The potential for the biological control of the floating aquatic fern, *Azolla filiculoides* Lamarck (red water fern /rooivaring) in South Africa.

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EXECUTIVE SUMMARY

Azolla filiculoides (red water fern) is native to South America and was first recorded in South Africa in 1940. The lack of natural enemies and the presence of enriched waters have contributed to its establishment throughout the country where dense infestations cause sever degradation of aquatic ecosystems and limit their utilization. The biology of *A. filiculoides* precludes it from being controlled manually or mechanically and chemical control in the aquatic environment is undesirable and should be avoided where possible. Biological control is the only sustainable control method for this weed.

In South Africa *A. filiculoides* and the supposed native *A. pinnata* (Kwazulu-Natal variety) sustained little observable damage from herbivorous insects. Relatively few insect herbivores occurred on these species, and at low individual densities. Since native insects are not important in the natural control of *A. filiculoides*, additional agents should be imported for the biological control of this weed. The absence of important local herbivores means that the possibility of interaction between these and potential biological control agents need not be a consideration in choosing agents. The poor fauna associated with *A. pinnata* (Natal) cast some doubts on the native origins of this plant.

The frond-feeding weevil, *Stenopelmus rufinasus* was imported into quarantine for testing as a potential natural enemy for *A. filiculoides*. Favourable biological characteristics of *S. rufinasus* include a high rate of increase, long-lived adults, a high per capita feeding rate and it would be capable of several generations per year. Host specificity of this insect was determined by adult starvation and oviposition tests on 26 plant species in 15 families. Feeding, oviposition and larval development was only recorded on the *Azolla* species tested (*A. filiculoides, A. pinnata* (collected in Kwazulu-Natal), *A. pinnata* (collected in Namibia) and *A. nilotica*). *A. filiculoides* proved to be significantly the most suitable host for the weevil. The low percentage larval survival on *A. nilotica* and *A. pinnata* (Namibia) would prevent the weevil from establishing on them in the field. However, the percentage larval survival on *A. pinnata* (Natal), despite being half of that on *A. filiculoides* was cause for concern. *A. pinnata* (Natal) has a weedy phenology in South Africa and is thus of low conservation status and therefore, in the unlikely event that some damage is inflicted on this plant in the field, it may be considered as a fair trade-off for the predicted impact of *S. rufinasus* on an aggressive exotic weed.

It was recommended that a thorough investigation into the taxonomic status and therefore possible origins of *A. pinnata* (Natal) be initiated prior to application for release of *S. rufinasus*. Furthermore it was recommended that an additional natural enemy for *A. filiculoides*, the flea beetle, *Pseudolampsis guttata* be imported for host specificity screening.

CHAPTER 1

INTRODUCTION

Azolla is a heterosporous aquatic fern genus which grows in symbiotic association with the heterocystous cyanobacterium (blue-green alga) *Anabaena azollae* Strasburger within the dorsal leaf lobe cavities (Ashton and Walmsley 1976, 1984). The alga can fix atmospheric nitrogen and is able to fulfil the nitrogen requirements of the fern, making it successful in nitrogen deficient waters (Ashton 1974, 1978). As a result, *Azolla* is an economically important fern, having been used in south-east Asia as a green manure associated with wetland rice cultivation for the last 200 years (Lumpkin & Plucknett 1982). However, the wider utilization of *Azolla* for agricultural purposes has been limited by various biological constraints including low tolerance to high temperatures and insect damage (Van Cat *et al.* 1989) and recently by the availability of commercial, ammonia-based fertilizers (Lumpkin & Plucknett 1982).

Taxonomy

The first classifications of *Azolla* species were based mainly on vegetative characteristics, in particular leaf form and size (Svenson 1944). This however, has lead to considerable confusion since the phenotypes of the genus are very plastic, varying under environmental influences (Ashton 1978; Watanabe & Berja 1983; Moretti & Gigliano 1988) and Zimmermann *et al.* (1989) attempted to reclassify the genus based on electrophoretic techniques and Nayak and Singh (1989) used a cytological approach. There are 25 fossil and seven extant species of *Azolla* recorded (Hills and Gopal 1967; Lumpkin & Plucknett 1980; Ashton & Walmsley 1984), these are divided into two sections, section *Azolla* (*Euazolla*) which contains *A. filiculoides* which is indigenous to South and western North American, but has been introduced to Europe, Southern Africa, China, Japan and southern Australia, and is the species most commonly used as a green manure; *A. caroliniana* Willd. which is indigenous to the eastern United States but is found elsewhere in North, Central and South America, and Europe, *A. mexicana* Presl. which is distributed from northern South America into North America extending to British Columbia, *A. ruba* R. Br. which is usually regarded as a variety of *A. filiculoides* and is recorded only in Australia and New Zealand; and *A. microphylla* Kaulf. which is recorded from western and northern South America, Central America and the West Indies, and section *Rhizosperma* which contains *A. pinnata* R.Br. which is distributed widely in tropical Africa, Australasia and south-east Asia and *A. nilotica* Decne. ex. Mett. which is found only in Africa from Egypt to South Africa (Stergianou & Fowler 1990).

