asia and the Pacific, with the highest level of control reported in Guam (Timbilla *et al*., 2003). In Sri Lanka, larvae of *P. pseudoinsulata* caused extensive defoliation and provided partial control of *C. odorata* (Kluge, 1990; Waterhouse, 1994). *Pareuchaetes pseudoinsulata* was tested for host specificity in South Africa and was released in 1988/9 in small numbers at different sites around greater Durban but failed to establish (Kluge and Caldwell, 1993b; Kluge, 1994). As in West Africa and India, the failure of *P. pseudoinsulata* in South Africa was attributed to predation by ants (Kluge, 1994). In 1998 much larger releases of *P. pseudoinsulata* were made in Limpopo Province but again, no establishment was achieved (Strathie and Zachariades, 2002). Poor climatic matching between Trinidad and South Africa may also have contributed to the failure of this agent (Zachariades *et al.*, 1999; Parasram, 2003).

The failure of *P. pseudoinsulata* to establish is not entirely surprising, given that an agent can be successful in one country but fail in another. Hoffmann (1995) supported this by indicating that, in assessing the success of a biological control agent, problems in its establishment could arise because performance could vary from country to country through ecological effects, intra-taxonomic variation e.g. *Dactylopius* species on South

African *Opuntia* species (Volchansky *et al*., 1999) or attack by natural enemies e.g. *P. pseudoinsulata* preyed on by ants (Waterhouse, 1994).

In 1989, a 'new-association' agent, *P. aurata aurata*, normally associated with *Chromolaena hookeriana* (Griseb.) King and Robinson (Asteraceae) (King and Robinson, 1987) was tested for host specificity and released in South Africa but also failed to establish (Kluge and Caldwell, 1993a; Zachariades *et al.*, 1999). One or more factors may have been responsible for this failure, including biotype and climatic incompatibility, predation, microsporidian diseases (Kluge and Caldwell, 1993a; Kluge, 1994; Zachariades *et al.*, 1999) and Allee effects (Zachariades and Strathie, 2006).

1.3.2 *Pareuchaetes insulata*

In addition to the other two *Pareuchaetes* species released, *P. insulata*, a moth with defoliating larvae and a distribution through much of Central America and the Caribbean (Cock and Holloway, 1982) was selected as an agent because it was believed that its continental origin might confer anti-predation abilities (Kluge and Caldwell, 1993b) and a culture from Florida was selected because climatic matching showed a reasonable compatibility between KwaZulu-Natal and southern Florida (Parasram, 2003; Byrne *et al.*, 2003; Zachariades and Strathie, 2006). On the other hand, the morphological form of *C. odorata* in Florida differs from the biotype invading southern Africa. *Pareuchaetes insulata* was tested for host specificity in the early 1990s and was found to be safe for release in South Africa (Kluge and Caldwell, 1993b). Permission for its release was granted and a culture was re-collected from southern Florida in December 2000. Following mass rearing by the South African Sugarcane Research Institute (SASRI), releases commenced early in 2001. Rearing of the insects by SASRI was implemented to

eliminate to a large extent the disease factor that might have been a reason why the other *Pareuchaetes* species failed to establish in South Africa (Strathie and Zachariades, 2002; Zachariades and Strathie, 2006).

Over a 2-year period, about 830,000 insects, mainly larvae, were released into the field at 17 sites in KZN (Strathie and Zachariades, 2002). Numbers of insects released at each site varied, as did the duration of releases and habitats in which releases were made. Cannonbrae plantation (Sappi Forests) near Umkomaas (30^{o} 13.236' S, 30^{o} 46.392' E) received by far the most insects (387,862), released over a 21-month period (Strathie and Zachariades, 2002). At all sites, persistence of the insects after releases as well as dispersal from the release sites was poor and therefore releases were terminated in March 2003. The Cannonbrae site was the only one where insects could still be found 6 months later, and even here, numbers persisting were extremely low. Most of the factors (see below) usually invoked to explain establishment failures had apparently been ruled out or at least minimized (Zachariades and Strathie, 2006). Climatic matching showed reasonable compatibility between KwaZulu-Natal and southern Florida (Parasram, 2003). The SASRI mass-rearing facilities had high standards of hygiene; hence, disease should not have contributed to the apparent failure of *P. insulata* (Walton, 2003). The large numbers of insects released should have compensated for any mortality caused by resident predators and parasitoids, leaving sufficient *P. insulata* adults to establish a population. Because of the large releases, dispersal of adults was also unlikely to be responsible for the failure of the agent to establish (Zachariades and Strathie, 2006). It seemed that only host/agent biotype incompatibility remained as an explanation for failure (Zachariades and Strathie, 2006).

In November 2002, separate cultures of *P. insulata* were introduced from Cuba and Jamaica, where plants identical to the southern African biotype are common (Zachariades and Strathie, 2006). Given that Cuba, Jamaica and Florida are isolated from one another geographically, are different climatically and have different morphological forms of *C. odorata*, it was hypothesized that insects from one or both of these islands would differ from the Florida population enough and might establish more readily on the weed in South Africa (Zachariades and Strathie, 2006). The Cuban culture was collected from a drier site than the Jamaican culture, but no climatic data are available. These populations were mass-reared by SASRI and in 2003, each was initially released at two sites in KZN (Zachariades and Strathie, 2006).

Compared with the release sites chosen in KZN for the Florida population, at one each of the release sites for the Cuban (Hluhluwe: 28^{o} 07.135' S, 32 ^{o} 66.308' E) and Jamaican (Umdoni: 30^{o} 23.057' S, 30^{o} 40.248' E) populations, initial results were promising. From smaller numbers released, damage seemed proportionally more, persisted longer after releases had been terminated, and was recorded further from the release point (Zachariades and Strathie, 2006). However, after the first winter the Cuban insects were not recovered. This may have been because the site was seasonally too dry for even the Cuban population. The Jamaican insects persisted for several years after releases ended, but were recovered in extremely low numbers (Zachariades and Strathie, 2006). This appeared to be following a pattern similar to that of the Cannonbrae (Florida) site (Zachariades and Strathie, 2006). The Umdoni site was destroyed following clearing activities in 2007.

In December 2004 a large outbreak of larvae was found 1km from the Cannonbrae release site, in a stand of young *C. odorata* plants (Zachariades and Strathie, 2006). This was a surprising find, as it was thought that the small numbers of *P. insulata* found on previous surveys indicated that the species was only persisting rather than establishing, and would eventually disappear from the site completely. It is likely that local adaptation of *P. insulata* occurred (Hufbauer, 2002; Hufbauer and Roderick, 2005; Goolsby *et al.*, 2006). Over the 2004/5 summers, numbers of larvae increased over an area of at least 4km^2 around the release site, and by autumn and winter 2005 (April-July) of *P. insulata* was found in good numbers at and around the release site, and patches of defoliated *C. odorata* were evident. *Pareuchaetes insulata* numbers continued to increase and the insect increased its range following good rains in the 2005/6 summers (Zachariades and Strathie, 2006). By April 2006, large areas of *C. odorata* in the nearby Mahlongwana valley had been defoliated and were dying and of *P. insulata* was distributed more than 20km along the coast on either side of the release site as well as 10km inland (Zachariades and Strathie, 2006). During the 2006/7and 2007/8 summers, numbers of larvae were much lower; therefore it appears that *P. insulata*, like *P. pseudoinsulata*, is an 'outbreak species' that sporadically achieves high population densities.

A site at Finningley Estates (30^{o}14.506' S, 30^o 46.392' E), only 2km from the Florida population at the Cannonbrae release site, received a large number of larvae from the Jamaican population. The Jamaican population at the Umdoni release site fell within the 20km radius to which the Florida population from Cannonbrae was found to have spread in April 2006. Hence, it is possible that adults from the Jamaica and Florida populations interbred in the field (Zachariades and Strathie, 2006). Because different genetic populations (biotypes or strains) of a weed biocontrol agent species have differed in their

ability to establish (Hoffmann *et al*., 2002), with some biotypes more suited than others, interbreeding between *P. insulata* populations from Jamaica and Florida may have negatively affected the established population. The possible existence of different biotypes of *P. insulata*, that respond differently to the South African biotype of *C. odorata*, and the implications of their crossbreeding in the field was thus a major focus of this study.

1.4 Cross-breeding of *Pareuchaetes insulata* populations

Cross-breeding or hybridisation is defined as the natural or experimental mating of individuals from different races or populations which have different adaptive norms (Gardener, 1968; Anderson and Stebbins, 1979). Cross-breeding experiments often include back-crossing, which is the cross of a hybrid to one of the parental types, with the offspring referred to as back-cross progeny (Gardener, 1968). Cross-breeding and back-crossing have been conducted in many living organisms, including plants, animals, insects, nematodes and mites, for very different purposes. As early as 1865, Gregor Mendel conducted cross-breeding experiments to obtain new variations in colour using peas, *Pisum* species (L.) (Henig, 2000). His results showed that the hybrids were not exactly intermediate between the parental varieties. Characteristics of the true parents only appeared in some of back-cross progeny (Henig, 2000). This is true of many hybridization events, highlighting the importance of back-crossing to produce an F2 generation when making an assessment of the effects of hybridization on populations (Hoffmann *et al*., 2002). In some vertebrates, cross-breeding can result in sterile offspring; for example, cross-breeding donkeys and horses resulted in sterile mule (Gardener, 1968).

Diamondback moth (DBM) *Plutella xylostella* (L.) (Lepidoptera: Yponomeutidae), the most important insect pest of crucifers, was found to be resistant to organophosphate insecticides and insect growth regulators were developed (Kobayashi *et al.*, 1990). To determine the mode of inheritance of resistance to insect growth regulators, cross-breeding and back-crossing experiments were conducted, using resistant and susceptible strains of DBM (Kobayashi *et al.*, 1990). In another example, to determine the diversity and distribution of entomopathogenic nematodes (EPNs) in Turkey, samples of Steinernematidae and Heterorhabditidae, two entomopathogenic nematode families, were collected from the field. Because nematodes of both families were found co-occurring and were indistinguishable, to confirm the identity of the species of *Steinernema* Travassos (Rhabditidae: Steinernematidae), *Steinernema* isolates that were collected with *Heterorhabditis* Poinar (Rhabditidae: Heterorhabditidae) were cross-bred with known *Steinernema* (Hazir *et al*., 2003).

Weed biological control programmes have also conducted cross-breeding experiments on agents, for various purposes. These include the production of hybrid biotypes that are better adapted than the parents to the target weed or environmental conditions in the introduced country (Volchansky *et al.*, 1999). Determination of the genetic basis of host specificity has also been assessed via hybridisation (Hoffmann *et al*., 2002). Another application is determining whether it is good practice to release more than one population of an agent and whether hybridization will impact on its efficacy (Hoffmann, 2003; this dissertation). In insect biological control, Assefa *et al.* (2006) mated pure strains of the sugarcane pest *Eldana saccharina* Walker (Lepidoptera: Pyralidae) from South Africa and Kenya. Resultant hybrids were later back-crossed with the South African parent

population to determine the genetic differences and fertility of crosses of the two populations, assuming that there were cryptic species within *E. saccharina*.

In weed biological control involving *Opuntia* species, cochineal insects (*Dactylopius* spp.) have been used worldwide (Hoffmann *et al.*, 2002). In South Africa, *Opuntia ficus-indica* (L.) Miller and *Opuntia stricta* Haworth (Cactaceae) have occurred in close proximity for years; however, *Dactylopius opuntiae* Cockerell (Homoptera: Dactylopiidae), which successfully controlled *O. ficus-indica*, failed to control *O. stricta* (Volchansky *et al.*, 1999). This problem was resolved by finding that there are different biotypes of *D. opuntiae* that are host specific to either *O. stricta* or *O. ficus-indica* (Volchansky *et al.*, 1999). Also, the cross-breeding of such biotypes may further negatively influence the success of their establishment and impact on the target weed. Hoffmann *et al.* (2002) conducted cross-breeding experiments on *D. opuntiae* from *O. stricta* and *O. ficus-indica*, to determine whether the two taxa might be considered separate genetic species and investigate the viability and host-preferences of progeny produced by crosses. Firstly pure strains were cross-bred, and to determine the viability of the F1 (hybrid) progeny, F2 crosses (back-crossing) were conducted.

During cross-breeding trials involving *Pareuchaetes* species from Central America, *P. insulata* populations collected from Yucatan in Mexico and Guanacaste in Costa Rica were cross-bred with each other and with *P. pseudoinsulata* from Trinidad to determine if *P. insulata* and *P. pseudoinsulata* are distinct species separated by a reproductive barrier (Cock and Holloway, 1982). Crosses of *P. insulata* from Yucatan and Guanacaste produced normal offspring which were taken through F2 and F3 generations, while crosses between the Trinidad *P. pseudoinsulata* males with *P. insulata* females from the

two Central American (Guanacaste and Yucatan) populations produced both fertile and sterile eggs, those that hatched developed and pupated normally. The Guanacaste x Trinidad cross produced normal adult progeny while the Yucatan x Trinidad cross produced weak adult progeny, with some of them dying within 24 hours of emergence (Cock and Holloway, 1982). These results implied that cross-breeding of different agent species within the same genus are undesirable as such crosses may produce unviable progeny and hence reduce the fitness of the stronger agent.

1.5 Aims and objectives of the study:

There are several questions that have arisen from the work undertaken so far on *P. insulata* and thus several aspects that require research. The Florida population of *P. insulata* has established in South Africa while there has been no confirmation of establishment of the Jamaica population. The latter is still being released at a site near Port Edward (31^{o} 04.398' S, 30^{o} 12.226' E), some 100km from the Cannonbrae site, making it important to evaluate any differences in fitness that may occur and thereby explain or predict the behaviour of the two populations in the field. The Cuban strain became inbred and/or diseased and was terminated; also it did not establish at any sites in South Africa (Zachariades and Strathie, 2006) and will therefore not be considered.

In investigating the Florida and Jamaica populations of *P. insulata*, the main objectives of this study were:

(a) To gain an understanding of their comparative biology (although such studies will not elucidate whether differences are (i) intrinsic, i.e. present in the countries of origin, due to differences in the way they respond to SA *C. odorata* or (ii) due to the history of the laboratory culture, including the size of the founder colony and

the length time they have been in culture). This will indicate whether one strain is more suited than the other for release into certain areas infested with *C. odorata*, which can dictate the nature of future mass-rearing initiatives and releases into those areas. Parameters that will be measured in studying their comparative biology include adult fecundity and longevity, egg viability, larval developmental duration, larval sizes and survival to pupation and adulthood. The relative feeding rates (i.e. leaf consumption) of the two populations will also be measured.

(b) To examine the effects of cross-breeding between the two populations by measuring relevant biological parameters (see above) in the hybrids for comparison with the parent populations and to thereby determine whether the fitness of the parent populations is decreased or increased by hybridisation.

(c) To examine the effects of back-crossing the hybrids with the parent stocks by measuring the same biological parameters as before to determine whether any deleterious effects from hybridization only manifest themselves in the F2 generation.

CHAPTER 2: GENERAL MATERIALS AND METHODS

2.1 Introduction

This chapter gives an overview of the materials and methods applied for the collection of data, and the statistical methods used to analyse the data in order to answer the research questions posed in Chapter 1. General materials and methods used in Chapters 3-4 are covered in this chapter to avoid unnecessary repetition where the same methods were applied. The study was conducted at the Entomology Department of the South African Sugarcane Research Institute (SASRI), Mount Edgecombe, South Africa, because both *P. insulata* cultures that were to be used, viz. (i) the Florida culture collected from the established site at Cannonbrae on the South Coast of KwaZulu-Natal province and henceforth abbreviated F and (ii) the Jamaica culture collected from Jamaica in 2002 which was subsequently integrated into or replaced with cultures collected in 2003, 2004 and 2005, and henceforth abbreviated J, were already being mass-reared there for field releases. This permitted access to a ready supply of insects, reared under consistent, hygienic conditions, for comparative trials. The studies were carried out from August 2006–January 2007 and from March-August 2007. Most of the general rearing materials and methods for this study are cited from Walton (2003) unless specified otherwise. Materials and methods used in 2007 were similar to those in 2006, except that methods that had failed in 2006 were replaced, and a few novel trials were conducted to collect additional data. Changes to methodology are indicated below.

2.2 Provision of plant material

2.2.1 Field collections

Cuttings of *C. odorata* that included healthy stems and leaves, for use in the various trials, were collected from the field around Mount Edgecombe and surrounding areas daily, but were not collected continuously from the same site. On returning to the laboratory at SASRI, the *C. odorata* cuttings were placed in a 100l bath filled with water to keep the material fresh.

2.2.2 Laboratory preparation

2l Freezete trays were filled with 70% Denol (Polychem Supplies, South Africa) for soaking and sterilizing the secateurs, knife and paintbrushes used for cutting plant material and transferring insects. Oasis® floral foam was cut into small blocks (10 x 5 x 8cm), which were soaked in a 100l bath filled with 20l water and 250ml Milton (22ml 12% sodium hypochlorite + 42g salt + 230ml water = 250ml Milton). The Oasis blocks were allowed to sink so that water was absorbed properly, after which about five to ten 20cm terminal branches of *C. odorata* were inserted into each block. D-Germ (chlorhexidine gluconate which contains 0.5g chlorhexidine with 70ml propylalcohol) provided in spray bottles was used, together with paper towels, to wipe down the Perspex cages and plastic oviposition jars used in the mating experiments, in order to reduce the chances of infection.

The laboratory conditions under which the insects were reared and trials carried out included 26^{o}C and 71% RH in 2006, while in 2007 these were changed to 27^{o}C and 78% RH.

2.3 Biology of parental (pure) populations

The biologies of the two pure populations were studied to obtain baseline data and to determine if there were differences in biology between the populations. It is possible that differences in biology could be related to differences in establishment success of *P. insulata* from Florida and that from Jamaica.

2.3.1 Adult fecundity and longevity

2.3.1.1 Mating

Data on fecundity were collected in both 2006 and 2007 by placing 10 adults eclosed during the previous night (5 males and 5 females) of the SA-established Florida (F) strain of *P. insulata*, taken from the SASRI laboratory culture, into a 30 x 30 x 30cm Perspex cage for 24 hours, and thereafter separating them into pairs, which were transferred into five 1l plastic oviposition jars. This procedure was followed in order to maximise mating possibilities on the first night and to track the number of eggs laid by individual females thereafter. Similarly, five males and females from the Jamaican (J) culture were mated in a Perspex cage and thereafter transferred to 1l plastic oviposition jars.

