BIOLOGICAL CONTROL OF RED WATER FERN IN SOUTH AFRICA

AJ McConnachie · MP Hill

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BIOLOGICAL CONTROL OF RED WATER FERN IN SOUTH AFRICA

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Joint Report to the Water Research Commission of the Projects:

"Potential biological control of *Azolla filiculoides*: Taxonomy of Azolla pinnata and re-importation of a sold adapted strain of *Stenopelmus refinasus*"

And

"Post-release evaluation of *Stenopelmus rufinasus* Gyllenhal (Coleoptera: Curculionidae) – a natural enemy released against red water fern, *Azolla filiculoides* Lamarck (Pteridophyta: Azollaceae) in South Africa

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AND

"Post-release evaluation of *Stenopelmus rufinasus* Gyllenhal (Coleoptera: Curculionidae) – a natural enemy released against red water fern, *Azolla filiculoides* Lamarck (Pteridophyta: Azollaceae) in South Africa"

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EXECUTIVE SUMMARY

Red water fern, *Azolla filiculoides* Lamarck (Azollaceae) is one of the five declared aquatic weeds in South Africa. This plant is native to South America and was first recorded in South Africa in 1948. Until the 1980s, the fern was confined to small streams and farm dams in the Colesburg area of the Northern Cape Province. However, a combination of phosphate-rich waters and the lack of natural enemies lead to its inevitable spread throughout the country. Dense mats of the weed (up to 30cm thick) severely degraded aquatic ecosystems and impacted all aspects of their utilization. The failure of mechanical control and the undesirability of herbicide control in the aquatic environment made red water fern an ideal candidate for biological control in South Africa.

The frond-feeding weevil, *Stenopelmus rufinasus* Gyllenhal (Coleoptera: Curculionidae) was collected from *Azolla caroliniana* Willd. in Florida, USA, and following host specificity screening it was released on *A. filiculoides* in South Africa in December 1997. Fears that this insect was a new association on *A. filiculoides* and therefore might not be all that effective and the fact that is was collected from a tropical area and might not be able to establish in the cooler regions of the country, prompted two courses of action. First, surveys were conducted on *A. filiculoides* in Argentina in an attempt to more closely match both the host species and climate compatibility. Second, an additional insect, the flea beetle, *Pseudolampsis guttata* (LeConte) (Coleoptera: Chrysomelidae) was collected on *A. caroliniana* in Florida and imported for host specificity screening.

However, both of these courses of action proved to be failures as the weevil collected in Argentina was a different species, *Stenopelmus brunneus* (Hustache), and the quarantine culture was subsequently destroyed without being released. In addition, *S. rufinasus* had not only established in some of the the coldest areas of South Africa but was having an impressive impact on the weed in these areas (see below).

The flea beetle (*P. guttata*) was, however, still screened for possible release. Favourable biological characteristics of this species included: long-lived and mobile

adults; short immature development times; high rate of increase, and high per capita feeding rates. In the laboratory, this species was a superior agent to the weevil (*S. rufinasus*). Host specificity screening, however, indicated that the flea beetle is an oligophagous species, capable of utilizing several species in the genus *Azolla* and could pose a threat to native, southern African species. The flea beetle was therefore rejected as a possible biological control agent for red water fern in South Africa.

The taxonomy of the genus *Azolla* remains difficult as it relies on differentiation of the reproductive structures. The morphology of the sporophyte is very plastic and can change dramatically depending on day length, temperature and, most importantly, water chemistry. The most reliable characteristic appears to be the structure of the sporocarps. For the purposes of the host specificity screening of the flea beetle, we had material identified by an expert from Portugal. In addition to this, we sent material to an expert in Hong Kong. Opinions of the experts differed in that the one from Portugal identified three taxa of *Azolla* in southern Africa: *Azolla filiculoides* (introduced), *Azolla pinnata* var. *africana* (from Zambia and Malawi) and *Azolla pinnata* var. *asiatica* (also introduced) from a pan in the Bluff Nature Reserve near Durban. The expert from Hong Kong confirmed the identification of *Azolla filiculoides* and *Azolla pinnata* var. *africana* but was uncertain of the identification of *Azolla pinnata* var. *asiatica*. Therefore the taxonomy of southern African *Azolla* species warrants further study.

The second part of this project was to conduct a thorough post-release evaluation of the weevil, *Stenopelmus rufinasus* on red water fern. The first release was made on a one hectare dam in a bird sanctuary in Pretoria in December 1997. Nine hundred weevils were released on the dam, which was 100% covered by a 5 cm thick mat of the weed. By February 1998 (2 months later) the red water fern mat had collapsed and from a $2m^2$ sample of decaying material in excess of 30 000 weevils were reared. This was an astonishing phenomenon, which has been successfully repeated throughout the country over the last four years.

To date, the weevils have been released (usually in batches of 100 adults) at some 112 sites throughout South Africa. The current information available on these sites is that the weevil has been responsible for clearing 91 of them completely. For the remaining 21

sites, either the weed has been washed away during flooding, they have not been revisited, or are in the final stages of control. All of the sites have cleared in less than one year. In addition to this, the weevil has migrated to other sites, sometimes up to 300km away from the point of release. It is uncertain if the weevil has been transported on weed by waterfowl, or if there has been short distance dispersal onto other dams with the weed, or if it is as a result of long-range dispersal by the adults. At 7% of the sites the weed has returned up to 2 years after the initial clearance. The weevil has located 90% of these and the weed is again under control.

In order to quantify the impact of the weevil on red water fern, field cage experiments were conduced at five sites in Gauteng Province during summer and winter. At each site, three floating cages (50cm x 50cm x 50cm) were erected and each was inoculated with 1kg of the fern. Two of the cages were gauze covered, while the third only had a small gauze skirt around the base. Ten male and 10 female weevils were introduced to the experimental cage while the other gauze covered cage served as a control for the effect of the weevils. The third cage served as a control for the affect of the gauze on the growth of the fern. Two samples of red water fern were taken from each cage weekly. One of the samples was used to determine the populations of the weevil while the other sample was used to quantify the impact of the weevil on the dry weight of the fern. At all five field sites, total weed clearance was achieved within a period of seven weeks in the summer trial and 14 weeks in the winter trial. The gauze had no significant effect on the growth of the fern which grew normally in both control cages. These cage experiments confirmed the field-release experiments (see above) in that weevil populations are capable of a rapid increase resulting in a dramatic crash of the fern mat.

Although the *S. rufinasus* that was released in South Africa originated in the tropical climes of southern Florida, USA, it was able to establish and have a major impact on the weed even in the coolest areas of South Africa (southern and eastern Free State). We therefore decided to undertake a series of laboratory trials to investigate the thermal tolerance of this weevil in order to be able to predict if there were any areas of the country where it might not establish. The critical thermal limits, or temperatures at which the insect became immobilized varied between 0^{0}C and 5^{o}C (lower limit) and between 45^{o}C and 48^{o}C (upper limit). The lethal temperature at

which 50% of the population was killed after prolonged exposure to that temperature was –12.1°C (lower limit) and 36.5°C (upper limit). The laboratory results confirmed what we had noted in the field, in that although many of the adults would become immobilised during the night in the winter months, and therefore might be exposed to predation and frost, the low winter temperatures would not prevent establishment. Futhermore, the eggs, larvae and pupae were buffered from the air temperatures in that they are endophytic (within the plant tissue). However, their duration of development is likely to be much longer during the winter months. The predictive strengths of two models (CLIMEX and degree-day) were also tested, with both confirming that the establishment of the weevil in South Africa would not be restricted by climate.

Biological control is often cited as the most cost effective weed control option and yet very few cost-benefit analyses have been conducted on weed biological control programmes. The fairly well defined nature of the *Azolla filiculoides* problem in South Africa coupled with the dramatic success of the weevil afforded us the opportunity to conduct a cost-benefit analysis of the programme to date. Questionnaires were sent to 30 land owners (farmers, municipalities, golf courses) that had been affected by the weed to assess the economic impact of the weed. Costs included loss of livestock, loss of irrigation potential, burning out of pumps, construction of alternative water supply facilities and the cost to control the weed, either mechanically or through the use of herbicides. The cost of the weed to aquatic biodiversity was impossible to calculate, but it was no doubt huge. The cost to develop the biological control programme was then offset against the total cost of the weed at all known sites in South Africa over the last 20 years. The benefit to cost ratio of the biological control programme on red water fern in South Africa was calculated at 2.5:1 for 2000, increasing to 13:1 in 2005 and 15:1 in 2010 as the annual costs of the programme decrease. This indicates an enormous return on investment into this research.

The biological control programme against red water fern is a unique example of biological weed control. Seldom, if ever, has there been a biological control programme that has resulted in such a rapid, and drastic decline in a weed population. Obviously long-term monitoring is required to determine the resurgence in the weed

populations and how the weevil is able to locate and reduce these. The initial data suggests that no matter where red water fern appears, the weevil will locate and control it.

The biological control of red water fern in South Africa has been as successful as the programmes on three other aquatic weeds, water lettuce, salvinia and parrot's feather, which leaves water hyacinth as the final aquatic weed to bring under effective biological control. However, the biggest challenge facing aquatic ecosystems in South Africa remains eutrophication, of which invasion of aquatic weeds is only a symptom. It is highly likely that in many of the systems in which red water fern has been controlled, unless the levels of eutrophication are reduced, other and possibly worse aquatic weeds will take hold.

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STORAGE OF DATA

Raw data used for the purposes of this study are lodged at the Plant Protection Research Institute.

CHAPTER 1

INTRODUCTION TO THE BIOLOGICAL CONTROL OF RED WATER FERN IN SOUTH AFRICA

1.1 Introduction

The genus *Azolla* incorporates heterosporous aquatic fern species which have a symbiotic association with the heterocystous cyanobacterium (blue-green alga) *Anabaena azollae* Strasburger which grows within the dorsal leaf lobe cavities of the fern (Peters & Calvert, 1987). The alga can fix atmospheric nitrogen and is able to fulfil the nitrogen requirements of the plant, making it successful in nitrogen deficient waters (Bar *et al.*, 1991).

Azolla filiculoides Lamarck (red water fern) is native to South America (Lumpkin & Plucknett, 1982) and was first recorded in South Africa in 1948 (Oosthuizen & Walters, 1961). It was introduced as a pond plant (Randall[1] pers. comm.). The fern was confined to small streams and farm dams in the Colesburg area (30° 52'16"S/25°19'22"E) for many years. However, phosphate enriched waters, the lack of natural enemies (Hill, 1998a) and dispersal between water bodies by man and waterfowl facilitated an increase in its distribution and abundance. In June 1996, the South Africa Plant Invaders Atlas database had records of *A. filiculoides* in 65 quarter degree squares in South Africa. By May 1998 the weed had been recorded in 152 quarter degree squares (Henderson, 1999). While this rapid increase is more likely to represent an increase in the sampling effort, it must also represent a growth in the abundance of the weed.

Azolla filiculoides is able to undergo rapid vegetative reproduction throughout the year by elongation and fragmentation of small fronds, and under ideal conditions, the daily rate of increase can exceed 15%, which translates to a doubling time for the weed of 5-7 days (Lumpkin & Plucknett, 1982). In addition, the fern can reproduce sexually via the production of spores, especially when the plant is stressed. The

[1] Dr. Rod Randall, Cape Nature Conservation, South Africa

spores can overwinter and are resistant to desiccation, allowing re-establishment of the fern after drought.

The increasing abundance of *A. filiculoides* in conservation, agricultural, recreational and suburban areas over the last twenty years was cause for concern. Among the major consequences of the dense mats (5-30 cm thick) of the weed on still and slow-moving water bodies in South Africa were: reduced quality of drinking water caused by bad odour, colour and turbidity; increase in waterborne, water-based and water-related diseases; increased siltation of rivers and dams; reduced water surface area for recreation (fishing, swimming and water-skiing) and water transport; deterioration of aquatic biological diversity (Gratwicke & Marshall, 2001); clogging of irrigation pumps; drowning of livestock; and reduced water flow in irrigation canals.

1.2 Control

Mechanical and herbicide control options have been suggested for red water fern. However, mechanical control is labour intensive. Small infestations of the weed in accessible areas can be removed with rakes and fine meshed nets, and used as cattle and pig fodder, or compost. The disadvantage of this method is that the rate of increase of the plant is such that a concerted effort is required to keep up with the daily production of even a small infestation, and if eradication was achieved, re-establishment of the weed from spores in the substrate of the water body would be inevitable. The herbicidal control of red water fern using the systemic glyphosate has been suggested (Steÿn *et al.*, 1979; Ashton, 1992) as well as paraquat and diquat (Axelsen & Julien, 1988). The disadvantages of herbicide control for *A. filiculoides* are that it is expensive, especially in view of the extensive follow-up programme required to eradicate plants continually germinating from spores; there is a danger of spray drift onto non-target vegetation and there is a need for well-trained personnel to conduct spraying. The impractical nature of mechanical control and undesirability of herbicide control in the aquatic environment suggested that *A. filiculoides* might be a suitable candidate for biological control.

The frond-feeding weevil, *Stenopelmus rufinasus* Gyllenhal (Coleoptera: Curculionidae) was imported to South Africa from Florida, USA in late 1995 (Hill, 1997). The weevil underwent host specificity testing in quarantine (Hill, 1998b) and

was cleared for release in late 1997 (Hill, 1999). At the time of release there was a lack of knowledge regarding its potential impact. Primarily, there was doubt whether this insect would be effective against *A. filiculoides* because it had been collected on *A. caroliniana* in Florida, and might not establish on *A. filiculoides* in cooler areas of the country as it originated in a tropical region. Therefore a survey was conducted on *A. filiculoides* in a cooler climate in Argentina to collect weevils which might be better suited for release in South Africa (Chapter 2). Furthermore, laboratory trials were conducted to establish the thermal tolerance of the weevil in order to predict where it might and might not establish (Chapter 4).

Seldom in the biological control of weeds has one agent been capable of reducing a weed population to below an acceptable threshold (Andow *et al.*, 1997). Usually a suite of natural enemy species are required to bring about complete biological control. Therefore the flea beetle, *Pseudolampsis guttata* (LeConte) (Coleoptera: Chrysomelidae) was collected from *A. caroliniana* in southern Florida and screened for release in South Africa (Chapter 3). The rationale might seem illogical in view of what is stated above, but at the time it was convenient to import the flea beetle and it appeared to have potential as a biological control agent. The flea beetle was, however, not host specific and was not released in South Africa.

The post release evaluation of a biological control agent is the real test of the success of a programme. Therefore, to quantify the impacts of the weevil in the field, a series of cage experiments were performed (Chapter 5). In addition, the weevil was released throughout South Africa and these release sites were monitored to assess the field impact and dispersal capabilities of the agent (Chapter 6). Both of these studies showed that the weevil was capable of rapidly reducing red water fern populations and dispersing to new sites.

Biological control is often referred to as the most cost-effective form of invading alien weed control as it is self sustainable. However, this is seldom quantified. The well defined nature of the red water fern problem in this country and the short duration of the project allowed us to conduct a cost-benefit analysis of the biological control programme on this fern (Chapter 7). The biological control programme against red water fern was highly successful amounting to a benefit-cost ratio of 2.5:1 in 2000,

increasing to 13:1 in 2005 and 15:1 in 2010 which compares favourably with other successful projects in other parts of the world. The concluding chapter of this study (Chapter 8) summarises the progress of the biological control programme on *A. filiculoides* and formulates recommendations on the long-term management of this weed in South Africa.

CHAPTER 2

SURVEY FOR POSSIBLE COLD-ADAPTED STRAIN/BIOTYPE OF *STENOPELMUS RUFINASUS* GYLLENHAL (COLEOPTERA: CURCULIONIDAE) IN ARGENTINA

2.1 Introduction

Stenopelmus rufinasus was released against red water fern in South Africa in late 1997 (Hill, 1999). This insect, however, was collected in Florida, USA, which has a fairly tropical climate, while the worst infestations of the weed occur in the cooler areas of South Africa (eastern and southern Free State (Hill, 1997)). There were, therefore, concerns that the weevil might not be able to cope with the with the cool winter temperatures in this area and that even if it did establish it might not control the weed. In addition, the weevil was collected from *A. caroliniana* and was therefore a "new association" (Hokkanen & Pimentel, 1984) on *A. filiculoides* in South Africa. There is some evidence to suggest that new associations make more effective biological control agents as there is a lack of homeostasis between the insect and the plant (Hokkanen, 1989). However, recently, Volchansky *et al.* (1999) have shown that the *Dactylopius opuntiae* (Cockerell) that was successful on *Opuntia ficus-indica* (L.) Miller (Cactaceae) and *Opuntia stricta* (Haworth) Haworth (Cactaceae) in Australia was only effective in the control of *O. ficus-indica* and not *O. stricta* in South Africa. It was only after the correct biotype of *D. opuntiae* was imported from *O. stricta* in Australia that biological control of *O. stricta* in South Africa was successful (Volchansky *et al.*, 1999). This study emphasised the importance of host plant and natural enemy biotype matching in the biological control of weeds. Consequently there were also fears that *S. rufinasus* might not establish on *A. filiculoides* due to host plant incompatibility, although it did perform well on the plant under laboratory conditions (Hill, 1998b).

Therefore, the aim of this study was to collect a population of the weevil from *A. filiculoides* growing in cooler areas in Argentina and thereby match both the climate and the species of the plant.

2.2 Materials and Methods

A nine day collecting trip was undertaken to Northern Argentina during December 1997. A total of 10 sites with *Azolla* spp. were visited. All sites were natural wetlands, seeps or small dams. At each site the plants were inspected for external feeders and dissected in the field for endophagous insects. Immature stages were maintained on host plant material until the adult stage emerged. *Azolla* samples were also returned to South Africa where 1/3 of the sample from each site was put through a Berlese funnel, 1/3 was sorted by hand and 1/3 was placed in an emergence box. Voucher specimens of insects, referred to by their accession numbers were lodged with the National Collection of Insects, in Pretoria.

2.3 Results

Unfortunately *A. filiculoides* was only located at two of the sites surveyed (Table 2.1). The identification of the other *Azolla* species found during the survey was uncertain, but was likely to be *A. microphylla* Kaulf. (Hills & Gopal, 1967).

Table 2.1 Sites in northern Argentina where *Azolla* spp. were surveyed for natural enemies.

Plant species	Site Description	Locality
Azolla filiculoides	Entre Rios Province 50 km South of Ceibas	33° 26'12"S / 58°35'23"W
Azolla sp.	Chaco Province Outskirts of Resistencia	27°25'59"S / 58°52'20"W
Azolla sp.	Chaco Province 5km west of Resistencia	27°24'43"S / 58°59'46"W
Azolla sp.	Chaco Province 20km west of Resistencia	27°07'37"S / 59°30'38"W
Azolla sp.	Chaco Province 15km west of Resistencia	27°22'37"S / 59°02'41"W
Azolla sp.	Chaco Province 40km north of Resistencia	27°07'07"S / 58°58'14"W
Azolla sp.	Chaco Province 50km north of Resistencia	27°34'01"S / 58°35'17"W
Azolla sp.	Formosa Province 40km west of Formosa	26°00'07"S / 58°24'45"W
Azolla sp.	Formosa Province 5km south of Formosa	26°12'05"S / 58°13'27"W
Azolla filiculoides	Buenos Aires Province 120km north of Buenos Aires	34°03'30"S / 59°17'47"W

Generally there was a depauperate insect fauna associated with the *Azolla* species in that only four insect herbivore species were collected (Table 2.2). This supports the work by Gomez (1978) and Lumpkin and Plucknett (1982) who suggest that few specialist herbivorous insect species have evolved on the genus *Azolla*.

2.3.1 Lygaeidae (AcSN 1981)

This small, black lygaeid was collected from the plant surface. It was collected at one of the *A.filiculoides* sites and five of the eight *Azolla* sp. sites. Although both adults and nymphs were collected, it is uncertain what they were feeding on. This species certainly does not inflict any noticeable damage to the plants and is of little value as a potential biological control agent.

2.3.2 *Paulinia acuminata* (De Geer, 1773) (Orthoptera: Pauliniidae)

This grasshopper was collected in four of the eight *Azolla* sp. sites. It was fairly abundant at these sites and both adults and nymphs were collected. It was imported into quarantine in South Africa and reared on *A. filiculoides* and submitted for identification. Once the identification had been confirmed, the culture was terminated. *Paulinia acuminata* has been released as a natural enemy of *Salvinia molesta* D.S. Mitchell (Salviniaceae) in Botswana, Fiji, India, Kenya, Sri Lanka, Zambia and Zimbabwe (Julien & Griffiths, 1998). Although it established in some areas, its impact on the weed was negligible and salvinia was brought under effective biological control worldwide with the release of *Cyrtobagous salvinae* Calder and Sands (Coleoptera: Curculionidae) (Julien & Griffiths, 1998).

The grasshopper cultured readily on *A. filiculoides* in the laboratory and it appears to be a generalist species attacking a number of aquatic and riparian plant species (Howard[1], pers. comm.). This species was therefore considered unsuitable for release in South Africa.

[1] Geoffrey Howard, IUCN, Nairobi, Kenya

2.3.3 *Pseudolampsis darwini* (Scherer) (Coleoptera: Chrysomelidae)

Adults of this flea beetle were reared out of both of the *A. filiculoides* sites. They were numerous in the samples taken and appear to be extremely damaging to the plants as the larvae are voracious, burrowing into the rhizome of the fern, eventually killing the plant. They pupate in a hollowed out cavity among the fronds.

This species was brought into quarantine in South Africa as we thought it was *P. guttata*, which we already had in culture from Florida (Chapter 3). Fortunately the cultures were kept separate until we could confirm the identity of this insect. Recently, *Pseudolampsis* was divided into two geographically isolated species, namely *P. guttata* from *A. caroliniana* in the USA and *P. darwini* from *A. filiculoides* in South America (Casari & Duckett, 1997). On confirmation of the identity of this insect as *P. darwini*, the culture was terminated to prevent any mixing of the populations. This insect has great potential as a biological control agent for red water fern in South Africa in that it is extremely damaging, it was collected from a temperate area in Argentina and as it was only collected from *A. filiculoides* and not from *Azolla* sp. which suggests that it might be host specific.

2.3.4 *Stenopelmus brunneus* (Hustache) (Coleoptera: Curculionidae)

This weevil was collected from both of the *A. filiculoides* sites but not from the *Azolla* sp. sites (Table 2.2). Once again, this species was numerous and very damaging to the plant. At both sites, larvae, pupae and adults were collected. We imported the weevil into quarantine in South Africa and submitted material for identification to a weevil expert, Charlie O' Brien in Florida, USA. It came as a surprise that the weevil we had collected was not *S. rufinasus*, but rather *S. brunneus*. According to O' Brien[2] (pers. comm.) *S. rufinasus* does not occur in South America. As in the *Pseudolampsis* example above, the culture of *S. brunneus* was terminated to prevent mixing it with *S. rufinasus*.

[2] Dr. Charles O'Brien, weevil taxonomist, Florida A & M University, Tallahassee, Florida

Table 2.2 Insect herbivores associated with *Azolla filiculoides* and *Azolla* sp. in northern Argentina.

Plant species	Number of samples	Insect species	Incidence[1]
Azolla filiculoides	2	**HETEROPTERA**	
		Lygaeidae (AcSN 1981)[2]	50
		COLEOPTERA	
		Chrysomelidae	
		Pseudolampsis darwini (Scherer)	100
		Curculionidae	
		Stenopelmus brunneus (Hustache)	100
Azolla sp.	8	**ORTHOPTERA**	
		Paulinidae	
		Paulinia acuminata (De Geer, 1773)	50
		HETEROPTERA	
		Lygaeidae (AcSN 1981)[2]	62.5

[1] Incidence is expressed as the percentage of samples in which the insect occurred

[2] Refers to the National Collection of Insects accession number.

2.4 Discussion

The purpose of this study was to collect a culture of *S. rufinasus* from *A. filiculoides* in the temperate areas of Argentina as it was felt that this culture might be more adapted to the control of red water fern in the cooler areas of South Africa. Unfortunately this search failed as *S. rufinasus* does not occur in South America. However, the survey was not a failure in that it produced two species, the weevil *S. brunneus* and the flea beetle, *P. darwini* that could be considered as biological control agents for red water fern. Favourable characteristics of these two species include the fact that they are extremely damaging to *A. filiculoides*, they were collected in a temperate region and are therefore likely to be cold adapted and they were not collected from other *Azolla* sp., which suggests that they may be host specific. The host specificity would need to be verified as the *Azolla* sp. populations were several hundreds of kilometers from the *A. filiculoides* sites and the beetles could be restricted by factors other than host specificity. Should *S. rufinasus* and *P. guttata* (see next chapter) not result in the long-term control of *A. filiculoides* in South Africa, *S. brunneus* and *P. darwini* could be considered for further screening.