There appears to be some confusion as to the status of *A. pinnata*. It was initially regarded as a complex of three species, *A. pinnata, A. africana* and *A. imbricata* but later reduced to one species with two varieties, *A. pinnata* var *imbricata* and *A. pinnata* var. *africana* (also called var. *pinnata*) (Sweet & Hills 1971; Stergianou & Fowler 1990). Nayak and Singh (1989) based on karyological and morphological results suggested that *A. pinnata* var. *africana* was sufficiently different form the other variety and should once again be accorded species status as *A. africana*.

The most recent revision of the section *Rhizosperma* by Saunders and Fowler (1992) showed considerable intraspecific variation in *A. pinnata*. Three main geographically related intraspecific groups were identified, viz. African, Asian and Australasian. The distinct morphology of the Asian variety has long been recognised and referred to as *A. pinnata* var. *imbricata* (Roxb. ex Griff.) Bonap. (e.g. Sweet & Hills 1971; Lumpkin & Plucknett 1982; Tan *et al.* 1986) or as a distinct species, *A. imbricata* Roxb. ex Griff. (Lin 1980). However, Saunders and Fowler (1992) found that the intergrity of the Asian varient of *A. pinnata* was less evident when considering specimens from the Indian subcontinent which had morphological characters closely resembling those of the African varient. They further proposed that the Asian varient be named *A. pinnata* subsp. *asiatica* R.M.K. Saunders & K. Fowler, the African varient *A. pinnata* subsp. *africana* (Desv.) R.M.K. Saunders & K. Fowler and the Australasian varient *A. pinnata* subsp. *pinnata* R. Brown.

Traditionally there are two species of *Azolla* recorded from South Africa, the introduced *A. filiculoides* and *A. pinnata* var. *pinnata* which has been recorded from three localities in Kwazulu-Natal but several localities in northern Namibia, Botswana and southern Zambia (Ashton & Walmsley 1984; Schelpe & Anthony 1986). During this study, *A. pinnata* was collected from Kwazulu-Natal and northern Namibia, the specimens were superficially so distinct that, for the puposes of this study, they have been referred to as *A. pinnata* (Natal) and *A. pinnata* (Namibia) until the taxonomic confusion surrounding them has been resolved.

Plant biology

Azolla filiculoides Lamarck is a small aquatic annual or perennial heterosporous fern, rarely larger than 25mm (O'Keeffe 1986) native to South America and western North America (Lumpkin & Plucknett 1980) but now widely distributed throughout the world, and is often regarded as a weed (refs. cited Ashton 1983). Each plant consists of a short, branched rhizome, bearing small, alternate, overlapping leaves and has roots that hang down in the water (Ashton 1974, 1978).

A. filiculoides is able to undergo rapid vegetative reproduction throughout the year by the elongation and fragmentation of the small fronds, and under ideal conditions, the daily rate of increase can exceed 15%, under ideal conditions doubling time of the fern can be 4-5 days (Lumpkin & Plucknett 1982). In addition, the fern can reproduce sexually via the production of spores during spring and summer which overwinter and are resistant to extreme desiccation, allowing for re-establishment of the fern after drought.

History in South Africa

A. filiculoides was first recorded in the Oorlogspoort River near Colesburg in 1948 (Oosthuizen & Walters 1961). Its method of introduction to the country is unknown, but was probably as an aquarium plant poured into the river (Jacot Guillarmod 1979). The fern was confined to small streams and farm dams in the Colesburg area for many years, but was then recorded near Upington in 1971, probably as a result of spores washed down the Orange river. It was then recorded in the bird sanctuary in Delta Park in 1974, from where it has spread by man, waterfowl and floods to many sites in the Jukskei, Crocodile and Elands rivers in the Johannesburg and Pretoria region (Ashton 1992). The National Botanical Institute has now recorded the weed at 64 localities throughout the country (Figure 1.1).