Leafy stalks of *C. odorata* were placed in Oasis blocks in the Perspex mating cage and in the plastic oviposition jars prior to releasing the *P. insulata* adults into them, to give the adults host-plant cues and a natural surface to encourage them to mate and oviposit on. For all pairs, four small sponges sprayed with a 50:50 honey: water solution were placed on the bottom of the cage. Similarly, for each mating pair, one sponge was placed in the plastic oviposition jar, for the adults to feed on. However, in some instances honey-water

leaking from the sponges trapped adults at the bottom of the oviposition jars, causing premature death. To avoid this, during the 2007 experiments sponges were replaced by small cotton wool plugs (Kluge and Caldwell, 1993a) in the plastic oviposition jars. These were similarly sprayed with honey-water.

2.3.1.2 Oviposition

The total number of eggs laid in the Perspex cage by the five females on the first night were counted, and kept until they hatched, and oviposition in the adult oviposition jars was checked daily. The *C. odorata* bouquets from each mating pair were removed, leaves with egg batches were detached from the stalks and the number of eggs in each batch was counted. These leaves were placed in Petri dishes and the eggs laid by the individual females were totalled separately. The lids of the Petri dishes were closed and labelled, and the date that the eggs were laid and the pair number that they were collected from were recorded. Eggs laid on the walls of the cage or plastic oviposition jars were marked with a date, counted, and left *in situ* until they hatched. New *C. odorata* bouquets were placed daily in the oviposition jars.

In 2007, the diameter of twenty eggs from each population was measured using a graticule under a Leica stereo light microscope and recorded.

2.3.1.3 Neonate hatching

The number of first-instar larvae hatching in Petri dishes and on the walls of the plastic oviposition jars was recorded daily. Enough fresh leaves were maintained in the Petri dish to sustain any larvae that hatched during the night. Eggs were kept under the same laboratory conditions as per mass-rearing methods (rooms at a constant 26^{o}C and 71%

RH (2006) and 27^{o}C and 78% RH (2007)). Hatching larvae were used to sustain the laboratory cultures.

2.3.1.4 Adult longevity

The dates on which mating pairs from each population were put together in a cage and their dates of death were recorded to determine adult longevity. The longevity of males and females was recorded separately. During the 2007 trials, on the date of death, the abdomens of the females were dissected to check if they contained a spermatophore from the males and if there were any eggs found, they were counted. Eggs laid and those retained in the ovarioles were analyzed separately to assess potential fecundity.

2.3.1.5 Statistical Analyses

Statistical analyses were used to test for differences in adult fecundity and longevity between the two populations. T-test, Mann-Whitney U tests and REML (Chi pr) tests were used to compare different aspects of adult fecundity. A Mann-Whitney U test was used to compare egg diameter between the F and J populations. A t-test was used to compare eggs retained in ovarioles. A t-test and restricted maximum likelihood estimation (REML) (Chi pr) tests were used to compare eggs laid, and to compare eggs hatched Mann-Whitney U tests were used. To compare adult longevity (including males and females between two populations), One-way ANOVA and REML (Chi pr) tests were used.

2.3.2 Larval survival and development

2.3.2.1 Larval growth parameters

In 2006, 50 freshly-hatched larvae from the F population and 50 from the J population were taken from the SASRI cultures and were monitored throughout their development on *C. odorata* leaves from hatching to pupation. Ten larvae were inoculated onto fresh *C. odorata* leaves in Petri dishes using a sterilized, paintbrush. Each of the 10 Petri dishes (five per population) was labelled with the *P. insulata* population, date hatched, number of larvae and the most recent date on which larvae had been changed over into a clean dish. The larvae were inspected daily to determine if they had moulted and were changed to fresh dishes with fresh leaves, every two days. Moulted skins were collected and the head-capsule width of each was measured crosswise. Once they reached the 3rd instar, surviving larvae were transferred to 2l Freezette trays with Oasis blocks stalked with fresh *C. odorata* bouquets, to give the larger larvae enough space and food, and were changed to new trays (with fresh bouquets) every two days until they pupated. Trays were kept upright to allow frass to fall to the bottom of the container, so as not to contaminate the developing larvae.

Any dead or sick larvae collected from the dishes/ trays were initially placed in a separate Petri dish to avoid contamination of the survivors, and the date of larval inoculation and the population of *P. insulata* was recorded. The sick or dead larvae were not handled thereafter, in order to prevent the spread of disease. Hands were sprayed with the anti-bacterial agent D-Germ and the paintbrushes and laboratory tables were also sterilized with this, so that healthy larvae were not infected. Sick or dead larvae were preserved for analyses of bacteria, fungi or viruses that might have been the cause of death.

2.3.2.2 Pupal development

As the final instar larvae were changed over, those which had pupated were collected and were placed in paper cups with autoclaved vermiculite to keep the pupae in hygienic conditions conducive to successful eclosion. Cups were numbered and labeled with the population, date hatched, date pupated and tray number. On the fifth day after transfer to the cups, sexes of the pupae from both populations were determined (see Fig. 3.6 later) under the microscope. Individual masses were measured, using a balance, to allow for differentiation between males and females in determining the fitness of the two populations. Paper cups with pupae were checked daily for eclosed adults. If there was eclosion, the number of the cup and the eclosion date were recorded. The eclosed adults were returned to the SASRI culture.

The larval- and pupal-development trials that were described previously were repeated, starting in May 2007, with some modifications. To determine development times of each larval instar and the pupae, as well as mortality rates, five replicates were again set up using a total of 50 larvae from each of the F and J populations. Ten newly hatched larvae were inoculated onto fresh *C. odorata* leaves in each Petri dish, and the 10 Petri dishes were labelled as before.

2.3.2.3 Larval leaf consumption

In order to obtain data on the mass of leaf material consumed by the larvae per day per Petri dish, all fresh *C. odorata* leaves were weighed together on a balance before putting them into the Petri dishes, to be inoculated with either neonate or growing larvae used in the trial. Every day after feeding, larvae were changed to new leaves and damaged leaves were removed and weighed immediately as a group. At the start of the trial, five

replicates (five Petri dishes) were set up with leaves without larvae of *P. insulata*, to act as controls (controlling for the loss of leaf mass due to water loss, rather than feeding). Similar numbers of leaves in the control Petri dishes were similarly changed and weighed daily.

On reaching the 4th instar, all surviving larvae were changed to 1l Freezette trays to provide them with enough space and food, and leaves continued to be weighed, as a group, before and after exposure to the larvae for feeding. To preserve moisture, the trays were lined with moistened filter paper; however, on the following day the filter paper was found to be dry, the leaves were also wilted, and most of the larvae appeared sick, especially those from the J population. To avoid further desiccation of leaves, wet Oasis blocks were put in trays with individual leaves and filter paper to preserve moisture. Similar numbers of control leaves were also treated in the same way; i.e. were transferred to 1l Freezette containers and weighed at the same time as the treatment leaves.

On reaching the 5th instar, larvae were reared according to ordinary mass-rearing methods; i.e. they were changed over to fresh leaves every 3 days and were placed in clean 2 l Freezette trays that had Oasis with fresh *C. odorata* bouquets. At this stage, leaves were no longer weighed as larvae were starting to cease feeding and undergo pupation. As the older larvae were changed over, the pupae were collected and labelled and the procedures that followed were similar to those described for the 2006 trial (see section 2.3.2.2). In both years, in order to obtain head capsules of the last instars, 5 larvae were sacrificed (killed) from each population as the head capsules were torn apart on pupating complicating finding of the actual size of the head capsules. For this purpose, 1 extra tray each with 10 larvae was kept aside from each population.

2.3.2.4 Statistical analyses

For statistical analyses, REML (Chi pr) tests and One-way ANOVA were used to compare sizes of the head capsules and the number of days larvae spent as each instar between the two populations. For comparison of pupal masses, REML (Chi pr) tests were used. One-way ANOVA followed by Fisher's protected test and Mann-Whitney U test were used to compare percentage pupation and percentage eclosion. Nonlinear regression analysis was used to assess leaf consumption over time. For life-cycle duration (from oviposition to pupation, from pupation to adult eclosion and from oviposition to adult eclosion) Mann-Whitney U were used.

2.4 Biology of cross-bred populations

2.4.1 Adult fecundity and longevity

2.4.1.1 Mating

In this section, the same methods were used as for the pure populations (section 2.3.1.1), except that in 2006, 10 F males were mated with 10 J females and 10 J males were also mated with 10 F females as above. Ten mating pairs were used instead of five in order to obtain enough hybrids to be used in later back-crossing trials as, in 2006, the larvae in the SASRI cultures became infected with a fungus previously identified as *Paecilomyces fumosoroseus* (Brown and Smith) (Deutoromycotina: Hyphomycetes) (J. Hatting, ARC-Small Grain Institute, Bethlehem and ARC-PPRI Mycology Unit) (Vidal *et al*., 1998; Lacey, 1999) and currently known as *Isaria fomosorosea* Wize (Hypocreales: Cordycipitaceae) (Luangsa-Ard *et al*., 2005; Meyer *et al*., 2008; Scorsetti *et al*., 2008).

2.4.1.2 Oviposition

Numbers of eggs laid by all females housed together in the Perspex cages were recorded and oviposition in the plastic oviposition jars was checked and recorded daily as for the pure population studies (section 2.3.1.2). Eggs laid on leaves were collected and put into Petri dishes on which were recorded the cross, date eggs were laid, number of eggs obtained and adult pair number they originated from. As in the pure population trials, eggs laid on the walls of the cage or plastic oviposition bottles were marked with a date, counted, and left *in situ* until they hatched.

2.4.1.3 Neonate hatching

Eggs were kept under the same laboratory conditions as per mass-rearing methods. The number of eggs hatching each day was recorded as reported in section 2.3.1.3.

2.4.1.4 Adult longevity

The dates on which the 10 pairs were put together in the cage and the dates of death from each of the cross-breeding trials were recorded to determine adult longevity, as before (section 2.3.1.4).

2.4.1.5 Repetition of trials

In 2007, a second series of the above trials was conducted and the procedures were the same as those used in 2006, except that only 5 F males were mated with 5 J females instead of using 10 mating pairs. Only 5 adult pairs were used due to the absence of fungus at the time. Similarly, 5 J males were mated with 5 F females. During the 2007

trials, once adult females had died, they were dissected to determine the presence of a spermatophore and unlaid eggs.

2.4.1.6 Statistical analyses

Statistical analyses were conducted to detect whether significant differences were present in the measured parameters between the pure-bred populations and the two cross-bred hybrid populations. Adult fecundity, including number of eggs laid and those retained in the ovarioles, and adult longevity was compared using One-way ANOVA. REML (Chi pr) tests followed by Fisher's test were used to compare the number of eggs that hatched.

2.4.2 Larval survival and development

2.4.2.1 Larval growth parameters

In 2006, with the presence of *I. fumosorosea*, larval developmental times were determined by monitoring the development of all newly-hatched larvae, in Petri dishes or trays. Larvae from the two crosses were confined according to the days on which they emerged, in order to keep larvae that hatched on the different dates separate. For the F♂ x J♀ cross, 15 batches of 25 larvae arose on the same day, followed by batches of 22, 13, 17, and 36 larvae from different days. For the J♂ x F♀ cross, the numbers of larvae included 11 batches of 25 arising on the same day, and then batches of 12, 19, 9, 11 and 36 larvae on different days. The high numbers of larvae originating from the two crosses (463 and 362) permitted enough progeny for the later back-crossing experiments. Newly-hatched larvae were kept in Petri dishes with fresh *C. odorata* leaves for two days. On the 3rd day after hatching, the larvae were transferred to fresh leaves placed in 2l Freezette trays.

The larval stocking rate was dependent on the number of larvae found on each day, but normally 25 larvae were inoculated per tray. However, if on a specific day there were less or more than 25 (e.g. 11 or 30) all of these were inoculated in one tray and not augmented by others from different days. Each tray was labeled with the crossed populations, date hatched, number of larvae per tray and the date changed.

The first instars from both crosses were transferred to new trays after seven days and not earlier, as they were very small, did not require too much food and could easily have been lost during transfer. At the 4th instar, 12 and 13 larvae from the set of 25 were inoculated into different trays to give them enough space and food and were labeled separately (e.g. 1a and 1b if originating from tray 1).

In 2007, in the absence of fungal contamination 300 newly-hatched larvae from both crosses were kept in Petri dishes with fresh *C. odorata* leaves for two days, with 25 larvae per Petri dish. On the 3rd day after hatching, the larvae were transferred to 2l Freezette trays at stocking rates of 25 larvae per tray and at the 4th instar these were also subdivided into batches of 12 and 13, as in 2006.

Thereafter, the methods used and the variables recorded were the same as in 2.3.2, except that, because the amount of work was prohibitive and because it was not considered necessary, head-capsule widths were not collected for measurement and the number of days taken for each moult was not recorded. Leaf masses before and after feeding were also not recorded.

2.4.2.2 Pupal development

Survival rates from newly-hatched larvae to pupation and to adult eclosion were recorded for comparison with the pure populations. The masses and sexes of pupae, the numbers of adults eclosed and the durations of larval development were also recorded. Larvae and pupae were kept under the same laboratory conditions as per mass-rearing methods.

2.4.2.3 Statistical analysis

The durations of larval and pupal development and pupal masses were compared using One-way ANOVA. Percentage pupation and eclosion were compared by ANOVA followed by Fisher's protected test. All data were compared to those of the pure populations. For life-cycle durations (from oviposition to pupae, from pupae to adulthood and from oviposition to adult eclosion) One-way ANOVA was used.

2.5 Biology of the population obtained from the back-cross of the hybrids with the parental (pure) populations

2.5.1 Adult fecundity and longevity

2.5.1.1 Mating

Five males each from the F and J populations were mated with five females of the FJ hybrid arising from the cross-breeding of F males and J females, while five females each from the F and J populations were mated with five males of the same hybrid (Table 2.1a). Similarly, five males each from the F and J populations were mated with five females of the JF hybrid arising from the cross-breeding of J males and F females, while five females each from the F and J populations were mated with five males of the same hybrid

(Table 2.1b). In both 2006 and 2007, five replicates (each consisting of a pair of adults) were conducted for each back-cross, except that in 2006 only four J males were mated with four FJ females, due to a lack of J males because of pathogenic fungal infection.

(a)

Pure strains		Hybrids		Back-crossing progeny	Number of larvae in 2006
5 F♂	X	5 FJ♀	⇒	F♂ X FJ♀	294
4 J♂	X	4 FJ♀	⇒	J♂ X FJ♀	67
5 F♀	X	5 FJ♂	⇒	F♀ X FJ♂	234
5 J♀	X	5 FJ♂	⇒	J♀ X FJ♂	135

(b)

Pure strains		Hybrids		Back-crossing progeny	Number of larvae in 2006
5 F♂	X	5 JF♀	⇒	F♂ X JF♀	200
5 J♂	X	5 JF♀	⇒	J♂ X JF♀	199
5 F♀	X	5 JF♂	⇒	F♀ X JF♂	75
5 J♀	X	5 JF♂	⇒	J♀ X JF♂	95

Table 2.1: (a) Back-crossing of males and females from pure strains with males and females from FJ hybrids and their back-crossing progeny. (b) Back-crossing of males and females from pure strains with males and females from JF hybrids and their back-crossing progeny.

2.5.1.2 Oviposition, neonate hatching and adult longevity

For each of these eight crosses, the number of eggs laid, retained in ovarioles and hatched was recorded as per sections 2.3 and 2.4. Adult longevity was also recorded

2.5.1.3 Statistical analyses

Statistical analyses to compare adult fecundity, egg viability, and the number of eggs retained in the ovarioles, between these eight back-crosses and the parent populations included One-way ANOVA followed by the Fisher's protected test. Adult longevity was also compared using One-way ANOVA followed by the Sidak test.

2.5.2 Larval survival and development

2.5.2.1 Larval growth parameters

In 2006, there was great variation in the number of hatching larvae produced by the different back-crosses because some of the back-crosses produced fewer eggs and hence fewer larvae (see Table 2.1 a, b). Hence, the numbers of newly-hatched larvae that were monitored through their development were unequal and all available larvae were reared to pupation and adult eclosion.

As before, 25 larvae were inoculated into 2l Freezette trays containing fresh bouquets of *C. odorata* and batches that were fewer than 25 were also inoculated e.g. the 294 larvae from the F♂ X FJ♀ back-cross comprised 25 larvae x 10 trays and one tray each of 11, 16, and 17 larvae. Larvae in the last three trays would have hatched on different days and hence were inoculated into separate trays. Also if there were 35 larvae found on one day they were also all inoculated into one tray. All larvae were monitored throughout their development until pupation, and survival rates were determined.

2.5.2.2 Pupal development

Larvae and pupae were reared under the same conditions previously described. The masses and the sexes of pupae, the numbers of adults eclosed, the durations of larval development and durations of life-cycle (from oviposition to pupation, from pupation to adult eclosion and from oviposition to adult eclosion) were recorded.

2.5.2.3 Statistical analyses

Statistical analyses for pupal masses and life cycle durations included One-way ANOVA, while for percentage pupation and eclosion, ANOVA followed by Fisher's protected test was used.

2.5.2.4 Repetition of trials

In 2007, rearing conditions in the laboratory were much better, owing to an absence of fungal infection, and larval development was re-tested as before using 125 newly-hatched larvae (25 larvae per tray x 5 replicates) from each of the eight back-crosses. Survival and duration of development until pupation and adult eclosion were recorded as before.

Data from the back-crosses were compared to those from the pure populations to determine the relative fitness of the different populations. Results from the cross-breeding and back-crossing trials obtained in 2006 were compared to those obtained from the pure populations in 2006, and the results obtained from the cross-breeding and back-crosses in 2007 were similarly compared to those obtained from the pure populations in 2007.

2.6 Population fitness index of the parental, cross-bred and back-crossed populations

Since several growth parameters, notably survival, duration of development and pupal mass, were used to determine the fitness of the immature stages of the different populations, it was desirable to incorporate these into a single overall estimate of fitness. This was achieved following the "host suitability index" used by Maw (1976). Although this index was originally designed to compare the fitness of the same insect population on

different host plants, in this context it was used to compare the fitness of different insect populations on the same host plant, and therefore called a “population fitness index”.