CHAPTER 3

LABORATORY HOST RANGE TESTING OF THE FLEA BEETLE, *PSEUDOLAMPSIS GUTTATA* (LECONTE) (COLEOPTERA: CHRYSOMELIDAE), A POTENTIAL NATURAL ENEMY FOR RED WATER FERN, *AZOLLA FILICULOIDES* LAMARCK (PTERIDOPHYTA: AZOLLACEAE) IN SOUTH AFRICA

3.1 Introduction

A single agent rarely effects complete control in the biological control of a weed, rather it requires a suite of natural enemies to reduce the weed populations to acceptable levels (Andow *et. al.*, 1997). Notable exceptions of this are the control of *Salvinia molesta* with *Cyrtobagous salvinae* (Cilliers, 1991a) and the control of water lettuce, *Pistia stratiotes* Linnaeus with the weevil, *Neohydronomus affinis* Hustache (Cilliers, 1991b). Therefore an additional agent was considered for red water fern and the opportunity arose to import the flea beetle, *P. guttata* for screening in South Africa prior to the release of *S. rufinasus*. The flea beetle was collected on *A. caroliniana* in Florida, USA and imported into South Africa in October 1997.

The native range of *P. guttata* appears to include the US states of South Carolina, Louisiana, Alabama and Florida (Balsbaugh & Kirk, 1968; Habeck, 1979). Balsbaugh (1969), however, concluded that *Distigmoptera darwini* Scherer, described from *A. filiculoides* in Uruguay and Brazil, was conspecific with *P. guttata*, suggesting that its native range also included much of South America. However, the species has now been divided into *P. guttata* from the USA and *P. darwini* from South America (Casari & Duckett, 1997; see Chapter 2).

In retrospect, as the target weed is *A. filiculoides*, it would have made sense for us to have tested *P. darwini* rather than *P. guttata*. But the separation of the of the two species was only published after we had imported and begun testing *P. guttata* from Florida.

The biology of *P. guttata* was described by Buckingham and Buckingham (1981). This insect appears suitable for use as a biological control agent as it has a high rate of

increase due to the high fecundity of the females, which produce on average 650 eggs per female in a 132 day oviposition period, and a short time of 24 to 34 days required from egg hatch to adult eclosion through three larval instars (Buckingham & Buckingham, 1981) and a high per capita feeding rate. Center *et al.* (1992) reported that this species was capable of destroying mats of *Azolla* in the southern USA and probably reduces the weedy potential of *A. filiculoides*, which is introduced to Florida.

Here we report on the laboratory host range of *P. guttata* and assess its suitability as a biological control agent for *A. filiculoides* in South Africa.

3.2 Materials and Methods

All studies were conducted in a quarantine glasshouse with fluctuating temperatures of 27 ± 2°C (day) and 20 ± 2°C (night) under natural light conditions, with a photoperiod of about 16 hours in summer and 12 hours in winter. Laboratory host range of *P. guttata* was determined by adult no-choice oviposition and larval starvation trials on a series of plant species selected on relatedness to *A. filiculoides* and habitat (Table 3.1).

3.2.1 Taxonomy of *Azolla* spp.

The taxonomy of the genus *Azolla* at species level is difficult as it relies on the morphology of the sporocarps (Saunders & Fowler, 1992) which are rare in nature, as these plants rely on vegetative reproduction. Two native species have been recorded from southern Africa, *A. nilotica* DeCasine ex. Mett., which is found throughout Africa (Stergianou & Fowler, 1990) and *A. pinnata* var. *africana* (Desv.) R.K.M. Saunders & K. Fowler, which has been recorded from several localities in KwaZulu-Natal Province (KZN) in South Africa and several other countries in southern Africa. We collected *A. pinnata* from KwaZulu-Natal Province, Zambia and Malawi for host specificity testing.

Azolla filiculoides, *A. pinnata* KZN, *A. pinnata* Zambia and *A. pinnata* Malawi were submitted to two *Azolla* taxonomists, Richard Saunders of the University of Hong Kong who revised the species *A. pinnata* (Saunders & Fowler, 1992) and Generosa Teixeira of the University of Lisbon in Portugal. Unfortunately the opinions of the

experts differed in that Teixeira identified three taxa, *A. filiculoides*. *A. pinnata* var. *africana* from Zambia and Malawi and *A. pinnata* var. *asiatica* (also introduced) from a pan in the Bluff Nature Reserve near Durban. Saunders confirmed the identification of *A. filiculoides* and *A. pinnata* var. *africana* but was uncertain of the identity of *A. pinnata* var. *asiatica* and suggested that we regard the material from KwaZulu-Natal, Malawi and Zambia as *A. pinnata*. The taxonomy of the southern African *Azolla* species warrants further study. We still felt that the cultures were clearly distinct in macromorphology and, for the purposes of this study, they have been referred to as *A. pinnata* KZN, *A. pinnata* Zambia and *A. pinnata* Malawi and treated separately.

3.2.2 Host specificity testing

Ten adult male *P. guttata* and ten females that had recently eclosed were confined to each of the test plant species for three days, after which they were removed. The number of adult progeny emerging from each test plant species and the duration of development was recorded. There were 10 replicates for each test plant species. The number of adults emerging and the duration of development of the larvae were compared between the plant species tested using a single factor analysis of variance, followed by Tukey HSD test.

3.3 Results

The host range of *P. guttata* was determined using 18 species in 10 families (Table 3.1). Feeding and oviposition was only recorded on the *Azolla* species and *Salvinia hastata* Desv., which is a floating fern indigenous to southern Africa. The data for the number of adults emerging and the duration of development of the larvae were normally distributed (emergence data: $d = 0.10$, $p > 0.20$, duration data $d = 0.11$, $p > 0.40$) which justified our use of an ANOVA. The number of adults emerging from the no-choice trials differed significantly between the test plant species ($F_{5, 54} = 59.29$, $p = 0.00$) (Table 3.2) with *A. filiculoides* being the preferred host, while *S. hastata* supported very little development. The number of days required for the larvae to develop on the *Azolla* species were similar but the beetles did require significantly longer to develop on *S. hastata* ($F_{5, 438} = 55.40$, $p = 0.00$) (Table 3.2).

Table 3.1 Results of the adult no-choice, feeding and oviposition trials with *Pseudolampsis guttata*.

Plant species	Common name	Feeding[a]	Oviposition[a]
BRYOPHYTA			
Ricciaceae		0	0
Ricciocarpus natans (L.) Corda			
Sphagnaceae			
Sphagnum sp.		0	0
PTERIDOPHYTA			
Isoetaceae			
Isoetes transvaalensis Jermy & Schelpe		0	0
Marseliaceae			
Marselia capensis A. Braun	Common fern	0	0
Marselia sp.		0	0
Azollaceae			
Azolla filiculoides Lamarck	Red water fern	+	+
Azolla pinnata (Kwazulu-Natal)		+	+
Azolla pinnata (Zambia)		+	+
Azolla pinnata (Malawi)		+	+
Azolla nilotica DeCasine ex. Mett.		+	+
Salviniaceae			
Salvinia molesta D.S. Mitchell	Kariba weed	0	0
Salvinia hastata Desv.		+	+
Thelypteriaceae			
Thelypteris confluens (Thumb.) Morton		0	0
ANGIOSPERMAE			
MONOCOTYLEDONAE			
Alismataceae			
Alisma plantago-aquaticum L.	Water alisma	0	0
Lemnaceae			
Lemna sp.	Duck weed	0	0
Wolffia sp.		0	0
Spirodela sp.		0	0
Araceae			
Pistia stratiotes L.	Water lettuce	0	0

[a] In the columns, + represents some feeding on the plants or evidence of oviposition while 0 represents no feeding or oviposition.

Table 3.2. Mean number of adult progeny of *Pseudolampsis guttata* recorded on species of *Azolla* and one species of *Salvinia* during adult no-choice experiments in which ten males and ten females were confined on each species for three days.

Host species	n[a]	Mean no. of adults/replicate [b,d]	n[c]	Mean duration of development (days) [b,c,d]
Azolla filiculoides	10	73.5 (13.4)a	124	24.4 (12.3)a
Azolla pinnata KZN	10	59.8 (15.8)ab	107	25.7 (9.9)a
Azolla pinnata Zambia	10	46.3 (7.9)bc	87	27.7 (15.2)a
Azolla pinnata Malawi	10	32.5 (11.7)c	56	26.4 (8.0)a
Azolla nilotica	10	15.8 (9.1)d	65	29.1 (10.2)a
Salvinia hastata	10	0.6 (3.3)e	6	42.4 (7.8)b

[a] There were ten replicates of ten males and ten females.

[b] Figures in parentheses represent the standard error.

[c] Development time from oviposition to adult eclosion in days.

[d] Mean in columns not followed by the same letter differ significantly at the 5% level of probability (ANOVA followed by Tukey HSD Multiple Range Test).

[e] The sample size of larvae used to determine mean duration of development.

3.4 Discussion

The results show that although *A. filiculoides* was the preferred host, the three *A. pinnata* populations would support development and probably a population of this beetle in the field. Therefore, *P. guttata* is regarded as an oligophage species, utilizing several species within the genus *Azolla*. Although its natural host is *A. caroliniana*, it was able to develop easily on *A. pinnata* during these trials, which is in a different section (*Rhizosperma*) to *A. filiculoides* and *A. caroliniana* both of which are in the section *Azolla* (Stergianou & Fowler, 1990). The concerns about attacks on native species of *Azolla* in southern Africa has resulted in our rejection of *P. guttata* as a potential natural enemy for *A. filiculoides* in South Africa.

CHAPTER 4

THERMAL PHYSIOLOGY AND PREDICTIVE MODELLING OF *STENOPELMUS RUFINASUS'* (GYLLENHAL) (COLEOPTERA: CURCULIONIDAE) POTENTIAL DISTRIBUTION IN SOUTH AFRICA

4.1 Introduction

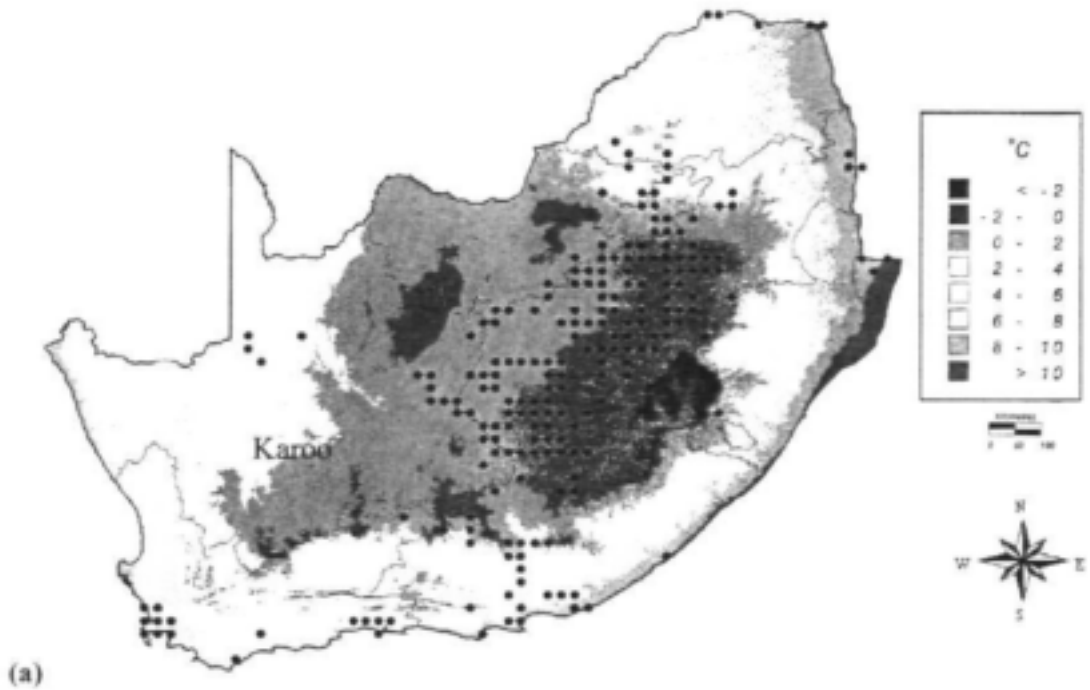
Temperature is one of the major factors influencing insect development (Stewart *et al.*, 1996), and has been implicated as a major contributing factor in the success or failure, of biological control programmes. McClay and Hughes (1995), Stewart *et al.* (1996), and Good *et al.* (1997) have shown that failure of establishment of several biological control agents could be directly attributed to climate incompatibility of the agent to its area of introduction. In an opinion survey conducted for the Silwood International Project on Biological Control of Weeds, it was suggested that climate incompatibility was responsible for reducing the effectiveness of biological control agents in known cases by up to 81% (Moran, 1985). However, success is rarely an all-or-nothing outcome in biological control because some agents establish, but fail to thrive under certain climatic conditions. Forno and Bourne (1986) showed that the damage to *Salvinia molesta* by three biological control agents, *Cyrtobagous salviniae* Calder and Sands (Coleoptera: Curculionidae), *Samea multiplicalis* Guenee (Lepidoptera: Pyralidae) and *Paulina acuminata* De Geer (Orthoptera: Acrididae), increased as temperature increased, while Room *et al.* (1989) described the rapid control of *S. molesta* in warm, tropical waters as opposed to slow control in cool elevated or temperate waters.

The failures and restricted successes of some biological control programmes represents an immense cost in terms of time and resources invested in foreign exploration, as well as quarantine host range testing, application for release and mass rearing of agents. The economic viability of such programmes would be substantially improved if reliable pre-release studies were able to predict the thermal characteristics of biological control agents and therefore their likelihood of establishment, prior to further investment. This study, however, was conducted at the post release stage of the programme, as there was uncertainty over what the weevil would do under novel

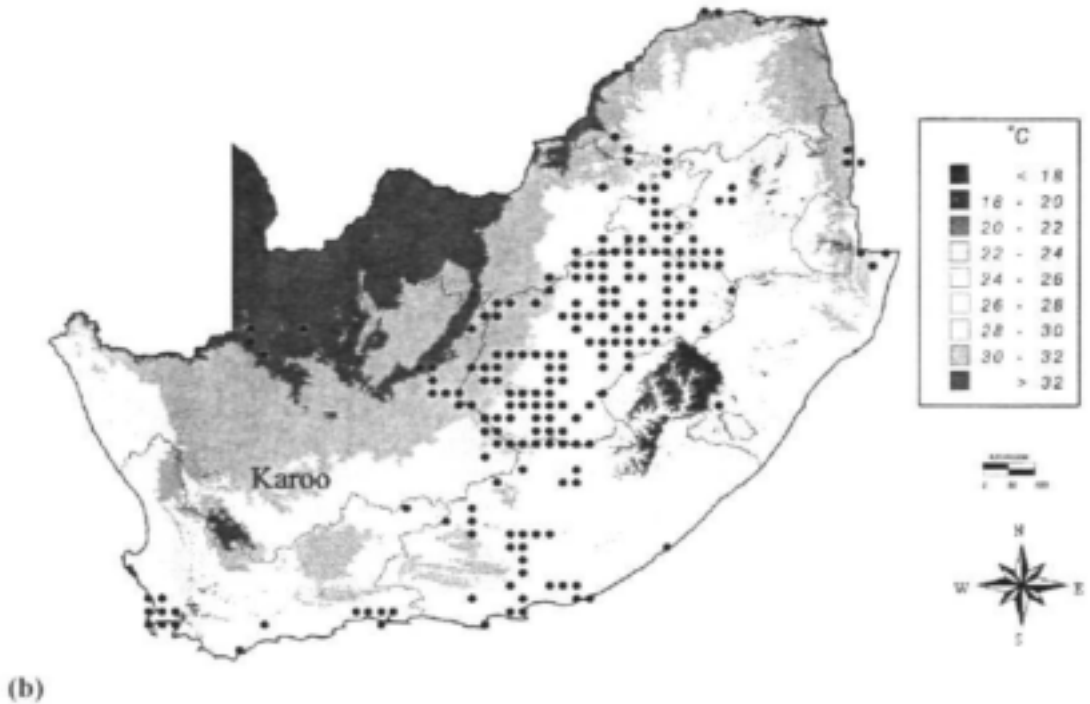
field conditions. Here, the thermal tolerance of *S. rufinasus* is investigated and its chances of establishment and potential range in South Africa predicted.

Azolla filiculoides has a temperate distribution in South Africa extending throughout most of the country with the exception of the dry Karoo region and, curiously, the humid low-lying areas of KwaZulu-Natal (Figure 4.1). It has a weedy phenology throughout its range, particularly in areas of relatively high summer and low winter temperatures, e.g. in the Eastern Cape and southern Free State provinces (Hill, 1997). In these areas, air temperature ranges between 11°C and 32°C in summer and -9°C and 12°C in winter (Schulze, 1997). Watanabe and Berja (1983) showed that the required temperatures for maximum growth of *A. caroliniana* and *A. filiculoides* (22°C) were significantly lower than that required for *A. pinnata* (33°C). This characteristic of *A. filiculoides* may account for its vigour in the cooler parts of its introduced range. In addition, de Waha Baillonville *et al.* (1991) found that *A. filiculoides* had a higher mean productivity in a sub desert tropic ecotype as opposed to a humid tropic ecotype. This could in part explain the absence of *A. filiculoides* in KwaZulu-Natal.

Stenopelmus rufinasus was released as a biological control agent on *A. filiculoides* in South Africa at the end of 1997. This weevil population originated from a tropical climate (Florida, USA) where it is found throughout the southern USA, with populations extending into central America (Hill, 1997). This tropical distribution fuelled concerns as to the ability of the weevil to establish and control *A. filiculoides*, especially in the temperate, high altitude South African sites, and prompted a survey for a cold-adapted strain of the weevil in South America (Chapter 3).



(a)



(b)

Figure 4.1 Distribution of *Azolla filiculoides* in South Africa (Henderson, 2001) overlayed on (**a**) mean daily minimum temperatures for July, and (**b**) mean daily maximum temperatures for December (Schulze, 1997).

Various thermal parameters and models are currently available to assist in the assessment of the climatic suitability of biological control agents. These are discussed below.

4.1.1 Thermal limits

4.1.1.1 Critical thermal maxima and minima, and lethal limits

Critical thermal maxima (CT_{MAX}) and minima (CT_{MIN}) describe temperatures at which animals go into torpor. During CT_{MAX} and CT_{MIN} experiments, animals are cooled or heated, usually at 1°C min^{-1}, until a point where loss of locomotory function occurs, but from which recovery is possible. Loss of locomotory function due to temperature extremes results in the organism becoming incapable of escaping conditions that may lead to death (Mitchell *et al.*, 1993). These critical limits are thought to define the ecological or behavioural temperature tolerance limits of a species (Fry, 1967; Kay & Whitford, 1978; Mitchell *et al.*, 1993). Lethal thermal limits (LT_{50}) describe the temperatures at which death occurs. Lower (LLT_{50}) and upper lethal (ULT_{50}) thermal limits define the temperature limits for the survival for an organism on exposure to low or high temperatures. During LLT_{50} and ULT_{50} experiments, animals are exposed to high or low temperatures for a fixed period of time, until a temperatures is reached from which they cannot recover (Bursell, 1964; Fry, 1967; Cloudsley-Thompson, 1970).

4.1.1.2 Developmental rates

Poikilothermic organisms generally develop at slower rates at cooler temperatures (Zalom *et al.*, 1983). This phenomenon has been extensively studied and modelled in insects (Wagner *et al.*, 1984; Lactin *et al.*, 1995), with the relationship between developmental rate and temperature generally being described as curvilinear (McClay, 1996). A lower threshold temperature (below which no development occurs) is followed by an increasing phase at higher temperatures (which is roughly linear), which is then followed by an upper threshold, above which the development rate decreases as the upper lethal temperature is approached (Liu *et al.*, 1995). An estimate of the lower developmental threshold (t) of a species can be obtained by projecting the straight-line segment of the curve until it intercepts the temperature (x) axis. This 'linear approximation' method is normally regarded to overestimate t.

However, since little development occurs at temperatures close to the threshold, this is regarded to be of little practical concern (Zalom *et al.*, 1983). Overall, the total heat accumulation necessary to complete development is considered to be a thermal constant (K). This measure of accumulated heat is known as 'physiological time', and provides a common reference for the developmental rate of poikilothermic organisms (Zalom *et al.*, 1983).

4.1.2 Temperature-development models

4.1.2.1 Degree-day model

Degree-days (°D) is the unit of measurement for physiological time where one °D is equal to one degree above the lower developmental threshold over 24 hours (Zalom *et al.*, 1983). Using historical weather records from specific geographical locations, available °D above any given threshold can be calculated and used to estimate whether these locations will provide sufficient physiological time for a particular insect species to complete its development (McClay, 1996). Maps of the number of generations of the insect species able to survive and reproduce within a year (based on the estimated degree-day totals) can then be generated and used to predict where the insect should be able to establish and where it might fail to establish (McClay & Hughes, 1995).

4.1.2.2 CLIMEX model

CLIMEX (CLIMEX programme ver. 1.1, CSIRO © Entomology) is a multiparameter, dynamic simulation model, which, in addition to using temperature parameters (as in the degree-day model), also includes humidity and precipitation to estimate potential distributions of animals and plants (McClay, 1996). The model was designed to both match climates of different localities, and enable the estimation of an animal or plant's geographic distribution and relative abundance within a given climatic region (Sutherst & Maywald, 1985). The model is based on the assumption that during a year most animal and plant populations experience a season which is favourable for population growth, and one that is unfavourable, that may jeopardise its persistence in a given area (McFadyen & Skarrat, 1996). The potential growth of a population during the favourable season is described by an annual population 'Growth Index' (GI_A), while the probability of the population surviving through the

unfavourable season is described by four stress indices (Cold, Hot, Wet, and Dry; Table 4.1; Maywald & Sutherst, 1991). To give an overall measure of favourability of a locality for permanent occupation by the population, the GI_A and Stress Indices are combined into an 'Ecoclimatic Index' (EI). Resulting EIs generated by the CLIMEX model can then be displayed as a map, table or graph.

Table 4.1 Growth-related and stress indices of the CLIMEX model used to estimate the potential for growth and survival of a population of a species at a given location (Maywald & Sutherst, 1997).

Growth-related indices [a]	Stress indices (SI) [b]
Annual Growth Index (GI_A)	Cold Stress (CS)
Weekly Growth Index (GI_W)	Heat Stress (HS)
Temperature Index (TI)	Dry Stress (DS)
Moisture Index (MI)	Wet Stress (WS)
Diapause Index (DI)	Stress interactions (SX) (Cold/Wet,
Light Index (LI)	Cold/Dry, Hot/Wet, Hot/Dry)

[a] weekly indices, which relate to seasonal activity patterns and relative abundance.

[b] yearly indices, which relate to conditions during the unfavourable season that limit the geographical distribution.

4.1.2.3 Microclimate

McClay and Hughes (1995) view the assumption that standard meteorological data can be used to represent the temperature actually experienced by insects in the field, to be a "drastic oversimplification". CLIMEX assumes that climate is the sole determining factor in the distribution of a species. Maywald and Sutherst (1997), however, acknowledge that potential distributions of species are also modified by other physical and biological factors, including microclimate. It is recommended that

the impact of these factors should be considered when assessing the predictions of any climate-based models (Maywald and Sutherst, 1997).

A series of laboratory trials were undertaken to investigate the thermal physiology of *S. rufinasus* to predict if there were any areas in South Africa where the weevil might not establish on *A. filiculoides* because of extremes in climate. The first aim of this study was to establish the upper and lower temperature limits of the weevil, including CT_{MAX} and CT_{MIN}, ULT_{50} and LLT_{50}, and to measure the effect of temperature on development rate. The second aim of the study involved the use of two temperature-based development models: the degree-day model and the CLIMEX model to predict the potential distribution of the weevil in South Africa. Since both of these models are based on the broad assumption that standard meteorological data is representative of the temperature actually experienced by insects in the field, the effect of microclimate on both models was also investigated.

4.2 Materials and Methods

4.2.1 Thermal limits

4.2.1.1 Critical thermal limits

The critical thermal limits of adult *S. rufinasus* were measured by placing 10 weevils individually into sealed glass vials (SAMCO™ 5 cm x 0.5 cm). For CT_{MIN} vials were sealed with Prestik™, while for CT_{MAX} the vials were sealed with moist cotton wool plugs to prevent evaporative cooling by the weevils. The temperature of the vials was progressively lowered (CT_{MIN}) or raised (CT_{MAX}) by 1°C min^{-1} from room temperature (25°C) in a programmable water bath (Haake F8) connected to a programmable temperature controller (Haake C25, 0.1°C accuracy). Vial temperatures were monitored with a thermocouple (YFE YF-160A Type-K; range: -50°C to 1300°C; accuracy 0.3°C). The tip of the thermocouple was inserted into a Prestik™ scale model of an adult weevil (thus acting as an operative thermometer) to monitor weevil body temperature during the course of the experiment. Weevils were observed every minute until locomotory function became impaired. Forty adult weevils (20 CT_{MIN}, 20 CT_{MAX}) were tested.