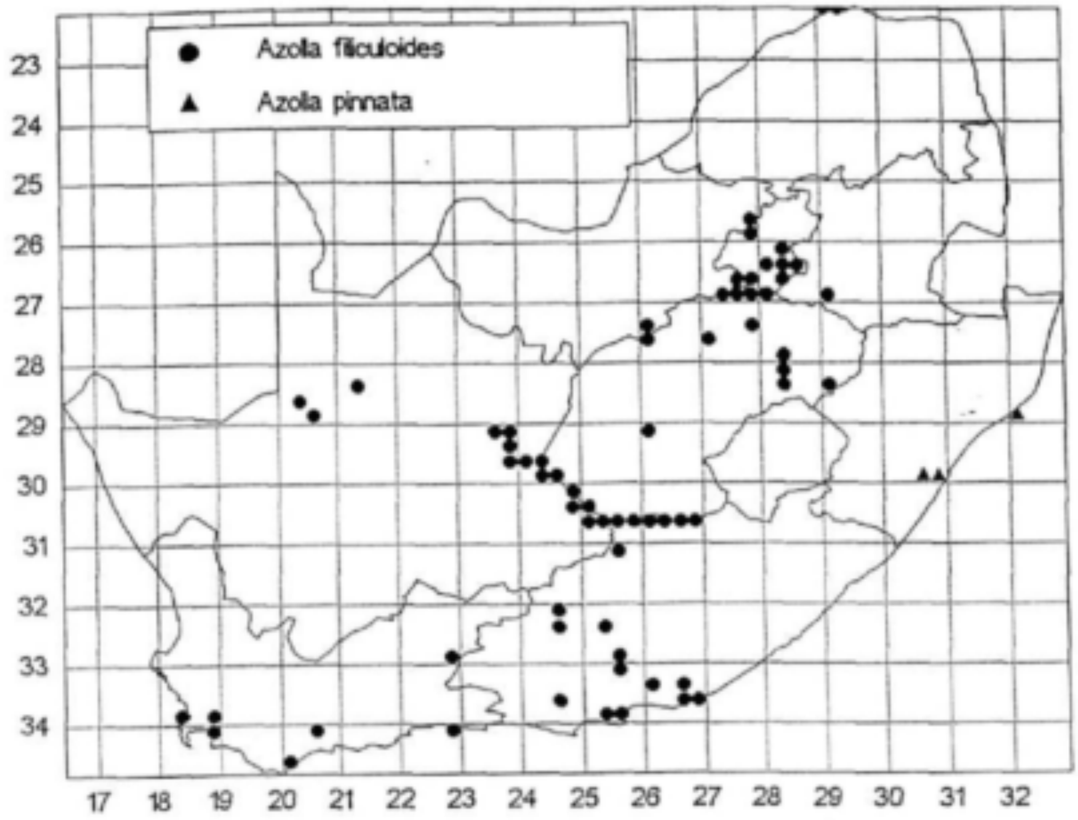

Figure 1.1 Distribution of *Azolla* species in South Africa.

Weed status of *Azolla filiculoides* in South Africa

The increasing abundance of *A. filiculoides* in conservation, agricultural, recreational and suburban areas over the last 10 years is cause for great concern. Among the major consequences of the dense mats (5-20cm thick) of the weed on still and slow moving water bodies in South Africa are:

1] Reduction in quality of drinking water caused by bad odours, colour and turbidity.

2] Promotion of the development of waterborne, water based and water related diseases.

3] Increased siltation of rivers and dams.

4] Loss of water by evapotranspiration through the weed's surface.

5] Reduction of useful water surface area for recreation (fishing, swimming and water skiing) and water transport.

6] Deterioration of aqua biodiversity (e.g. the near extinction of the Eastern Cape Rocky, *Sandelia bainsii* due to a dense mat of the weed covering one of its last habitats).

7] Clogging of irrigation pumps.

8] Drowning of livestock.

9] Reduction in the water flow in canals used for irrigation.

Utilization of *Azolla filiculoides*

The genus *Azolla* has been used as a green manure in rice paddies in China and Vietnam for in excess of 200 years (Lumpkin & Plucknett 1980, 1982). The two main species used in this practice are *A. filiculoides* and *A. pinnata* var. *imbricata*. There is a large volume of literature dedicated to the use of *Azolla* as a green manure in rice paddies, this is excellently reviewed in Lumpkin and Plucknett's (1982) book on the subject. The most widely used system is to monocrop *Azolla* and rice whereby the *Azolla* is floated into the paddy prior to rice planting, the paddy is drained after 6-8 weeks and the *Azolla* then ploughed into the soil. This improves the soil quality by increasing organic nitrogen levels, it may also improve water holding capacity and cation exchange capacity of the soil. Under these conditions, *Azolla* can contribute as much as 180kg of organic nitrogen/hectare/year to the soil and increase rice yields buy 100%. It is also possible to intercrop the *Azolla* and rice (not drain the paddy) which means that the organic nitrogen is available later in the season but the disadvantage is that the *Azolla* can compete for phosphates with young rice seedlings. The techniques required to utilize *Azolla* as a green manure are complicated and labour intensive and only really viable in regions where commercially produced nitrogen-based fertilizers are expensive.