The index incorporated data, recorded during monitoring of the development of the pure populations, crosses and back-crosses, to assess the comparative fitness of these populations, as follows:

$$Population\ Fitness\ Index = \frac{Mean\ mass\ of\ \ pupae \times \%\ pupation}{Mean\ development\ time\ to\ \ pupation}$$

Because male and female pupae differ in their mean masses, female pupae were used in the above formula for consistency. Higher scores, derived from the above formula, were indicative of higher levels of fitness.

CHAPTER 3: DIFFERENCES IN THE BIOLOGY OF THE FLORIDA AND JAMAICAN POPULATIONS OF *PAREUCHAETES INSULATA*

3.1 Introduction

A primary aim of this study was to gain an understanding of the comparative biology of the Florida and Jamaican laboratory populations of *P. insulata* and quantify any differences in their response to feeding on South African *C. odorata*. This was aimed at determining whether one population is potentially more suited than the other for establishment and impact on the weed under South African conditions. Differences were assessed using several parameters, including adult fecundity and longevity, larval feeding rates (i.e. leaf tissue consumption), larval developmental duration (by days spent as each instar), sizes of head capsules, survival to pupation and adulthood and pupal masses.

There is limited published information on the biology of different species of *Pareuchaetes*. Adults of *Pareuchaetes* species are golden yellow to dirty brown (Fig. 3.1) with longitudinal rows of small black abdominal spots both dorsally and laterally, and can only be differentiated by examining the female genitalia. In *P. pseudoinsulata* and *P. insulata* the forewings are slightly darker than the hind wings (Cock and Holloway, 1982). *Pareuchaetes insulata*, like other *Pareuchaetes* species studied, is nocturnal, with mating occurring on the first night after adult eclosion (Kluge and Caldwell, 1993a,b). Conner *et al.* (1985) showed that in *P. insulata* and several arctiid moths the females attract the males and copulation usually occurs between 02h00 and 04h00. Besides South Africa, *P. insulata* has not been reared and released elsewhere in the world and very little information is available on its biology. Therefore, this chapter provides details of the moth's biology with particular emphasis on differences between the South African-adapted Florida (F) and the Jamaica (J) populations.

Figure 3.1. Adults of *P. insulata* (R) and *P. pseudoinsulata* (L).

3.2 Biology of parental (pure) populations

3.2.1 Adult fecundity

In 2006, *P. insulata* from the F and J populations differed significantly in the mean number of eggs produced (n = 5 pairs each, P = 0.038, t = 2.48, t-test). In both populations, mating started on the first night and eggs were found on the first day after placing the pairs together in the cage. There was a significant difference in the number of eggs laid on different days between the F and J populations (P = 0.018, REML Chi pr), although not on every day (Fig. 3.2a). On day 1, the females from five pairs of J adults each laid an average of 23 eggs while females from the F pairs each laid an average of 3.2 eggs, from which an average of 21.8 (95% of those eggs laid) and 1.8 (56% of those eggs laid) hatched from the J and F egg batches, respectively. On the second day, when individual pairs were put in plastic oviposition jars, F females initially laid more eggs than J females (Fig. 3.2a). Oviposition peaked on the second day for F females, with a mean (± SD) of 117.2 ± 101.49 eggs/female. For J females the peak was on the fourth day with a mean of 45.4 ± 55.47 eggs/female (Fig. 3.2a). Values of means used in Fig. 3.2a were statistically back-transformed for non-normal data and the above values are

hence different to those shown in the caption of Fig. 3.2a. On the 8th day, only J females laid eggs, the F females having ceased oviposition on day 7.

The total number of eggs laid by each of the five pairs put in plastic oviposition jars (i.e. from the second day on) ranged from 198 to 433 for the F population and from 64 to 263 for the J population and averaged 291.2 ± 103.4 and 144.6 ± 75.4 eggs per female for the F and J populations, respectively. Including the eggs laid in the cage on day 1, the mean number of eggs laid per female throughout their lifespan were 294.4 and 169.6 for the F and J populations, respectively. Regarding egg viability, 169.2 ± 51.5 (mean ± SD) eggs hatched per F female from day 2 on (58% of the mean laid) which was significantly higher than the average of 48.6 ± 67.9 eggs hatched per J female (33% of the mean laid) (P < 0.001, u = 1537.0, Mann-Whitney U test). However, for the total eggs laid throughout the females' lifespan (i.e. including the data from day 1), 58% hatched from the F population and 42% from the J population.

In 2007, on the first night, of the mean of 96.4 eggs laid per female in the cage containing five pairs of F adults, a mean of 95.4 eggs (99%) hatched. No eggs were found in the cage with the five J pairs. From day 2 on, the total number of eggs laid per F female ranged from 346 to 595 and from 143 to 506 per J female, with a mean (± SD) of 437.6 ± 97.0 and 268.0 ± 152.7 eggs per F and J female, respectively (n = 5 pairs, P = 0.02, REML Chi pr log transformation). Consequently, the mean number of eggs laid per female throughout their lifespan was 534.0 and 268.4 for the F and J populations, respectively. The J females had more eggs retained in their ovarioles than the F females, with a mean of 5.2 ± 4.4 (range: 0-12 eggs) for F females and 24.4 ± 33.8 (range: 0-73)

for J females, although no statistically significant difference was detected (P = 0.771, t = -0.30, T-test log10 [x+1] transformation).

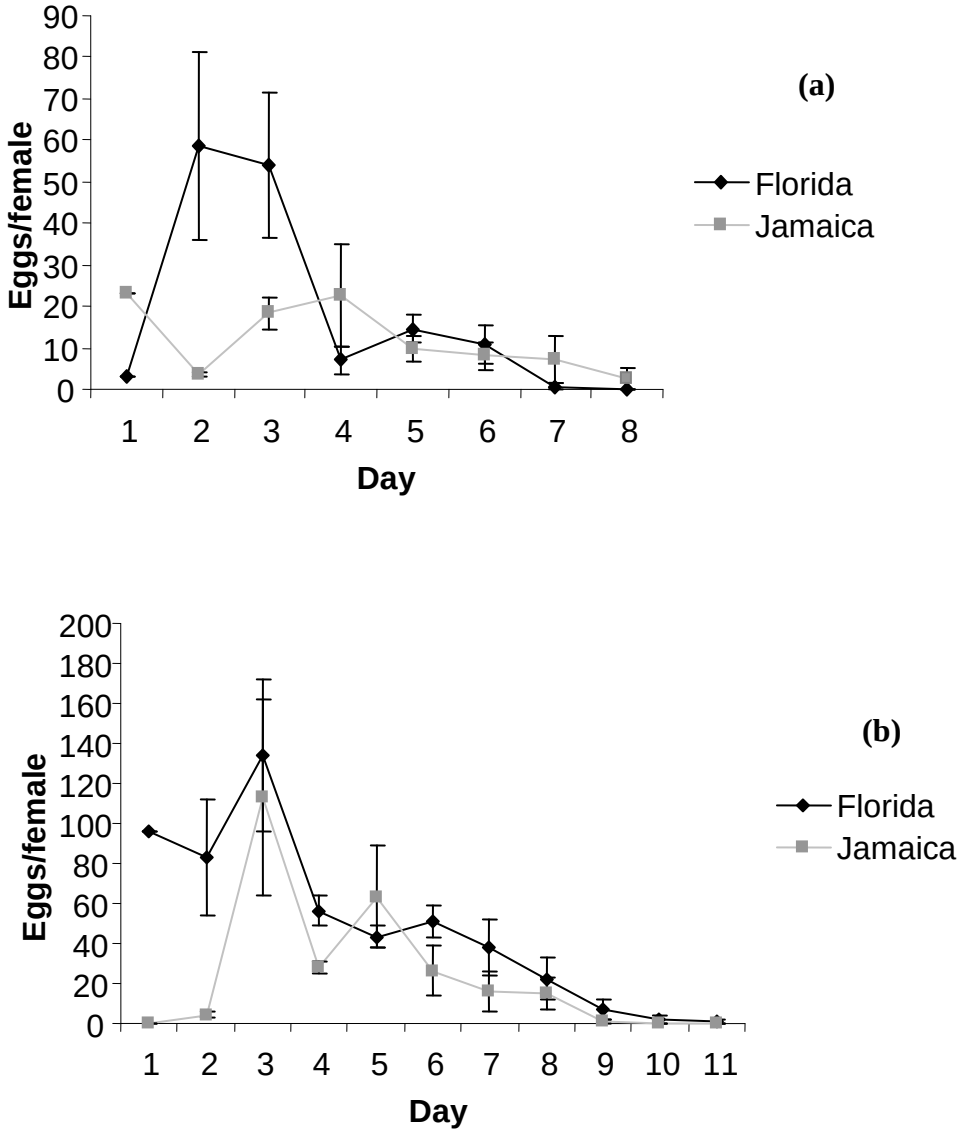


Figure 3.2. The mean ± 1SE number of eggs per day laid by F and J females over several days during (a) the 2006 trials, when there was a significant difference in egg production between the two populations (P = 0.018, REML Chi pr); (b) the 2007 trials, when there was no significant difference in egg production between the two populations (P = 0.617, REML Chi pr). For day 1 SE could not be calculated because the data were pooled.

Unlike the results from 2006 (Fig. 3.2a), there was no significant difference in the number of eggs laid on different days between the F and J females (P = 0.617, REML Chi pr). Although on day 2 and day 4 the number of eggs laid by the F population was very different to that of the J population, no statistical difference could be detected (Fig. 3.2b). Both populations laid spherical eggs with a mean (± SD) diameter of 0.89 $\pm$ 0.02 and 0.86 $\pm$ 0.03 mm (n = 20) for F and J eggs, respectively. Eggs laid by J females were significantly smaller than those of F females (P < 0.001, u = 84.5, Mann-Whitney U test).

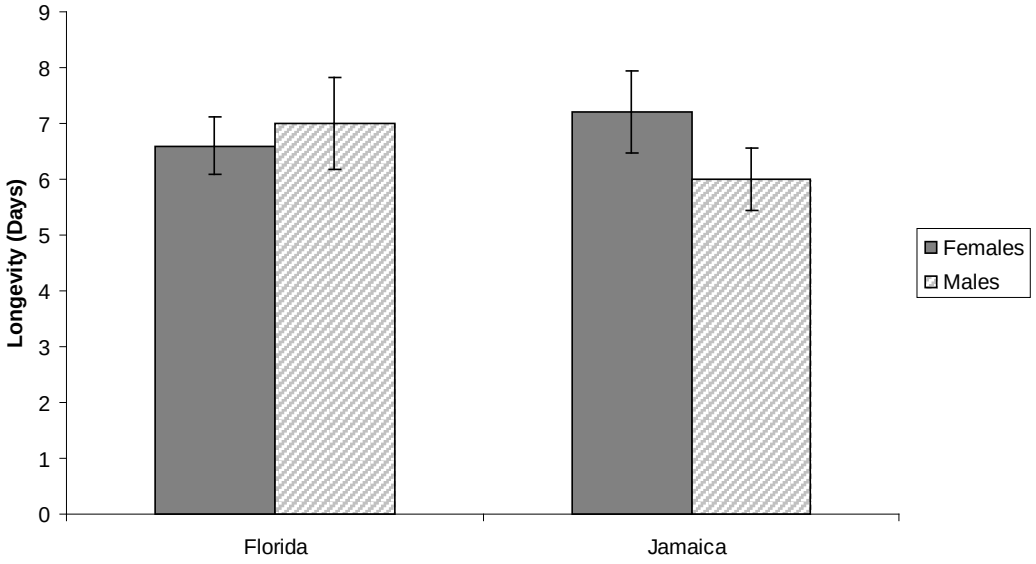
In 2007, the mean number of eggs that hatched from those laid in the oviposition jars by F females from day 2 on was 324.6 ± 211.04 (74% of those laid) compared to 157.4 ± 193.42 (59% of those laid) for J females. Including eggs laid in the cage on day 1 (i.e. all eggs laid throughout the females' lifespan), 79% hatched from the F population and 59% from the J population. Although more F than J eggs hatched, the difference was not significant (P = 0.302, u = 7.0, Mann-Whitney U test). The number of F eggs that hatched ranged from 0-564 per female from day 2 on compared to 0-468 for J eggs. The F population had four out of five females mated and with spermatophores, whereas the J population had three out of five females mated and with spermatophores. In both populations, the mated and unmated females laid eggs; however, the unmated females laid infertile eggs that shrivelled while still yellow.

3.2.2 Adult longevity

In 2006, adults from the F and J populations of *P. insulata* had similar longevity (6-7 days; n = 10, P = 0.189, ANOVA) (Fig. 3.3a) and there was no significant difference in longevity between the sexes within either population (P = 0.499, ANOVA). However, in 2007, F adults lived significantly longer than J adults (7-9 days; n = 10, P = 0.015,

REML Chi pr) (Fig. 3.3.b), but there was no significant difference in longevity between the sexes within either population (P = 0.689, REML Chi pr).

(a)



(b)

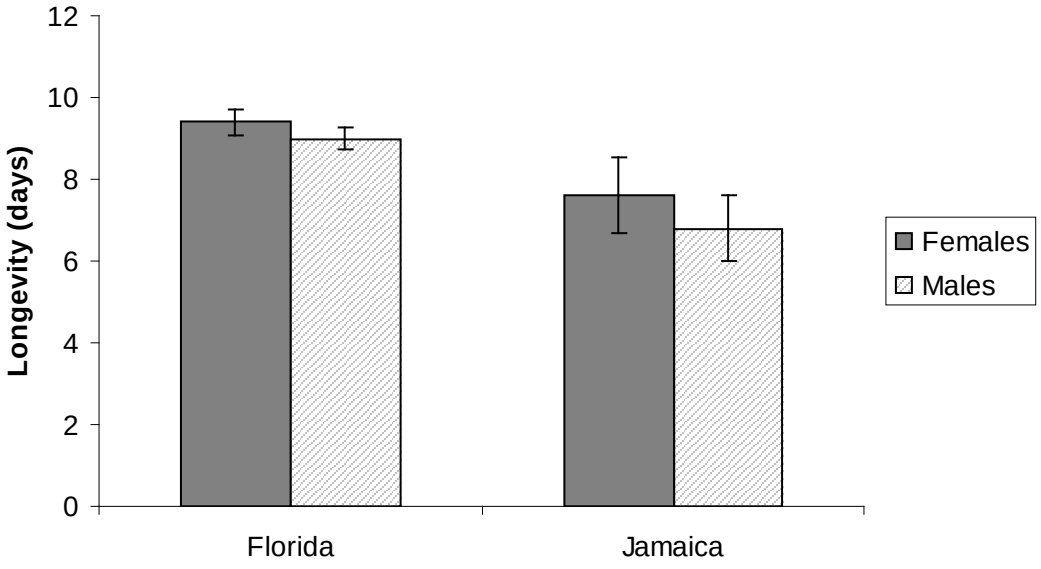


Figure 3.3. Mean (± SE) longevity of five females and five males of the two populations of *P. insulata* in (a) 2006 and (b) 2007.

3.3 Larval survival and development

3.3.1 Durations and sizes of larval instars

The larvae from the F and J populations were similar in appearance. All eggs from both populations took four days to hatch and turned grey before hatching. On the fourth day, larval setae were visible through the chorion of the eggs, when examined under the microscope, and probably gave the eggs their grey colour. Head capsules appeared as dark objects through the chorion. Larval movements were noticed and the ready-to-hatch larvae chewed the chorion until they formed exit holes. After emerging from the chorion, the newly-hatched larva returned its head into the chorion and fed on the remaining contents of the egg. It then wandered around until finding food, i.e. the leaf of *C. odorata* provided.

Figure 3.4. Second instar larvae of *P. insulata* showing orange colour in segment 1, 3, 7 and 9.

In 2006, there were six instars for both F and J larvae (Table 3.1). Newly-hatched larvae had cream, white or grey body segments with a prothoracic shield on the first segment and shiny black setae. Prothoracic, abdominal and anal prolegs were visible in the newly-hatched larvae and were grey like the body. After feeding, segments 2, 5 and 8 became

pale orange. After four days, segments 2, 5 and 8 (and sometimes 6) in the 1st instar larvae were orange, with white stripes running along the length of the abdomen dorso-laterally and ventro-laterally (n = 10 larva examined). Second and 3rd instars differed from 1st instars in that segments 1, 3, 7 and 9 were orange (n = 10) (Fig. 3.4). The 4th, 5th and 6th instars (n = 5) also had segments 1, 3, 7 and sometimes 9 orange.

Table 3.1. Mean ± SD head capsule width (mm) of each instar of F and J larvae reared on *Chromolaena odorata* during 2006 and 2007. Number of samples for each instar indicated in brackets. In 2007 all F larvae pupated at the 5th instar.

	2006		2007	
Instar	F	J	F	J
First	0.40 ± 0.00 (50)	0.40 ± 0.00 (50)	0.40 ± 0.00 (50)	0.40 ± 0.00 (50)
Second	0.56 ± 0.02 (50)	0.59 ± 0.02 (50)	0.59 ± 0.02 (50)	0.59 ± 0.02 (50)
Third	0.81 ± 0.07 (50)	0.92 ± 0.04 (49)	0.97 ± 0.03 (50)	0.92 ± 0.04 (49)
Fourth	1.12 ± 0.08 (26)	1.41 ± 0.16 (46)	1.51 ± 0.06 (49)	1.41 ± 0.16 (37)
Fifth	1.60 ± 0.2 (40)	1.64 ± 0.30 (40)	2.38 ± 0.05 (5)	1.64 ± 0.23 (17)
Sixth	2.32 ± 0.22 (5)	2.47 ± 0.15 (5)	*	2.47 ± 0.15 (5)

***** Only five instars recorded.

In 2006, the size of the head capsules differed significantly between instars, for both populations ($P < 0.001$, REML Chi pr). First instar larvae from the F and J populations had equally sized head capsules, but from the 2nd instar on, J larvae had larger head capsules ($P < 0.001$, REML Chi pr) (Table 3.1). Initially, the duration of the larval instars was the same for both populations; however, from the 4th instar on F larvae took significantly longer to develop ($P < 0.001$, REML Chi pr). The 6th instars were reached at 24 and 21 days for the F and J populations, respectively (Table 3.2A and Fig. 3.5a).