4.2.1.2 Lethal temperatures

Lethal temperature experiments were conducted on adult *S. rufinasus*. Groups of 20 weevils per test temperature were placed individually into glass vials (as above). The temperature of the vials was progressively lowered (LLT_{50}) or raised (ULT_{50}) by 1°C min^{-1} from room temperature to the experimental temperature, in a programmable water bath (as above). Weevil temperatures were monitored throughout the experiment (as above). The following temperatures were tested: -12°C to 0°C (in 2°C increments); -16°C to -12°C (in 0.5°C increments for finer resolution) (LLT_{50}), and 30°C to 42°C (in 1°C increments) (ULT_{50}). Following two hours exposure at the experimental temperature, the vials were removed from the water bath and the weevils placed in petri dishes with moist filter paper and a single *A. filiculoides* plant. The weevils were given a recovery period of 24 hours after which the number of dead weevils was recorded. Following the methods of Klok and Chown (1997), weevils that showed only slight movement of antennae and legs at the end of the given recovery period were considered incapable of recovering and therefore dead. The LLT_{50} and ULT_{50} were calculated from the survival data by probit analysis (Finney, 1962).

4.2.1.3 Developmental rates

Twenty five pairs of *S. rufinasus* adults were placed on fresh *A. filiculoides* for 12 h. Eggs were dissected from the *Azolla* fronds, placed individually in 5 cm diameter petri dishes on moist filter paper and placed in a constant temperature incubator. Five temperature treatments were run concurrently (n = 30 eggs / treatment). Temperature records for each treatment were obtained using a datalogger (MCS 120-02EX, MC Systems, Steenberg, South Africa, 0.1°C accuracy), with a thermocouple probe situated in a petri dish. All incubators were on a 12 h photoperiod throughout the experiment. The dishes were checked every 12 h and the time to hatching, moulting for each instar, pupation and eclosion was recorded. Larval instars were identified according to the dimensions recorded by Hill (1997), by measuring the head capsule width with an ocular micrometer attached to a dissecting microscope. *Stenopelmus rufinasus* larvae were fed whole *A. filiculoides* plants *ad libitum*.

The developmental rate of the weevil was calculated from the average number of days from egg-hatch to adult emergence. The traditional 'linear approximation' method was used to plot the inverse of the developmental duration (developmental rate) against temperature, where y = a + bx. K was estimated by calculating the inverse of the gradient of the slope and t was estimated from the x-intercept.

4.2.2 Temperature-development models

4.2.2.1 Degree-day model

Daily maximum and minimum temperature records were obtained from the CLIMEX database for 134 locations throughout South Africa. The thermal constant and developmental threshold estimated from the degree-day model were used to calculate accumulated °D for each year and each location according to the equation:

$$K = \sum \left\{ \frac{\left(T_{max} + T_{min}\right)}{2} - t \right\} \quad \text{(if } T_{min} < t\text{, } t \text{ was used)}$$

The mean annual °D total was then calculated for each location. A contour map of the number of generations of *S. rufinasus* for South Africa was generated using ARCVIEW (ARCVIEW GIS programme ver. 3.2, Environmental Systems Research Institute Inc.). A continuous surface between weather stations was interpolated using the Inverse Distance Weighted (IDW) algorhythm[1], and then grouped into class intervals (number of generations).

4.2.2.2 CLIMEX model

The climatic requirements of an organism being modelled in CLIMEX are inferred from its known geographical distribution, and threshold temperatures of the species can be used to further fine-tune the model parameter values (Sutherst & Maywald, 1985). Data obtained from the thermal limits of *S. rufinasus* were thus incorporated into the CLIMEX model. The assumption was made that *S. rufinasus* was not restricted by moisture in its habitat as it is an aquatic insect. The moisture parameters, however, could not be excluded from the CLIMEX programme, and were therefore

[1] The IDW interpolator assumes that each point has a local influence that diminishes with distance. It weights the points closer to the processing cell greater than those farther away. A specified number of points, or optionally all points within a specified radius, can be used to determine the output value for each location (ARCVIEW GIS programme ver. 3.2, Environmental Systems Research Institute Inc.).

made as broad as possible. The stress indices, dry stress and wet stress, were also excluded because of the aquatic habit of the weevil. The programme's wet tropical template was used to define cold stress and heat stress, as *S. rufinasus* was collected in the humid, tropical ecotype of Florida, U.S.A. The model was then refined by matching the predicted distribution of the weevil in its country of origin. Once the parameter values had been estimated, annual ecoclimatic indices (EIs) were derived using these indices and meteorological data from 134 South African weather stations. The EI is scaled between 0 (totally unsuitable) and 100 (optimum) and is derived as the annual mean of weekly values of GI reduced by the stress indices (Maywald & Sutherst, 1997).

4.2.2.3 Microclimate

A datalogger (see developmental rates) was set up in the field (Wits University, Johannesburg) to record air, *Azolla* mat and water temperature over a seven day winter period during which below freezing air temperatures were experienced. Thermocouple probes were inserted 15cm below the water surface (water temperature), in the middle of a 10cm thick mat of *A. filiculoides* (mat temperature) and in a Stevenson screen (air temperature). Temperatures were logged every 15 minutes. Corresponding periods where air temperature was less than mat temperature were selected and their relationship examined using regression analysis. The straight-line equation obtained was used to transform air temperature data (minima and maxima) from 134 South African localities to obtain the corresponding mat temperature data. These transformed data were used to examine the effect of microclimate (mat temperature) on the degree-day and CLIMEX models. All transformed data (temperature, number of generations and EI) were compared statistically to the original data using t-tests.

4.3 Results

4.3.1 Thermal limits

4.3.1.1 Critical thermal limits

Loss of locomotory function for adult *S. rufinasus* ranged between 0°C and 5°C (lower thermal limits) and 45°C and 48°C (upper thermal limits). The mean CTMIN was 1.3 ± 0.2°C; (mean ± SE, n = 20, test range = 0°C to 7°C). The mean CTMAX was 47.5 ± 0.1°C; (mean ± SE, n = 20, test range = 41°C to 48°C).

4.3.1.2 Lethal temperatures

No adult weevil deaths occurred after 2 h exposure to temperatures between 0 and -2°C. The majority of adult weevils survived temperatures between –4 and -12°C, while 100% mortality was recorded between -13 and -16°C. The LLT_{50} for *S. rufinasus* was -12.1°C. In ULT_{50} trials, all adult weevils survived temperatures between 30°C and 34°C, while 100% mortality occurred between 41°C and 48°C. The ULT_{50} was 36.5°C.

4.3.1.3 Developmental rates

Stenopelmus rufinasus successfully completed development from egg-hatch to adult emergence at 14.5°C, 18.9°C, 23.5°C, 29.4°C and 31.9°C (Table 4.2). The length of time for development of the different weevil stages decreased as temperature increased within the temperature range tested (Fig. 4.2). A linear regression analysis was applied to the developmental points within the above range, with t estimated at 9.2°C ($R^2 = 0.95$; $p = 0$) and K at 256.4 °D.

Table 4.2 Developmental time from egg to adult for *Stenopelmus rufinasus* at five constant temperatures.

	Mean duration (days) ± SD, (N), and % total development time at indicated temperature				
Stage	14.5°C	18.9°C	23.5°C	29.4°C	31.9°C
Egg	16.6 ± 0.8 (19) 30.4	8.2 ± 0.4 (22) 29.0	4.7 ± 0.5 (24) 28.5	3.4 ± 0.5 (20) 26.6	3.6 ± 0.5 (20) 29.8
L1	7.8 ± 1.0 (18) 14.4	4.5 ± 0.8 (20) 15.7	2.5 ± 0.5 (17) 15.3	2.3 ± 0.7 (19) 17.6	1.9 ± 0.5 (19) 15.8
L2	5.3 ± 1.5 (18) 9.7	3.7 ± 0.6 (20) 13.1	2.1 ± 1.2 (17) 12.5	1.5 ± 0.5 (19) 11.5	1.4 ± 0.5 (19) 11.4
L3	8 ± 1.3 (18) 14.7	3.6 ± 0.5 (20) 12.5	2.4 ± 0.6 (17) 14.6	2.0 ± 0.7 (19) 15.2	1.5 ± 0.5 (19) 12.8
Pupa	16.8 ± 1.2 (18) 30.9	8.4 ± 0.8 (20) 29.7	4.8 ± 0.5 (17) 29.2	3.7 ± 0.8 (19) 28.7	3.6 ± 0.6 (19) 30.3
Total	54.6 ± 3.5 (18)	28.3 ± 1.8 (20)	16.5 ± 1.5 (17)	12.8 ± 1.2 (19)	12.0 ± 1.0 (19)

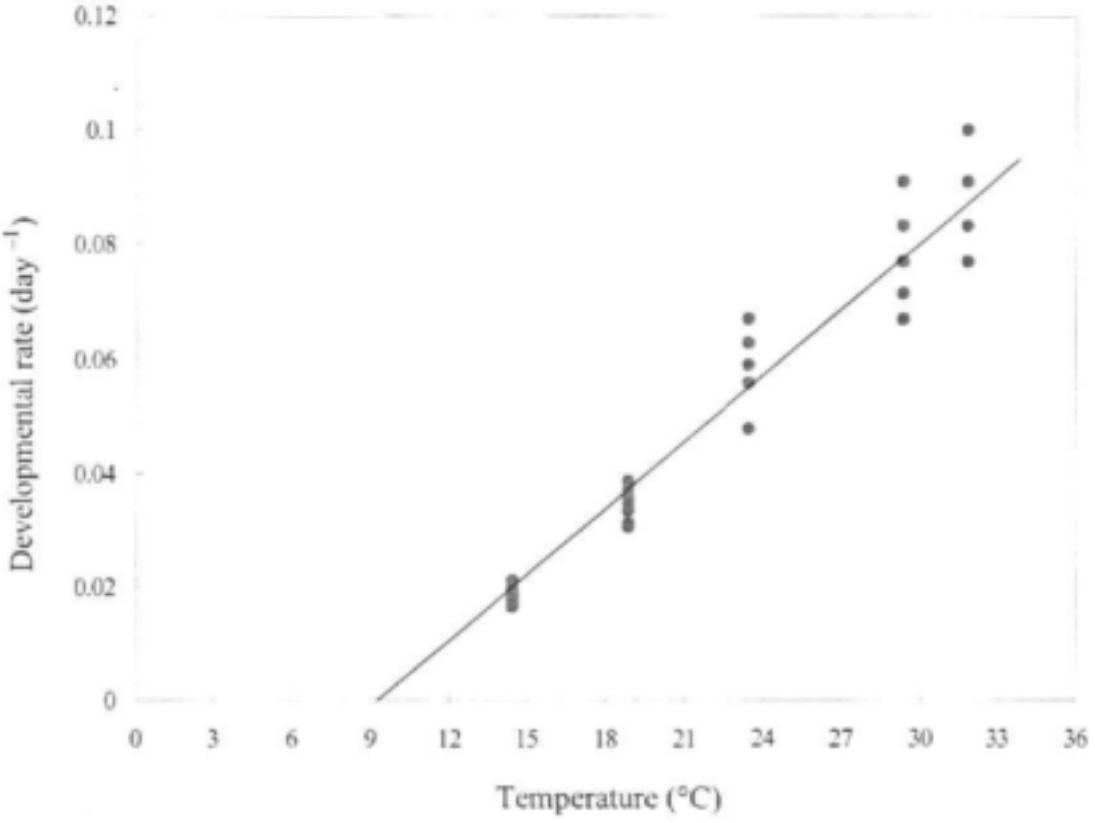


Figure 4.2 Linear regression of development rate against rearing temperature of *Stenopelmus rufinasus* from (egg to adult) at five different constant temperatures. Equation of line: $y = 0.0039x - 0.0358$, $R^2 = 0.95$.

4.3.2 Temperature-development models

4.3.2.1 Degree-day model

Given the values of t and k calculated for *S. rufinasus* all areas where the weevil was released in South Africa have sufficient °D for at least 5.6 generations per year (Table Mountain, Cape Town, Eastern Cape Province), and at most 20.1 generations per year (Messina, Northern Provine) (Fig. 4.3). The majority of weevil releases have occurred in areas where the model predicts between 10.4 and 12 generations per year.

4.3.2.2 CLIMEX model

Measured and inferred physiological parameters for *S. rufinasus* were incorporated into the CLIMEX model (Table 4.3). The native range of *S. rufinasus* includes the tropical ecotypes of the southern and western U.S.A. (Leconte, 1876) extending from Florida to as far as the central valley and coastal San Luis Obispo county, California (Richerson & Grigarick, 1967). This known distribution was confirmed by the

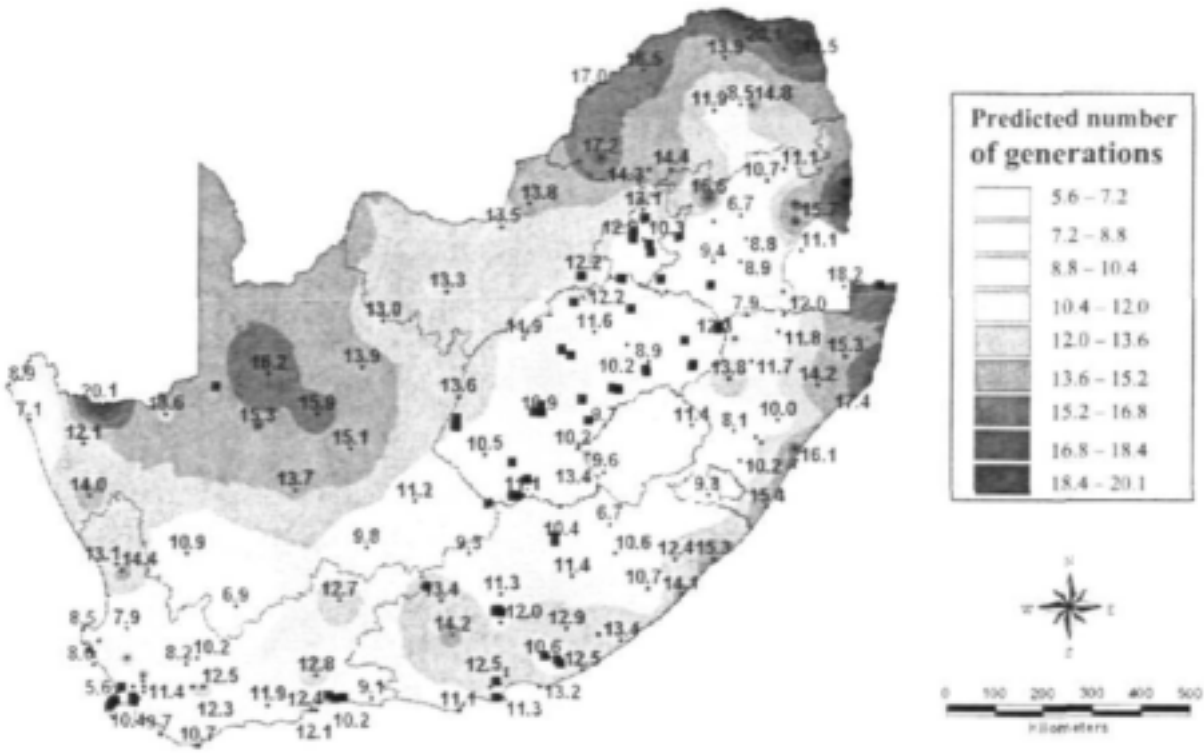


Figure 4.3 Potential number of generations per year of *Stenopelmus rufinasus* in South Africa. Blue squares - localities at which *Stenopelmus rufinasus* has been released and established; black dots – localities for which degree-days were estimated and used in plotting contours.

CLIMEX model for *S. rufinasus* (Fig. 4.4), which predicted that the weevil would be able to occur in the states of Florida, Alabama, Louisiana, Mississipi, Texas, Arizona and California. The predicted distribution of the weevil for South Africa showed a high probability of the weevil occurring throughout the country (Fig. 4.5). These localities have conditions that are suited to the modelled parameters of the weevil. At a few localities scattered through the interior of the country, the probability of the weevil's occurrence is reduced (smaller solid circles) or excluded (crosses). In total, 13 localities were predicted to not be suitable for weevil establishment (EI = 0), with the cold stress index responsible for all failures. One such locality is the town of Bethlehem, Free State Province. *Stenoplemus rufinasus*, however, was released and established at this locality in February 1999. Over a period of eight months, which spanned the South African winter, the weevil was able to control the infestation of *A. filiculoides* in Bethlehem (Appendix A).

Table 4.3 CLIMEX growth and stress indices used for *Stenoplemus rufinasus*.

Parameter	Explanation
Temperature (°C):	
DV0 = 9.2	Lower threshold for development (t of *S. rufinasus*)
DV1 = 25.0	Lower level of optimum range (obtained from developmental rate graph)
DV2 = 31.9	Upper level of optimum range (obtained from developmental rate graph)
DV3 = 36.5	Level above which no growth occurs (ULT_{50} of *S. rufinasus*)
PDD = 256.4	Annual minimum degree-days (°D) of *S. rufinasus* (K)
Moisture:	
SM0 = 0.000	*Stenopelmus rufinasus* is assumed not to be restricted by
SM1 = 0.001	moisture in its habitat as it is aquatic. Parameter can not
SM2 = 9.999	be excluded from programme, therefore, range made as wide
SM3 = 10.000	as possible.
Cold stress:	
TTCS = 0.000	
THCS = 0.000	Wet tropical template values used as *Stenopelmus rufinasus*
DTCS = 25.000	originated from Florida, U.S.A.
DHCS = 0.0015	
Heat Stress	
TTHS = 36.00	
THHS = 0.001	Wet tropical template values used as *Stenopelmus rufinasus*
DTHS = 0.000	originated from Florida, U.S.A.
DHHS = 0.000	

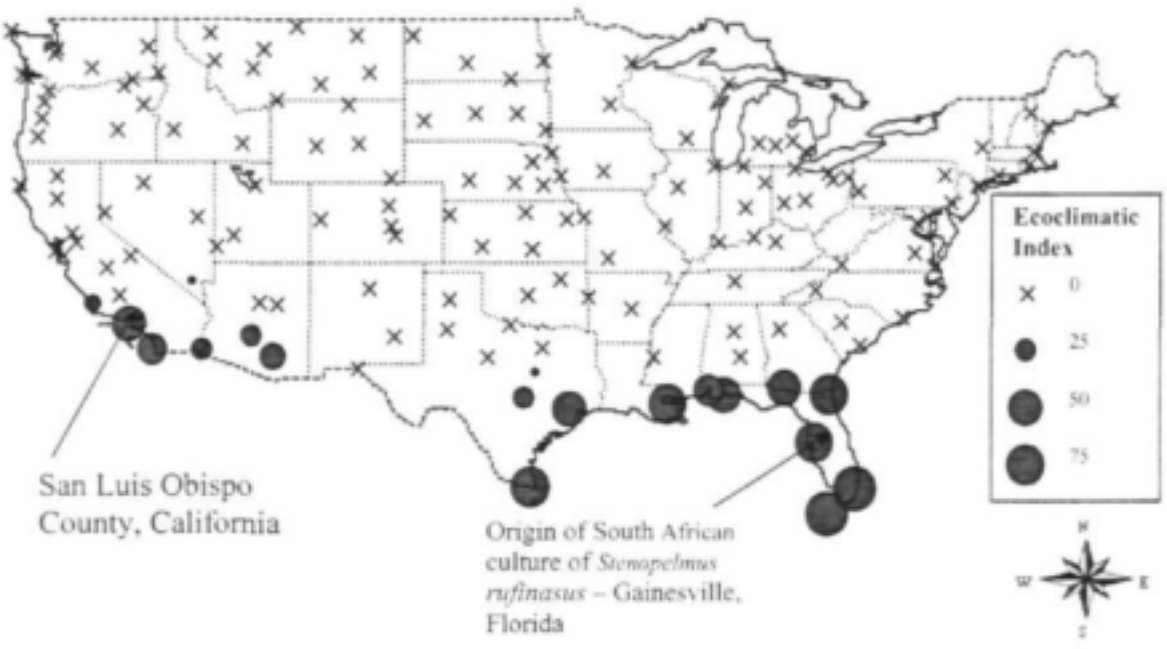


Figure 4.4 CLIMEX generated map of the relative climatic suitability of the U.S.A. for *Stenopelmus rufinasus*. Areas of the red circles (ecoclimatic index) are proportional to the suitability of each location. Crosses indicate localities that are unsuitable for *S. rufinasus* (ecoclimatic index = 0).

Figure 4.5 CLIMEX generated map of the relative suitability of South Africa for *Stenopelmus rufinasus*. Areas of the red circles (ecoclimatic index) are proportional to the suitability of each location. Present distribution of the weevil is shown (grey area), which is limited by the distribution of the plant. Crosses indicate localities that are unsuitable for *S. rufinasus* (ecoclimatic index = 0).

4.3.2.3 Microclimate

A linear regression anaylsis was applied to the air temperature and *Azolla* mat temperature data (Fig. 4.6). A strong positive linear relationship was evident between the two variables ($R^2 = 0.93$; $p = 0.00$) . Mat temperature remained well above 0°C, while the corresponding air temperature dropped below freezing. The regression equation ($y = 0.81x + 5.46$) was used to modify the CLIMEX summer and winter temperature data from 134 South African localities, so that it was representative of the microclimatic temperatures most probably experienced by the weevil in the field.

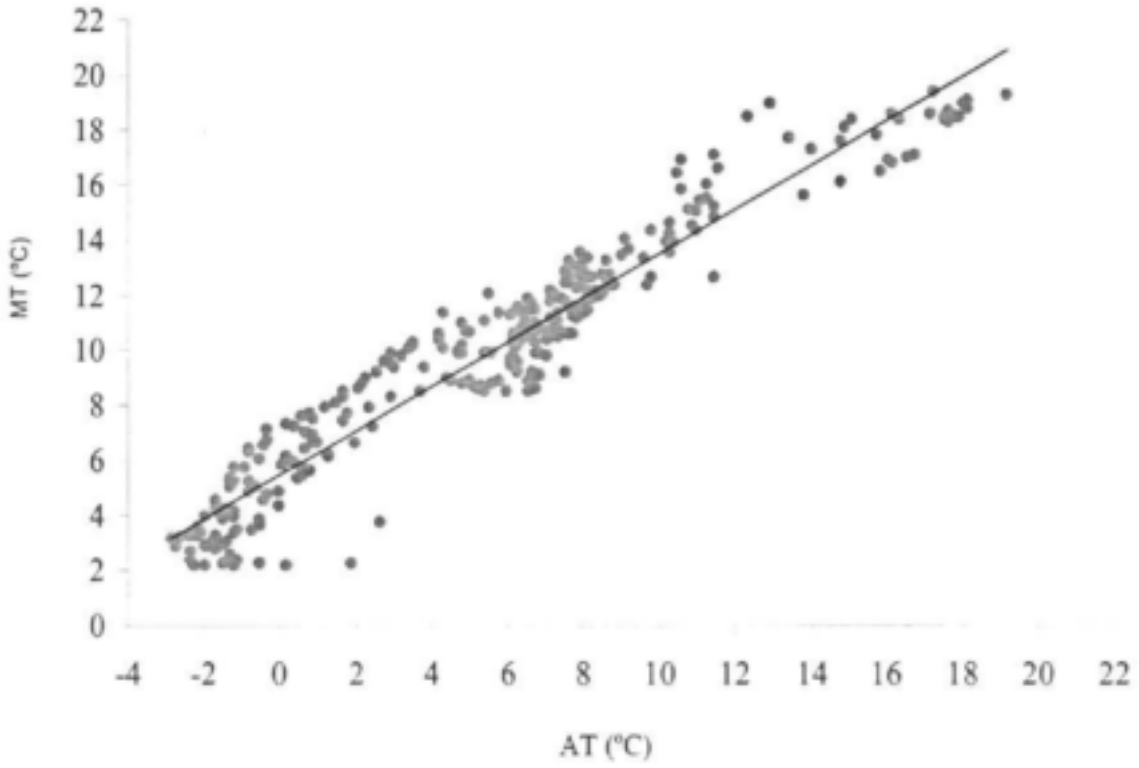


Figure 4.6 Regression plot of air temperature (AT) against *Azolla filiculoides* mat temperature (MT). Equation of line: $y = 0.81x + 5.46$, $R^2 = 0.93$.

Temperature data modified for microclimate was found to be significantly different to the original temperature data (min. temperatures: $t_{3214} = -5.44$, $p < 0.001$, max. temperatures: $t_{3214} = -20.84$, $p < 0.001$). The effect of microclimate with regards to the degree-day (Fig. 4.7) and CLIMEX models (Fig. 4.8) was significantly different. The number of weevil generations predicted to be produced per annum using

temperature data modified for microclimate was found to be significantly greater than the original degree-day prediction using unmodified weather station data ($t_{266} = -7.85$, $p < 0.001$). Similarly, the effect of temperature data modified for microclimate on the CLIMEX model showed a significant improvement in the EI ($t_{266} = -8.70$, $p < 0.001$) for the weevil in South Africa, which predicts the distribution of the weevil to include all localities in South Africa.