Azolla can also be used as a fodder for swine, poultry, cattle and fish, but cannot be used as the only protein source and should be supplemented with other feeds. The advantages are that it has a high nutrient content, it grows quickly on natural water bodies, it is available throughout the year and does not need processing. *Azolla* can also be composted, used as an ornamental on ponds and fish tanks, people eat it in India in fried dishes, and it can be used in mosquito control, a complete mat disrupts larval development, however, an incomplete mat increases mosquito problems as it affords the larvae protection from predation.

Taking into consideration the potential for utilization of *A. filiculoides* and its severe impact on aquatic ecosystems in South Africa is there potential for conflict of interest between those wanting to utilize the plant and those wanting to eradicate the weed? In real terms, the potential for utilization of *A. filiculoides* in South Africa is very small. South Africa produces very little rice, all produced in dry land agriculture, no paddy rice is grown in this country. Control methods, whether they be chemical, mechanical or biological will never result in the eradication of the target weed and, any utilization programme will never control the weed. Therefore there would be no potential conflict of interest with the control of *A. filiculoides* in South Africa.

Control of *Azolla filiculoides*

Techniques for the control of *A. filiculoides* fall into three broad categories. These are mechanical control, chemical control and biological control.

Mechanical Control

This is a labour intensive method that has the advantage of being ecologically benign. 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 as compost. The disadvantages of this method are that under ideal conditions, the weed can double itself every 4 to 5 days (Lumpkin & Plucknett 1982) so a concerted effort would be needed to keep up with the daily production of even a small infestation, and if eradication was achieved, re-establishment of the weed from spores resident in the substrate of the water body would be inevitable.

The weed is susceptible to fragmentation by physical disturbances and the fragments are highly sensitive to high light intensity and are killed by direct sunlight. In view of this, Ashton (1992) proposed a mechanical agitator or stirrer to provide enough turbulence to break up the plants. However, the costs of such a control method, even on a small scale would be prohibitive.

Chemical Control

The chemical control of *A. filiculoides* using the systemic glyphosate has been suggested by several authors (Steÿn *et al.* 1979; Ashton 1992) as well as paraquat and diquat (Axelsen & Julien 1988) and kerosene mixed with a surfactant (Diatloff & Lee 1979), and Ashton (1992) has also suggested the use of a, as yet untried, chemical, Bayluside. While one does not question the rapid and obvious impact of chemical control, this method does have certain disadvantages:

a) It is expensive, especially in view of the extensive follow-up programme needed to eradicate the plants continually germinating from spores.

b) Treated plants left in the water cause massive deoxygenation of the system, thereby negatively affecting the water quality.

c) There is a danger of spray drift onto non-target vegetation.

d) The water can't be used for irrigation or stock until the herbicide has broken down.

e) There is a need for well-trained personnel.

Biological Control

Ashton (1992), on the grounds of insufficient research and risk involved, recommended that biological control of *A. filiculoides* not be considered. In view of the difficulties involved with mechanical control and the expense, risk and variable results of of chemical control programmes, biological control is the only sustainable method for controlling this increasingly invasive weed.

Two insect species have been identified as possible candidates for the biological control of this weed. Both are frond-feeding beetles, *Pseudolampsis guttata* (Leconte) (Chrysomelidae) and *Stenopelmus rufinasus* Gyllenhal (Curculionidae) recorded from *A. filiculoides* in Argentina (S. Neser pers. comm.) and *A. caroliniana* in Florida (Richerson & Grigarick 1967; Habeck 1979; Buckingham & Buckingham 1981). Both species do extensive damage to the plants in the country of origin, and it is expected that without their natural enemies they might control the weed in South Africa.

AIMS OF RESEARCH

The primary aim of this research was to assess the potential for the biological control of *Azolla filiculoides* in South Africa. More specifically the aims included a pre-introductory survey of the insect fauna associated with *A. filiculoides* and *A. pinnata* (Natal), and to import and determine the life history and host specificity of a natural enemy of *A. filiculoides* from its region of origin, *Stenoplemus rufinasus* Gyllenhal (Coleoptera: Curculionidae).

CHAPTER 2

INSECT HERBIVORE FAUNA ASSOCIATED WITH *AZOLLA FILICULOIDES* IN SOUTH AFRICA

Introduction

Introduced plant species provide ideal opportunities to study the recruitment of native phytophagous insects. One would expect indigenous plant species to support a rich herbivore fauna, and introduced or exotic plant species to support a poor herbivore fauna (Southwood 1961; Southwood *et al.* 1982). However, insects are often capable of finding and colonizing species of introduced plants quickly (100 years or less) (Strong 1974). The rate of colonization by phytophages is affected by the range of the introduced plant species, and the taxonomic, phenological, biochemical and morphological match between the introduced and native plant species (Strong *et al.* 1984). *A. pinnata* is reportedly a native species in South Africa and therefore should support a suite of specialized phytophages that would have evolved with the plant. As related plant species often present very similar cues to insects (Connor *et al.* 1980) one might expect *A. filiculoides* to support a phytophage fauna similar to that of the native *A. pinnata*. However, the *A. pinnata* that occurs most commonly in the Kwazulu-Natal region of South Africa, *A. pinnata* (Natal) could be native to Australasia and south-east Asia (Nayak & Singh 1989), and a very early introduction to South Africa. If this were the case one would expect it to support a depauperate insect fauna comprising a few aquatic plant generalist species and few or no specialists, similar to what one would expect of *A. filiculoides* (Strong *et al.* 1984).