In 2007, there was a difference in the number of instars between J and F larvae, six compared to five respectively (Table 3.1, Fig. 3.5B). As in 2006, all eggs took four days to hatch. Initially, i.e. for 1st and 2nd instars, larvae from both populations had similar

sizes of head capsules, but from the 3rd instar on there was a highly significant difference in the sizes of the head capsules between the populations ($P < 0.001$, ANOVA). In 2007, F larvae had larger head capsules, in contrast to 2006 where the J larvae were larger (Table 3.1). In 2007, the J population had exactly the same sizes of head capsules as in 2006. Initially, in 2007, there was no difference in the number of days spent by larvae at each instar between the two populations (Fig. 3.5B).

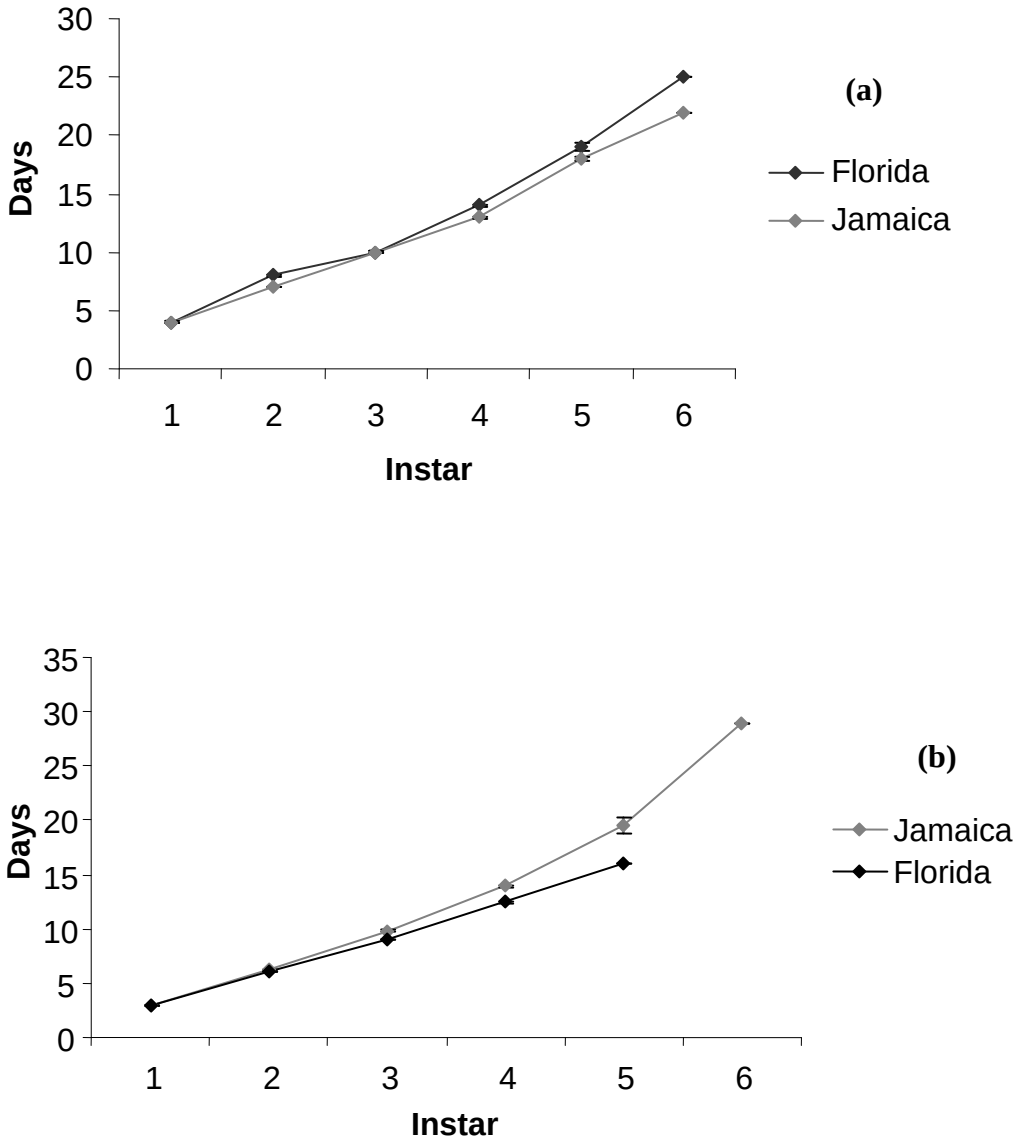


Figure 3.5. Cumulative mean (± 1SE) duration of development of *Pareuchaetes insulata* larval instars from F and J populations during trials in 2006 (a) and 2007 (b).

However, from the 4th instar on, the F larvae developed significantly faster than the J larvae ($P < 0.001$, ANOVA) (Table 3.2B). While all F larvae took only 16 days to reach the 5th instar and thereafter pupated after 19-21 days, only two of J larvae reached the 5th instar on the 16th day. Out of 13 J larvae that pupated, seven did so as 5th instar larvae after 19-21 days, as in the F population. Those larvae that developed through to the 6th instar took 23-26 days to reach the 5th instar. One of these larvae moulted into 6th instar after 29 days and pupated after 39 days, but did not eclose. Other 6th instar larvae died without pupating. This trend was opposite to that observed in 2006 where F larvae developed more slowly than J larvae (Fig. 3.5a).

3.3.2 Characteristics of pupae

Pupation occurred in a flimsy cocoon spun between leaves on the plant. The pupae from both populations were dark brown (Fig. 3.6). Females were characterized by a linear genital opening situated close to the intersegmental membrane of the last segment of the pupal abdomen and male by genital openings situated in the middle of the last segment (Fig. 3.6). In 2006, the mean mass of F female pupae was 0.22 ± 0.04mg (n = 19) and that of male pupae was 0.16 ± 0.05mg (n = 14). J females had a mass of 0.24 ± 0.05mg (n = 29) and males a mass of 0.16 ± 0.01mg (n = 17). Females from either population were always significantly heavier than males ($P < 0.001$, REML Chi pr), but there were no differences in pupal mass of the same sexes between the two populations ($P = 0.257$, REML Chi pr). In the same year, there was a significant difference ($P < 0.001$, ANOVA) in the pupation rate of larvae from the F (70 ± 0.0%, n = 35) and J (92 ± 0.0%, n = 46) populations. Adult eclosion occurred in 51.4 ± 33.5% of F pupae and 92.0 ± 8.4% of J pupae and these differences were significant ($P < 0.001$, ANOVA). At 26°C and 73%

RH, adults eclosed from their pupae after 10.0 ± 1.9 days (n = 20) in the F population and after 9.0 ± 1.9 days (n = 40) in the J population; there was no significant difference between the populations (P = 0.316, u = 9.0, Mann-Whitney U test) (Table 3.2A). The complete life-cycle took an average of 38.0 ± 3.3 days (n = 20) for the F population and 38.0 ± 2.9 days (n = 40) for the J population (Table 3.2A), i.e. was virtually identical between the two (P = 0.715, u = 24.5, Mann-Whitney U test).

Table 3.2: Cumulative mean (± SD), and range of the immature stages of Florida and Jamaica populations of *Pareuchaetes insulata* on *Chromolaena odorata* in the laboratory during 2006 (A) and 2007 (B). Numbers of samples for each stage are indicated in brackets. In 2007 all F larvae pupated at the 5th instar (*).

A

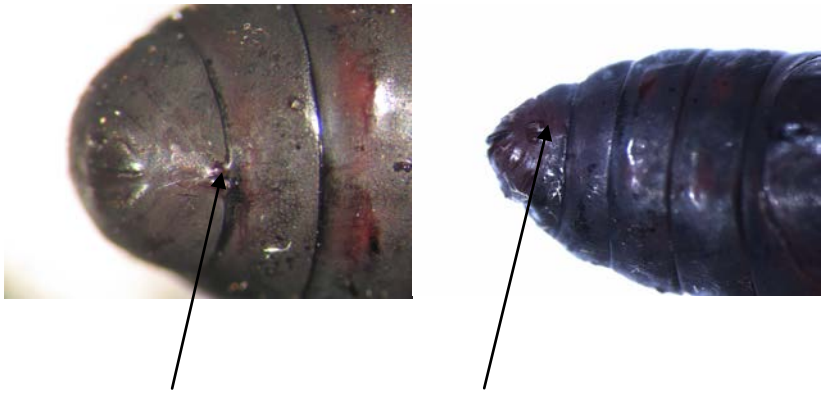
	Duration (days)		Range	
Stage[1]	Florida	Jamaica	Florida	Jamaica
First	3 ± 0.0 (50)	3 ± 0.41 (50)	3	3-4
Second	6 ± 0.55 (50)	6 ± 0.48 (50)	6-8	6-7
Third	9 ± 0.43 (50)	9 ± 0.43 (50)	9-10	9-10
Fourth	13 ± 0.50 (26)	12 ± 0.44 (46)	12-13	12-13
Fifth	18 ± 2.66 (40)	17 ± 1.77 (40)	14-21	14-19
Date hatched-pupation[2]	27 ± 4.03 (35)	26 ± 5.01 (45)	22-31	19-33
Oviposition-pupation	31 ± 4.03 (35)	30 ± 5.01 (45)	26-35	23-37
Pupation-eclosion	10 ± 1.92 (20)	9 ± 1.87 (40)	9-12	6-11
Oviposition-eclosion	38 ± 3.28 (20)	38 ± 2.87 (40)	34-43	34-42

B

	Duration (days)		Range	
Stage[1]	Florida	Jamaica	Florida	Jamaica
First	3 ± 0.0 (50)	3 ± 0.00 (50)	3	3
Second	6 ± 0.00 (50)	6 ± 0.39 (50)	6	6-7
Third	9 ± 0.14 (50)	10 ± 0.60 (49)	9-10	9-11
Fourth	12 ± 0.79 (49)	14 ± 1.54 (37)	12-14	13-15
Fifth	*	20 ± 3.22 (17)	*	16-26
Date hatched-pupation[3]	20 ± 1 (41)	26.2 ± 8.07 (13)	19-21	19-39
Oviposition-pupation	24 ± 1.00 (41)	30 ± 8.07 (13)	23-25	23-43
Pupation-eclosion	10 ± 1.53 (26)	10 ± 1.83 (7)	9-12	8-12
Oviposition-eclosion	35 ± 2.08 (26)	35 ± 1.53 (7)	33-37	34-37

[1]Duration of larval instar is from date of inoculation of newly hatched larvae until the date of moulting into next instar.
[2]Equivalent to length of time between date of inoculation and the end of 6th instar.
[3]Equivalent to length of time between date of inoculation and end date of 5th instar for Florida population, and of 5th and 6th instars for Jamaica population.

In 2007, pupal masses of both F and J were significantly lower than those in 2006. Female pupae from the F and J populations had similar mean masses of 0.19 ± 0.02mg (n = 23) and 0.19 ± 0.05mg (n = 4), respectively (P = 0.954, REML Chi pr). Male pupae from the F and J populations also had similar mean masses of 0.14 ± 0.02mg (n = 18) and 0.15 ± 0.02mg (n = 10), respectively (P = 0.954, REML Chi pr). As in 2006, females from either population were always heavier than males ($P < 0.001$, REML Chi pr). There was high larval mortality in the J population compared to the F population; while a mean of 84.0 ± 0.9% F larvae pupated, only 26.0 ± 4.2% of J larvae did so. However, this difference was not statistically significant (P = 0.151, u = 5.0, Mann-Whitney U test) because of high variability caused by 100% pupation in one of the J replicates. For the F population, 61.1 ± 1.2% (n = 26) of the pupae eclosed, while for the J population, only 10.0 ± 0.5% (n = 10) eclosed (P = 0.013, u = 7.0, Mann-Whitney U test). At 27^{o}C and 78% RH, F pupae eclosed after an average of 10.0 ± 1.5 days as pupae (n = 26) and J pupae eclosed after 10.0 ± 1.8 days (n = 7) and there was no significance difference between the F and J population (P = 0.750, u = 6.5, Mann-Whitney U test) (Table 3.2B). Overall, F and J populations completed their life-cycle in an average of 35.0 ± 2.1 days (n = 26) and 35.0 ± 1.5 days (n = 7), respectively, which was about 3 days quicker than recorded in 2006 (probably because laboratory conditions were 1^{o}C warmer in 2007) and there was no significant difference between the two populations (P = 0.800, u = 3.0, Mann-Whitney U test). According to Table 3.2B the F larvae developed to pupation faster than the J larvae; however, the complete life-cycle was similar to the two populations because the J larvae that pupated later (e.g. at 39 days) did not eclose.



(a) Female genital opening **(b)** Male genital opening

Figure 3.6. Differences in external anatomy between *Pareuchaetes insulata* female (a) and male (b) pupae. Females are characterized by a linear genital opening situated close to the last segment of the pupa and males by genital openings situated in the middle of the last segment.

3.3.3. Population fitness and larval leaf consumption on *Chromolaena odorata*

The Population Fitness Index suggested that the two populations responded differently when reared on South Africa *C. odorata* (Table 3.3), although the differences were not consistent. In 2006, the J population scored 44% higher than the F population but in 2007, it scored substantially lower (73%) than the F population (Table 3.3). These differing results are likely to have been caused by laboratory effects, notably fungal infection in 2006 and inbreeding in 2007 (see Discussion).

Table 3.3. Population Fitness Index (PFI) of the Florida and Jamaica populations of *Pareuchaetes insulata* when reared on *Chromolaena odorata*.

Year	Population	PFI score
2006	Florida	0.5
	Jamaica	0.72
2007	Florida	0.67
	Jamaica	0.18

In addition to the Population Fitness Index, leaf consumption during 2007 also gave an indication of the performance of the two populations on *C. odorata*. In the first few days, F and J larvae fed at the same rate (Fig. 3.7). However, from day 8 onwards F larvae fed at a higher rate (higher curve and different slope) than J larvae ($P < 0.001$, non-linear regression analysis) although both populations displayed a similar general feeding pattern (exponential curve) (Fig. 3.7) ($P = 0.320$, non-linear regression analysis). By the end of the trial i.e. on the 16^{th} day, only 29 larvae were left from the J population, while the F population still had 50 larvae. Larval mortality, which started from day 12 on, was taken into account by dividing the amount of leaf tissue consumed by the remaining number of larvae per Petri dish (no larval mortality was recorded during the trial for the F population). Therefore, mortality was not directly accountable for lower feeding rates but may have been an indicator of the poor health of the J population. Even on days 1-8, when both populations had a full complement of 50 larvae, feeding by F larvae was generally higher than that of J larvae, particularly on day 8 (Fig. 3.7). Hence, these results show that the F population performed better than the J population. During the moulting period, larvae from both populations did not feed and resumed feeding after shedding their skin, accounting for day-to-day variation in the data.

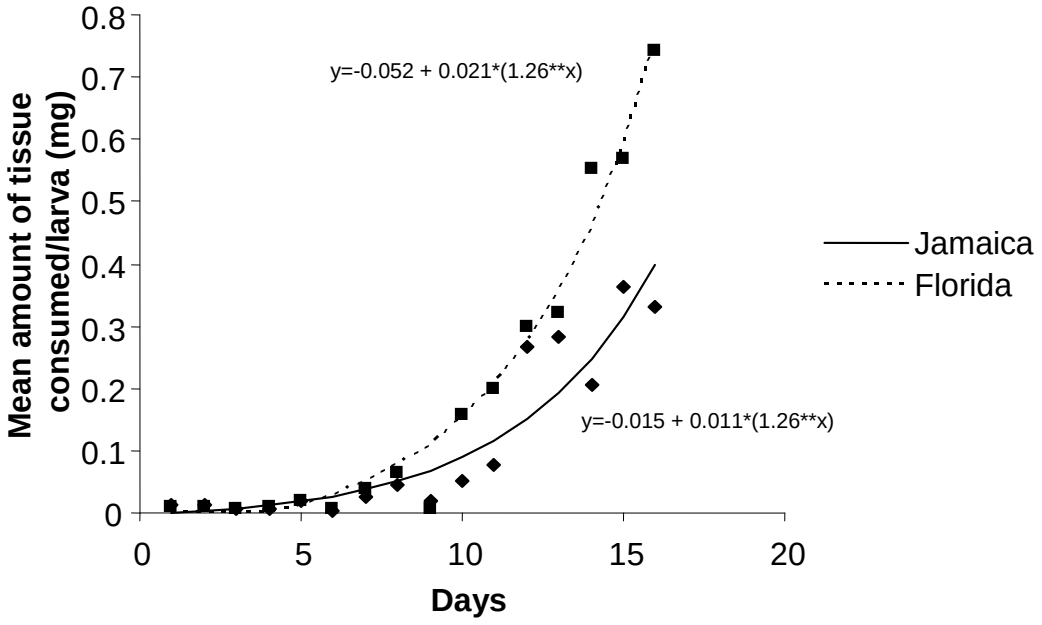


Figure 3.7. Exponential curves of leaf consumption for Florida and Jamaica larvae over time (r^2 = 0.94), in 2007. Florida larvae fed at a higher rate than Jamaica larvae ($P < 0.001$, non-linear regression analysis) and both populations displayed a similar general feeding pattern ($P = 0.320$, non-linear regression analysis).

3.4 Discussion

The current study shows that, based on an overall assessment of several life-history parameters in 2007, the F population generally had higher fitness than the J population (Table 3.4), and would therefore probably have a better chance of establishing. However, in 2006, although the J population showed lower fitness in terms of fecundity and egg viability, the J larvae had larger head capsules compared to F population larvae which also took longer to develop than the J larvae; the J population also showed better survival to pupation and adulthood than the F population. Only in 2007 did the J population suffer higher mortality than the F population. At times, the J population also showed better fitness in other aspects of its biology; for example, in 2006, it displayed a higher score in

the PFI than the F population (Table 3.3). In contrast, the F population displayed better survival, higher leaf consumption rates and a higher PFI score on *C. odorata* during 2007.

Table 3.4. Overall life-history parameters examined when comparing the biology of *Pareuchaetes insulata* from Florida and Jamaica reared on *Chromolaena odorata* in the laboratory.