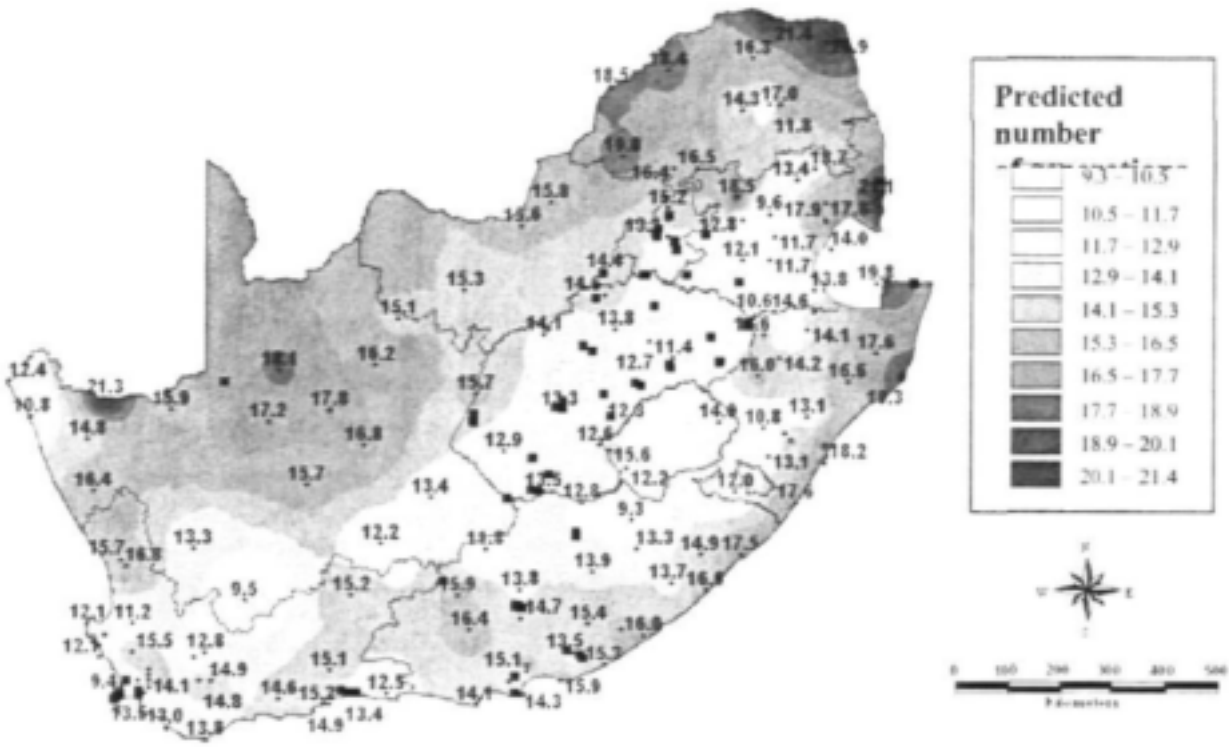


Figure 4.7 Potential number of generations per year (using transformed temperature data) of *Stenopelmus rufinasus* in South Africa. Blue squares - localities at which *Stenopelmus rufinasus* has been released and established; black dots – localities for which degree-days were estimated and used in plotting contours.

Figure 4.8 CLIMEX generated map (using transformed temperature data) of the relative suitability of South Africa for *Stenopelmus rufinasus*. Areas of the red circles (ecoclimatic index) are proportional to the suitability of each location. Present distribution of the weevil is shown (grey area), which is limited by the distribution of the plant.

4.4 Discussion

Physiologically, *S. rufinasus* is tolerant of a wide range of temperatures. In terms of its locomotory limits (CT_{MIN} and CT_{MAX}) the weevil would be able to maintain locomotory function within a 46°C range (1.3°C to 47.4°C). This range is comparable with other studies e.g. *Eccritotarsus catarinensis* (Heteroptera; Miridae), a tropical bug from Brazil, maintains locomotion within a 48°C range (Coetzee, 2003); while *Spodoptera exempta* (Lepidoptera: Noctuidae), a sub-tropical caterpillar from South Africa, maintains locomotion within a 40°C range (Klok & Chown, 1997). The lethal thermal limits of *S. rufinasus* (LLT_{50} and ULT_{50}) are impressive for an insect that originates in the tropics as its lethal temperature range is approximately 48°C (-12.1°C to 36.5°C). The weevil's LLT_{50} of -12.1°C is surprisingly low for a tropical insect. *Hodotermes mossambicus* (Isoptera: Hodotermitidae), for example, a sutropical termite has a LLT_{50} of 2.8°C (Mitchell *et al.*, 1993); while *E. catarinensis*, a tropical bug has a LLT_{50} of –3.6°C (Coetzee, 2003); and *Gratiana spadicea* (Coleoptera:

Chrysomelidae), a subtropical tortoise beetle, has a LLT_{50} of -7.1°C (Byrne *et al.*, 2002). Other studies, however, have documented insects with LLT_{50}s well below -10°C. These include *Pringleophaga marioni* (Lepidoptera: Tineidae), a sub-antartic caterpillar, which has a LLT_{50} of -12°C (Klok & Chown, 1997); and *Thrips palmi* (Thysanoptera: Thripidae), a thrips, which has an LT_{50} of -18.8°C (McDonald *et al.*, 2000). As the upper thermal limits of insects are usually above the average environmental temperatures, Byrne *et al.* (2003) suggested that an insect's lower thermal limits (in particular the LT_{50}) show some utility for estimating its chances of surviving extreme winter conditions. The thermal limits of *S. rufinasus*, especially its low LLT_{50}, suggested that there would be few localities in South Africa which would thermally limit its survival, but further modelling techniques were required to make predictions about its development and establishment..

Two temperature-based development models (degree-day and CLIMEX) were used in this study to predict the potential distribution of *S. rufinasus* in South Africa. Both models required the input of various thermal parameters of the weevil. The choice of which parameters to use, however, was challenging. While the locomotory and lethal thermal limits of the weevil were relatively easy to measure under laboratory conditions, other studies have utilised these parameters to explain the over wintering capabilities of insects (Papadopoulos *et al.*, 1996; Olsen *et al.*, 1998; McDonald *et al.*, 2000), and not to model potential distributions. Compared to the locomotory and lethal thermal limits, determination of the developmental parameters (K and t) of *S. rufinasus* required both time and effort. However, this empirical physiological data has been successfully used in degree-day models to predict whether insects can establish at particular localities (e.g. McClay & Hughes, 1995; McClay, 1996). Similarly, the CLIMEX model has been used to predict the potential distribution of invasive species (e.g. Tribe & Richardson, 1994; Julien *et al.*, 1995; McFadyen & Skarratt, 1996; Vera *et al.*, 2002). Both the degree-day and the CLIMEX model have as their shortcoming the failure to incorporate the effects of microclimate in their analyses. Once accounted for, however, both models strongly supported the predictions made from the lower thermal limits of *S. rufinasus*, that the weevil should be able to establish and complete favourable numbers of generations throughout the range of its introduction.

Byrne *et al.* (2003) viewed the degree-day model to be satisfying from the perspective that the results are sensible, and useful for different geographical areas. This was also the case in modelling the number of generations that *S. rufinasus* would be able to complete in South Africa during the course of a year. The model estimated between 5 and 20 generations per year (Fig. 4.4), with the widespread establishment of the weevil (Chapter 6) confirming the model's predictions. Degree-day models have proven to be informative from a variety of aspects. These include; the scheduling of pest management actions; monitoring pest activity (Zalom *et al.*, 1983); avoiding the wastage of effort in trying to establish biological control agents which are not adapted to the climatic conditions in their introduced range; optimising release strategies for biological control agents which failed to establish from initial releases; providing encouragement to continue with releases of agents whose potential was previously unknown; and in facilitating field monitoring (McClay, 1996). The model has also been modified to reflect number of generations of the organism being studied during different seasons. Coetzee (2003) found the use of a modified degree-day model, which focussed on the cooler winter months, to better predict the potential distribution of *E. catarinensis* in South Africa.

The validity of the CLIMEX model developed for this study, was tested by generating a predictive map of the weevil's distribution in North America. The model postulated the weevils range to extend along the southern and lower western reaches of North America (Fig. 2.4), which corresponds with the general distribution available from the literature. The assumption was, therefore, made that the parameters used in the model were satisfactory. However, CLIMEX's predicted distribution of *S. rufinasus* for South Africa, suggested that there were several localities in the country where the weevil would not occur (Fig. 4.5), which contradicted field establishment data (Chapter 6). All 13 localities where non-establishment was predicted, were as a result of cold stress. These results were attributed to the tropical template cold and heat stress indexes used in the model. One might then argue that these values needed to be 'tweaked' in order for the predicted distribution to more closely match the field establishment data. However, it was felt that a more fundamental factor was responsible for the mismatch. CLIMEX assumes the distribution of a species to be solely determined by climate (Sutherst & Maywald, 1985). However, Maywald & Sutherst (1997) conceded that this 'potential' distribution is often modified by

physical and biological factors such as soil type, microclimatic factors, topography, food quality and availability, parasites, predators and pathogens. They also urged that the impact of these modifiers should be considered when making assessments of CLIMEX predictions. Roltsch *et al.* (1999) regarded the lack of consideration of microclimate to one of the major shortcomings of the degree-day model. Therefore, the effect of microclimate on both the degree-day and CLIMEX models was investigated.

Many studies have investigated the effect of microclimate on arthropod development and distributions (Ferro *et* al., 1979; Ferro & Southwick, 1984; Wilhoit *et al.*, 1991; Chown & Crafford, 1992; Gibbs *et al.*, 2003; Irwin & Lee, 2003). Several studies share the view that until appropriate microclimatic measurements are recorded and related to standard meteorological data and the population dynamics of the test species, temperature development models will only be looking at a correlation rather than a cause-effect relationship (Ferro *et al.*, 1979; Wilhoit *et al.*, 1991). *Stenopelmus rufinasus* occupies a buffered niche or microclimate in the *A. filiculoides* mat, where, even when air temperatures drop below zero the mat temperature stays above freezing point – probably as a result of a buffering effect from the water, or as a result of photochemical and thermochemical events of the metabolic and physiological processes of the plant (Gates, 1968). This microclimate allows the weevil to develop and control infestations of red water fern in areas of South Africa that possibly experience air temperatures unsuitable for the development of the weevil. Wilhoit *et al.* (1991) conducted a study that looked at estimating manure temperatures from air temperatures, and then using the results to model *Musca domestica* L. (Diptera: Muscidae) populations, which use the manure as a larval habitat. Their investigations found that predicted population sizes using estimated manure temperatures were closer to the sizes using actual manure temperatures than the sizes using actual air temperatures. Similarly, the predicted distribution of *S. rufinasus* using estimated mat temperatures in the CLIMEX and degree-day models, correlated better with confirmed field distributions and population dynamics, than the same models using standard meteorological air temperatures.

Several recommendations emerge from this study with regards to determining the predictive distributions of biological control agents. First, CT_{MIN} and LLT_{50} appear to

be the most useful of the thermal limit tests conducted. Consideration of this data in view of the extremes of climate in the proposed area of introduction, should give a broad idea of the chances of agent survival (Byrne *et al.*, 2003). Second, the degree-day model was the more satisfying of the two models tested. Even before the effects of microclimate were taken into account, the model predicted that *S. rufinasus* would be able to establish throughout South Africa. The data required for the model (developmental rate of the agent) may be costly in terms of time and money (McClay, 1996), however, its use may ultimately translate into future savings in avoiding costs associated with climate-incompatibility failures-to-establish. Third, only through comprehensive micro-climatological studies, will the relationship between standard meteorological measurements, microclimatic measurements and insect population dynamics be resolved (Ferro *et al.*, 1979). This study measured only one parameter of microclimate – temperature. The measurement of factors such as humidity, wind speed and radiation would further define the microclimate / meteorological data relationship, ultimately improving the predictive strength distribution models.

Thermally, *S. rufinasus* appears to be climatically compatible with the extremes in climate experienced in South Africa. Confirmation of this prediction, however, needs to be verified under controlled field conditions. Therefore field trials were conducted in cages during the South African winter and summer (Chapter 5), to investigate the ability of the weevil to establish and develop in these seasons, and to investigate the level of control exerted on *A. filiculoides* in the field.

CHAPTER 5

FIELD-CAGE ASSESSMENT OF THE ESTABLISHMENT AND POTENTIAL IMPACT OF *STENOPELMUS RUFINASUS* ON *AZOLLA FILICULOIDES* IN SOUTH AFRICA

5.1 Introduction

The assessment of the effects of natural enemies in the regulation of their host plant densities is of key interest to biological control practitioners (Luck *et al.*, 1999). Such biological assessments should not only serve to quantify the impact of the natural enemy on its target plant, but also provide an insight into the population dynamics of the agent in the country of introduction (Harris, 1980). Far too often though, assessments are only carried out after agent release or establishment, and even then only monitor the presence and spread of the agents (McFadyen, 1998). As a result, the reasons for biological control successes, or more importantly failures, are often not clear (Luck *et al.*, 1999). The more data gathered from pre-release evaluations, however, the easier it will become to make the right decisions about the future directions of the project and ultimately its success (Farrell and Lonsdale, 1997).

Defining and describing success in biological control is a contentious issue (Julien, 1997; McFadyen, 1998). Laing and Hamia (1976) and Hoffmann (1995) proposed simple descriptive methods, which rate success from negligible to complete, while Moran and Zimmermann (1984) recommended more complex quantitative methods, and Julien (1997) suggested a combination of qualitative and quantitative methods. As the issue of describing success is more pertinent to the post-release phase of a programme, it will be discussed in more depth in Chapter 4. However, pre-release studies may be useful in terms of allowing the researcher to determine what level of success may be anticipated using a particular natural enemy or combination of enemies. For example, post-release studies on the water hyacinth weevils, *Neochetina eichhorniae* and *N. bruchi*, indicated that control was more successful when both species were released together rather than individually (Harley, 1990).

Pre-release evaluations are valuable in predicting the ability of the agent to establish in the country of introduction. In some biological control programmes, field establishment has taken many years (McFadyen, 1998; Vittelli *et al.*, 1996) and, therefore, pre-release assessments could identify difficult agents, and possibly shed light on alternative techniques to improve their chances of establishment. Once established, however, successful biological control agents take on average 10 to 12 years to affect the target weed to such an extent that the population is reduced to levels below the pest status threshold (Lawton, 1984), and research projects are seldom funded for this length of time. Determination of the impact of natural enemies on their target plant populations has been variable and has included the measurement of agent impact on plant morphometrics (i.e. number, quality and size of leaves, stems, roots etc.) (Center & Durden, 1981; Room & Fernando, 1992; Anderson *et al.*, 1999), density (Hoffmann & Moran, 1998), biomass (Harley *et al.*, 1984; Room *et al.*, 1984), percentage cover (Harley *et al.*, 1990); and seed production (Hoffmann & Moran, 1999; Impson *et al.*, 1999). Pre-release studies also present a good opportunity to develop and refine the techniques required for measuring agent impact on the target, which should prove valuable in large-scale post-release studies.

Cages are frequently used to evaluate biological control agents under field conditions (Ashby, 1974; Faeth & Simberloff, 1981; Julien *et al.*, 1987; Nechols *et al.*, 1996) and may include (usually pre-release studies) or exclude (usually post-release studies) the organism being tested. Essentially the use of cages can be viewed as an intermediate step between the laboratory and the field, but data obtained should be interpreted with care. Criticisms of the method include the inhibition of predator or prey movement, the interference of oviposition behaviour, the lack of predator free controls, the altering of microclimate through shading and inhibition of airflow, and extreme changes in solar radiation (Luck *et al.*, 1999). Nonetheless, cages are still considered a useful technique with which to evaluate the efficacy of biological control agents (van Driesche & Bellows, 1996).

Predictions from Chapter 4 suggested that the weevil was thermally suited to the climatic extremes of the introduced range of the weed. However, there was uncertainty as to whether these theoretical predictions would translate into actual field establishment and

reproduction of the weevil during the South African summers and winters. In addition, the exact impact the weevil on the weed was previously unquantified. The following aims were, therefore, addressed with the use of field inclusion cages: First to investigate the establishment, reproductive potential and population dynamics of *Stenopelmus rufinasus* on *Azolla filiculoides* during a South African summer and winter; second, to quantify the impact of the weevil on the weed; and third, to test the predictive capacity of the thermal parameters and models (Chapter 4), using the population dynamics data.

5.2 Materials and Methods

5.2.1 Field-cages

Trials were conducted in floating, inclusion cages at five different field sites in Gauteng Province (Table 5.1) during summer 1999 (November, December) and winter 2000 (June, July, August). Three cages were placed at each site. Cages were constructed from 1.4 cm and 5 cm diameter PVC tubing and measured 50 x 50 x 50 cm (Fig. 5.1). Two-litre soft drink bottles, half filled with water, were attached to each corner of the cage as floats. Two of the cages (treatment and closed control) were covered on the five upper surfaces with gauze (1mm mesh diameter) (Fig. 5.1a), while the third (open control) had a 20cm gauze skirt around the bottom to retain the weed (Fig. 5.1b). One kilogram (wet weight) of *A. filiculoides* was placed in each cage. Ten pairs of weevils were introduced to the treatment cages at the same time as the weed, while the other two served as controls. The open control cage served as a control for the effect of the gauze on the weed growth. The cages were anchored one meter apart from each other.

5.2.2 Sampling

Due to the small, variable size of the *Azolla* macrophyte, and the ease with which it fragments, the effect of the weevil on the growth of the weed (plant vigour) was measured by comparing the density (difference in dry weight) of samples from treatment and control cages. Sampling of plant density was carried out using a small scoop with a mesh bottom. The size of the scoop was determined by testing the sample consistency ($n = 20$) of two differently sized scoops (15 cm^2 and 24 cm^2). The smaller of the two provided samples with the least variability (dry weight), and was therefore adopted as the standard measure. Two samples (15 cm^2 each) of the weed were taken from each of the cages once a week until the *Azolla* in the treatment cages was completely cleared. One

sample was hand-sorted to determine the number of weevil eggs, larvae, pupae and adults present. The other sample was placed in a drying oven at 50°C for 48 hours. The first samples, in week zero, were taken before the weevils were released into the cage.

Table 5.1 Field sites used in both summer and winter field-cage trials to quantify the impact of *Stenopelmus rufinasus* on *Azolla filiculoides*. All sites fall within a summer rainfall region that is characterised by cold winters and frost.

Site description	Locality	Previously covered with *Azolla*
Pond – Wits University, Johannesburg	26°11'52"S / 28°01'53"E 1692 m	N
Water hazard – Parkview Golf Course, Johannesburg	26°10'20"S / 28°00'54"E 1666 m	Y
Dam - Delta Park Bird Sanctuary, Johannesburg	26°07'49"S / 28°00'50"E 1580 m	Y
Dam – Chicken Farm, Johannesburg	25°57'34"S / 28°02'15"E 1420 m	Y
Water Reservoir – PPRI, Rietondale, Pretoria	25°43'55"S / 28°14'32"E 1291 m	N

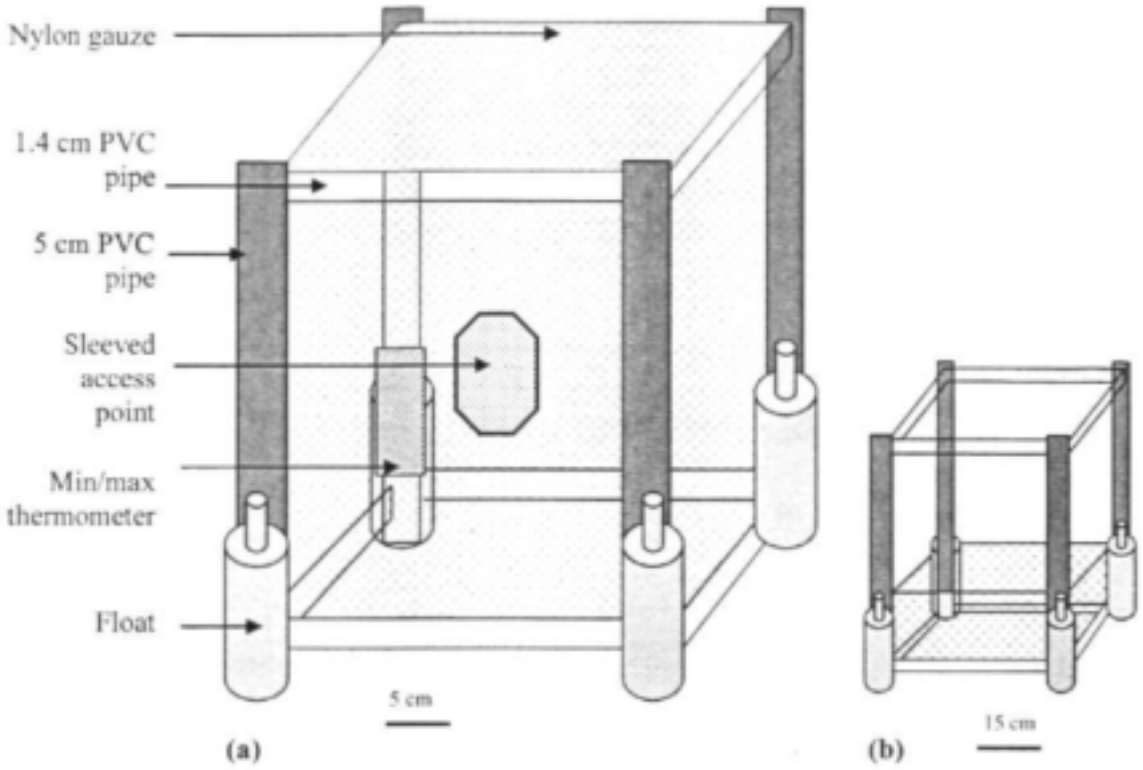


Figure 5.1 Floating field-cages used to monitor *Stenopelmus rufinasus* impact on *Azolla filiculoides*. (a) Cage design with full gauze covering ('treatment' and 'closed control'); (b) Cage design with 20cm gauze skirt ('open control').

5.2.3 Temperature, pH and phosphorous

The minimum and maximum temperatures were recorded weekly for each site by means of min. / max. thermometers in the treatment cages. The water pH and phosphate concentrations for each site were also recorded weekly using a pH meter (Orion, model 420A) and spectrophotometer (Hanna C100 multiparameter bench spectrophotometer, Hanna Instruments) respectively.

5.2.4 Predictive capabilities of the degree-day model

The number of generations of *S. rufinasus* in the cage populations was estimated from the fluctuation of the different numbers of life stages for Johannesburg and Pretoria, for both summer and winter. The approximate number of days for one generation for each season was then calculated, and used to calculate how many generations would be completed during the entire summer (December, January, February) and winter (June, July, August) periods. These values were then compared to the predicted values obtained from the degree-day model for the same seasonal periods (Chapter 4).

5.2.5 Statistics

Plant vigour and insect population dynamics data for summer and winter trials were analysed using repeated measures ANOVA and post hoc Tukey pair-wise comparisons.

5.3 Results

No significant differences in plant vigour were found between the five field sites in summer (repeated measures ANOVA $F_{4,10} = 0.59$; $p = 0.68$) or winter (repeated measures ANOVA $F_{4,10} = 1.24$; $p = 0.36$). Data for all five sites were therefore pooled. At all five of the field sites, total weed clearance was achieved within a period of seven weeks in the summer trial and 14 weeks in the winter trial. Upon clearance of the red water fern, secondary weed infestations of other aquatic plant species occurred at all five of the experimental sites. These included *Lemna* sp., *Spirodela* sp. or *Wolffia* sp. (Lemnaceae).

During the summer trials, the mean (±SE) summer minimum and maximum air temperatures inside the treatment cages were 13.5 ± 0.3°C and 37.1 ± 0.6°C respectively. Temperatures as high as 45°C were recorded at some of the sites in the experimental cage. The mean winter minimum and maximum air temperatures (± SE) were 5.1 ± 0.6°C and 32.3 ± 0.6°C respectively. Temperatures as low as −5°C were recorded at some of the sites.

5.3.1 Plant vigour

The weight of the plant material in all the treatment cages during summer was found to steadily increase until week three, thereafter it rapidly decreased until it disappeared by week seven (Fig. 5.2). The plant vigour of the treatment cages differed significantly from that of both types of control cages (repeated measures ANOVA, $F_{96, 23} = 7.46$, $p << 0.001$) over the seven weeks. The weight of the material in both types of control cages steadily increased over the eight-week period, fluctuating between 0.12g / 15 cm^2 and 0.17g / 15 cm^2. The plant vigour of the open control cages did not differ significantly from the closed controls (repeated measures ANOVA, $F_{77, 1} = 0.02$, $p = 0.88$) over the seven weeks, showing that the gauze had no effect on plant vigour.

During winter, the weight of the plant material in the treatment cages steadily increased until week five, after which it gradually decreased until it was cleared by week 14 (Fig. 5.3). The plant vigour of the treatment cages differed significantly from that of both types of control cages (repeated measures ANOVA, $F_{180, 44} = 7.28$, $p << 0.001$) over the 14 weeks. The mean weight of the material in both types of control cages fluctuated between 0.1g / 15 cm^2 and 0.26g / 15 cm^2 over the 14 week period, with a gradual increase until week 5, and then a slow decline until week 13. This dip in the sample weights occurred as a result of an outbreak of aphids in both cage types. The open control cages did not differ significantly from the closed controls (repeated measures ANOVA, $F_{147, 1} = 2.88$, $p > 0.09$) over the 14 weeks, once again showing that the gauze had no effect on plant vigour.