The primary aim of this chapter was to conduct a survey of the insect herbivores associated with *A. filiculoides*, in order to determine the contribution of native insect species to the natural control of the weed, and hence the types of imported natural enemies required to supplement their impact. Another aim was to determine whether any natural herbivores of *A. filiculoides* had already entered the country accidentally, as was the case with *Trichapion lativentre* (Bèguin-Billecocq) on *Sesbania punicea* (Cavanillies) Bentham (Fabaceae) (Hoffmann & Moran 1988). The final aim was to conduct a survey of the fauna associated with *A. pinnata* (Natal) in Kwazulu-Natal in an attempt to try and determine the possible origins of this plant in South Africa.

Materials and methods

Azolla filiculoides was sampled monthly at a number of sites in South Africa between March 1995 and December 1996 and opportunistically at other sites, and *A. pinnata* (Natal) at a site in Kwazulu-Natal (Table 2.1). At each site the *Azolla* was sampled with 2l ice cream containers with gauze covered holes in the bottom. The containers were placed under the *Azolla* and lifted with the water draining out through the gauze. Two such samples were taken at each site, the *Azolla* material was then taken back to the laboratory and the samples from each site were divided into three equal sub samples. One sub sample was placed in a Berlese funnel where the *Azolla* was allowed to dry under lights and the insects were collected in an alcohol-filled jar at the bottom of the funnel, one sub sample was sorted manually under a dissection microscope to collect loose herbivores, and the final sub sample was placed in an emergence box to allow all immature insects to develop and emerge.

Table 2.1 Localities where *Azolla filiculoides* and *Azolla pinnata* (Natal) were collected to determine the insect herbivore fauna associated with them in South Africa.

Azolla filiculoides

Site	Locality*	Sampling intensity
Sasolburg	Dam M 26°46'14"S 27°49'58"E Dam E/1 26°45'38"S 27°46"52"E Dam D 26°45'28"S 27°48"00"E Dam L 26°45'43"S 27°49'50"E	Monthly: May '95-Dec '96 Monthly: May '95-Dec '96 Monthly: June '95-Dec '96 Monthly: May '95-Dec '96
Parys (Vaal River)	Aser 26°44'S 27°35' E Schuttes Eiland 26°58'S 27°26'E Feesgronde 26°53'S 27°29' E	Monthly: March '95-Dec '96 Monthly: March '95-Dec '96 Monthly: March '95-Dec '96
Pretoria	VOPI, Roodeplaat (small dam)	Monthly: March '95-Dec '96
Brits (Crocodile River)	Rennie 25°39'S 27°47'E	Monthly: March '95-Dec'96
Eastern Cape (Blaaukraans River)	Belmont Valley 33°19'30"S 26°35'30"E Manley Falts 33°20'15"S 26°43'30"E Halfway House 33°25'30"S 26°45'10"E	Monthly: Jan '96-Oct '96 Monthly: Jan '96-Oct '96 Monthly: Jan '96-Oct '96
Zambia (Kafue River)	Kafue Marina 15°47'01"S 28°09'16"E	Feb '96, April '96, Sept '96, Dec '96
Bloemfontein (Modder River)	28°48'20"S 26°06'30"E	Aug '95, Sept '95, Jan '96, April '96

***Azolla pinnata* (Natal)**

Site	Locality	Sampling intensity
Hammersdale (Umgeni River)	Inlet to the Dam 29°48'20" S 30°39'56"E	Monthly: June '95-Nov '96

* The samples collected from the Sasolburg, Parys and the Eastern Cape localities were pooled for each of those sites as the localities were in close proximity.

Results

A filiculoides and *A. pinnata* (Natal) supported an extremely impoverished herbivore fauna in South Africa and only four regularly occurring phytophages were recorded on these plant species (Tables 2.2 and 2.3). All of the herbivores recorded were generalists, commonly found on other aquatic plant species.

Table 2.2. Insect herbivore species associated with *Azolla filiculoides* at various sites in South Africa. Species recorded only once were excluded. Incidence measured as a percentage of the total number of samples in which the species occurred.