Parameter	2006*	2007*
Adult fecundity	F > J	F > J
Egg viability	F > J	F > J
Longevity	F = J	F > J
Sizes of head capsules	J > F	F > J
Duration of larval instars	F > J	J > F
Pupal mass	F = J	F = J
Percentage pupation	J > F	F > J
Percentage eclosion	J > F	F > J
Population fitness index	J > F	F > J
Leaf consumption	N/A	F > J
Eggs retained in ovarioles	N/A	F = J
Egg diameter	N/A	F > J

* > indicates a significant difference ($P < 0.05$) while = indicates no significant difference ($P > 0.05$) between populations. N/A indicates where comparative data were not available.

F female adults of *P. insulata* had higher fecundity and the eggs had higher viability than J females and eggs in both 2006 and 2007. However, the difference detected between the number of J and F eggs hatched in 2007 was not statistically significant, which was attributed to the high variability between replicates. The mean number of eggs retained in the ovarioles of J female adults was higher than that for F female adults, with the statistically non-significant difference also resulting from high variability between replicates. The difference in fecundity and egg viability may have influenced the establishment success of the two populations in South Africa. However, other factors can also explain the difference in establishment success, including the large number of larvae (around 388 000) released at the single established site (Cannonbrae), environmental

conditions closer matched to the country of origin (Parasram, 2003) that allowed the Florida population to establish at this site, and the larger number of sites at which the Florida population was released compared to the Jamaica population. Calvo and Molina (2005) indicated that fecundity is an important life history trait in understanding lepidopteran population dynamics, as it is one of the bases from which population changes are determined through the incidence of environmental factors such as change in temperature or quality of the host plant. Poor climate matching could manifest through factors other than, or in addition to, lowered fecundity e.g. increased egg and larval mortality (van Lenteren *et al.*, 2003). The climate of Florida was shown to be reasonably similar to that of KwaZulu-Natal (Parasram, 2003) whereas those of Cuba and Jamaica were of lower similarity (Robertson *et al.*, 2008). Therefore, *P insulata* from Florida was probably better adapted to the KwaZulu-Natal climate than *P. insulata* from Jamaica (Zachariades and Strathie, 2006), and if climate did affect fecundity and/or other life-history parameters, might thus have a better chance of establishing than the Jamaican *P. insulata*. Indeed, it was recognized before the Jamaican and Cuban cultures were imported that Jamaica, and possibly even Cuba, provided poorer climatic matches with South Africa than Florida (Zachariades and Strathie, 2006). These two populations were nonetheless introduced because at that time the culture from Florida was believed to have failed to establish, and it was thought that close matching between the *P. insulata* population and the *C. odorata* host-plants (it is thought that the SA *C. odorata* originates from one of these two islands) might be more important than climate matching (Zachariades and Strathie, 2006). However, since all the trials for the current study were conducted in the laboratory with a controlled climate, no direct link can be made between the lower fecundity of the J population, and poor climate matching resulting in non-establishment in the field. It seems unlikely, although not impossible, that the lower

fecundity rates and egg viability displayed by J females in this study could have been due to climatic conditions in the laboratory that favoured the F over the J population.

In lepidopteran species, adult size is often the main determinant of female fecundity i.e. larger females have higher fecundity than smaller females (Calvo and Molina, 2005; Poykko, 2005). However, our data did not support this pattern because, for both populations, 1st trial (= 2006) female adults from heavier pupae had lower fecundity than 2nd trial (= 2007) female adults from lighter pupae. In addition, the J larvae in 2006 were bigger than the F larvae and took less time to develop, but still had lower egg output and viability. This could be attributed to the fact that in 2006, the adult longevity results were biased by the premature death of adults. In 2007, *P. insulata* adults from the F population had higher longevity than the adults from the J population, although there were no differences in longevity between the sexes within each population.

The current data support those from earlier work conducted on *Pareuchaetes* species, including the biology and host-specificity testing of *P. aurata aurata* (Kluge and Caldwell, 1993a) and the host-specificity testing of Florida-collected *P. insulata* (Kluge and Caldwell, 1993b). Eggs from both species were pale yellow, had a mean diameter of about 0.9mm (although F and J eggs were 0.89mm and 0.86mm, respectively, and statistically different) and turned grey before hatching, while the hatching larvae developed through six instars. The duration of the life-cycle in my study ranged from 34-43 days for the F population and 34-42 days for the J population in 2006, compared with 33-37 days and 34-37 days (26-28^{o}C and 71-78% RH) for the F and J populations, respectively in 2007. Kluge and Caldwell (1993b) reported a somewhat slower

development for *P. insulata*, with a range of 40-60 days (22-26^{o}C and 60-80% RH), which was likely caused by lower night temperatures used during their study.

In 2006, larvae from both populations developed to the 6th instar before pupating. However, in 2007, the number of larval instars was reduced to five in the F population while larvae from the J population had both fifth and sixth instars pupating. Out of 13 J larvae that pupated, seven did so between 19-21 days, as did F larvae, providing further evidence that these J larvae pupated at the 5th instar, while four pupated at 23 days and the other two pupated between 29 and 39 days, with the latter not emerging to adulthood. Reduction in the pupal masses for both populations during 2007 was also indicative of a reduction in the number of larval instars for the J population, bearing in mind that there was no statistical difference in pupal masses of females and males between the two populations. Kluge and Caldwell (1993a, b) do not mention variation in the number of larval instars for either *P. aurata aurata* or *P. insulata*.

Variation in the number of larval instars is common in the Lepidoptera but has not been considered as an important trait in their life history (Poykko, 2005). As for other Lepidoptera, the variation in *P. insulata* could be influenced by biotic or abiotic factors such as temperature, day length and nutritional quality of the diet (Calvo and Molina, 2005). The first set of trials was conducted in late winter/summer (August 2006–January 2007) when the plants are growing vigorously and thus had large leaves and no flowers, whereas the second set was conducted in late summer/winter (March-August 2007) when the leaves of *C. odorata* were smaller and flowers were dominating the plants. This agrees with Liggitt (1983) that *C. odorata* flowers in winter, and that the onset of flowering prevents further growth of vegetative parts (McFadyen,

1991) including leaves which are the food source for larvae of *P. insulata*. Hence, it is possible that early pupation or variation in the number of instars was triggered by the decrease in nutritional quality of *C. odorata*. The reduction in number of instars possibly resulted in reduced pupal mass in 2007, as Calvo and Molina (2005) recorded that body size in insects is modified or determined by environmental factors affecting larval development. Contrary to expectation, *P. insulata* from both populations were healthier in the laboratory in early autumn and winter compared to spring and summer, as, in the latter seasons, the laboratory cultures tended to be more highly affected by the fungus *I. fumosorosea*. It is known that *Pareuchaetes* species are susceptible to diseases in the laboratory, and that this affects the viability of released insects. Previously, Kluge and Caldwell (1993a,b) found a microsporidian disease identified as *Nosema* sp in their cultures of *Pareuchaetes* species.

The J population used for the trials has been in the laboratory since its arrival in 2002 (from an original founder colony of 25 larvae collected in the field in Jamaica) and was only augmented with 38 larvae collected in 2003, 59 larvae in 2004 and 10 larvae with one batch of eggs collected from Jamaica in 2005 (C. Zachariades, ARC-PPRI, pers. comm.). High mortality in the J population in 2007 could be attributed to the fact that the culture had recovered from a single egg batch at the SASRI laboratory at the end of 2006, with the remainder of the population having been killed by the fungus. Therefore, in 2007 the J population was highly inbred (all siblings), which in most lepidopteran species results in reduced female fertility and unviable progeny (Saccheri *et al*., 1996; Higashiura *et al*., 1999; Saccheri *et al*., 2005). Also, because of inbreeding, the J population could only show limited resistance to environmental changes such as food quality. In contrast, the insects comprising the F population were regularly collected from the site at

Cannonbrae where the Florida population had established. At the end of 2006, the fungus destroyed the entire F laboratory culture, and a new culture was collected from the field at Cannonbrae.

On reaching 4th instar, larvae were transferred to 1l Freezette trays lined with moistened filter papers to preserve moisture, but the filter papers were found dry after the first day of transferring the larvae, during the 2007 trials, hence, both populations were affected by desiccation; however, only the F population managed to survive through to adulthood in good numbers with 61% of pupae eclosed. The J population had only 10% of pupae eclosing and that could be attributed to inbreeding which led to a lower resistance to stress than in the F larvae.

Two factors, fungal infection in 2006 and inbreeding in 2007, could explain the ambiguous results in Table 3.3, whereby the J population appeared fitter in 2006 but considerably weaker than the F population in 2007. Therefore, before one can make definitive conclusions about which insect population is better adapted, it would be advisable to repeat these trials using recently collected cultures of *P. insulata* from Florida and Jamaica, with similar numbers of larvae collected from the field in these areas.

In any event, given the above constraints, these results emphasize the importance of keeping laboratory cultures that are being used for trials or field releases, free of disease. Also, they emphasize the importance of maintaining genetic diversity in such cultures and avoiding the development of weakened laboratory strains. Failure to achieve the above culturing conditions can lead to ambiguous test results, as was likely the case in

this study (i.e. the 2006 versus the 2007 data). Given that differences between the two populations (albeit artificial) were shown, the implications of mixing populations with differing levels of fitness were investigated in Chapter 4.

CHAPTER 4: COMPARATIVE BIOLOGY OF PURE-BRED, CROSS-BRED AND BACK-CROSSED POPULATIONS OF *PAREUCHAETES INSULATA*

4.1 Introduction

Given the differences that were demonstrated between the two populations for *P. insulata* in Chapter 3, the first aim of this part of the study was to examine the effects of cross-breeding between the two populations. This was done by measuring key fitness factors in the hybrids for comparison with the parent populations and thereby determining whether the fitness of the parent populations was decreased, increased or unchanged by hybridisation. The second aim was to examine the effects of back-crossing the hybrid progeny with the parent stocks by measuring the same fitness factors. It was possible that the results of these trials could explain patterns of *P. insulata* establishment and their impact on *C. odorata* populations in South Africa. In addition, they could resolve the question of whether or not it is good practice to release different populations of a biocontrol agent in the same country or region. This chapter firstly presents the results of hybridization between F and J populations of *P. insulata* in the laboratory with comparisons to the parent populations studied in Chapter 3. Secondly, the results of back-crossing the hybrid populations with the pure parent populations are considered.

4.2 Biology of cross-bred populations

4.2.1 Adult fecundity and egg viability

At the beginning of the 2006 crossbreeding trials, the fungus *I. fumosorosea* was widespread in the laboratory cultures, being particularly virulent during the development from larvae to pupae. Hence most of the results were biased due to the fungus, rather than

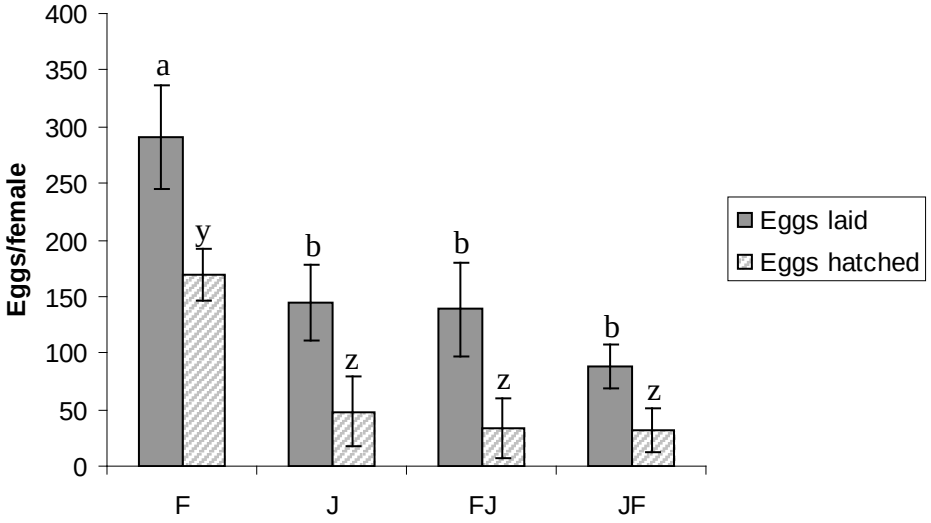
the true situation; some of these were omitted. Mating of the F♂ X J♀ (FJ) and J♂ X F♀ (JF) crosses started on the first night after adult eclosion, as in the parent populations. On day 1 when put together in the cage, the females from ten FJ pairs each laid an average of 27.1 eggs while females from the ten JF pairs each laid an average of 10.5 eggs, from which an average of 19.4 larvae (72%) and 9.0 larvae (86%) hatched from the FJ and JF egg batches, respectively. Eggs from the FJ crosses were laid over a period of 11 nights, whereas the duration of egg laying in the JF crosses was 7 nights. When the individual pairs were put in plastic oviposition bottles, female adults from both FJ and JF crosses displayed reduced fecundity (eggs laid) compared to the F females (P = 0.005, ANOVA, log10 transformation for non-normal data), rather following J population trends in fecundity (P = 0.118, ANOVA, log10 transformation) (Fig. 4.1a, Table 4.1). Similarly, the egg viability of both crosses was reduced compared to that of the F population (P = 0.05, REML Chi pr), but was similar to that of the J population with lower numbers of eggs hatched (Fig. 4.1a, Table 4.1) (P > 0.05, ANOVA, Fisher's protected test at 5% l.s.d. = 28.57).

In 2007, mating again started on the first night of the trials and the mean number of eggs laid by each of the FJ females was 33.0, from which 27.8 eggs (84%) hatched; the five JF females did not lay any eggs on the first night, as for the J population. Eggs from the FJ crosses were laid over a period of 9 nights and the eggs from JF crosses over 7 nights. When individual pairs were put in plastic oviposition jars, FJ and JF females had reduced fecundity compared to the F females (P = 0.01, ANOVA), but the difference in fecundity was not significant between these females and those of the J population (P = 0.07, ANOVA) (Fig. 4.1b, Table 4.1). The FJ and JF crosses also followed the trend of the J females in that fewer eggs hatched than for the F females (Fig. 4.1b); however, a

statistically significant difference between FJ, JF, J and F could not be detected ($P = 0.72$, REML Chi pr angular transformation of % hatched) due to high variability between replicates. The mean (± SD) number of eggs retained in the ovarioles of females from F, J, FJ and JF populations averaged 5.2 ± 4.38 (range: 0-12), 24.4 ± 33.78 (0-73), 30 ± 55.94 (2-150) and 18.8 ± 25.49 (0-94), respectively. However, statistically significant differences could not be detected here ($P = 0.716$, ANOVA); this was also attributed to high variability between replicates. The FJ crosses had four fertile females with spermatophores; however, one of the replicates had very few eggs that hatched and the spermatophore found in the female was loaded with whitish contents, showing that not all sperm were used during fertilization. Three of the females from the JF crosses were fertile and the spermatophores were empty, meaning that the sperm had been used to fertilize eggs.

As in the parent populations, the eggs arising from both crosses took four days to hatch. However, in 2007, although the cross proceeded normally between the F♂ and the J♀ (FJ) and produced fertile eggs (indicated because some of the eggs turned grey on the fourth day as per normal), some subsequently turned maroon and failed to hatch. However, only a small proportion of eggs did so, from one egg batch of one pair. The mating between the J♂ and the F♀ (JF) proceeded normally.

(a)



(b)

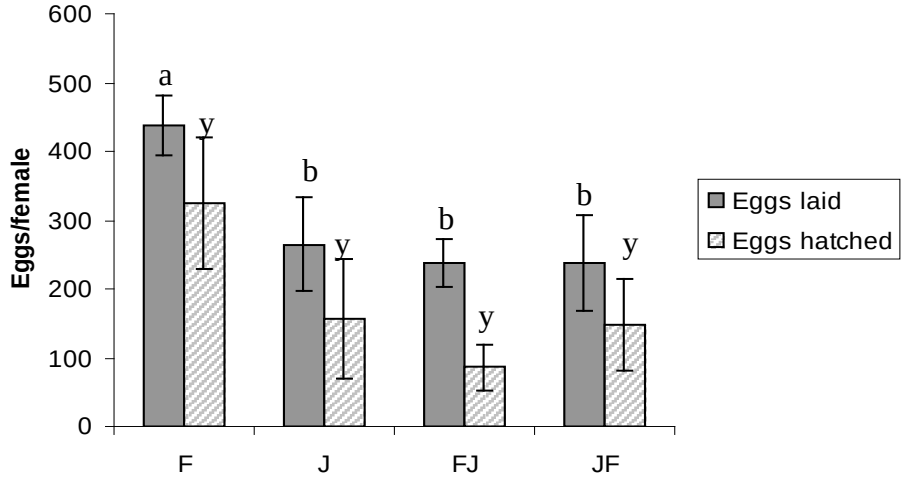


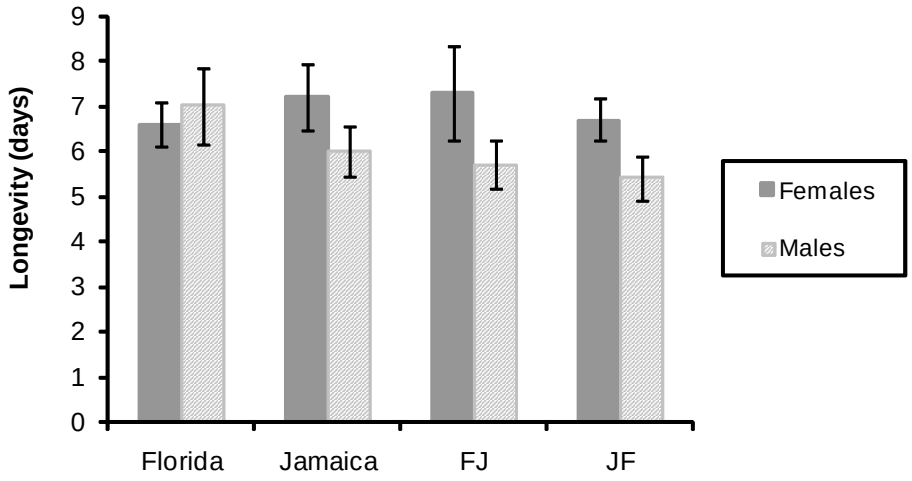
Figure 4.1. Mean (± SE) number of eggs laid and hatched from females of the pure populations of F and J, and cross-bred females (FJ and JF), during trials conducted in 2006 (a) and 2007 (b). Different letters indicate significant differences. Statistical comparisons (ANOVA, log10 transformation for non-normal data and ANOVA followed by l.s.d.) are made between populations.