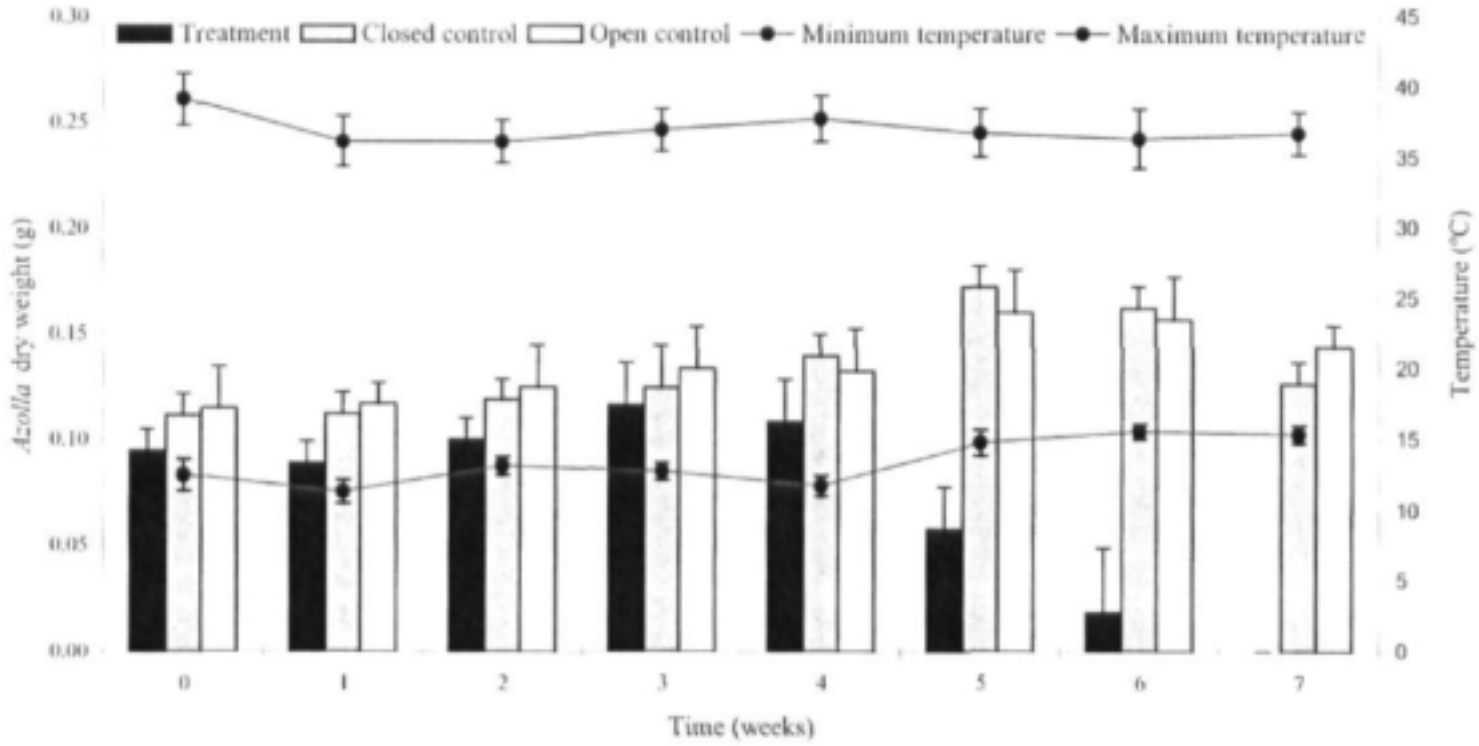


Figure 5.2 The impact of the weevil, *Stenopelmus rufinasus*, on the vigour of *Azolla filiculoides* from field-cages during summer 1999. Each column represents the mean dry weight of plant material from five field sites. Means presented + SE. Temperatures (min. and max.) are means from five sites. Means presented ± SE.

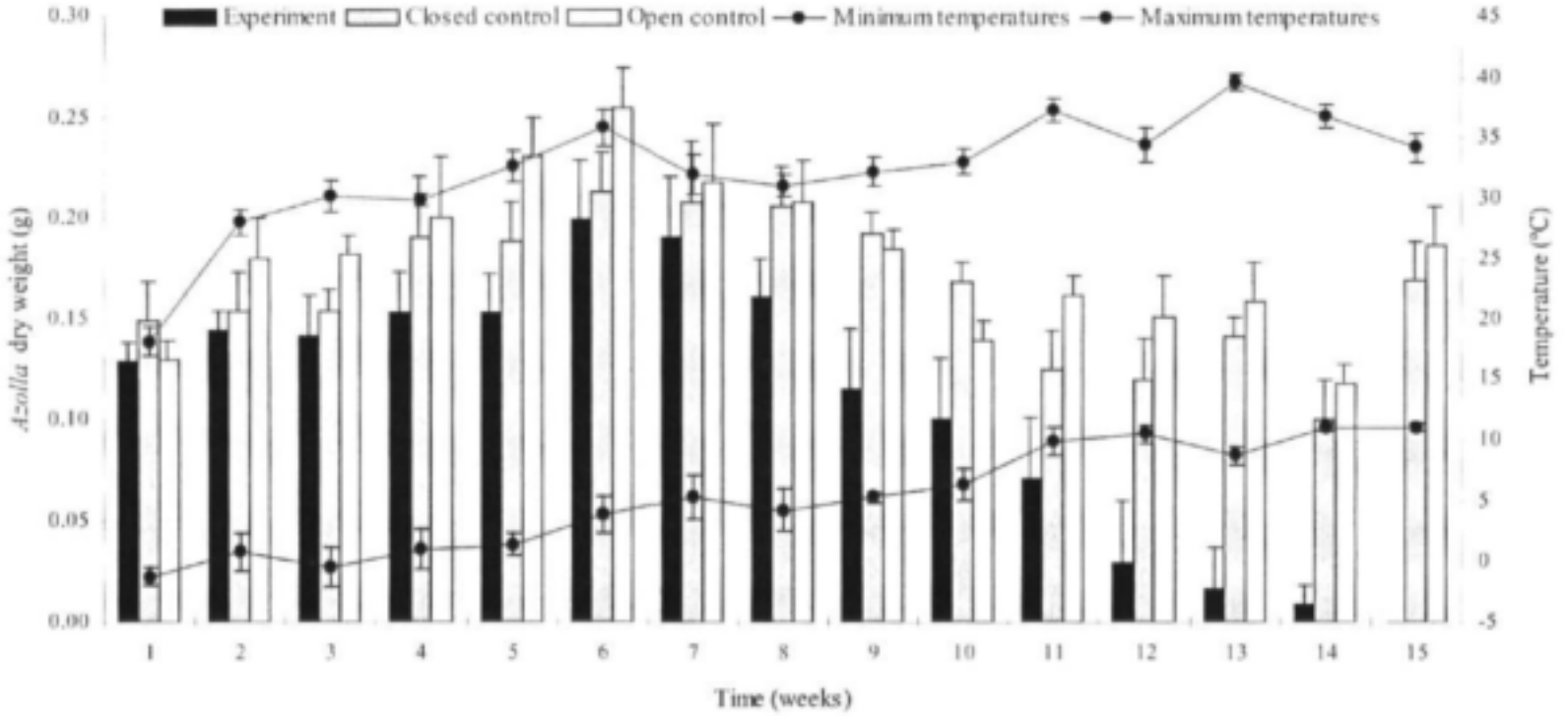


Figure 5.3 The impact of the weevil, *Stenopelmus rufinasus*, on the vigour of *Azolla filiculoides* from field-cages during winter 2000. Each column represents the mean dry weight of plant material from five field sites. Means presented + SE. Temperatures (min. and max.) are means from five sites. Means presented ± SE.

5.3.2 Population dynamics of the weevil

During the summer trials, the mean number of weevil life stages per sample increased steadily until week four (Fig. 5.4). The number of eggs laid per 15 cm^2 sample reached a peak of 9.8 in the fourth week. The mean maximum number of first (14) and second instar larvae (22) per sample were documented in week two, while third instar larvae peaked at very low means of 0.8 per sample in the fourth and fifth weeks. Pupation peaked at a mean of 11.2 pupae per sample at week three, while the mean maximum number of adults (10.6) per sample was recorded at week four. Estimation of the first generation (G1) was at week three, and the second generation (G2) at week five. These estimates were based on temperature-linked developmental data (Chapter 4) and by careful analysis of the population dynamic data. Extrapolating the mean number of weevils (all life stages combined) at week five, to the entire surface area of *Azolla* (in the treatment cage) suggests that there were 26 267 individuals on 0.25 m^2 of plant material. After week five, there was a decline in the number of individuals until week seven when, in the absence of the plant, none were recorded. The remaining larvae and adults had moved onto the sides of the cages where they remained (adults) or died of starvation (larvae).

The winter trials revealed a slow and steady increase of weevils until week six (Fig. 5.5). The mean number of eggs laid per 15 cm^2 sample reached a peak of 4.4 in the fourth week. The mean maximum number of first (3.2) and second instar larvae (2.4) per sample were documented in week 12 and 13 respectively, while third instar larvae peaked at a very low mean of 1.2 per sample in the fifth, seventh and thirteenth weeks. Pupation peaked at a mean of 4.4 pupae per sample at week nine, while the mean maximum number of adults (3.4) per sample was recorded at week eight and nine. Estimation of the first generation (G1) was predicted at week five, the second generation (G2) at week nine, and the third generation (G3) at week thirteen. As with the summer trial, these generation estimates were based on temperature-linked developmental data (Chapter 4) and by careful analysis of the population dynamic data. Extrapolating the mean number of weevils (all life stages combined) at week six to the entire surface area of *Azolla* (in the treatment cage) suggests that there was a total of 1 867 individuals at that time period. From week seven onwards, the total life stage counts gradually declined until week 14 when none were recorded. At this point all the *Azolla* had been cleared in the experimental cage, and once again, the

remaining larvae and adults had moved onto the sides of the cages where they remained (adults) or died of starvation (larvae).

Overall, the population dynamics of *S. rufinasus* during the summer and winter trials showed similar patterns, with the exception that winter trends were extended over double the time period. The rate of increase and total number of weevil feeding stages (first, second and third instars, and adults) found during summer trials was considerably greater than during the winter trials. There was a rapid increase in weevil feeding stages during summer trials, reaching a mean peak of 14.6 individuals per sample at week four (≈ 132 weevils / g) (Fig. 5.6a). A more gradual increase of feeding stages was evident during winter trials, reaching a mean peak of 7.8 individuals per sample at week 12 (≈ 18 weevils / g) (Fig. 5.6b). The impact of the weevil feeding stages on the mean summer *Azolla* dry weights per sample was noticeable, with a peak plant density of only 0.12 g / 15 cm^2, as opposed to the winter peak of 0.2 g / 15 cm^2.

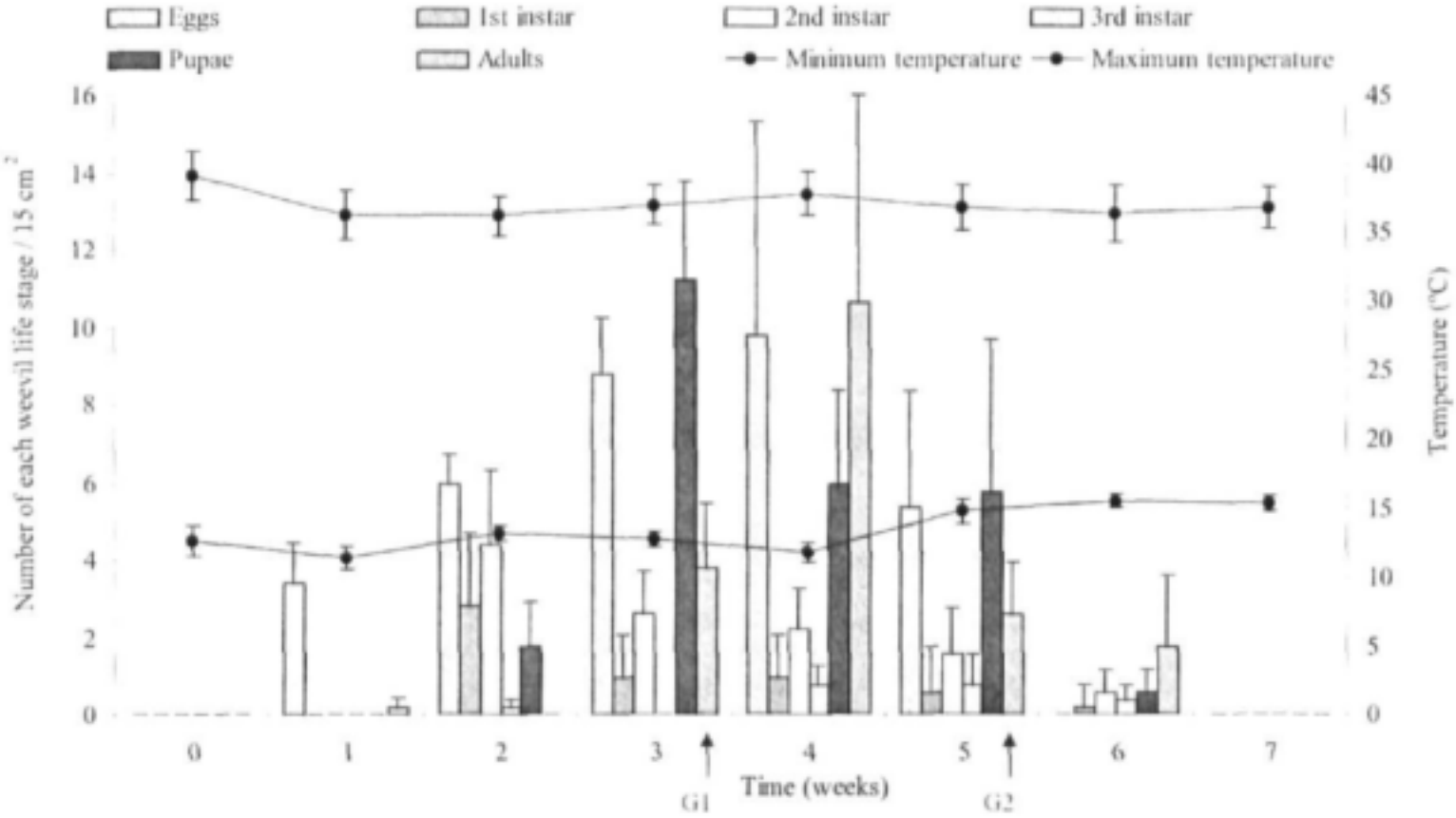


Figure 5.4. *Stenopelmus rufinasus* life stages from a 15cm^2 sample of *Azolla filiculoides* during field-cage experiments in summer 1999. Each column represents the mean number of life stages from five field sites. Means presented + SE. G1 – first generation; G2 – second generation. Temperatures (min. and max.) are the means from the five sites. Means presented ± SE.

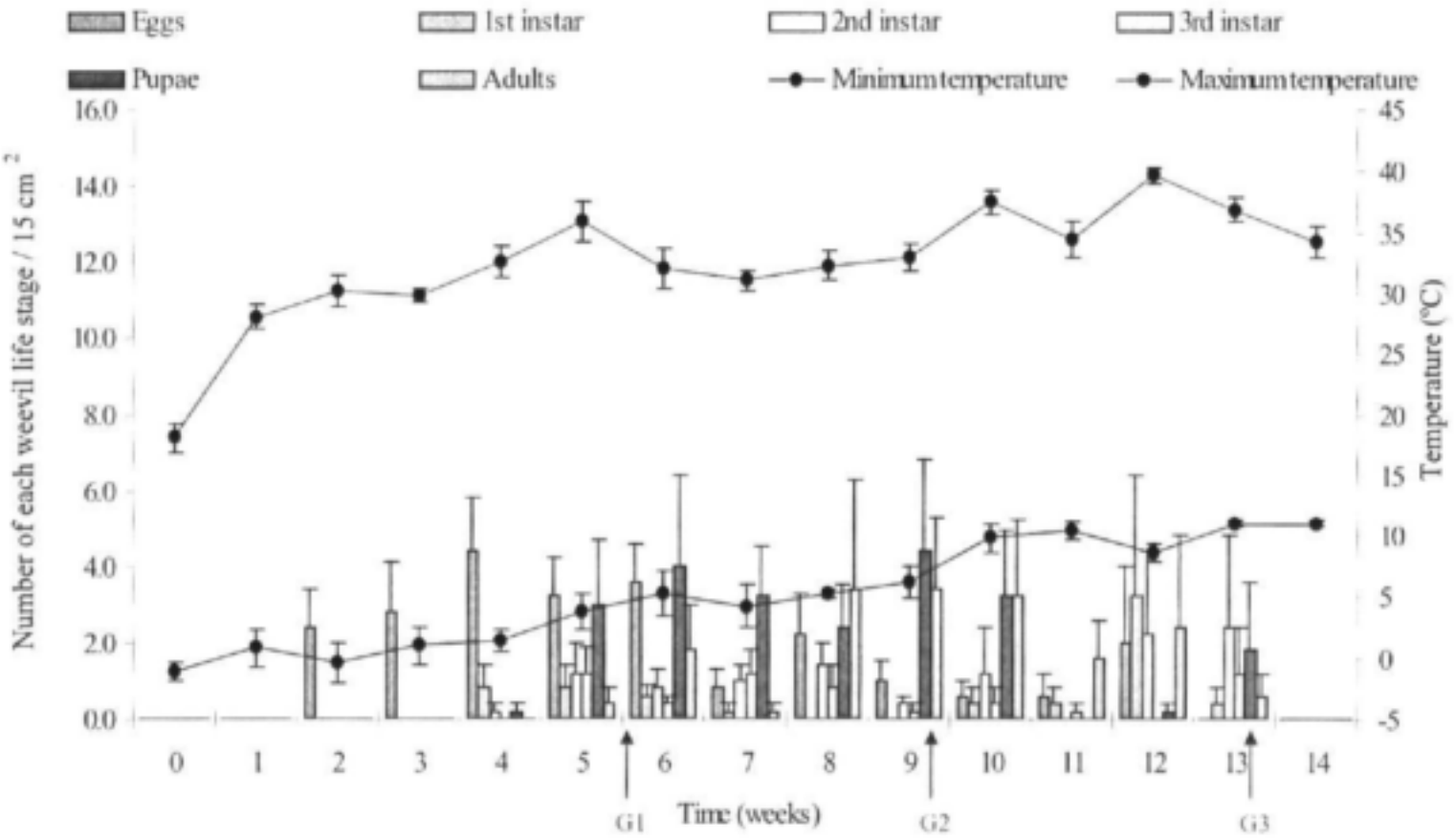
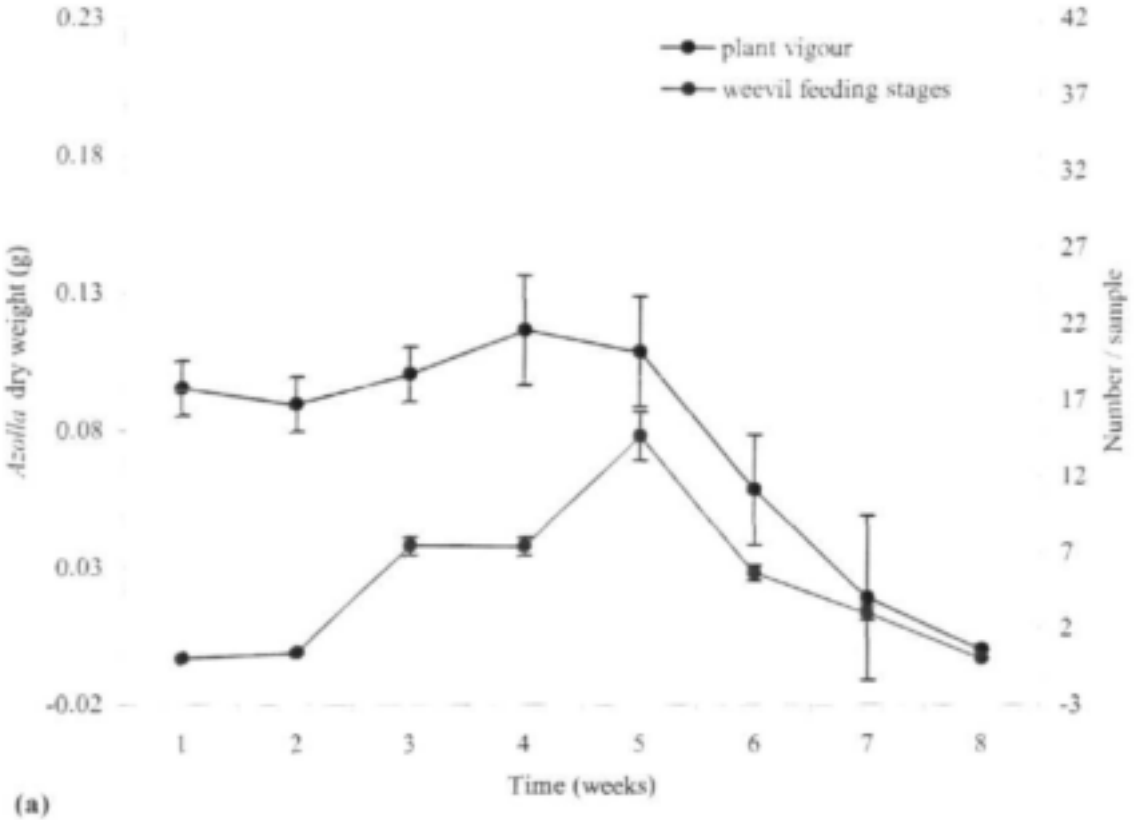
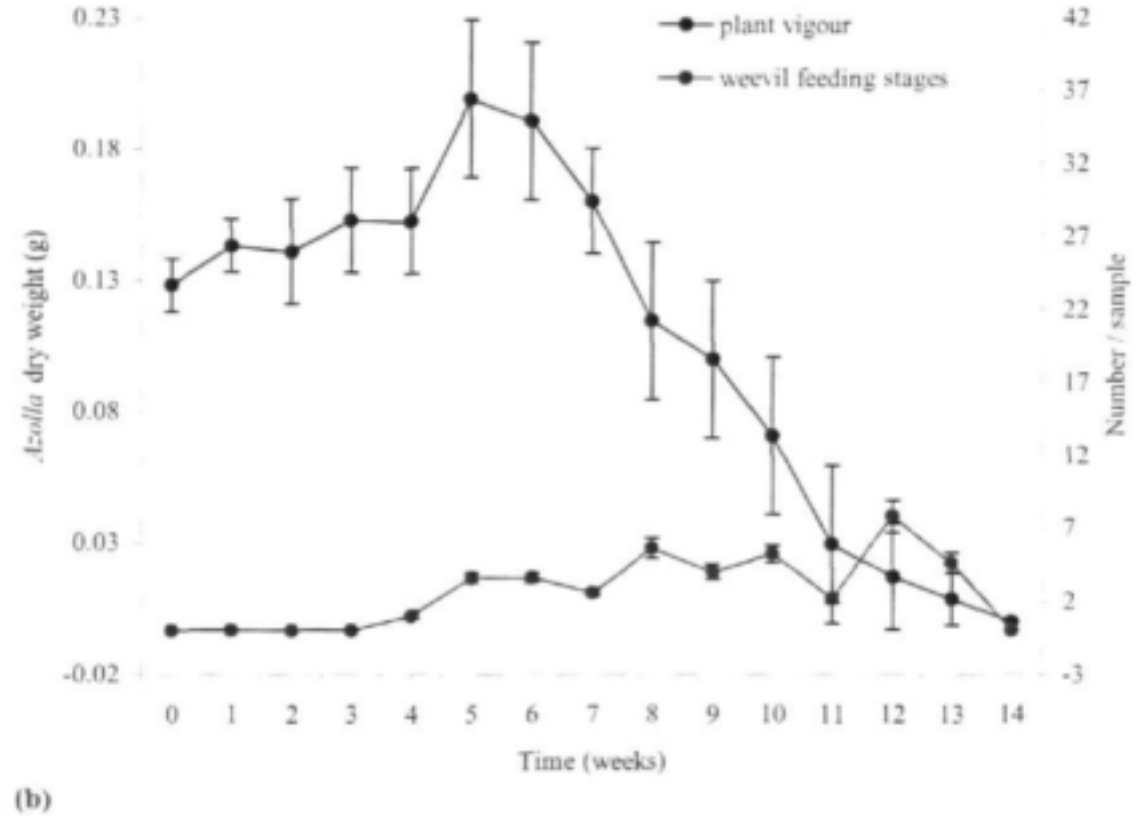


Figure 5.5 *Stenopelmus rufinasus* life stages from a 15cm^2 sample of *Azolla filiculoides* during field-cage experiments in winter 2000. Each column represents the mean number of life stages from five field sites. Means presented + SE. G1 – first generation; G2 – second generation; G3 – third generation. Temperatures (min. and max.) are the means from the five sites. Means presented ± SE.