Insect Species	Incidence at Site						
	Sasol-burg	Parys	Eastern Cape	Pretoria	Brits	Bloem-fontein	Zambia
HOMOPTERA							
Aphididae							
Rhopalosiphum nymhaeae L.	100.0	72.7	42.6	77.3	81.8	50.0	50.0
DIPTERA							
Chironomidae							
Chironomus sp.	15.0	6.7	8.7	6.4	10.9	0.0	0.0
Cecidomyiidae							
Unknown sp.	30.0	31.8	12.2	13.6	18.2	0.0	0.0
LEPIDOPTERA							
Pyralidae							
Nymphula obliteralis Walker	45.0	63.6	0.0	68.2	9.1	25.0	0.0

The waterlily aphid, *Rhopalosiphum nymphaeae* L. was the most abundant species in the samples, and occurred in the highest number of samples. It is reported to have a cosmopolitan distribution and has been recorded on at least 27 other species of aquatic plants (Center *et al.* 1992). Despite its abundance on both *Azolla* species in many of the samples, it did not appear to be particularly damaging to the plants.

The waterlily leafcutter, *Nymphula (Synclita) obliteralis* Walker was the most damaging insect recorded on the *Azolla*, capable of destroying large amounts of especially *A. filiculoides*. It has been recorded from in excess of 60 other aquatic plant species (Center *et al.* 1992). The eggs are laid singularly on the surface of the fronds, the larvae then form a case of *Azolla*. The larvae extrude the anterior portion of their bodies out of the case while feeding to reach the surrounding plants. The larvae then attach the case to the *Azolla*, either above or below the surface of the water, spin a cocoon in the case in which they then pupate.

Larvae of the Chronomidae and Cecidomyiidae were infrequently found and then in low numbers, and appear to have little or no impact on the *Azolla* plants.

There was variation in the incidence of the herbivore species between sites, but the same species occurred at most of the sites, although *N. obliteralis* was not recorded from the Eastern Cape. The Bloemfontein and Zambian sites suffered from low sample sizes and these results should be interpreted with care. Of significance is that the *A. pinnata* (Natal) collected from Kwazulu-Natal supported the same group of generalist herbivore species as *A. filiculoides* (Table 2.3) but the aphid, *R. nymphaeae* was less abundant.

A great number of other insect and mite species were collected from the *Azolla* samples, these were all predaceous, saprophagous and detritus feeders which are usually associated with aquatic plant species. Three families of Collembola (Sminthuridae, Poduridae and Isotomidae) occurred in excess of 90% of the samples, these would presumably feed on the decaying *Azolla* and associated fungi and other micro-organisms. *Plea piccanina* Hutch. (Heteroptera: Pleidae) was also very common, preying mainly on collembola on the *Azolla*. Several species of beetles in the families Dytiscidae, Hydraenidae and Gyrinidae were collected, these were all either predaceous or fed on decaying organic material. Tipulidae (craneflies) and Culicidae (mosquitoes) were frequently reared from the *Azolla* species.

Table 2.3 Insect herbivore species associated with *Azolla pinnata* (Natal) at Hammersdale, Kwazulu-Natal. Incidence measured as a percentage of the total number of samples in which the species occurred

Insect species	Incidence
HOMOPTERA	
Aphididae	
Rhopalosiphum nymhaeae L.	33.3
DIPTERA	
Chironomidae	
Chironomus sp.	11.1
Cecidomyiidae	
Unknown sp.	13.6
LEPIDOPTERA	
Pyralidae	
Nymphula obliteralis Walker	31.8

Discussion

A. filiculoides and *A. pinnata* (Natal) have impoverished herbivore faunas in South Africa, evidenced by the fact that although *N. obliteralis* was damaging, both species were naturally vigorous and largely free of major insect damage, which indicated that native herbivores did not contribute to the natural control of the weed.

There have been several other surveys of insects associated with *Azolla* spp. Esterhizen (1993) surveyed the insects associated with *A. filiculoides* in the Modder river and Renosterspruit near Bloemfontein, South Africa. He recorded 7 phytophagous species, of which the aphid, *R. nymphaeae* was the most abundant. His results compare similarly with this study in that he showed the weed to be attacked by a small group of generalist insects, and then infrequently and in low numbers, causing minimal damage to the plants. Calilung and Lit (1986) recorded 8 phytophagous species on several *Azolla* species in the Philippines, they found two *Nymphula* species and two chironomid species that caused severe damage to the plants and enabled *A. filiculoides* to be utilized as a green manure in rice paddy's and prevented it from becoming a weed. Gomez (1978) investigated the insect interactions with *A. mexicana* in Costa Rica and finding very little concluded that ferns, as a result of their secondary chemical compounds, many acting as insect deterrents, would naturally support few insect herbivore species.