4.2.2 Adult longevity

In 2006, the longevity of *P. insulata* adults used for the F♂ x J♀ (FJ) and J♂ x F♀ (JF) crosses was similar to those used in the pure matings (*P* = 0.463, ANOVA) (Fig. 4.2a, Table 4.2). There was also no significant difference in longevity between males and females used for the FJ and JF crosses compared to males and females used in the pure matings (*P* = 0.725, ANOVA, log10 transformation for non-normal data).

In 2007, P. insulata adults from the F population displayed significantly higher longevity relative to the adults used for the FJ cross (P = 0.020, ANOVA log10 transformation for non-normal data) (Fig. 4.2b, Table 4.2). However, there was no statistically significant difference in the longevity of adults from the F population and those used in the JF crosses, or between the J population and those used for the FJ and JF crosses (P = 0.074, ANOVA, log10 transformation for non-normal data) (Fig. 4.2b, Table 4.2). There was also no significant difference in the longevity of males and females (when analysing genders separately) used for the FJ and JF crosses as in the males and females used in the pure mating (F and J), i.e. males and females had similar longevity (P = 0.162, ANOVA log10 transformation for non-normal data).

(a)



(b)

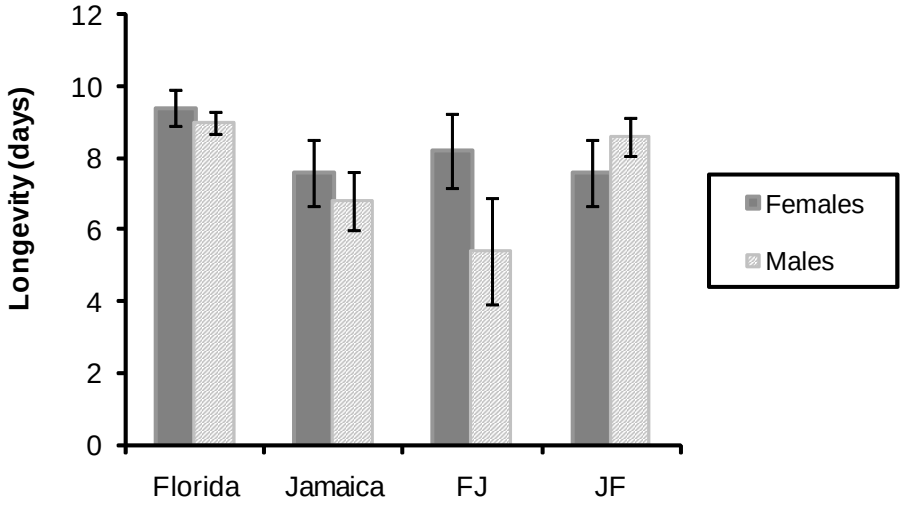


Figure 4.2. Mean (± SE) longevity of females and males from the pure mating (F and J) versus the cross-mating (FJ and JF), during trials conducted in 2006 (a) and 2007 (b).

4.2.3 Larval survival and development

In 2006, the hybrid larvae had significantly slower development (from oviposition to pupae) compared to the parent populations (P = 0.033, ANOVA) (Table 4.3). Percentage pupation in the hybrids was reduced significantly compared to that of the parent populations (P < 0.001, ANOVA angular transformation for non-normal data of %

pupation) (Table 4.4). There was no significant difference in pupal masses of female and male progeny of both crosses compared to those of the parent populations (P = 0.156, ANOVA). As in the parent populations, female pupae of the hybrids were heavier than the male pupae (P < 0.001, ANOVA) (Table 4.3). At 26^{o}C and 71% RH, the adult progeny from the FJ and JF crosses eclosed within an average of 10 ± 3.16 and 11 ± 1.87 (mean ± SD) days, respectively, after pupation. These were not significantly different from the parent populations (P = 0.563, ANOVA) (Table 4.3). The J population displayed a higher percentage eclosion compared to the F population, FJ and JF hybrids (P < 0.01, ANOVA, angular transformation for non-normal data of % eclosion); however, there was no statistical difference between the F population and the FJ hybrids (P > 0.05, ANOVA, Fisher's protected least significant difference test at 5%). The JF hybrids displayed the lowest percentage eclosion but this was similar to the FJ hybrids (P > 0.05, ANOVA, Fisher's protected least significant difference test at 5%) (Table 4.4). Development time from date of oviposition to adult eclosion was longer for the hybrids compared to the parent populations (P < 0.01, ANOVA) (Table 4.3).

In 2007, there was no significant difference in the development of hybrid larvae (both FJ and JF) (from oviposition to pupation) compared to parent populations (P = 0.17, ANOVA) (Table 4.3). There were notable reductions in pupal masses of the hybrids, compared to 2006 (Table 4.3). There were no significant differences between pupal masses, within gender, of female and male progeny of the crosses in relation to the pupal masses of females and males of the parent populations (P = 0.429, ANOVA). The female pupae were heavier than the male pupae in both hybrids and parent populations (P < 0.001, ANOVA) (Table 4.3). The JF hybrids displayed significantly higher percentage pupation (Table 4.4) than the J population (P < 0.001, ANOVA angular transformation)

but this was similar to the F population ($P > 0.05$, ANOVA, Fisher's protected least significant difference test at 5%). The FJ hybrids had an intermediate percentage pupation between parent populations ($P > 0.05$, ANOVA, Fisher's protected least significant difference test at 5%) (Table 4.4). At 27 ^{o}C and 75% RH, the adult progeny from the FJ and JF crosses eclosed within an average of 11 ± 3.28 and 11 ± 2.94 (mean ± SD) days, respectively after pupation, as in the parent populations ($P = 0.82$, ANOVA) (Table 4.3). The J population displayed a significantly lower percentage eclosion compared to both FJ and JF hybrids ($P < 0.001$, ANOVA); however, there was no significant difference in the percentage eclosion between the hybrids and the F population ($P > 0.05$, ANOVA, Fisher's protected least significant difference test at 5%) (Table 4.4). As in 2006, the developmental time from oviposition to adulthood was longer in the hybrids compared to the parent populations ($P = 0.01$, ANOVA) (Table 4.3). In 2007, there was no significant difference in eclosion of males and females as sex ratios were similar between the parent population and hybrids when the sexes were considered together (i.e. females eclosed in the same numbers as males, with no skewed sex ratio) (Table 4.5). Because of the high mortality rates, insufficient data are available for a proper comparison of the sex ratios of the hybrids for the 2006 trials.

In 2006, the Population Fitness Index (PFI) suggested that both FJ and JF hybrids of *P. insulata* developed poorly on *C. odorata*. Both hybrids had a similar score, but they attained significantly lower scores compared to the J and F populations (Table 4.4). In 2007, the JF hybrids attained a similar score to the F population. The FJ hybrids attained a score higher than that for the J population and lower than for the F population and FJ hybrids, but none of these differences were significant (Table 4.4.).

4.3 Biology of the population obtained from the back-cross of the hybrids with the parental (pure) populations

4.3.1 Adult fecundity and egg viability

In 2006, no eggs were deposited on the first night from any of the FJ or JF back-crosses; eggs were only found after individual pairs were placed together in the plastic oviposition jars. Females from the FJ♂ X F♀, FJ♀ X F♂, FJ♀ X J♂ and FJ♂ X J♀ back-crosses laid eggs over a period of 10, 4, 5 and 6 nights, respectively. The F female adults displayed significantly higher fecundity compared to all four FJ back-crosses ($P < 0.001$, ANOVA, log10 transformation for non-normal data) while females of the FJ back-crosses followed the fecundity trends of the J female adults ($P > 0.05$, ANOVA, Fisher's protected test at 5% l.s.d. = 122.2) (Table 4.1). There was no significant difference in egg viability (i.e. numbers of larvae that hatched from the eggs) between the four FJ back-crosses and the J population ($P = 0.055$, ANOVA). However, egg viability of the FJ back-crosses was significantly reduced relative to that of the F females except for that of FJ♂ X F♀ back-crosses ($P < 0.05$, ANOVA, Fisher's protected test at 5% l.s.d = 99.2). Eggs from the JF♀ X F♂, JF♂ X F♀ and JF♀ X J♂ back-crosses were all laid over a period of 6 nights while eggs from the JF♂ X J♀ back-cross were laid over 7 nights. With one exception (JF♀ X F♂), female adults from the F population showed significantly higher fecundity compared to those used in the JF back-crosses ($P = 0.05$, ANOVA, log10 transformation for non-normal data); the females used in all JF back-crosses followed the trend of the J population females with lower fecundity ($P = 0.323$, ANOVA). Similarly, all the JF back-crosses had significantly reduced egg viability compared to the F females ($P = 0.009$, ANOVA) but similar egg viability to that of the J female adults ($P > 0.05$, ANOVA, Fisher's protected test at 5% l.s.d. = 79.5) (Table 4.1).

In 2007, on the first night the FJ♀ X F♂ back-crosses laid an average of 16 eggs, all of which hatched, the FJ♂ X J♀ back-crosses laid an average of 60.8, all of which hatched, while the FJ♂ X F♀ and the FJ♀ X J♂ back-crosses laid no eggs. Eggs from the FJ♂ X F♀ and FJ♂ X J♀ back-crosses were laid over 7 nights, the FJ♀ X F♂ back-crosses over a period of 8 nights and eggs from the FJ♀ X J♂ over 6 nights. When individual pairs were put in plastic oviposition jars, the fecundity of the FJ♂ X F♀ back-crosses was intermediate between the parent populations ($P > 0.05$, ANOVA, Fisher's protected test at 5% l.s.d. = 154.6). The FJ♂ X J♀, FJ♀ X J♂ and FJ♀ X F♂ back-crosses displayed significantly reduced fecundity compared to the F population ($P = 0.02$, ANOVA), but similar fecundity to that of the J population females ($P > 0.05$, ANOVA, Fisher's protected test at 5% l.s.d. = 154.6) (Table 4.1). There was no significant difference in the number of eggs retained in the ovarioles between the females used in the FJ back-crosses and those from the pure populations, due to high variability in these numbers ($P = 0.840$, ANOVA $(x + 1)^{-0.5}$ transformation to stabilise type variances) (Table 4.1). Egg viability (hatch rates) of the FJ♂ X F♀ back-crosses was intermediate between the parent populations, while the FJ♂ X J♀, FJ♀ X J♂ and FJ♀ X F♂ back-crosses showed reduced egg viability compared to the F population, but similar to the J population females. However, these differences between the back-crossed and pure populations were not significant ($P = 0.84$, ANOVA) due to high variability between replicates (Table 4.1). The FJ♂ X F♀ and FJ♂ X J♀ back-crosses had five fertile females with empty spermatophores (i.e. all sperm were used in fertilization), the FJ♀ X F♂ back-crosses had four fertile females with spermatophores, one of which was loaded (i.e. not all sperm were used in fertilization), and the FJ♀ X J♂ back-cross had two fertile females with spermatophores of which one was loaded.

On the first night, the JF♀ X F♂ and JF♂ X F♀ back-crosses did not lay any eggs, while the JF♀ X J♂ back-crosses laid an average of 35.0 eggs, of which an average of 28.2 eggs (81%) hatched. The JF♂ X J♀ back-crosses laid an average of 56.8 eggs, of which an average of 51.0 eggs (90%) hatched. Eggs from the JF♀ X F♂, JF♀ X J♂, JF♂ X J♀ and JF♂ X F♀ back-crosses were laid over a period of 8, 7, 5 and 9 nights, respectively. When the individual pairs were put in plastic oviposition jars, the JF♀ X F♂ and JF♂ X F♀ back-crosses had intermediate fecundity between the two parent populations ($P > 0.05$, ANOVA, Fisher's protected test at 5% l.s.d. = 154) (Table 4.1). The JF♂ X J♀ and JF♀ X J♂ back-crosses had significantly reduced fecundity compared to the F female adults ($P = 0.01$, ANOVA) but similar fecundity to the J female adults ($P > 0.05$, ANOVA, Fisher's protected at 5% l.s.d. = 154) (Table 4.1). Similarly, the number of eggs hatched from the JF♀ X F♂ and JF♂ X F♀ back-crosses was intermediate between the parent populations, while the JF♂ X J♀ and JF♀ X J♂ back-crosses had fewer eggs hatched compared to both F and J female adults, although these differences were not significant. There were no significant differences in egg viability between the back-crossed and parent populations ($P = 0.202$, ANOVA) due to high variability between replicates. In one pair of the JF♀ X J♂ back-crosses, the female 'knotted' (i.e. remained stuck after copulation) with a male after having laid some eggs; the male then died and the female remained attached until she died. There was no significant difference in the number of eggs retained in the ovarioles of the JF back-crosses compared to the parent populations ($P = 0.297$, ANOVA, $(x + 1)^{-0.5}$ transformation to stabilise type variances) (Table 4.1). The JF♀ X F♂ and JF♀ X J♂ back-crosses had four fertile females with spermatophores, one of which was loaded (i.e. not all sperm were used in fertilization),

whereas the JF♂ X F♀ and JF♂ X J♀ back-crosses had five fertile females with empty spermatophores (i.e. all sperm were used in fertilization).

4.3.2 Adult longevity

In 2006, *P. insulata* adults used in the FJ♀ X F♂ and FJ♂ X J♀ back-crosses lived for significantly fewer days than adults used in the pure-population matings ($P < 0.001$, ANOVA) (Table 4.2). The female adults from the FJ♂ X F♀ back-crosses lived significantly longer than the males and longer than the female and male adults of the other three FJ back-crosses as well as the parent populations ($P = 0.002$, ANOVA). The adults used in the FJ♀ X J♂ and FJ♂ X F♀ back-crosses had similar longevity compared to that of the parent populations ($P = 0.069$, ANOVA). *Pareuchaetes insulata* adults from the JF♂ X J♀ back-crosses had significantly reduced longevity compared to the parent populations ($P = 0.01$, ANOVA) (Table 4.2). However, the adults from the JF♀ X F♂, JF♂ X F♀ and JF♀ X J♂ back-crosses displayed similar longevity to the parent populations ($P = 0.351$, ANOVA). There was no significant difference in the longevity of male relative to female adults between the parent populations and all four JF back-crosses ($P = 0.506$, ANOVA) (Table 4.2).

In 2007, *P insulata* adults used in all four FJ back-crosses displayed significantly lower longevity compared to the F adults used in the pure population matings ($P = 0.008$, ANOVA) but their longevity was similar to the J adults used in the pure population matings ($P = 0.973$, ANOVA) (Table 4.2). There was no significant difference in the longevity of males and females used in the FJ back-crosses ($P = 0.347$, ANOVA), as with the parent populations; i.e. females had similar longevity to males (Table 4.2). *P. insulata* from all four JF back-crosses displayed significantly lower longevity compared to the F

population adults (*P* = 0.004, ANOVA) but displayed similar longevity to the J population adults (*P* > 0.05, ANOVA, Fisher's protected test at 5% l.s.d = 1.44) (Table 4.2). Females used in the JF♀ X F♂ and JF♂ X J♀ back-crosses had significantly higher longevity than the males (*P* = 0.049, ANOVA). There was no interaction in female and male longevity of *P. insulata* between parent populations and JF♂ X F♀ and JF♀ XJ ♂ back-crosses (*P* = 0.593,ANOVA) i.e. females and males had similar longevity.

Table 4.1. Mean (± SD) egg fecundity (number laid and retained in the ovarioles) and embryonic viability (number of eggs hatched) of the pure-bred, cross-bred and back-crossed progeny of *Pareuchaetes insulata*.

Mating pair	No. of replicates	No. of eggs laid[1,2]	Lifetime fecundity[3]	No. of eggs hatched[1,2]	Total mean no. of eggs hatched[3]	% hatched[3]	No. eggs retained in ovarioles
2006: Pure-bred and cross-bred							
Florida ♂ X Florida ♀	5	291.2 ± 103.40[a]	294.4	169.2 ± 51.46[a]	171	58	*
Jamaica ♂ X Jamaica ♀	5	144.6 ± 75.42[b]	167.6	48.4 ± 68.10[b]	70.2	42	*
Florida ♂ X Jamaica ♀ (FJ)	10	139 ± 131.18[b]	166.1	33.2 ± 85.11[b]	52.6	32	*
Jamaica ♂ X Florida ♀ (JF)	10	88.7 ± 60.97[b]	99.2	31.3 ± 61.90[b]	40.3	41	*
2006: Back-crossed							
FJ♂ X F♀	5	159.8 ± 95.84[b]	159.8	79.6 ± 112.26[ab]	79.6	50	*
FJ♀ X F♂	5	72.8 ± 88.30[b]	72.8	39 ± 83.89[b]	39	54	*
FJ♀ X J♂	4	68.8 ± 40.79[b]	68.8	19.8 ± 39.5[b]	19.8	29	*
FJ♂ X J♀	5	108.8 ± 122.32[b]	108.8	31.6 ± 70.66[b]	31.6	29	*
JF♀ X F♂	5	107.4 ± 100.56[ab]	107.4	57 ± 72.33[b]	57	53	*
JF♂ X F♀	5	99.8 ± 87.98[b]	99.8	19.8 ± 44.27[b]	19.8	20	*
JF♂ X J♀	5	101.6 ± 75.92[b]	101.6	29.8 ± 61.19[b]	29.8	29	*
JF♀ X J♂	5	99.2 ± 64.87[b]	99.2	46.4 ± 63.57[b]	46.4	47	*
2007: Pure-bred and cross-bred							
Florida ♂ X Florida ♀	5	437.6 ± 97.02[a]	534	324.6 ± 211.04[a]	420	79	5.2 ± 4.38[a]
Jamaica ♂ X Jamaica ♀	5	264.8 ± 152.74[b]	264.8	157.4 ± 193.42[a]	157.4	59	24.4 ± 33.78[a]
Florida ♂ X Jamaica ♀ (FJ)	5	236.4 ± 77.97[b]	269.4	86.2 ± 76.06[a]	114	42	18.8 ± 25.49[a]
Jamaica ♂ X Florida ♀ (JF)	5	238.4 ± 155.94[b]	238.4	147.2 ± 150.22[a]	147.2	62	30 ± 55.94[a]
2007: Back-crossed							
FJ♂ X F♀	5	353.8 ± 73.41[ab]	353.8	276 ± 70.50[a]	276	78	9.2 ± 12.48[a]
FJ♀ X F♂	5	151.2 ± 85.59[b]	167.2	82 ± 75.26[a]	98	59	2.6 ± 1.82[a]
FJ♀ X J♂	5	200 ± 167.25[b]	200	105.8 ± 187.57[a]	105.8	53	26 ± 51.12[a]
FJ♂ X J♀	5	236.6 ± 125.48[b]	297.4	173.4 ± 106.58[a]	234.2	79	1.2 ± 1.64[a]
JF♀ X F♂	5	362.6 ± 155.20[ab]	362.6	241.6 ± 221.08[a]	241.6	67	28.8 ± 28.42[a]
JF♂ X F♀	5	312.6 ± 94.14[ab]	312.6	243.4 ± 106.03[a]	243.4	78	3.6 ± 2.19[a]
JF♂ X J♀	5	222.2 ± 131.14[b]	180.4	104 ± 35.16[a]	160.8	89	4.4 ± 4.83[a]
JF♀ X J♂	5	145.4 ± 39.82[b]	202.2	100.2 ± 134.59[a]	157	78	3.6 ± 4.93[a]

[1]Values with different letters within the same column, within the same year, are significantly different to each other (see text for the *P* values and more details).
[2]From day 2 on, when separated into pairs.
[3]Includes day 1, when all 10 adults were kept together, therefore no SD could be calculated.
*Values not available

Table 4.2. Mean (± SD) adult longevity of the pure-bred, cross-bred and back-crossed progeny of *Pareuchaetes insulata*.