(a)



(b)

Figure 5.6 Impact of *Stenopelmus rufinasus* feeding stages (L1-L3 and adults) on *Azolla filiculoides* mean dry weight per 15cm^2 sample during (**a**) summer and (**b**) winter. Means presented from five field sites ± SE.

5.3.3 Predictive capabilities of the degree-day model

Using the population dynamics data obtained from the summer and winter cage trials, predictions of the number of weevil generations made by the degree-day model were tested (see Chapter 4. The number of generations predicted for Johannesburg and Pretoria were obtained from summer (Fig. 5.7a) and winter (Fig. 5.7b) maps. The model predicted 3.9 generations for Johannesburg and 4.4 generations for Pretoria during summer (December - February). Summer field-cage data (Fig. 5.4) suggested that the weevils would be able to complete 6.5 generations for the same time period for both localities. During winter (June – August), the model predicted 0.8 generations for Johannesburg and 0.9 generations for Pretoria. Field-cage data, however, showed that the weevil would be able to complete 3.2 generations during that time period for both localities.

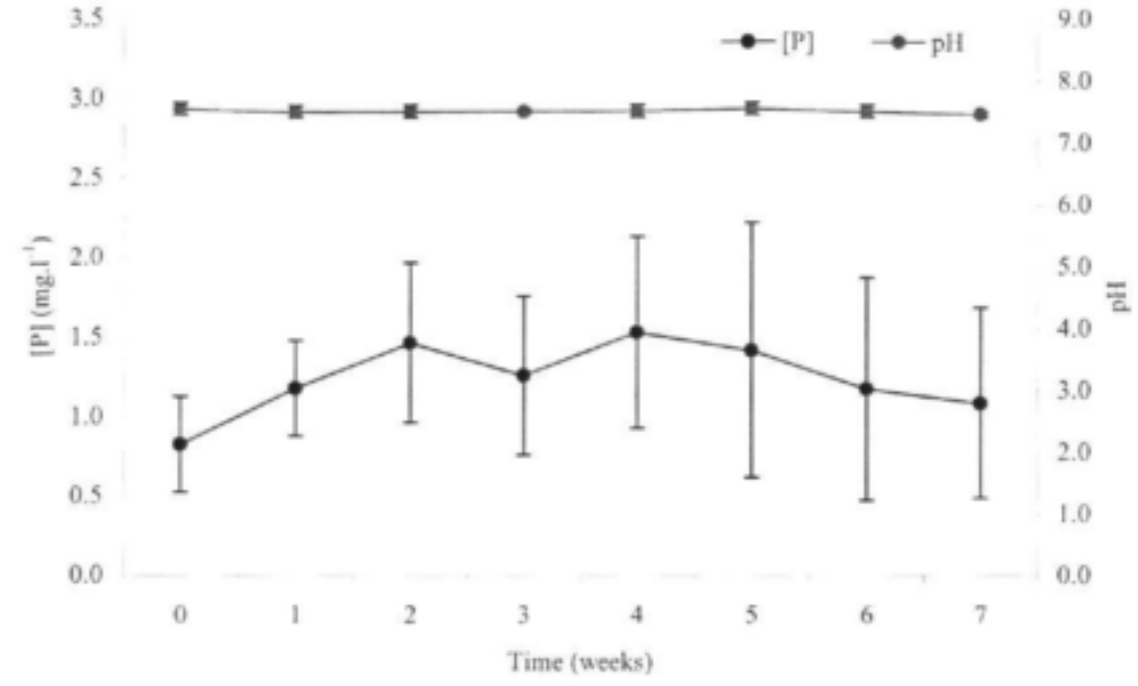
5.3.4 pH and phosphorous

The mean summer phosphate concentration (± S.E.) for the five sites was 1.2 ± 0.2 mg.l^{-1} and the mean pH (± S.E.) was 7.6 ± 0.3 (Fig. 5.8a). The mean winter phosphate concentration for the five sites was 0.7 ± 0.1 mg.l^{-1} and the mean pH was 7.2 ± 0.1 (Fig. 5.8b).

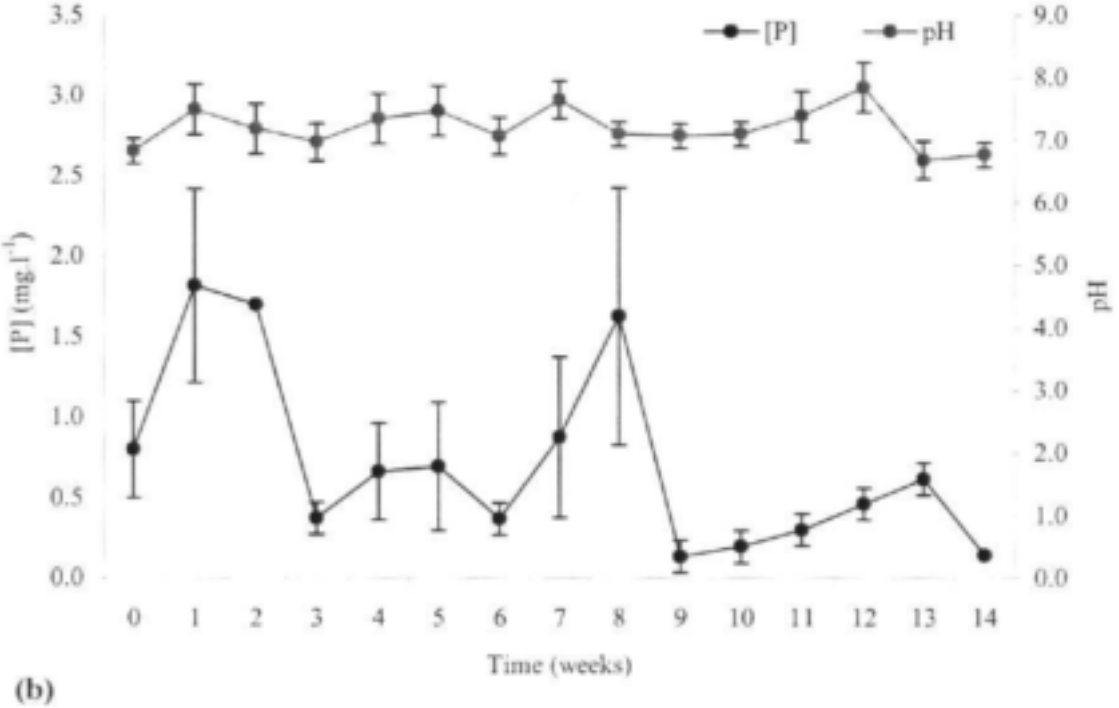
(a)

(b)

Figure 5.7 Degree- day predictions of the potential number of generations of *Stenopelmus rufinasus* during (**a**) summer (December – February) and (**b**) winter (June - August). Blue dot – Pretoria, red dot – Johannesburg.



(a)



(b)

Figure 5.8 Field phosphorous and pH levels recorded during (**a**) summer and (**b**) winter field-cage trials. Means presented from five field sites ± SE.

5.4 DISCUSSION

Relatively little use has been made of cages in assessing the effect of herbivores on pest plants, while cages have been extensively used in the biological control of insect pests (Van Driesche & Bellows, 1996). Despite the negative attributes of field-cages, they have been successfully implemented in weed biological control to aid in the establishment of agents (Thomas & Room, 1984; Wright, 1997), and investigate the effects of temperature and nutrients on biological control agents and their hosts (Julien *et al*., 1987; Room & Fernando, 1992). The confined area of a cage allows for close observation and a rapid build up of agents. In addition, they facilitate the manipulation of various experimental variables and the ease and consistency with which populations can be sub-sampled during an experiment. However, elements such as a lack of predators and an altered microclimate may confound the accuracy of the data obtained through the use of field-cages.

Various studies have shown that the failure of establishment of biological control agents could be directly attributed to climate incompatibility of the agent to its area of introduction (McClay & Hughes, 1995, Stewart *et al*., 1996; Good *et al*., 1997). The use of field-cages, therefore, as part of the pre-release phase of this programme, facilitated the controlled investigation of the climatic predictions made in Chapter 4. The thermal parameters and models used in Chapter 4 predicted that the establishment and distribution of *S. rufinasus* would not be restricted by temperature. These predictions were confirmed in both the summer and winter cage trials (Fig. 5.4 and 5.5). Cage air temperatures of 45°C were recorded during the summer trial, while the winter trial experienced temperatures as low as -5°C. Despite these temperature extremes, survival and development of all weevil life stages were recorded. Only the thermal parameters of adult weevils were tested in Chapter 4. The finding that these parameters appear to be representative of all life stages, suggests that the adult stage may be a good indicator of the weevil's thermal characteristics.

The difference of weed clearance times (7 weeks) between summer and winter can be attributed directly to the temperature-linked development of *S. rufinasus* (Chapter 4). A curious finding from the field-cage trials, however, was that a significantly reduced density of weevils (18 / g: Fig. 5.6b) was required to cause a decline in *Azolla* density in winter (week five) as compared to that required in summer (132 / g: Fig. 5.6a) in

week 3. This difference could be attributed to the phenomenally rapid rate of increase of the weevils in summer. However, the possibility also exists that the damage inflicted on *A. filiculoides* by the weevil feeding stages, and subsequent decline in weed density, is not just reliant on mechanical feeding damage i.e. the weevil may also be transmitting a pathogen to the plant while feeding, which contributes to the decline in plant vigour.

The modified degree-day model used in this study (5.7a and b) appears to have underestimated the number of generations that *S. rufinasus* actually experienced during summer and winter field-cage trials (Fig. 5.4 and 5.5). These differences are most likely attributed to the effects of microclimate – the weevils are not only experiencing buffered temperatures in the *Azolla* mat (see Chapter 4), but also the gauzed cages would obviously have had some effect on air movement and humidity in the cage. As suggested in Chapter 4, however, the effects of microclimate need to be measured and then used to suitably modify standard meteorological data so that it is representative of the conditions actually experienced by the insect. Only then will the accuracy of predictive models be improved.

Winter field phosphorous and pH levels were found to be far more stochastic than summer levels (Figure 5.8 a and b). This may be accounted for by fluctuating water levels in winter (due to dry, windy conditions) and hence varying P concentrations from week-to-week. The mean field phosphorous concentrations recorded at field sites during both summer (1.2 ± 0.2 mg.l^{-1}) and winter (0.7 ± 0.1 mg.l^{-1}) was fairly low, considering that laboratory studies have found *A. filiculoides* to grow optimally (maximum rate of increase) at phosphorous levels of 20ppm (Cary & Weerts, 1992). The weed, however, has been present in South Africa for over 55 years and may have adapted to growing in waters with lower phosphorous levels. The highest density achieved by *A.filiculoides* was during the winter trial (0.23 g / 15 cm^2: Fig. 5.3) – a density one-and-a-half times that recorded in the summer trial (0.16 g / 15 cm^2: Fig. 5.2). This superior winter growth could be attributed to several factors, the most likely of which was that a higher abundance of aphids was noted during summer compared to winter trials. Cary and Weerts (1992) also noted that *A. filiculoides* shows optimal growth at pH values of 5 and 7. The mean field pH values were just outside the optimal range both during summer (7.6 ± 0.3) and winter (7.2 ± 0.1).

Phenotypically, *A. filiculoides* is very plastic, varying under environmental influences (Ashton, 1978; Watanabe & Berja, 1983; Moretti & Gigliano, 1988). In addition, spore production only occurs in summer, and even then only under particular environmental conditions (Ashton, 1982). As a result, measuring the impact of *S. rufinasus* on the weed using plant morphometrics or spore production was not feasible. The decision to use plant density (as dry weight per unit sampling area) as a measure of the impact of the weevil on plant vigour, followed other successful aquatic weed studies, which used a similar technique (e.g. Harley *et al.*, 1984; Room *et al.*, 1984). The results of this field-cage study confirmed the findings of Hill (1998b), who showed the weevil to be a voracious feeder capable of rapidly destroying mats of the weed while still in quarantine culture. Although the trials in this study were conducted under the fairly restricted conditions of field-cages, the phenomenon of a rapid increase in the weevil population, followed by a complete collapse of the mat, has also been reported from field sites in Florida, U.S.A. (Center[1], pers. comm.).

Hoffmann (1990) emphasised that success in weed biological control could only be claimed when, through suitable evaluation, the impact of the agents had caused a decrease in the weed density or inhibited the spread of the weed. He further emphasised that it was erroneous to equate establishment and visible damage by the agent with success, as the impact of herbivore damage on the population dynamics of plants was often not apparent. There are many examples of aquatic weed biological control where success has been measured in terms of biomass and surface area reduction (Room *et al.*, 1981; Room & Thomas, 1985, 1986; Cilliers, 1991a, b; Cilliers, 1999; Julien *et al*, 1999; Buckingham, 2002). By definition, biological control will not eradicate a weed. However, Julien (1997) acknowledged the occurrence of local extinctions. According to Hoffmann's (1995) definitions of success, the impact of *S. rufinasus* on *A. filiculoides* during field-cage trials would predict complete success under field conditions. However, the complete extermination of plant material in the cage trials of this study suggests that, under natural field conditions, success might more accurately be described as 'local extinctions'.

[1] Dr. Ted Center, United States Department of Agriculture, Florida.

The use of field-cages in providing an intermediate link between the laboratory and the field has provided valuable insight into the establishment and population dynamics of *S. rufinasus* and the resulting impact on the vigour of *A. filiculoides*. Furthermore, they have provided a means of testing the predictive capabilities of the models examined in Chapter 4, and have offered insight with regards to the level of success that might be achieved at infestations of the weed. However, after noting the shortcomings of cages, the findings of this study should be treated with caution until they are verified on a large scale under natural field conditions (Chapter 6).

CHAPTER 6

FIELD ASSESSMENT OF THE IMPACT OF *STENOPELMUS RUFINASUS* ON *AZOLLA FILICULOIDES* IN SOUTHERN AFRICA

6.1 Introduction

The degree of control that a biological agent will exert on its target weed under natural field conditions is difficult to predict from laboratory studies (Forno & Julien, 2000) because favourable results recorded under quarantine or artificial field conditions (e.g. cages) are not always reproduced in the open field for various reasons. First, the agent may not be climatically suited to the area of introduction. Optimal temperature, light and humidity used under artificial conditions may deliver favourable results, however, field temperatures may negatively impact the performance of the agent. Second, the agent could be adversely affected by predators and parasitoids that were excluded during cage trials (Luck *et al.*, 1999). Insecticides or herbicides may add a further barrier to the ability of the biological control agent to elicit control (Ueckermann & Hill, 2001).

From the thermal physiology and predictive modelling work on *Stenopelmus rufinasus*, temperature is known not to be a limiting factor in the establishment and distribution of the weevil in South Africa (Chapter 4). Field-cage trials showed that *S. rufinasus* was able to establish and completely exterminate small populations of *Azolla filiculoides* during both summer and winter (Chapter 5), suggesting that it might be capable of local extinctions of the weed under natural field conditions. The final test for a new biological control agent, however, has to be regarded as its performance on field populations of the target plant throughout its range, in varying climatic and topographical conditions. Quantifying the field performance of the control agent, however, can be a persistent challenge for biological control researchers (Harris, 1997).

Forno and Julien (2000) felt that methods for measuring the success of biological control agents released on weeds are theoretically sound. Hoffmann (1995) proposed several definitions for success: (a) 'complete' – where no other control method is required; (b) 'substantial' – where other control methods are required, but to reduced

extent; and (c) 'negligible' – where despite agent damage, weed control is still dependent on other control techniques. Moran and Zimmermann (1984) suggested more complex quantitative methods of defining success, which score the degree and area of impact of each agent, together with the relevant importance and distribution of each target weed species in each country. Julien (1997) reccomended a combination of qualitative (using sociological and environmental descriptors) and quantitative methods (using ecological and economic data) including the use of cost-benefit analyses in describing agent success.

It was proposed by McFadyen (1998) that Hoffmann's (1995) definitions of success be adopted by the international biological control community in order to standardise its description. Julien (1997), however, felt that while descriptions like Hoffmann's (1995) were useful in some situations, they oversimplified the reality that includes variation in time and space i.e. because biological control is driven by interactions with the environment, it is dynamic and variable. Thus levels of control might vary between seasons and there may be periods of control that are inadequate. Similarily, factors such as climate, may preclude control throughout a weeds range (see McClay & Hughes, 1995). Also, levels of control may be adequate for certain activities but not for others (e.g. a reduction in water hyacinth may improve water transport but not net fishing). Julien (1997) thus suggested that knowledge of spatial and temporal variability was essential for realistic assessments of the level of control that has been achieved.

In addition to adopting the most suitable methods for measuring the success for a particular biological control programme, Harris (1991) stressed the need to differentiate between three types of success: First, 'biological success', which is a measure of how well a particular plant resource is utilised over an infested area; second, 'impact on the target plant', which is a measure of the reduction of biomass or reproductive potential of the weed at sites favourable to the agent; and third, 'control success', which following the reduction in weed biomass, quantifies economic, environmental and human gains. Forno and Julien (2000) suggest that 'biological success' of agents is best evaluated individually, while 'impact on the target plant' and 'control success' can be assimilated into a measure of 'impact success'.

Post-release evaluation of biological control agents is at best *ad hoc*, if it happens at all. The *A. filiculoides* programme fortuitously brought together money and manpower, with a rapidly establishing and effective agent which has allowed the dramatic events of this very successful work to be summarised. The aim of this study was to assess the biological and impact success of *S. rufinasus* under natural field conditions in controlling *A. filiculoides* since its release in South Africa.

6.2 Materials and Methods

Weevils were mass reared at the Plant Protection Research Institute (PPRI), Rietondale, Pretoria and at the University of the Witwatersrand (Wits), Johannesburg, and released at 112 sites between 1997 and 2002. This is about 40 sites less than the total number of *A. filiculoides* infested sites listed by Henderson (1999). Ongoing laboratory and field experiments, however, indicated that *S. rufinasus* was able to disperse unaided and that those sites where weevils were not released would soon be colonised. Weevils were released either directly (during field trips), or mailed via the national postal service to the affected water-users upon request. Release site details were captured using a 'release questionnaire' (Appendix A). Initially, weevils were released in batches of 500. However, this number depleted laboratory cultures, and 100 weevils was found to be sufficient to establish a viable field population. Weevils were most often collected as copulating pairs in the mass-rearing facility, so an assumption was made that equal numbers of males and females were released. Where possible, sites were visited twice annually over a four-year period. When site visits were not feasible, telephonic contact was maintained with the respective landowners to ascertain the status of the weed. All sites were visited at least once during the study. A record was kept of weevil establishment and the impact of the weevils on the weed (i.e. changes in area of the water body covered, time taken for the weed to disappear, re-appearance of the weed and re-colonisation by the weevil). Details of weevil population dynamics and their effects on plant vigour were not collected at the field sites because of replicating the counting and emergence procedures while in the field. The effects of the weevils on the weed were recorded using 'before' and 'after' fixed point photographs at 20 sites.

Forno and Julien (2000) proposed a scoring system to rate the impact success of phytophagous arthropods following their establishment as biological control agents against aquatic weeds (Table 6.1). This system was adapted from Goeden (1983) and Harris (1991) and is applied specifically to aquatic weeds. It was used in this study to compare the impact of *S. rufinasus* on *A. filiculoides* with other biological control agents that have been used against aquatic weeds elsewhere in the world.

Table 6.1 A scoring system to assess the impact success of biological control agents on aquatic weeds (From Forno & Julien, 2000).

Criterion		Score
1. *Environment limitations*		
A	Restricted by habitat and/or climate	2
B	Not restricted by habitat and/or climate	4
2. *Implications for management*		
A	No change in the losses caused by the weed and/or in management practices	0
B	Biological control integrated with other control options results in economic or environmental gain in most areas	2
C	Biological control alone results in economic or environmental gain in most areas	4
3. *Duration until control achieved*		
A	No control	0
B	Control achieved in 5 or more years	2
C	Control achieved in less than 5 years	4
4. *Impact on biomass and area covered*		
A	No reduction in biomass, or the area covered or infested	0
B	Reduction in biomass but no change in the area covered in most infested areas	2
C	Area covered reduced to an acceptable level in less than 50% of the infested areas	4
D	Area covered reduced to acceptable level in 50% or more of infested areas	6

6.3 Results

Stenopelmus rufinasus was released at 112 *Azolla*-infested sites around South Africa (total surface area of 208.5 ha), and (7 ha) in Zimbabwe (Table 6.2). Before the insects were fully established, the weed infestations at 13 sites were washed away by floods, and a dam at one site was drained. Eighty-one percent of all the field sites (91 sites; 203.5 ha), were completely cleared of *A. filiculoides* by the weevils in an average of 6.9 (±4.3) months per site. The status of the remaining seven sites was not determined. Following destruction, no *A. filiculoides* plants were located at any of the sites that had been cleared by *S. rufinasus* (Fig. 6.1a–f). At some sites, secondary infestations of *Lemna* sp. (Lemnaceae), *Spirodela* sp. (Lemnaceae) and *Wolffia* sp. (Lemnaceae) were apparent (Fig. 6.1f), indicating that these sites were eutrophic, probably as a result of human activities (Pieterse, 1993).

Table 6.2 Records of *Azolla filiculoides* in southern Africa where *Stenopelmus rufinasus* has been released, showing the success rate and time to reach control.

Province / Country	Area of *Azolla* infestation (ha)	No. of weevils released	No. sites controlled (%)	Area of *Azolla* cleared (ha)	Mean time to control (months ± S.D.)
Eastern Cape	77.3	4000	17 (89.5)	76.3	4.6 ± 3.4
Free State	52.1	8500	33 (84.6)	49.1	7.4 ± 3.8
Gauteng	20.5	4600	13 (92.90)	19.0	7.1 ± 4.9
KwaZulu Natal	3.0	500	3 (60)	3.0	7.4 ± 0.0
Limpopo	7.5	400	3 (75)	6.5	11.8 ± 7.2
Mpumalanga	20.1	1600	5 (41.7)	16.6	5.5 ± 4.2
Northern Cape	13.0	600	2 (50)	11.0	9.0 ± 4.8
Western Cape	15.0	4200	12 (100)	15.0	6.4 ± 4.8
Chiredzi (Zimbabwe)	7.0	300	3 (100)	7.0	10.8 ± 0.0
TOTALS	**215.5**	**24700**	**91 (81.3)**	**203.5**	**6.9 ± 4.3**

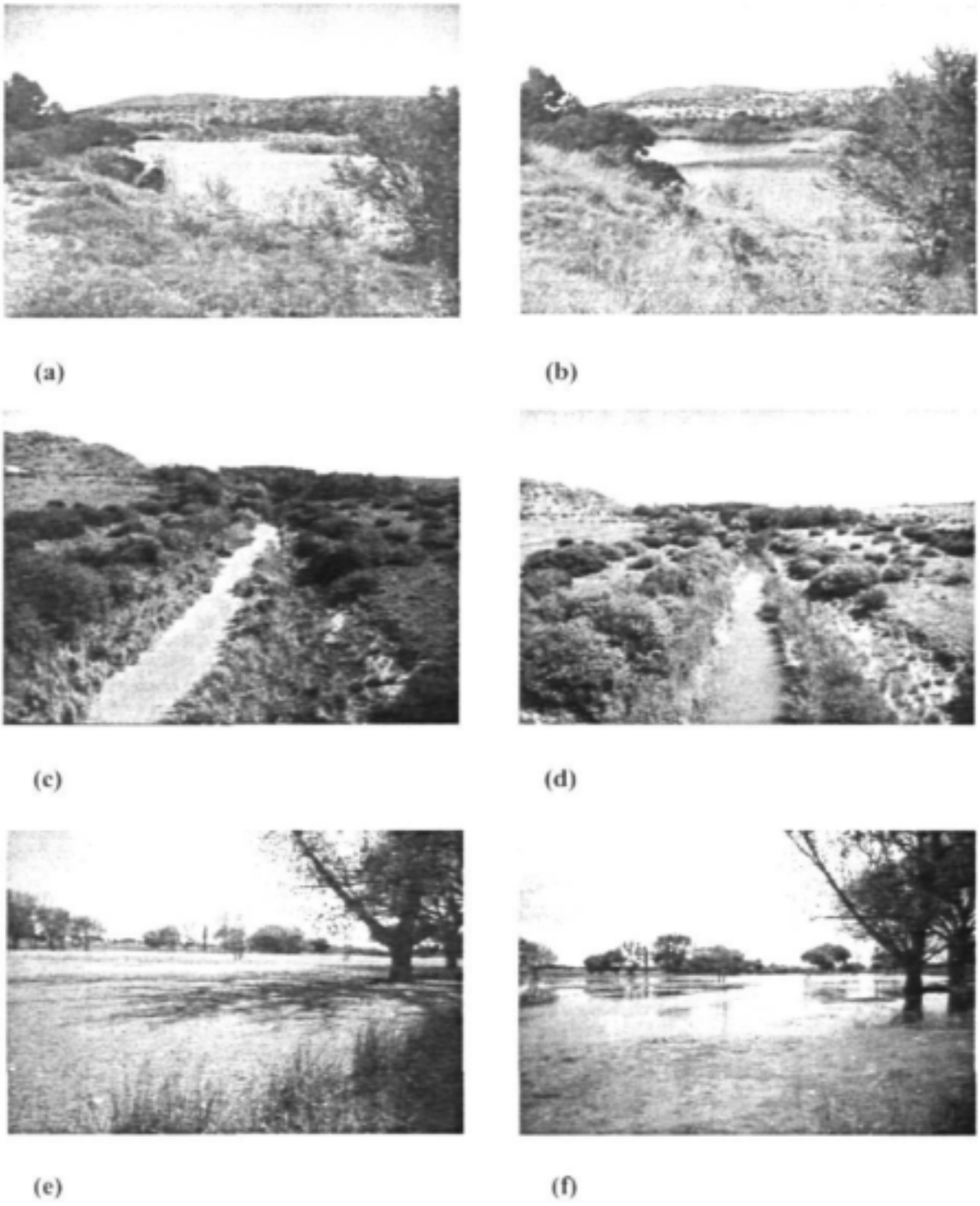


Figure 6.1 Before and after photographs of the impact of *Stenopelmus rufinaus* on *Azolla filiculoides* in the field in South Africa. **(a)** and **(b)**: Witmos, Eastern Cape Province – 312 days to clearance. **(c)** and **(d)**: Slykspruit River, Free State Province – 271 days to clearance. **(e)** and **(f)**: Sasolburg Nature Reserve Dam, Free State Province – 270 days to clearance. Note secondary infestation of *Wolffia* sp., *Spirodela* sp. and *Lemna* sp. in foreground of **(f)**.