Furthermore, that the two *Azolla* species support a insect fauna characterised by a few, generalist species indicates that they are out of their native range where insects would have had an opportunity to evolve on them, and that there is no local (South African) pool of oligophagous species capable of colonizing either *Azolla* species.

The situation in South Africa contrasts that of Central Asia, where insect herbivores limit the potential of several *Azolla* species being cultivated as a green manure. Lumpkin and Plucknett (1982) recorded six Lepidoptera species, five midge species, three beetle species, two grasshopper species and several unidentified aphid and mite species that cause severe damage to *A. filiculoides* and *A. pinnata* var. *imbricata* being utilized in rice paddies in China and Vietnam. Once again the majority of these species were generalist herbivores on aquatic plants, but the three beetle species were far more specialised in their host plant requirements and it is two of these (*Stenopelmus rufinasus* Gyllenhal (Curculionidae) and *Pseudolampsis guttata* (Leconte) (Chrysomelidae) that are currently being considered as potential natural enemies for *A. filiculoides* in South Africa.

Conclusions

A. filiculoides is under very little herbivore pressure in South Africa which enhances its ability to be invasive. No unintentionally introduced natural herbivores of *A. filiculoides* were found in South Africa. The plants were largely undamaged by insects which indicated that native herbivores are not important in the natural control of this weed. No local insects are thus important enough to impose any constraints on the type of biological control agent imported from the Americas.

The depauperate insect fauna associated with *A. pinnata* (Natal) in South Africa reconfirms the notion that this plant might not be in its native range. This study however, had several constraints, there were small sample sizes from several areas, especially for *A. pinnata* (Natal). More importantly, a survey of the insects associated with the native southern African *A. pinnata* (Namibia) R. Br. and *A. nilotica* DeCaisne ex Mett. needs to be undertaken to determine the pool of *Azolla* oligophages available to colonize both *A. filiculoides* and *A. pinnata* (Natal).

CHAPTER 3

LIFE HISTORY AND HOST SPECIFICITY OF *STENOPELMUS RUFINASUS* GYLLENHAL (COLEOPTERA: CURCULIONIDAE), A POTENTIAL NATURAL ENEMY OF *AZOLLA FILICULOIDES* IN SOUTH AFRICA.

Introduction

Azolla filiculoides is becoming an increasingly important weed on water bodies in South Africa. Its apparent lack of utility in South Africa, its increasing abundance in agricultural, recreational and suburban situations, its alien status and the failure of mechanical control and undesirability of chemical control suggest that it might be a suitable candidate for biological control.

The pre-introductory survey of the fauna associated with *A. filiculoides* in South Africa, and host records from around the globe revealed that the genus *Azolla* is attacked by generalist herbivorous insects and that very few specialist insect species have evolved on these plants. However, two beetle species, the weevil, *Stenopelmus rufinasus* and the flea beetle, *Pseudolampsis guttata* appear to have specialized on the genus *Azolla* (Richerson & Grigarick 1967; Habeck 1979) and have been identified as potential biological control agents for *A. filiculoides* in South Africa.

The weevil, *S. rufinasus* is indigenous to southern and western United States of America (LeConte 1876) where it occurs on the native *A. caroliniana* and the introduced *A. filiculoides* (Richerson & Grigarick 1967), but it has also been collected from *A. filiculoides* in Argentina (S. Neser pers. comm.) and has been accidentally introduced to Europe with imported *Azolla* (Janson 1921).

This species was first described by Gyllenhal (1836). LeConte (1876) placed it in the tribe Erirhinini, the members of which are mostly aquatic or semi-aquatic, and in the tribe Stenopelmi, which is monotypic and contains only *S. rufinasus*. This is encouraging for biological control as it suggests that the insect has had a long association with *Azolla* and is therefore likely to be specific, certainly to the genus. This species was redescribed as *Degorsia champenoisi* by Bedel (1901) who mistakenly thought it indigenous to France.

Richerson and Grigarick (1967) described the life history of *S. rufinasus* and reported that it is capable of devastating mats of *Azolla* in the southern USA. It also probably reduces the weedy potential of these plants in this region (Center *et al.* 1992). Lumpkin and Plucknett (1982) regarded *S. rufinasus* as one of the most damaging pests of *Azolla*.

Stenopelmus rufinasus was imported from Florida, USA into quarantine in South Africa in late 1995. Reported here is the life history and host specificity of the weevil under quarantine laboratory conditions.

Materials and methods

All studies were conducted in a quarantine laboratory with fluctuating temperatures of 27 ± 2°C (day) and 20 ± 2°C (night) and a 14-16 hour photoperiod. Biological observations were conducted on whole, actively growing plants floating in gauze-covered glass aquaria (300mm x 250mm x 250mm). Fresh plant material was added as required. Adult longevity was determined by placing a recently eclosed female with two recently eclosed males in a plastic, gauze-covered pill vial (diameter 55mm, depth 50mm) filled with water and fresh *A. filiculoides* plants. The weevils were placed in new vials every three days until they became senescent and died. All adults emerging from the vials were collected daily to indicate fertility of the female. There were 30 replicates.