Mating pair	No. of replicates	Adult longevity (days)		
		Female[1]	Male[1]	Adults[1,2]
2006: Pure-bred and cross-bred				
Florida ♂ X Florida ♀	5	6.6 ± 1.14 [a]	7.0 ± 1.87 [a]	6.8 ± 1.48[a]
Jamaica ♂ X Jamaica ♀	5	7.2 ± 1.64[a]	6.0 ± 1.22[a]	6.6 ± 1.51[a]
Florida ♂ X Jamaica ♀ (FJ)	10	7.3 ± 3.30[a]	5.7 ± 1.70[a]	6.5 ± 2.69[a]
Jamaica ♂ X Florida ♀ (JF)	10	6.7 ± 1.49[a]	5.4 ± 1.51[a]	6.1 ± 1.61[a]
2006: Back-crossed				
FJ♂ X F♀	5	9.2 ± 1.14[c,y]	4.6 ± 2.41[a,z]	6.9 ± 3.03[a]
FJ♀ X F♂	5	4 ± 1.00[b]	2.8 ± 1.31[b]	3.4 ± 1.26[b]
FJ♀ X J♂	4	5.8 ± 2.50[a]	5.0 ± 2.94[a]	4.3 ± 2.56[a]
FJ♂ X J♀	5	5.0 ± 2.55[b]	3.0 ± 0.00[b]	4.0 ± 2.00[b]
JF♀ X F♂	5	4.2 ± 1.92[a]	5.8 ± 1.48[a]	5.0 ± 1.83[a]
JF♂ X F♀	5	6.8 ± 1.30[a]	5.2 ± 2.05[a]	6.0 ± 1.83[a]
JF♂ X J♀	5	4.6 ± 2.70[b]	3.8 ± 1.79[b]	4.2 ± 2.20[b]
JF♀ X J♂	5	5.2 ± 1.92[a]	5.0 ± 1.00[a]	5.1 ± 1.45[a]
2007: Pure-bred and cross-bred				
Florida ♂ X Florida ♀	5	9.4 ± 1.14[a]	9.0 ± 0.71[a]	9.2 ± 0.92[a]
Jamaica ♂ X Jamaica ♀	5	7.6 ± 2.07[b]	6.8 ± 1.79[b]	7.2 ± 1.87[b]
Florida ♂ X Jamaica ♀ (FJ)	5	8.2 ± 2.28[b]	5.4 ± 3.29[b]	6.8 ± 3.05[b]
Jamaica ♂ X Florida ♀ (JF)	5	7.6 ± 2.07[a]	8.6 ± 1.14[a]	8.1 ± 1.66[ab]
2007: Back-crossed				
FJ♂ X F♀	5	7.4 ± 1.14[b]	6.8 ± 1.48[b]	7.1 ± 1.29[b]
FJ♀ X F♂	5	6.6 ± 1.14[b]	6.6 ± 1.52[b]	6.6 ± 1.26[b]
FJ♀ X J♂	5	7.8 ± 2.28[b]	7.6 ± 1.52[b]	7.7 ± 1.83[b]
FJ♂ X J♀	5	7.4 ± 1.52[b]	7.6 ± 0.89[b]	7.5 ± 1.18[b]
JF♀ X F♂	5	7.4 ± 1.67[b,y]	5.4 ± 1.14[b,z]	6.3 ± 1.58[b]
JF♂ X F♀	5	7.0 ± 1.22[b]	7.8 ± 1.64[b]	7.4 ± 1.43[b]
JF♂ X J♀	5	7.4 ± 1.95[b,y]	5.6 ± 1.14[b,z]	6.5 ± 1.78[b]
JF♀ X J♂	5	7.2 ± 1.79[b]	7.6 ± 2.07[b]	7.4 ± 1.84[b]

[1]Values with different letters within the same column (a-c) and across columns (y-z), within the same year, are significantly different to each other.
[1,2]Values representing combined genders.

Table 4.3. Mean (± SD) pupal masses and duration of development of the pure-bred, cross-bred and back-crossed progeny of *Pareuchaetes insulata*. Sample size in parentheses.

Mating pair	Pupal mass (mg)[1]		Duration of development (days)[1]		
	Female	Male	Oviposition-pupation	Pupation-eclosion date	Oviposition-eclosion date
2006: Pure-bred and cross-bred					
Florida ♂ X Florida ♀	0.22 ± 0.04 (19)[a]	0.16 ± 0.05 (14)[a]	31 ± 4.03 (35)[a]	10 ± 1.92 (20)[a]	38 ± 3.28 (20)[a]
Jamaica ♂ X Jamaica ♀	0.24 ± 0.05 (29)[a]	0.16 ± 0.01 (17)[a]	30 ± 5.01 (45)[a]	9 ± 1.87 (40)[a]	38 ± 2.87 (40)[a]
Florida ♂ X Jamaica ♀ (FJ)	0.23 ± 0.13 (39)[a]	0.17 ± 0.01 (55)[a]	35 ± 4.09 (94)[b]	10 ± 3.16 (31)[a]	43 ± 2.74 (31)[b]
Jamaica ♂ x Florida ♀ (JF)	0.24 ± 0.04 (30)[a]	0.18 ± 0.05 (42)[a]	35 ± 3.39 (72)[b]	11 ± 1.87 (21)[a]	43 ± 2.73 (21)[b]
2006: Back-crossed					
FJ♂ X F♀	0.11 ± 0.00 (1)[a]	*	40 ± 0.00 (1)[b]	*	*
FJ♀ X F♂	0.23 ± 0.42 (7)[a]	0.15 ± 0.46 (8)[a]	34 ± 4.21 (15)[b]	10 ± 2.08 (4)[a]	45 ± 1.53 (4)[b]
FJ♀ X J♂	0.22 ± 0.06 (6)[a]	0.19 ± 0.05 (8)[a]	37 ± 2.52 (14)[b]	*	*
FJ♂ X J♀	*	*	*	*	*
JF♀ X F♂	0.22 ± 0.02 (3)[a]	0.15 ± 0.05 (4)[a]	40 ± 2.74 (7)[b]	12 ± 0.00 (1)[a]	52 ± 0.00 (1)[b]
JF♂ X F♀	*	*	*	*	*
JF♂ X J♀	*	*	*	*	*
JF♀ X J♂	0.21 ± 0.07 (3)[a]	0.19 ± 0.07 (3)[a]	32 ± 2.83 (6)[b]	*	*
2007: Pure-bred and cross-bred					
Florida ♂ X Florida ♀	0.19 ± 0.02 (23)[a]	0.14 ± 0.02 (18)[a]	24 ± 1.00 (41)[a]	10 ± 1.53 (26)[a]	35 ± 2.08 (26)[a]
Jamaica ♂ X Jamaica ♀	0.19 ± 0.05 (4)[a]	0.15 ± 0.02 (10)[a]	30 ± 8.07 (14)[ab]	10 ± 1.83 (7)[a]	35 ± 1.53 (7)[ab]
Florida ♂ X Jamaica ♀ (FJ)	0.20 ± 0.03 (72)[a]	0.15 ± 0.02 (73)[a]	32 ± 5.22 (145)[a]	11 ± 3.28 (93)[a]	42 ± 4.72 (93)[b]
Jamaica ♂ X Florida ♀ (JF)	0.21 ± 0.03 (127)[a]	0.15 ± 0.02 (114)[a]	30 ± 4.27 (241)[a]	11 ± 2.94 (196)[a]	41 ± 5.65 (196)[b]
2007: Back-crossed					
FJ♂ X F♀	0.19 ± 0.03 (42)[a]	0.14 ± 0.03 (46)[a]	30 ± 5.23 (88)[b]	9 ± 1.87 (73)[a]	40 ± 3.89 (73)[b]
FJ♀ X F♂	0.18 ± 0.05 (19)[a]	0.16 ± 0.02 (17)[a]	35 ± 5.71 (36)[b]	9 ± 1 (11)[a]	46 ± 3.88 (11)[b]
FJ♀ X J♂	0.19 ± 0.05 (59)[a]	0.15 ± 0.03 (58)[a]	30 ± 5.41 (117)[b]	10 ±1.58 (90)[a]	37 ± 3.56 (90)[b]
FJ♂ X J♀	0.17 ± 0.03 (35)[a]	0.13 ± 0.03 (37)[a]	32 ± 5.89 (72)[b]	9 ± 2.73 (24)[a]	38 ± 3.36 (24)[b]
JF♀ X F♂	0.19 ± 0.03 (58)[a]	0.13 ± 0.02 (34)[a]	30 ± 5.13 (92)[a]	9 ± 1.58 (66)[a]	38 ± 3.32 (66)[ab]
JF♂ X F♀	0.21 ± 0.04 (59)[a]	0.16 ± 0.02 (40)[a]	27 ± 4.32 (99)[a]	9 ± 1.58 (79)[a]	37 ± 3.32 (79)[ab]
JF♂ X J♀	0.19 ± 0.03 (28)[a]	0.14 ± 0.03 (29)[a]	32 ± 3.87 (57)[a]	10 ± 2.56 (28)[a]	43 ± 5.83 (28)[ab]
JF♀ X J♂	0.18 ± 0.04 (39)[a]	0.13 ± 0.03 (47)[a]	33 ± 7.21 (86)[a]	9 ± 1.58 (28)[a]	40 ± 3.48 (28)[ab]

[1]Values with different letters within the same column, within the same year, are significantly different to each other (see text for the *P* values and more details).
*Values not available (no survival).

Table 4.4. Percentage survival to pupation and eclosion and population fitness index (PFI) scores of the pure-bred, cross-bred and back-crossed progeny of *Pareuchaetes insulata* on *Chromolaena odorata*.

Mating pair	% pupation[1]	% eclosion[1]	PFI score[1]
2006: Pure-bred and cross-bred			
Florida ♂ X Florida ♀	70^{b}	51^{b}	0.50^{a}
Jamaica ♂ X Jamaica ♀	92^{a}	90^{a}	0.72^{b}
Florida ♂ X Jamaica ♀ (FJ)	23^{c}	25^{bc}	0.12^{c}
Jamaica ♂ X Florida ♀ (JF)	18^{c}	17^{c}	0.17^{c}
2006: Back-crossed			
FJ♂ X F♀	0.42^{d}	0^{c}	0.11^{c}
FJ♀ X F♂	7^{d}	9^{c}	0.13^{c}
FJ♀ X J♂	22^{c}	0^{c}	0.11^{c}
FJ♂ X J♀	0^{d}	0^{c}	0^{c}
JF♀ X F♂	5.42^{c}	1^{c}	0.17^{c}
JF♂ X F♀	0^{c}	0^{c}	0^{c}
JF♂ X J♀	0^{c}	0^{c}	0^{c}
JF♀ X J♂	3.24^{c}	0^{c}	0.21^{c}
2007: Pure-bred and cross-bred			
Florida ♂ X Florida ♀	84^{ab}	61^{ab}	0.67^{a}
Jamaica ♂ X Jamaica ♀	28^{c}	10^{c}	0.18^{b}
Florida ♂ X Jamaica ♀ (FJ)	56^{bc}	35^{b}	0.35^{ab}
Jamaica ♂ X Florida ♀ (JF)	93^{a}	71^{a}	0.65^{a}
2007: Back-crossed			
FJ♂ X F♀	83^{ab}	78^{a}	0.53^{a}
FJ♀ X F♂	44^{c}	21^{c}	0.23^{b}
FJ♀ X J♂	100^{a}	69^{a}	0.63^{a}
FJ♂ X J♀	78^{ab}	29^{c}	0.41^{ab}
JF♀ X F♂	81^{a}	68^{ab}	0.51^{a}
JF♂ X F♀	81^{a}	85^{a}	0.63^{a}
JF♂ X J♀	69^{a}	36^{c}	0.49^{a}
JF♀ X J♂	83^{a}	27^{c}	0.38^{ab}

[1]Values with different letters within each column, within each year, are significantly different to each other (see text for the *P* values and more details). Hybrids and back-crosses are compared to pure matings within each year.
Comparisons are made between pure matings versus cross matings and between pure matings versus FJ backcrosses and pure matings versus JF backcrosses within same year.

Table 4.5. Sex ratios arising from the numbers of females and males that eclosed following the development of larvae arising from the pure-bred, cross-bred and back-crossed populations of *Pareuchaetes insulata*.

Mating pair	No.♀ eclosed	No.♂ eclosed	Sex ratio ♀: ♂	Chi square	*P* value
Florida	16	10	1:0.62	1.385	NS
Jamaica	3	4	0.75:1	0.143	NS
FJ	55	38	1:0.69	3.108	NS
JF	108	88	1:0.81	2.041	NS
FJ♂ X F♀	36	37	0.97:1	0.014	NS
FJ♀ X F♂	8	3	1:0.38	2.273	<0.05
FJ♀ X J♂	38	52	0.73:1	2.178	NS
FJ♂ X J♀	17	7	1:0.41	4.167	<0.05
JF♀ X F♂	41	25	1:0.61	3.879	NS
JF♂ X F♀	48	31	1:0.65	3.658	NS
JF♀ X J♂	17	11	1:0.65	1.286	NS
JF♂ X J♀	17	11	1:0.65	1.286	NS

4.3.3 Larval survival and development

In both 2006 and 2007, eggs took 4 days to hatch, as did those in the parent populations. However, in 2006 larvae from all the back-crosses displayed delayed development compared to the F ($P = 0.047$, ANOVA), and the J ($P = 0.011$, ANOVA) populations i.e. larvae took longer to reach pupation than in each of the parent populations (F and J). In 2007 larvae from the FJ back-crosses took longer to develop than the F population ($P = 0.042$, ANOVA) but a similar length of time to the J population ($P = 0.615$, ANOVA). There was no significant difference in larval development times between the JF back-crosses compared to the F population ($P = 0.073$, ANOVA) and the J population ($P = 0.904$) (Table 4.3). In 2006, there were no significant differences in pupal masses between the progeny of the parent populations and those of the four FJ back-crosses ($P = 0.175$, ANOVA). There were also no significant differences in pupal masses between the progeny of the JF back-crosses and

those of the parent populations (P = 0.32, ANOVA) (Table 4.3). The female pupae from the FJ and JF back-crosses were significantly heavier than the males, as in the parent populations (P < 0.001, ANOVA) (Table 4.3). Similarly in 2007, there was no interaction in pupal masses of *P. insulata* from the FJ back-crosses (P = 0.938, ANOVA) and JF back-crosses (P = 0.889, ANOVA), i.e. female and male pupal masses were similar in all crosses as in the parent populations, and the female pupae from both the FJ and JF back-crosses were heavier than those of the males (P < 0.001, ANOVA) (Table 4.3).

In 2006, there was a significant difference in percentage pupation and percentage eclosion between the progeny of the pure populations and those of all FJ back-crosses (P < 0.001, ANOVA Fisher's protected test at 5% l.s.d. = 14.39 and 21.13, for percentage pupation and eclosion, respectively) (Table 4.4). Similarly, the progeny of all JF back-crosses displayed significantly lower pupation and eclosion rates compared to the parent populations (P < 0.001, ANOVA, Fisher's protected test at 5% l.s.d = 15.57 and 34.95 for percentage pupation and eclosion, respectively). In 2007, the percentage pupation of progeny of the F population was not significantly different to that of the FJ♀ X J♂, FJ♂ X F♀ and FJ♂ X J♀ back-crosses (P > 0.05, ANOVA, Fisher's protected test at 5% l.s.d. = 28.40) but was significantly different to the progeny from the FJ♀ X F♂ back-crosses that displayed a lower percentage pupation that was similar to that recorded in the J population (P < 0.05, ANOVA, Fisher's protected test at 5% l.s.d. = 28.40) (Table 4.4). Contrarily, for percentage eclosion in 2007, progeny from the F population were only similar to progeny from the FJ♂ X F♀ and FJ♀ X J♂ back-crosses, while progeny from the FJ♂ X J♀ and FJ♀ X F♂ back-crosses were similar to those of the J population (P > 0.05, ANOVA, Fisher's protected test at 5% l.s.d. = 24.23). Progeny from all four JF back-crosses were not significantly

different in percentage pupation from the F population ($P > 0.05$, ANOVA, Fisher's protected test l.s.d. = 27.49), but differed significantly from the J population, which displayed a lower percentage pupation ($P < 0.001$, ANOVA, Fisher's protected test l.s.d. = 27.49) (Table 4.4). Contrarily, progeny from the JF♂ X F♀ and JF♀ X F♂ back-crosses displayed a higher percentage eclosion compared to the J population but similar to the F population, and the percentage eclosion of those of the JF♂ X J♀ and JF♀ X J♂ back-crosses were similar to the J population ($P > 0.05$, ANOVA, Fisher's protected test at 5% l.s.d. = 18.98) (Table 4.4).