After destruction by *S. rufinasus*, *A. filiculoides* re-appeared at only 22 release sites, mostly in the Free State Province (Table 6.3). The average time to re-appearance of the weed was 12.2 (±7.2) months. These re-appearances could have been due to the germination of spores (Ashton, 1982) or due to the movement of whole plant material by waterfowl or other agents. Each of the 22 sites was eventually recolonised by *S. rufinasus*, unaided, and subsequently the weed was cleared again from 18 of these sites. The remaining four sites have not been revisited. This is similar to the situation recorded in southern USA where the weevils disperse after causing local extinction of their host plant, but recolonise the original sites once the plant populations recover (Center[1], personal communication). In addition, once the weed mat has collapsed, quiescent adults have been found on other aquatic vegetation and have been able to stay alive for over two months without food (McConnachie, unpublished data). These individuals are a potential source for populations of weevils that recolonise re-appearing mats of the host plant.

This weed control programme against *A. filiculoides* using *S. rufinasus*, scored 18 on the Forno & Julien (2000) impact scale, and ranks highest of all other aquatic weed control projects reported (Table 6.4).

[1] Dr. T. Center, senior researcher – aquatic weeds, United States Department of Agriculture, Fort Lauderdale, Florida.

Table 6.3 Sites at which *Azolla filiculoides* re-appeared subsequent to clearing by *Stenopelmus rufinasus*. All 22 of these sites were re-located by the weevils and the extent of the damage they inflicted on the host plant is also recorded.

Locality	Co-ordinates	Time to re-apperance (months)	Damage to plant after re-location by weevil
Eastern Cape Province			
Graaf Reinet	32°03'42"S 4°14'02"E	6	Damaged
Witmos (Cradock)	32°31'12"S 25°36'48"E	18	Controlled
Molteno	31°12'34"S 26°35'17"E	13	Controlled
Free State Province			
Bethulie (Zoetvlei)	30°30'24"S 5°53'33"E	18	Damaged
Bethulie (Iona)	30°29'51"S 5°47'12"E	15	Controlled
Harrismith GC	28°15'47"S 29°07'44"E	12	Controlled
Harrismith Dam	28°16'52"S 29°06'43"E	13	Controlled
Slykspruit	30°13'55"S 26°05'49"E	12	Controlled
Smithfield	3026 BA	14	Controlled
Viljoenskroon	27°10'21"S 26°55'30"E	20	Controlled
Westminster	29°12'56"S 7°12'58"E	17	Controlled
Gauteng Province			
Marievale	26°21'27"S 28°30'55"E	2	Controlled
Midrand	25°57'36"S 28°09'51"E	7	Controlled
Limpopo Province			
Rust de Winter	25°13'42"S 28°29'48"E	3	Controlled
Mpumalanga Province			
Bethal	26°27'05"S 29°27'41"E	6	Controlled
Messina	2230 AC	5	Controlled
Potchestroom	26°43'75"S 27°04'02"E	3	Controlled
Northern Cape Province			
Colesburg	30°37'57"S 5°21'21"E	7	Damaged
Western Cape Province			
George East	33°59'47"S 2°31'18"E	9	Damaged
Steenberg	34°04'35"S 8°25'38"E	19	Controlled
Noordhoek	34°07'14"S 8°22'54"E	31	Controlled
Wynberg	34°01'24"S 8°29'23"E	19	Controlled
Summary:	**Reoccurence at 22 sites**	**[a]12.2 (±7.2) months**	**18 sites controlled**

[a] mean (±S.D.)

"damaged" – weed turns purple; visible larval feeding damage.

Table 6.4 Assessment of the impact success of phytophagous arthropods following establishment on their target weeds (Forno & Julien, 2000). Scores are averaged over the countries where the agents established. Shaded area highlights the scores for *Stenopelmus rufinasus* impact on *Azolla filiculoides*.

Target weed and agent	Years of introduction	No. of countries	Environmental limitations	Implications for management	Duration until control achieved	Impact on biomass and area covered	Average scores for impact success
Red water fern							
Stenopelmus rufinasus	1997	2	4.0	4.0	4.0	6.0	18.0
Salvinia							
Cyrtobagous salviniae	1980-96	12	3.7	4.0	4.0	6.0	17.7
Cyrtobagous singularis	1971-79	3	4.0	0.0	0.0	0.0	4.0
Paulinia acuminata	1969-75	5	4.0	0.0	0.0	0.0	4.0
Samea multiplicalis	1976-81	3	3.0	0.0	0.0	0.0	3.0
Water lettuce							
Neohydronomus affinis	1982-96	7	3.4	3.7	4.0	5.1	16.2
Water Hyacinth							
Neochetina bruchi	1974-93	8	3.3	3.8	4.0	4.8	15.9
Neochetina eichhorniae	1971-91	14	3.4	2.4	2.4	3.0	11.2
Niphograpta albiguttalis	1977	2	3.0	0.0	0.0	2.0	5.0
Orthogalumna terebrantis	1971-86	2	4.0	0.0	0.0	0.0	4.0
Hydrilla							
Bagous hydrillae	1991	1	2.0	0.0	0.0	0.0	2.0
Hydrellia balciunasi	1989	1	2.0	0.0	0.0	0.0	2.0
Hydrellia pakistanae	1987	1	2.0	0.0	0.0	0.0	2.0
Alligator weed							
Agasicles hygrophila	1964-86	5	2.4	3.2	3.2	4.4	13.2
Amynothrips andersoni	1967	1	4.0	0.0	0.0	0.0	4.0
Arcola malloi	1971-77	2	3.0	2.0	2.0	2.0	9.0

6.4 Discussion

Two aspects of this biological control programme seem to be unusual with respect to other weed control efforts. First, *S. rufinasus* literally eradicated the weed at most of the release sites that were not affected by flooding. No *A. filiculoides* plants could be located at any of these sites after control. Complete eradication of the target weed is very rare in biological control, and it is perhaps more accurate to refer to the observations in this study as 'local extinctions' of the weed. In the biological control of insect pests, local extinctions are considered to deliver satisfactory control in model systems, as opposed to the classical stable-target-equilibrium model in biological control theory (Murdoch *et* al., 1985). Second, biological control usually requires a number of years before its effects are fully realised (McFadyen, 1998). In this programme, water bodies were cleared of *A. filiculoides* in seven months on average. Cilliers (1991a, b) reported similar clearance times in South Africa for water lettuce, *Pistia stratiotes*, ranging from 9 – 10 months, and slightly longer clearance times for water fern, *Salvinia molesta*, ranging from 13 – 48 months.

Using the Forno & Julien (2000) impact scale, this programme ranks highest of all other aquatic weed control projects reported. It even scored even higher than *Cyrtobagous salviniae* Calder and Sands (Coleoptera: Curculionidae) on *S. molesta*, and *Neohydronomus affinis* Hustache (Coleoptera: Curculionidae) on *P. stratiotes*, which are widely regarded as two of the most successful weed biological control programmes worldwide (McFadyen, 1998). One may be inclined to view such a high ranking with caution, considering the other projects listed (Table 6.4) are established projects where each agent has been released in a number of countries over an extended period of time. However, there are some criteria which have not been scored, where *S. rufinasus* ranks highly.

On average, the other biological control agents listed have been introduced against their respective aquatic weeds over a period of 9.1 years (± 7.8yrs) (clmn 2, Table 6.4). *Stenopelmus rufinasus*, however, was introduced in one year and has caused local extinction of the weed so effectively that there has been no need for further

introductions. This criterion is not weighted and possibly should be as it indicates, to some degree, the effort required to establish the agent. *Stenopelmus rufinasus* has been released in relatively few countries compared to some of the other programmes (clmn 3, Table 6.4), and if weighted, *S. rufinasus* would score poorly on this criterion. The environmental limitations (clmn 4, Table 6.4) score for *S. rufinasus* is supported by physiological data that show the weevil copes with extremes of climate (Chapter 4). In the field, the weevil has established on, and controlled, red water fern in a wide variety of habitats ranging from high elevation (>1500m) to sub-tropical and coastal regions. Economic and environmental gains were achieved exclusively by biological control in this programme, justifying the high score for the mangement criterion (clmn 5, Table 4.4). Rapid control (clmn 6, Table 6.4) and impact on biomass (clmn 7, Table 6.4) are two criteria where the biological control of *A. filiculoides* by *S. rufinasus* cannot score high enough. However, the notion of 'control' should be clarified. Populations of the weed were not only reduced to acceptable levels; the weevil caused local extinctions. No individual plants could be located at any of the controlled sites. The strong likelihood of the weevil / *Azolla* relationship being a new association, as well as the possibility of a weevil transmitted pathogen aiding the control effort (Saacks, 2002), are thought to be key determinants in this result. The Forno and Julien (2000) scoring system ranks the impact (degree of success) of biological control agents on aquatic weeds in their introduced range. However, they acknowledge that this predictive process is difficult, and that the criteria chosen offer scope to improve agent selection and assessment in future programmes and so enhance levels of success in the biological control of water weeds.

This study has used a combination of qualitative and quantitative methods to describe successful biological control. The economic benefits of the *A. filiculoides* biological control program are reported in Chapter 7, and together with these data, show that *S. rufinasus* is now well established and self-sustaining throughout South Africa, and that *A. filiculoides* has been eliminated as a threat to southern African aquatic ecosystems. This study, however, has also shown that red water fern has re-appeared at 22 sites around the country, probably as a result of spores left behind from previous infestations. The question that begs to be answered here is: Why have so many of the

sites, where the weevil caused local extinction of the weed, not experienced a re-appearance of the weed? Other aquatic weeds like water hyacinth have seeds which are viable for 5-20 years (Matthews *et al.*, 1977), and once conditions are suitable, these propogules will germinate and re-infest water bodies. This may be the case for *A. filiculoides*, where viable spores are present in the sediment of a water body, waiting for suitable conditions to germinate. Or, the weed may simply not be producing viable spores.

CHAPTER 7

ECONOMIC EVALUATION OF THE SUCCESSFUL BIOLOGICAL CONTROL OF *AZOLLA FILICULOIDES* IN SOUTH AFRICA

7.1 Introduction

Biological control of weeds is generally considered successful when the target plant population has been significantly reduced and no additional control methods are required, as is now the case of *A. filiculoides* in South Africa. Success is usually described using ecological criteria, which are difficult to quantify, or descriptions of sociological or environmental benefits (Julien & White, 1997). The reduction of a weed can be measured in terms of an increase in crop production and / or reduced costs of other control measures (Julien & White, 1997). For example, where alligatorweed, *Alternanthera philoxeroides* (Mart.) Griseb. (Amaranthaceae), was locally controlled on a river in Australia, the local council saved A$8,000 per year on herbicide applications (Julien, 1981). Such savings, however, have not always been quantified.

A commonly used procedure in the assessment of biological control projects since the early 1930s (Huffaker *et al.*, 1976), is the calculation of benefit-cost ratios. The decision rule for this protocol implies that a biological control activity is economically viable if the ratio of the present value of benefits to the present value of costs exceeds one. Nevertheless, it should be noted that such a decision rule does not give information on the economic viability of possible alternative control projects, and these should ideally also be compared according to the same decision rule before selecting an option. An analysis on the cost-effectiveness of alternative control options, therefore, would be beneficial prior to calculating benefit-cost ratios to obtain the relative ranking of these control options. If one option is already more cost-effective than the alternatives, and it is expected that benefits would also be higher, a cost-effectiveness analysis would be sufficient to generate a ranking on which option to use. Benefit-cost calculations, however, have the additional advantage of expressing the costs of control in terms of the efficacy of control, and thus in terms of the potential economic losses that will be avoided.

The positive benefit-cost ratios for many projects indicate the efficacy of classical biological control, and in some cases, indicate high economic viability (Table 7.1). These studies, however, cannot be compared directly with each other, unless the same cost and benefit categories were used. Despite these methodological differences, benefit-cost ratios have become increasingly important in describing the success and potential of the biological control method (Headley, 1985). The successes achieved with classical biological control, however, cannot always be depicted in terms of benefit-cost ratios. Often specific project costs and benefits are sketchy or lacking (Andres, 1977). A good part of this can be attributed to the difficulty of assigning values to the many intangible benefits and losses from the weeds themselves (Andres, 1977; Dahlsten *et al.*, 2000) and the expected rate of spread of these species (De Wit *et al.*, 2001). In addition, biologists often seek counsel from economists with experimental results that do not lend themselves to economic evaluation (Headley, 1985). This is evident in the methods of early studies (e.g., Box, 1960, Melville, 1959; Simmonds, 1960), which clearly focussed on the biology of the control effort rather than the economic details. Headley (1985) noted that without economic evaluation as an objective, scientific economic evaluation would continually fall victim to *ad hoc* procedures to estimate the values of missing parameters. More recent studies (e.g., Doeleman, 1989; Chippendale, 1992; Coombs *et al.*, 1996; Dahlsten *et al.*, 2000; CRC, 2001), however, have followed methodical economic approaches in the calculation of their respective benefit-cost ratios. The aim of the study, was to determine the economic viability of the biological control programme of *A. filiculoides* in South Africa.

Table 7.1 Examples of benefit-cost results of some successful biological control projects.

Pest species controlled	Region	Date of control	Benefits / annum (US$)	Costs (US$)	Benefit - Cost ratio	Reference
INSECTS						
Diatraea saccharalis (sugarcane borer)	West Indies	1945	41,250	21,250	1.9:1	Box (1960)
Planococcus kenyae (coffee mealy bug)	Kenya	1939	1,250,000	75,000	16.7:1	Melville (1959)
Aspidiotus destructor (coconut scale)	West Africa	1956	180,000	10,000	18:1	Simmonds (1960)
Ctenarytaina eucalypti (blue gum psyllid)	USA	2000	558,000 to 1,488,000	62,000	9:1 to 24:1	Dahlsten *et al.* (2000)
WEEDS: TERRESTRIAL						
Opuntia megacantha (prickly pear)	South Africa	1950	237,500	42,500	5.6:1	Pettey (1950)
Xanthium occidentale (noogora burr)	Australia	1991	A$16,750,000	A$7,200,000	2.3:1	Chippendale (1992)
Senecio jacobaea (tansy ragwort)	Oregon	1996	16,200,000	1,200,000	13:1	Coombs *et al.* (1996)
Chrysanthemoides monilifera *ssp. rotundata* (Bitou bush)	Australia	2000	A$45,000,000	A$2,200,000	20.7:1	CRC (2001)
WEEDS: AQUATIC						
Alternanthera philoxeroides (alligator weed)	USA	1976	*	*	8:1	Andres (1977)
Salvinia molesta (Kariba weed)	Sri Lanka	1989	8 million	150,944	53:1	Doeleman (1989)

* Values not available A$ = Australian dollar

7.2 Materials and Methods

7.2.1 Questionnaire

We developed a questionnaire which was completed by personal visits with 30 randomly selected individuals/organizations affected by the fern. The questionnaire required data on the direct costs of the weed to the respondent (Appendix C). This included stock losses, the costs of replacing water pumps, the costs of setting up an alternative water supply, and the loss of recreational activities. The respondents estimated surface area of their water bodies and percentage infested. Duration of the infestation was also recorded.

7.2.2 Evaluating economic viability of biological control

The average costs per hectare per year of the weed per respondent was calculated from the questionnaire. As a result of biological control, these avoided costs (or benefits of control) were assumed to be constant for the time period 1995-2000 and adjusted to year 2000 South African Rands (ZAR) using Statistics South Africa's most recent producer price index (PPI). The costs to develop the biological control agent, including salaries, overheads, and operational costs were obtained from the Plant Protection Research Institute, Pretoria. These control costs were also adjusted using the PPI and expressed in constant, year 2000 ZAR. All amounts were converted to United States Dollars (US$) at a ZAR / US$ exchange rate of 10:1. The US$ figures were not adjusted for purchasing power or varying levels of income between the RSA and USA. Once these adjustments were made, average costs and benefits per hectare were calculated for the period 1995-2000. A rate-of-spread model was used to estimate the area that will be invaded with and without biological control in the future. This model is based on the well-known thesis that invasions occur on the pattern of a sigmoidal curve. Historic data points on the hectares that were invaded with *A. filiculoides* and the maximum that could be invaded on data produced in the South African Water Social Accounting Matrix (WSAM) was used to fit a statistically meaningful sigmoid relationship (Le Maitre[1], pers. comm.). A full discussion on the methodology can be found in van Wilgen *et al.* (2004). It was assumed that the economic value of future benefits will increase at 3% per annum. It was further

[1] David Le Maitre, CSIR Division of Water, Environment and Forestry Technology, Stellenbosch, South Africa.

assumed that future costs of control will be 20% of the average costs during the period 1995-2000, a conservatively high figure for *A. filiculoides*, but one used as a proxy for the costs of maintaining biological control on different alien species in the future (van Wilgen *et al.*, 2004).

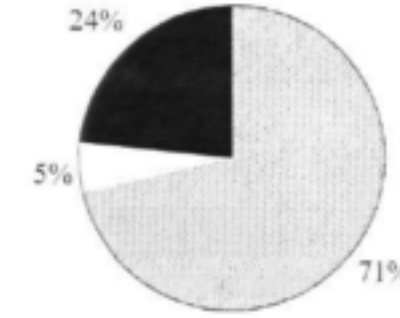
7.3 Results

7.3.1 Respondent demography

Of the 30 respondents, the majority were involved in farming (Fig. 7.1a). Recreational water-use was the next largest grouping followed by a small number of municipal users. Within the farming category, crop, cattle, and sheep farming were the main activities (Fig. 7.1b). Recreational water-users comprised mainly golf courses, ecotourism, hunting, housing estates and fishing (Fig. 7.1c).

7.3.2 Cost to respondents

Most of the 30 respondents had attempted to control *A. filiculoides* either manually using nets and rakes, or with the use of glyphosate-based herbicides. All were of the opinion that these attempts were futile due to the rapid regrowth of the weed. Losses to the agricultural community involved the replacement costs of irrigation pumps that had blocked and burnt out (at an average of US$63 per respondent per year) and the drowning of livestock (at an average of US$186 per respondent per year). One sheep farmer in the Free State Province estimated losses of 40 sheep per year (@ US$30 per sheep), which had drowned after walking into weed-infested dams perceiving them as pasture. Red water fern was found on many golf courses in South Africa. Course managers felt that they had incurred significant direct losses of customers and therefore income, due to aesthetic water features being covered by unsightly, thick mats of the weed. These and other miscellaneous costs (loss of property sales in housing estates bordering infested water bodies, labour costs to clean pump filters, loss of farming productivity, decline in recreational fishing, and helicopter monitoring of infested dams in game reserves) amounted to an average of US$533 per respondent, but should be interpreted with caution as the standard deviation is very high (Table 7.2).



(a) ☐ Farming ☐ Municipal ■ Recreational

(b) ☐ Dairy ▤ Sheep ▨ Cattle ■ Crop ☐ Goats ⊡ Chicken

(c) ▤ Hunting ☐ Golf ▨ Housing ■ Ecotourism ⊡ Fishing

Figure 7.1 (a)-(c): Demography of questionnaire respondents: (**a**) Major activity of respondents (n=30). (**b**) Agriculture categories (n=21). (**c**) Recreational categories (n=9).

The cost of constructing alternative water supply facilities is very high (Table 7.2). Most farmers found that livestock would not drink from infested water bodies as the weed gives water a bad odour. In addition, irrigation water was rendered unsuitable due to root material from the weed blocking sprinkler nozzles, and as a result farmers were forced to sink boreholes to ensure clean water supplies. In an extreme case, the town of Warden (Free State Province) was forced to construct an alternative water supply reservoir costing US$120,000. It is, however, not clear if these water works were constructed solely because of *A. filiculoides* impacts. As a result, a conservative approach was taken and final benefit-cost ratios were calculated without the costs of constructing alternative water facilities.

Increased water loss due to increased evapotranspiration from aquatic weeds has been recorded for other species (Brenzy *et al*., 1973; Lallana *et al*., 1987; Boyd, 1987). This is, however, not the case with *A. filiculoides* (A.J. McConnachie, unpublished data) and was therefore disregarded.

Table 7.2 Summary of costs of *Azolla filiculoides* accruing to water-users, as determined by questionnaire.

Assessment	Question	Mean Response	S.D.	*n*
Water user	Total property size (hectare)	2 665	9 619	29
	% of property covered by water	7	10	26
	Water use (liters / day)	258 963	644 799	21
Extent of weed	% of dam infested by weed	85	31	30
	Area covered by weed (hectare)	2	2	30
	Time period of infestation (years)	5	7	30
	Azolla invasion (hectare / year)	1.3	2	30
Costs		**Mean response (US$)**	**S.D.**	***n***
Current control costs	Labour cost (mechanical control)	1 004	1 464	30
	Herbicide cost (chemical control)	134	3 308	30
Damage costs	Damage to: Livestock	186	694	30
	Pumps	63	199	30
	Miscellaneous	533	2 204	30
Replacement costs	Construction of alternative water facilities	7 158	24 926	30
Total cost (including alternative water facilities)		**7 940**	**24 995**	
Total damage cost (excluding alternative water facilities)		**782**	**2 024**	
***Azolla* damage cost per hectare per year (excluding alternative water facilities)**		**589**	**7 984**	

7.3.3 Cost of the biological control programme

The total cost of developing the biological control of *A. filiculoides* using *S. rufinasus* for the period 1995-2000 was US$46 962 (Table 7.3) translating into an average annual cost of the weed of US$276 per hectare[2]. A total of 170 ha was controlled through this programme. This is lower than the mean direct operational costs of alternative control reported by the respondents per year which amounted to US$1 005 (mechanical control) and US$136 (chemical control) (Table 7.2). More than half of

[2]Excluding start-up investment costs of USD$7 700 in 1995. These costs were excluded to make them comparable to the operational costs of alternative options of mechanical and chemical control.

the respondents used both mechanical and chemical control, and it is apparent that these methods, or a combination of these methods, were not effective. This, basically, means that lower benefits are achieved at higher costs when these options are compared to the biological control option. It can be concluded that a biological control programme on *A. filiculoides* is significantly more cost-effective than mechanical and chemical control options. On average, private welfare losses that could have been avoided through a biological control programme of *A. filiculoides* did occur. As standard deviations are very high, such a conclusion would, however, need more site-specific analysis.

Table 7.3 The total cost of developing and releasing *Stenopelmus rufinasus* against *Azolla filiculoides* in constant 2000 prices (1995–2000).

Cost type	Category	Value (US$)
Salaries	Proportion time/year on *A. filiculoides*	24 931
Infrastructure	Capital items	13 203
Survey costs	Travel and accommodation	8 828
TOTAL		**46 962**
TOTAL area controlled (hectares)		**170**
Average cost per hectare / year		**276**

7.3.4 Cost-benefit analysis

With the exception of 1995, when no hectares of the fern were cleared by the biological control programme, the average cost per hectare was US$276. When the investment costs of 1995 were added, the average costs for the six years (1995-2000) were US$1,511 per hectare. As indicated by results from the survey, the average benefits per hectare of the biological control programme over the same period amounted to US$450 per hectare. This analysis is not complete without referring to the present value of the future cost and benefits from a biological control programme. When evaluated from 1995 onwards, with the inclusion of investment costs, benefit-cost ratios for the biological control of *A. filiculoides* increased from 2.5:1 in 2000, to 13:1 in 2005, and 15:1 in 2010. These results do not imply that it is beneficial to shift

the focus from current to future control, but rather indicate that the value of economic losses that could have been avoided, would have risen substantially over time if nothing was done. The decision rule is based on whether the net present value (NPV) of a biological control programme is positive. When the net benefit per hectare from 1995 onwards was calculated, the NPV is US$1,093 per hectare. For the whole of South Africa, the NPV, also from 1995 onwards, of the biological control programme is US$206 million. These positive values indicate the savings from the *A. filiculoides* biological control programme.