Developmental durations from pupation to adult eclosion and for the entire life cycle (from oviposition to adult eclosion) of all eight back-crosses for both 2006 and 2007 are summarised in Table 4.3. Because of the high mortality rate in 2006, insufficient data were available for a meaningful statistical comparison of the developmental period from egg hatching to adult eclosion and pupation to adult eclosion for the progeny of JF and FJ back-crosses (Table 4.3) and of the sex ratios for both the 2006 JF and FJ back-crossed progeny (Table 4.5). In 2007, the development period from oviposition to adult eclosion was longer for progeny from the FJ back-crosses compared to those of the F ($P = 0.013$, ANOVA) and J ($P = 0.028$, ANOVA) populations. The development period from oviposition to adult eclosion was longer for JF back-crosses compared to that of the F population ($P = 0.045$, ANOVA) but there was no significant difference relative to the J population ($P = 0.09$, ANOVA) (Table 4.3). In addition, there was no significant difference in adult eclosion when the sexes were considered together i.e. the number of females that eclosed was equal to the numbers of males that eclosed (Table 4.5). Only the FJ♀ X F♂ and FJ♂ X J♀ back-crosses showed a significantly female-biased sex ratio, while the sex ratios approached equality in the other back-crosses, the hybrids and in the pure populations (Table 4.5).

In 2006, the Population Fitness Index of the progeny from the FJ back-crosses showed that the FJ♂ X F♀, FJ♀ X F♂ and FJ♀ X J♂ back-crosses developed poorly on *C. odorata* and attained significantly lower scores compared to the progeny of the parent populations, while there was no survival to adulthood of the FJ♂ X J♀ back-cross progeny (Table 4.4). Progeny from the JF♀ X F♂ and JF♀ X J♂ back-crosses also developed poorly on *C. odorata* and attained lower scores compared to the parent populations, while progeny from the JF♂ X F♀ and JF♂ X J♀ back-crosses did not survive to pupation (Table 4.4). In 2007, progeny from the FJ♂X F♀, FJ♀ X J♂, JF♂ X F♀, JF♀ X F♂ and JF♂ X J♀ back-crosses attained similar scores to that of the F population for the index of population fitness (Table 4.4). Those from the FJ♂ X J♀ and JF♀ X J♂ back-crosses attained scores that were intermediate to both parent populations (not significantly different from either), and progeny from the FJ♀ X F♂ back-cross attained a score similar to that of the J population (Table 4.4).

4.4 Discussion

In both 2006 and 2007, the crosses between the F males and J females (FJ) and between the J males and F females (JF) proceeded normally; the two populations mated freely and produced normal offspring, which developed to adulthood. Most of the eggs obtained in 2007 from the FJ crosses hatched; some of those that did not hatch did not shrivel while still yellow (as do infertile eggs laid by a virgin female), but started to develop, became grey in colour as is normal shortly before hatching, but failed to hatch and subsequently turned maroon. Cock and Holloway (1982) recorded similar observations when crosses were made between *P. pseudoinsulata* males from Trinidad and *P. insulata* females from Yucatan and Guanacaste (Central American strains). As a consequence, it was confirmed that *P. insulata* and *P. pseudoinsulata* are distinct

species separated by a reproductive barrier. However, this phenomenon was not observed in eggs from the JF hybrids in the current study, nor in those from the back-crosses. It seems that this single FJ mating replicate was an anomaly; studies of genetic differentiation using mitochondrial DNA conducted on *P. insulata* from Florida, Cuba and Jamaica showed no genetic differences between the three populations (Assefa, 2007). Both crosses, in both years, followed the J population patterns in terms of fecundity and egg viability and showed reduced fecundity and egg viability relative to the F population. Similarly, in 2006 the FJ and JF back-crosses followed the J population patterns in fecundity and egg viability. In 2007, one of the FJ back-crosses had fecundity intermediate to the parent populations, while the other three FJ back-crosses showed reduced fecundity as in the J population. Two JF back-crosses had intermediate fecundity and egg viability between the parent populations while the other two showed reduced fecundity and egg viability as in the J population. When conducting experiments using hybrids and back-crossed larvae of two species of tiger swallowtail butterflies, *Papilio canadensis* Rothschild and Jordan and *Papilio glaucus* Linnaeus (Lepidoptera: Papilionidae), which had different levels of esterase detoxification enzymes, Scriber *et al* (1999) found that the back-crossed larvae had intermediate levels of these. Such intermediate traits are frequently found in the progeny of two different parent populations or closely related species that have been hybridized and back-crossed, although the F1 hybrids and F2 back-crosses progeny may not be exactly intermediate between the parent populations (Henig, 2000).

Regarding egg viability of females and availability of spermatophores, in 2007 the FJ crosses had four fertile females and with spermatophores, while the JF crosses had three fertile females and with spermatophores. The FJ♂ X F♀ and FJ♂ X J♀ back-

crosses had all five females fertile and with spermatophores, while FJ♀ X F♂ back-cross had four fertile females and FJ♀X J♂ back-crosses had only two fertile females with spermatophores. On the other hand, two JF back-crosses had five fertile females with spermatophores and the other two back-crosses had four fertile females with spermatophores. From all the matings, the availability of spermatophores probably determined the fertility of the females because eggs from the females with spermatophores hatched, while the ones from females without spermatophores did not hatch; instead they shrivelled while still yellow, as do eggs laid by a virgin female (Cock and Holloway, 1982). There were virgin females in some replicates even though all females were paired with males. According to Wedell (2005), the decision of Lepidoptera females to mate may be influenced by several factors including their readiness to mate (i.e. reaching reproductive maturity), and the females often choose healthier (fitter) males (i.e. having nutrients that increase fecundity). In this case the unmated females may not have found the males suitable for mating, even though exposed to five males on the first night.

Females with loaded spermatophores in their genital tract had very few eggs hatched compared to females with empty spermatophores. Studies conducted on insects including Diptera and Lepidoptera (especially the Arctiidae to which *P. insulata* belongs) showed that polyandry (multiple mating between one female and several males) could be a possible reason for sperm not leaving the spermatophore or fertilizing female eggs (LaMunyon and Eisner, 1993, 1994; Tram and Wolfner, 1998, Wedell, 2005). During mating, the males of some species ejaculate pheromones that cause mated females to become less attractive to other males (Tram and Wolfner, 1998; Wedell, 2005). For example, LaMunyon (2001) indicated that some male Lepidoptera

may influence sperm competition by producing a non-fertilizing sperm called apyrene sperm that has been shown to delay female remating. However, in other insects such as *Drosophila melanogaster* Linnaeus (Diptera: Drosophilidae) the female antiaphrodisiacs can be short-lived, leading to remating which allows sperm from different mates to compete for fertilization (Tram and Wolfner, 1998). Similarly, in Lepidoptera these antiaphrodisiacs can have a transient effect (Wedell, 2005). For example, the female adults of *Utetheisa ornatrix* Linnaeus (Lepidoptera: Arctiidae) can also mate with several males (LaMunyon and Eisner, 1993) but one set of sperm is used at the expense of the others and females often use the sperm from larger partners (LaMunyon and Eisner, 1994). Usually, the sperm of earlier mating is either displaced by the fresh sperm or incapacitated by the seminal fluid produced by the accessory glands of the last male, and the earlier matings avoid this effect in females by triggering oviposition and repressing the acceptance of males for remating, therefore increasing the number of eggs fertilised by the first sperm prior to the next mating (Xue and Noll 2000; Wedell, 2005). Multiple mating is therefore possible in *Pareuchaetes insulata* and sperm observed in females with loaded spermatophores were probably the incapacitated ones from previous matings, and the few eggs that hatched were fertilized by one of the matings. In addition, LaMunyon (2001) observed that in the noctuid moth *Heliothis virescens* Fabricius (Lepidoptera: Noctuidae), sperm may fail to fertilise eggs because they do not leave the spermatophore (also possible in *P. insulata*) or are swept from the reproductive tract by passing eggs.

In 2006, the adults used in both crosses displayed similar longevity to those used in pure matings; however, in 2007 adults used in the JF crosses had similar longevity to the adults used for F X F matings, while adults used in the FJ crosses had longevity

similar to those used in J X J matings. In 2006, adults used in the FJ♀ X F♂ and FJ♂ X J♀ back-crosses showed reduced longevity compared to those used in pure matings, whereas the adults from the FJ♀ X J♂ and FJ♂ X F♀ back-crosses had longevity similar to those used in pure matings. In 2007, the FJ and JF back-crosses followed the J X J matings (the population with the lower adult longevity). These results showed that the longevity of the adults used in the crosses may be similar to that of either the parent populations or be reduced relative to the parent populations; i.e. no consistent trends were apparent. As the two pure populations have shown different strength in fecundity and egg viability with the J population being weaker, it was important to see the longevity of the hybrid adult progeny when mated with the parent stocks.

In 2006, the high mortality rate due to the *I. fumosorosea* fungus resulted in lower pupation and eclosion in the progeny of the crosses and back-crosses and hence reduced PFI relative to the parent populations. However, for the larvae that pupated, the pupal masses were similar to the parent populations. In 2007, traits of both parent populations were reflected in the survival rates of the crossed and back-crossed progeny, in that these progeny displayed PFI scores and percentages of pupation and eclosion that were either similar to one population, higher/lower than both or intermediate between the two populations, i.e. no consistency again. In 2007, although head capsules and number of days spent per each instar were not used to determine numbers of instars in hybrid and back-crossed progeny, numbers of instars were reduced or variable in these progeny; this was indicated in reduced pupal masses, as with the parent populations. However, in 2007 some of the FJ hybrid larvae lagged behind in development, and finally died before pupating, when their siblings were far ahead in development as was recorded in the J population. Studies conducted in

different insect species showed that delayed development is caused by several factors including activity of juvenile hormones (Yoichi, 1990), changes in temperature (Alapanida *et al*., 2002), insecticides (Wu *et al*., 2002) or poor diet (Babic *et al.*, 2008). Oliver (1979) showed that in some lepidopteran hybrid progeny, abnormally long developmental periods may be caused by growth hormone irregularities, which may be presumably due to cross-inviability. It is possible that my data supported his hypothesis, which explains that within each interpopulation crossing and back-crossing, there is great individual variation in responses, with some individuals not completing the embryonic development while others may complete development to as far as pupation or adulthood before inviability is revealed (Oliver, 1979). This was evident in JF hybrids where many pupae eclosed but some of the adults died a few hours after eclosion. Although some of the crossed and back-crossed progeny showed a high percentage pupation (Table 4.3), reduction in percentage eclosion showed that mortality/inviability occurred or was expressed in the pupal stages. Oliver (1972) showed both skewed and unskewed sex ratios when he worked on hybrid viability within and between some Lepidoptera species. Like Oliver (1972), my data showed mainly unskewed sex ratios, as both sexes were mostly equally represented ($P > 0.05$) after the crosses and back-crosses, relative to the parent populations (Table 4.5). Only the FJ♀ X F♂ and FJ♂ X J♀ back-crosses showed significantly skewed sex ratios ($P < 0.05$) in favour of females. The latter agree with the hypothesis of Oliver (1979) that occasionally the adult sex ratios of crossed and back-crossed populations in some Lepidoptera are heavily skewed in favour of females. According to Haldane's rule (Haldane, 1922), the affected sex, i.e. where there are fewer of them (in this case males), is usually the heterogametic sex.

Some of the comparisons made in relation to progeny from the crosses and back-crosses showed features that were similar to those of the F population, which showed better fitness mainly in 2007 than the J population, and some features were intermediate between the two populations. However, in most cases the crosses and back-crosses followed the trends of the weakest population. These results also showed that the two populations can mate successfully and produce normal adults, which support our hypothesis that the two populations may have interbred in the field, and probably some of the damage noticed at the intersection zone where the two populations might have overlapped could be attributed to the hybrids of these populations. On the other hand, they showed that when different populations of the same agent species have different levels of fitness, even if this is due to inbreeding of one of them (in this case J) in the laboratory, their hybridization may negatively affect the fitness of stronger populations (in this case F). This emphasizes the importance of refreshing cultures, especially of Lepidoptera, with fresh field material on a regular basis to boost genetic variability. Although Assefa (2007) found no differences between the mitochondrial DNA of J and F populations, some of the observed variations between the pure strains and the hybrids and the back-crosses may nevertheless be due to intrinsic genetic variation between populations rather than only decreased genetic fitness due to the history of the culture (e.g. inbreeding or other laboratory artifacts). These results, therefore, also reinforce the caution of Hoffmann *et al.* (2002) that the possible consequences of mixing genotypes of a biocontrol agent species should be investigated before different populations are released in the same country.

In conclusion, the results of this chapter caution against the mixing of distinct populations, or even distinct laboratory cultures, of a biocontrol agent, either in the laboratory or in the field, in the country of introduction. For whatever reason, natural or artificial, different populations or laboratory cultures may have differing levels of fitness and hybridization may lead to a disproportionate loss of fitness in the stronger one.

CHAPTER 5: GENERAL CONCLUSIONS AND RECOMMENDATIONS

The comparative biology and the cross-breeding of populations of the biocontrol agent *Pareuchaetes insulata* originating in Florida, USA, and Jamaica were studied in the laboratory. Insects for the Florida culture were collected from an established field site in South Africa, whereas those for the Jamaica culture had been collected from the field in Jamaica and reared through several generations in the laboratory, for release in South Africa. Life-history traits likely to give an indication of relative fitness of each population (pure-bred, cross-bred and back-crossed) were measured over two consecutive years. Hybridization experiments included crossing both F males and J females and J males and F females, in case fitness was affected differently. Similarly, back-crossing of the resulting hybrid progeny with parental stock was conducted using all possible combinations (a total of eight; see Tables 4.4 and 4.5), to also determine whether fitness is differentially affected.

In the overall assessment of several life-history parameters in this study, the F population generally had higher fitness than the J population, and would therefore probably have a better chance of establishing. However, factors other than this may have been important in accounting for the establishment success of the F population and the failure of the J population, including the large number of larvae released at the F site that established, the long period over which they were released, the correct set of environmental conditions at this site, and the larger number of sites (18 in total) at which the F population was released compared to the J population (4 in total). Although the J population showed lower fitness in terms of fecundity and egg viability, in both years, in 2006 the J larvae had larger head capsules compared to F population

larvae, which also took longer to develop than the J larvae, implying lower fitness in the F population. In 2006, the J larvae also showed better survival to pupation and adulthood and the J population displayed a higher score in the Population Fitness Index than the F population. Only in 2007 did the J population suffer higher mortality than the F population. In addition to higher fecundity and egg viability, the F population displayed better adult longevity, survival rates, higher leaf consumption rates and a higher Population Fitness Index score than the J population on *C. odorata* during 2007.

The J population used for the trials had been in the laboratory since being collected in the field in Jamaica in 2002 and augmented in 2003, 2004 and 2005. Decreases in the values of several fitness parameters from 2006 to 2007, such as higher mortality of larvae and pupae, and slower larval development could be attributed to the fact that the J culture had recovered from a single egg batch at the SASRI laboratory at the end of 2006, with the remainder of the population having been killed by the fungus *I. fumosorosea*. Therefore, in 2007 the J population was highly inbred, which in most lepidopteran species results in reduced female fertility and unviable progeny (Saccheri *et al*., 1996). In contrast, the insects comprising the F population were regularly collected from the site at Cannonbrae where the Florida population had established. Also, having survived in the field in South Africa for several years, it is likely that the Florida population is now better adapted to local conditions, including the SA biotype of *C. odorata*. At the end of 2006, the fungus destroyed the entire F laboratory culture, and a new culture was collected from the field at Cannonbrae. Because of inbreeding, it is likely that the J population had limited resistance to environmental changes (Higashiura *et al*., 1999) such as food quality and climate. For example, both populations' larvae were desiccated in the laboratory when the moistened filter paper

used to preserved moisture dried out during the 2007 trials and only the F population subsequently survived through to adulthood.

The F and J populations mated successfully and produced normal F1 hybrid results. Similarly, back-crossing the hybrid progeny with parental populations resulted in the production of F2 progeny and adults for all mating combinations. This supports our hypothesis that the two populations may have interbred in the field, and that some of the damage of *C. odorata* in this 'hybrid zone' could probably be attributed to the hybrid larvae of these populations. In hybridisation and back-crossing experiments conducted in both years, high variability in measured life-history/fitness parameters was evident among the two hybrids and the eight back-crosses. These parameters varied in value between being similar to the two parental populations, intermediate between them and being higher/lower than either parental population. However in most cases the hybrid and back-crossed progeny followed the trends of the weaker J population. For example, even when the hybrid and back-crossed progeny showed higher pupation rates, the eclosion rate was reduced. These results showed that when different populations of the same agent species have different levels of fitness, their hybridization may negatively affect the fitness of stronger populations, to the extent that the fitness of the hybrids is as low as the weaker population, rather than being intermediate to the two (Henig, 2000). The lower fitness of the J population, particularly in 2007, that may have been caused by various reasons already mentioned, may thus have reduced the fitness of the established F population where the two came into contact.

The results presented in this study do not allow distinction to be made between (i) intrinsic differences in measured biological parameters between populations, (ii) those arising as a response to feeding on SA *C. odorata*, and (iii) those arising through the history of the laboratory culture. In order to achieve this, it would be necessary to repeat these trials using recently collected cultures of *P. insulata* from Florida and Jamaica, with similar numbers of larvae collected from the field in these areas; and to cross-expose these cultures to both the SA *C. odorata* biotype to plants collected in Florida.

This study emphasises the importance of keeping colonies being held for mass-rearing free of disease. It is not known where the fungus that afflicted the cultures originated, but it may well have been picked up from the *C. odorata* cuttings brought into the laboratory from the field for feeding larvae. If this is the case, a change in this aspect may be desirable (e.g. sterilization of cuttings prior to feeding (Boughton & Pemberton, 2008) or using potted plants of *C. odorata*). The study also emphasises the importance of maintaining genetic diversity and possibly avoiding the development of laboratory strains by regular re-collection of fresh field material culturing.

Finally, the results of the study caution against the mixing of distinct populations or laboratory cultures of species of a single agent, either in the laboratory or in the field in the country of introduction. Whatever the reason, different populations and cultures may have different levels of fitness, and hybridization may lead to disproportionate loss of fitness for the stronger one. However, should such mixing of populations be considered, then the possible outcomes should be investigated by studies such as this, prior to its implementation.

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