7.3.5 Sensitivity analysis

As high standard deviations were recorded for the questionnaire data, sensitivity analysis was required. When the standard deviation of *Azolla* damages per hectare (US$7 984) was used in the analysis, the damages increased to a NPV of US$122 147 per hectare and a NPV of the biological control programme to the country as a whole of US$2.9 billion. When data of the landowner with the lowest reported damages were used as the baseline for the analysis, the NPV was negative US$8 106 per hectare and a loss of US$3.1 million to the country as a whole.

7.4 Discussion

Two aspects of this biological control project were unique in facilitating economic analysis. The first is the rate at which the weed was controlled. Successful biological control efforts are not usually observed within the period of a year (Andow *et al.*, 1997). All of the field sites in this project were cleared within a year of the release of the weevil at that site. Second, unlike terrestrial weeds, *A. filiculoides* occupies well-defined areas in rivers, lakes, and dams. This allowed for accurate estimation of the extent of the invasion of the weed. Third, some important components were unavoidably omitted from this analysis – mostly off-site and on-site biodiversity and water losses. Other attempts have been made to quantify various components of biodiversity in monetary terms (van Kooten & Bulte, 2000). The invasion of aquatic ecosystems by *A. filiculoides* is known to have negatively affected biodiversity (Gratwicke & Marshall, 2001). Blaaukranz Nature Reserve, one of the last remaining habitats of the eastern Cape rocky (*Sandelia bainsii* Castelnau 1861; Anabantidae), an endangered fish, had become totally overgrown with *A. filiculoides*. The Albany Museum (Grahamstown, South Africa) launched a public awareness campaign to help

manually remove the weed every week, using volunteers with tennis rackets. Due to the rapid regrowth of *A. filiculoides*, however, this removal was not sufficient to keep the site clear. Had the biological control project not been successful, *S. bansii* faced extinction. Despite these negative impacts on biodiversity, monetary values were not estimated for these impacts and therefore were not included in the calculation of the benefit-cost ratio.

Water is a scarce commodity in southern Africa (Versveld *et al.*, 1998), and any action that improves access to, and the quality of, existing water resources is likely to have a positive economic value. These impacts were also not taken into account.

There are no direct economic benefits from *A. filiculoides* that need to be included in the evaluation. Since rice is not grown in the region, the control of *A. filiculoides*, which is used as a green manure in Asian rice paddies (Lumpkin & Plucknett, 1980), has at this stage no apparent drawbacks.

The above impacts would only increase the benefit-cost ratios of biological control. When both on-site and off-site (market and non-market) values, most often external to the land-owner, are included, benefit-cost ratios can be much higher (e.g., De Wit *et al.*, 2001; van Wilgen *et al.*, 2004). In this study, only direct financial costs, as borne by the land-owner, were used in the analysis and still demonstrate the viability of the biological control programme.

The sensitivity analysis figures indicate that one should interpret the results of this study with caution. On average, biological control will benefit the country, but extreme variations can be expected at a site-specific level. This means that, on a national level, the financing of this biological control programme was justified, but that such a programme could possibly have been implemented at higher benefits if better up-front prioritisation of dealing with the problem on a site-specific level was possible.

The development of an economic approach to evaluating environmental management programmes, plans, and projects is helpful when evaluating alternative methods of environmental management and policy. Although the limitations of cost-benefit

analysis are well documented, it is still a very useful method to present the impacts of a project on the environment in a systematic way (Hanley & Spash, 1993). Through such an analysis, limited funds can be allocated more efficiently across competing environmental management alternatives, in this case alternative control programmes for different species. Biological control projects can be ranked and compared with other means of control to provide a more comprehensive picture of where funds could be best spent to achieve maximum private and social welfare.

Now that the economic viability of a biological control programme has been highlighted, the policy question remains: Who remains responsible for its implementation? In the case of *A. filiculoides*, and in most other cases where invasive species are controlled, the South African government carries the investment and operational costs of these programmes, while benefits accrue to private, public and communal land-owners, many water users, and specific ecosystems. In a world of more needs than resources, such programmes do carry an opportunity cost to the government. These are the benefits of the next best alternative investment foregone, so it can be argued that, given their economic viability, biological control programmes should be self-financed. The important question is whether financial benefits are actually achieved, as is the case with *A. filiculoides*. It is apparent that land-owners are already willing to pay for alternative control options at higher costs and lower benefits than biological control options on *A. filiculoides*. While the control of *A. filiculoides* was remarkably effective, ecologically speaking, and most benefits have already been internalized, some lessons for other biological control programmes do apply. For instance, there is certainly scope to further explore inventive financial mechanisms to ensure the sustainability of biological control projects. It is recommended that more research should be directed to the viability of creating a fund for the biological control of invasive species. Contributors would include government (possibly as a research and development provider), private land-owners, national and international institutions whose vision is to preserve the integrity of ecosystems, and those responsible for the spread of such invasive species in the first place. Once established, such a fund could play a crucial role in minimizing massive private and social welfare losses incurred by the spread of alien invasive species.

CHAPTER 8

GENERAL DISCUSSION

The biological control programmes on aquatic weeds, including salvinia, water lettuce, water hyacinth, alligator weed, parrot's feather and hydrilla have generally been very successful (McFadyen 1998) and that on red water fern is no exception. The biological control programme against *Azolla filiculoides* in South Africa is, to date, one of the most dramatic examples of biological control in the 80-year history of the science in this country. In fact it can be regarded as a fairly unique project worldwide. The rapid increase in weevil populations followed by the local extinction of the weed in a very short period of time makes this programme unique. The interaction between *Stenopelmus rufinasus* and its host appears to be far more stochastic than programmes on other weeds. The weevil cues into dense mats of the weed, resulting in rapid population increases due to high fecundity and short generation times. The high feeding rates cause extensive damage to the mats causing them to sink, leaving no residual red water fern populations. The insect population then undergoes massive larval and pupal mortality with the sinking of the mat, but the adults are capable of dispersing to locate other *Azolla* mats.

When this study was initiated, there were concerns that the weevil would not establish and control the weed in the cooler parts of the country, that one agent was probably not sufficient, and would probably not disperse particularly well. Here we have shown that these fears were unfounded. However, it is still relatively early in the biological control programme and it remains to be seen if this level of control will be sustained and if the weevil does not recruit native parasitoids that might reduce its effectiveness.

Some six years after the first releases of *S. rufinasus* in *A. filiculoides*, the weed no longer poses a threat to the aquatic resources of South Africa. However, with the decline of red water fern mats, other aquatic plant species (*Lemna* sp., *Wolffia* sp., *Spirodela* sp. and algae) have taken its place. This is a good indication that while we might have solved the red water fern problem, this was simply a symptom of a far bigger problem, that of

eutrophication or enrichment of aquatic ecosystems with nitrates and phosphates. It is imperative that the biological control of any aquatic weed be linked to an integrated control approach, which has as its foundation, the control of nutrients.

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APPENDIX A

Release Questionnaire

With the completion and return of this questionnaire, you will be contributing to the research and mapping of biological control agents in southern Africa. Please find enclosed a self-addressed envelope for the speedy return of this questionnaire.

Complete by sender. Date sent: .....

Insect species sent: .....

Number of insect: .....

Complete by client: Name: .....

Residential address: .....

..... Code: .....

Telephone number: ( ).....Cell number : .....

Fax number: ( ).....E-mail address:.....

.....

Place of release (please be as specific as possible and include names of places, rivers and dams. Also add a sketch map with notes on direction and distance - use the allocated space on the next page for this purpose.): .....

.....

.....

.....

.....

(Space allocated for sketch of release site)

Time and date of release: .....

Climatic condition with release (circle): Clear / partly cloudy / overcast skies?

⇒ Temperature (warm / cold): .....

⇒ Is it raining? .....

Condition of the insects with release (healthy)? .....

.....

Any further comments: .....

.....

.....

Regards,

On behalf of the Plant Protection Research Institute (PPRI-ARC) and the University of the Witwatersrand.

Andrew McConnachie, Martin Hill & Marcus Byrne

APPENDIX B

Release database

Table. Records of field *Azolla*-infested water bodies in southern Africa where *Stenopelmus rufinasus* has been released, showing the success rate and time to control at these sites.

Locality	Co-ordinates	No. of weevils released	Date released	Status	Date controlled	Control time
Eastern Cape Province						
Cradock	32°31'S 25°36'E	300 (p)	29.01.99	c	12.12.99	312
Cradock	32°29'55"S 25°30'12"E	(im)	11.02.99	c	04.00	41
Cradock	32°27'55"S 25°30'39"E	(im)	11.02.99	c	04.00	41
Cradock	32°28'18"S 25°33'15"E	(im)	11.02.99	c	04.00	41
Cradock	32°31'S 25°36'E	200 (p)	20.10.99	c	02.00	100
Graaf-Reinet	32°03'42"S 24°14'02"E	100 (p)	08.12.99	c	03.03.00	86
Grahamstown (Bleukrans River)	33°19'46"S 26°38'16"E	300 (t)	21.10.98	c	05.99	200
Grahamstown	33°23'28"S 26°42'26"E	500 (t)	11.11.98	c	02.99	90
Grahamstown	33°15'26"S 26°25'10"E	300 (t)	28.01.99	c	11.99	300
Humansdorp	3424 BB	100 (p)	08.04.99	c	08.99	120
Jeffries Bay	*	100 (p)	12.99	c	06.03.00	90
Middleburg	31°17'37"S 26°35'17"E	100 (p)	24.02.00	wa	*	n.a
Molteno	31°12'34"S 26°35'17"E	100 (p)	21.05.99	c	10.99	130
Molteno	31°12'34"S 26°35'17"E	400 (p)	12.11.2001	c	1.02	49
Uitenhage	33°41'52"S 25°20'19"E	500 (p)	15.09.98	c	20.02.99	147
Uitenhage	3325 CD	200 (p)	08.12.99	c	4.00	113
Uitenhage (Chelsea)	33°59'S 25°36'E	100 (p)	29.08.00	c	1.01	124
Witmos	32°31'12"S 25°36'48"E	500 (p)	12.02.98	c	25.02.99	379
WFW	*	200 (p)	23.11.01	te	*	*
Free State Province						
Aliwal North	3026 DA	(im)	19.02.99	c	9.99	193
Bethlehem	28°22'10"S 28°16'15"E	(im)	02.02.99	c	10.99	238
Bethlehem	28°18'50"S 28°15'25"E	(im)	02.02.99	c	10.99	238
Bethlehem	2828 AB	(im)	20.02.99	c	07.99	160
Bethulie (Zoetvlei)	30°30'24"S 25°53'33"E	500 (t)	11.09.98	c	02.99	150
Bethulie (Irma)	30°29'51"S 25°47'12"E	100 (p)	19.09.98	c	05.99	220
Bloemfontein (Bot. Gdns.)	29°03'01"S 26°12'44"E	500 (p)	17.09.98	c	01.02.00	470
Bloemfontein	28°56'32"S 26°20'01"E	500 (p)	11.09.98	c	08.99	335
Bloemfontein	26°03'32"S 28°51'27"E	(im)	29.01.99	c	10.99	270
Bloemfontein	29°04'22"S 26°20'43"E	(im)	29.01.99	c	09.99	240

Bultfontein	26°19'25"S 28°21'30"E	(im)	01 02 99	c	10 99	270
Ficksburg	28°40'27"S 27°45'35"E	500 (p)	19 02 98	c	15 02 99	361
Ficksburg	28°40'S 27°45'E	100	9 10 00	c	1 01	83
Harrismith GC	28°15'47"S 29°7'44"E	600 (p)	04 02 99	c	12 99	326
Harrismith Dam	28°16'52"S 29°06'43"E	300 (t)	16 02 99	c	12 99	314
Heilbron	27°17'20"S 27°58'45"E	(im)	02 02 99	c	02 00	365
Jacobsdaal	29°09'21"S 24°46'24"E	200 (p)	20 10 99	wa	*	*
Jacobsdaal	29°17'37"S 24°45'44"E	200 (p)	20 10 99	wa	*	*
Jacobsdaal	29°17'50"S 24°45'41"E	200 (p)	20 10 99	wa	*	*
Jacobsdaal	29°18'32"S 24°46'41"E	200 (p)	20 10 99	wa	*	*
Jacobsdaal	29°14'55"S 24°46'26"E	200	20 10 99	wa	*	*
Klip River	27°35'01"S 29°35'58"E	(im)	29 03 99	c	09 99	158
Lindley	2727 DD	100 (p)	31 05 99	c	11 99	150
Marquard	28°38'51"S 27°39'03"E	300 (p)	28 01 99	c	04 99	92
Memel	27°38'42"S 29°34'59"E	100 (p)	28 03 99	c	06 99	63
Sasolburg 1	26°46'18"S 27°49'59"E	500 (t)	29 01 98	c	27 10 98	270
Sasolburg 2	26°45'18"S 27°47'24"E	100 (t)	27 10 98	c	02 99	108
Slykspruit	30°13'55"S 26°05'49"E	(im)	30 01 99	c	10 99	271
Smithfield	3026 BA	200 (p)	19 02 99	c	06 09 99	120
Trompsburg	29°55'46"S 25°48'48"E	(im)	29 01 99	c	12 99	330
Viljoenskroon	27°10'21"S 26°55'30"E	500 (t)	17 02 98	c	14 02 99	363
Villiers	26°46'S 28°32'E	500 (p)	19 02 98	c	14 6 98	115
Virginia GC 1	28°05'26"S 26°52'20"E	500 (t)	19 02 98	c	14 02 99	360
Virginia GC 2	28°05'26"S 26°52'20"E	500 (t)	19 02 98	dd	*	*
Warden	27°50'39"S 28°57'55"E	500 (p)	8 04 98	c	10 98	180
Welkom	27°59'46"S 26°42'08"E	500 (t)	18 02 98	c	14 02 99	360
Winburg	28°30'S 27°05'E	100 (p)	12 02 98	c	4 5 98	81
Westminster	29°12'56"S 27°12'58"E	(im)	30 01 99	c	03 99	30
Westminster	29°12'33"S 27°12'51"E	(im)	30 01 99	c	03 99	30
Gauteng Province						
Benoni	26°11'S 28°19'E	500 (p)	06 02 98	c	11 04 99	430
Benoni	26°10'S 28°19'E	500 (p)	17 09 98	c	15 11 98	58
Brits	2527 DB	200 (p)	21 10 99	c	02 00	130
Fourways 1	26°01'56"S 28°00'37"E	50 (t)	28 4 01	c	07 01	183
Fourways 2	26°01'57"S 28°00'37"E	50 (t)	28 4 01	c	07 01	183
Groetvlei	26°05'S 28°01'E	500 (p)	19 02 98	c	18 05 98	90
Inanda	25°57'22"S 28°02'11"E	500 (t)	10 12 98	wa	*	*
Magaliesberg	2527 CA	100 (p)	01 12 99	c	05 05 01	519
Pretoria (ARBS)	25°43'42"S 28°14'11"E	900 (t)	05 12 97	c	03 98	86
Roodeplaat	*	500 (t)	29 08 00	c	08 01	336
Standerton	2629 CD	100 (p)	25 03 98	c	01 99	246
Standerton	2629 CD	100 (p)	31 05 99	c	12 99	180

Standerton	2629 CD	100 (p)	14 12 99	c	02 00	45
Val	26°53'S 29°27'E	500 (p)	20 02 98	c	09 98	280
KwaZulu-Natal Province						
KwaNgwanase (Tembe El.Pk.)	26°53'S 32°34'E	100 (p)	17 11 99	wa	*	*
KwaNgwanase (Tembe El.Pk.)	26°53'S 32°34'E	100 (p)	17 11 99	wa	*	*
Thiba (Tembe El.Pk.)	26°53'07"S 32°34'09"E	100 (p)	17 09 00	c	04 01	223
Thiba(Tembe Elephant Park)	26°53'35"S 32°34'11"E	100 (p)	17 09 00	c	04 01	223
Nyoni(Tembe Elephant Park)	26°53'20"S 32°35'08"E	100 (p)	17 09 00	c	04 01	223
Limpopo Province						
Limpopo	*	100 (p)	14 12 99	wa	*	*
Rust de Winter (Dithole Nature Reserve)	*	100 (p)	15 02 00	c	05 00	105
Warmbaths	2428 CD	100 (p)	12 05 99	c	08 00	473
Warmbaths	2428 CD	100 (p)	01 12 99	c	03 01	485
Mpumalanga Province						
Hoopstad	2725 DD	200 (p)	20 10 99	c	01 00	90
Lower Sabie Sunset dam	25°07'08"S 31°54'37"E	300 (t)	28 11 98	c	27 11 99	356
Messina	2230 AC	100 (p)	27 05 99	c	24 01 00	240
Messina (Overvlakte)	2230 AC	20 (p)	14 10 99	u	*	*
Messina (Popallin Ranch)	2230 AC	80 (p)	14 10 99	u	*	*
Messina (Popallin Ranch)	2230 AC	200 (p)	21 10 99	u	*	*
Messina (Overvlakte)	2230 AC	200 (p)	21 10 99	u	*	*
Messina (Plaas Simple)	2230 AC	100 (p)	8 2 00	wa	*	*
Messina (Plaas Overvlakte)	2230 AC	100 (p)	8 2 00	wa	*	*
Middelburg (Skilderkrans)	2529CD	100 (p)	24 02 00	wa	*	*
Potchestroom	26°43'75"S 27°04'02"E	100 (p)	9 05 00	c	08 00	70
Rietspruit GC	*	100 (p)	14 12 99	c	3 00	76
Northern Cape Province						
Augrabies National Park	28°36'55"S 20°19' 19"E	100 (p)	7 09 00	u	*	*
Augrabies National Park	28°36'55"S 20°19'19"E	100 (p)	1 12 00 00	u	*	*
Colesburg	30°37'57"S 25°21'21"E	100 (p)	19 02 99	c	01 03 00	371
Kimberly	*	300 (p)	14/11/00	c	5/01	167

Western Cape Province						
Cape Town	34 04'35"S 18 25'36"E	200 (p)	22 10 99	c	04 03 00	132
Durbanville	33°48'05"S 18°35'06"E	500 (p)	20 02 98	c	20 02 99	366
Faure	3418 BB	200 (p)	19 10 99	c	11 99	14
George	33°57'04"S 22°24'55"E	500 (p)	16 03 98	c	11 98	240
George East	33 59'47"S 22 31'18"E	100 (t)	15 12 99	c	12 00	350
George (Hoogekraal)	3322 CD	100 (t)	17 02 00	c	03 00	15
Noordhoek	34 07'14"S 18 22'54"E	500 (p)	19 02 98	c	16 04 98	58
Sedgefield	33 59'04"S 22 46'34"E	500 (p)	19 02 98	c	23 02 99	361
Somerset West	34 02'30"S 18 49'00"E	300 (p)	12 01 99	c	04 99	90
Stellenbosch	33 57'36"S 18 48'24"E	500 (p)	18 09 98	c	09 99	365
Wynberg	34 01'24"S 18 29'23"E	300 (p)	23 01 99	c	03 99	60
Zeekoevlei	34 04'56"S 18 24'55"E	500 (p)	19 02 98	c	04 11 98	255
Zimbabwe						
Chiredzi (Lojaan)	21 03'S 31 53'E	100 (p)	25 06 99	c	18 04 00	323
Chiredzi (Nduna)	21 03'S 31 53'E	100 (p)	25 06 99	c	18 04 00	323
Chiredzi (Hlamba)	21 03'S 31 53'E	100 (p)	25 06 99	c	18 04 00	323
TOTALS	*	**24 700 adults released**	*	**91 of 112 sites cleared to date**	*	**206.81 (± 127.6) = mean # days (sd) to clearance**

APPENDIX C

Resource Economics Questionnaire

With the completion and return of this questionnaire, you will be contributing to the cost assessment of the red water fern / rooivaring, Azolla filliculoides, in South Africa. Please find enclosed a self-addressed envelope for the speedy return of this questionnaire.

Name : .....

Residential address: .....

.....

.....Code : .....

Telephone number:.....Cell number: .....

Fax number: .....E-mail address: .....

Place of release (please be as specific as possible and include names of places, rivers and dams. Also add a sketch map with notes on direction and distance - use the space on the next page) .....

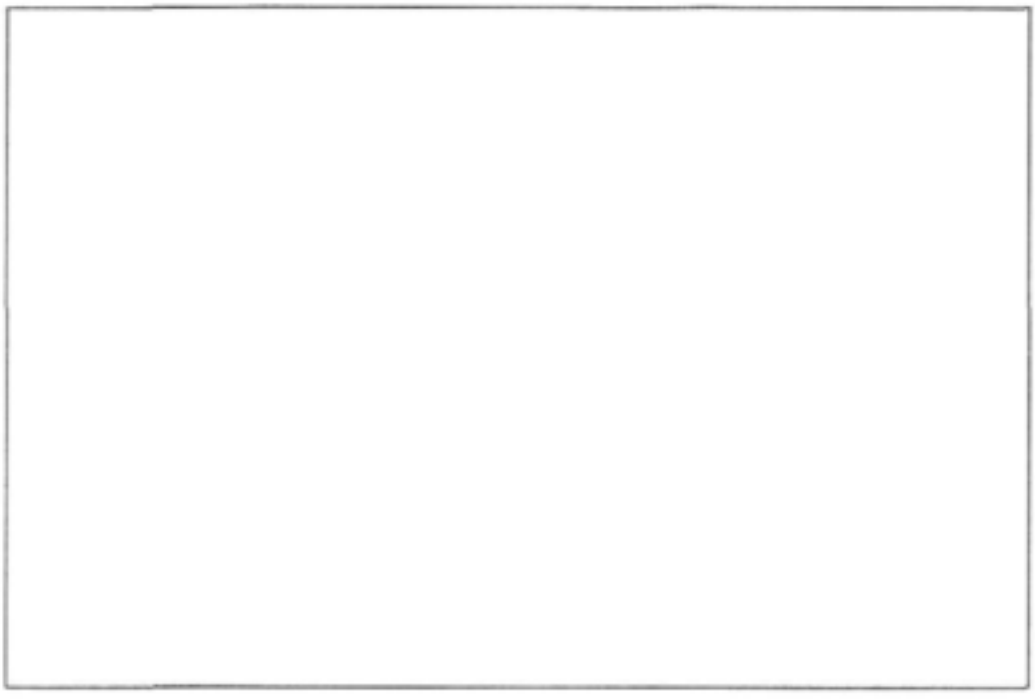
.....

.....

How long has the water body been infested?.....

What is the purpose of the land on which the infested water body is located?.....

.....



(Space allocated for sketch of release site)

What is the size of the land?.....

What are your water costs per unit? .....

.....

What is your daily average water consumption? .....

.....

How much ground water are you using? .....

.....

Approximate area covered by water fern: .....

.....

How long has water body been infested for? .....

How has the water fern affected the quality of the water? .....

.....

.....

What is the estimated total labour cost for removing the weed per day? .....

.....

If herbicides (chemical control) have been used on the infestation, what has their approximate cost been per application? .....

.....

What is the total cost incurred by the weed in terms of pump / livestock / miscellaneous damage? .....

.....

Have you incurred any other costs through having to construct alternative water constructions? If so, at what price? .....

.....

Hypothetically speaking, if we approached you and said that we would be able to take care of your *Azolla* infestation by the next day, how much would you be willing to pay?.....

Any further comments?.....

.....

.....

Regards

On behalf of the Plant Protection Research Institute (PPRI-ARC) and the University of the Witwatersrand.

Andrew McConnachie, Martin Hill and Marcus Byrne

Other related WRC reports available:

Impact of herbicides used in water hyacinth control on natural enemies released against the weed for biological control

Ueckermann C; Hill MP

This project was undertaken to provide a means of augmenting relatively sparse information on the water use of commercial timber species, particularly *Eucalyptus grandis*, in relation to root development. Initially, the use of laboratory rhizotron facilities for this purpose had been anticipated. However, the perceived difficulty in extrapolating laboratory results to plantation conditions led to the rhizotron approach being abandoned in favour of a field-study approach. A thorough investigation was consequently undertaken into the stem steady state energy balance (SSS) technique for monitoring the rate of sap flow through the stems and roots of trees. With the necessary refinement, the technique proved useful in obtaining accurate sap-flow measurements in plantation trees with diameters measuring up to 120 mm. Simultaneous measurements of sap flow through the stem, lateral roots and the tap root proved feasible, illustrating how transpiration (stem flow) responds to root severing or the drying-out of certain soil layers. It was shown that the SSS technique, used in combination with Bowen ratio or eddy correlation techniques for evaporation measurement, might provide a successful means of partitioning total evapotranspiration into its soil surface and plant components.

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