Host specificity of *S. rufinasus* was determined by adult starvation and oviposition trials on a series of test plants selected on relatedness to *A. filiculoides* and economic importance. Ten males and females that had recently eclosed and not yet fed as adults, were confined to each of the test plant species for 7 days after which they were removed and mortality and presence of feeding activity recorded. The test plant species were then monitored daily for larval emergence, development and ultimately the number of adults emerging from each test plant species was recorded. There were ten replicates for each test plant species. The number of adults emerging was compared between the plant species tested using a Kruskal-Wallis single factor analysis of variance by ranks followed by Dunn's multiple range test (Zar 1974), where applicable. All means are quoted with standard deviations.

Results

Life history of *Stenopelmus rufinasus*

Egg

The female chews a hole into the tip of one of the fronds into which a single, yellow-orange egg is laid. The exposed tip of the egg is covered with a cap of frass. The eggs are 0.31 ± 0.02 mm ($n = 48$) in length and 0.19 ± 0.02 mm ($n = 48$) in width. The mean incubation period of the eggs kept at a constant 25°C was 4.18 ± 1.38 days ($n = 65$).

Larvae

Stenopelmus rufinasus undergoes 3 larval instars, all of which feed voraciously on the fronds of *A. filiculoides*. The larvae are typically legless and range in body colour from yellow-orange to a dark red, depending on the colour of the *Azolla* on which they are feeding. The head capsule of the larva is black, a divided prothoracic shield is present behind the head. The larvae range in size from about 1mm in the neonates to 3.6mm in the mature third instar larvae (Table 3.1). Duration of each larval stage was 2 to 3 days.

The first instar larvae mined the upper lobes of the fronds. Second and third instars fed externally and were far more obvious. Third instars often produced a droplet of frass on the dorsal surface which effectively concealed the larva. Older larvae were capable of consuming several plants per day.

Pupae

Pupation occurred in a black, ovoid chamber measuring about 2.1mm x 1.1mm constructed in an *A. filiculoides* plant above the surface of the water. The larva selected a pupation site on the leaf surface and began to prepare the chamber by chewing a depression in the leaves and constructing the chamber around itself. The material used to construct the cell was an anal secretion. The pupation period was 4 to 6 days (Table 3.1). The duration of immature stages, egg to adult eclosion from pupation ranged between 16 and 23 days.

Adults

The adults are small, measuring about 1.7mm in length, the females are slightly larger than the males, but not significantly larger (Table 3.1). The adults are a grey-black colour, and covered with red, black and white scales in a variable pattern. The legs and tip of the rostrum are reddish. The sexes are superficially similar, but the first abdominal sternite is flat or slightly concave at the midline in the males, strongly convex in the females.

Copulation can occur immediately after eclosion. The females have pre-oviposition period of 1.36 ± 0.72 days ($n = 30$) after which they lay eggs frequently, up to 10 per day. Both males and females are fairly long-lived (55-60 days, Table 3.1) and the females are very fertile, producing a mean of 324.68 ± 101.67 viable offspring per female (n = 30, range 128 - 474 viable offspring per female). The sex ratio of 9711 emerging adults was 1 : 0.98 (males : females).

Table 3.1. Size and duration of immature and adult stages of *Stenopelmus rufinasus* on *Azolla filiculoides* in the laboratory.

Stage	n	B.L. / mm (± SD)[a]	H.C.W. / mm (± SD)[b]	Duration/days (± SD)[c]
I	79	1.00 (0.22)	0.17 (0.02)	2.09 (1.28)
II	84	1.87 (0.39)	0.27 (0.03)	2.20 (1.00)
III	77	3.62 (0.57)	0.34 (0.06)	2.94 (1.35)
Pupa[d]	118	2.10 (0.24)	1.12 (0.09)	4.82 (1.72)
Adults (Male)[e]	60	1.64 (0.12) a	0.52 (0.01)a	56.88 (10.16)a
Adult (Female)[e]	30	1.70 (0.14) a	0.57 (0.03)a	59.20 (8.77)a

[a]B.L., body length, [b]H.C.W., head capsule width
[c]Develop time in days for each stage.
[d]For pupal measurements B.L. = length of pupal cell, H.C.W. = width of pupal cell
[e]Means of adult measurements in columns not followed by the same letter differ significantly at the 5% level (Mann-Whitney test)

The adults were never recorded flying. Dissection of 393 adults (160 males and 263 females) revealed that functional hind wings were present, but that wing musculature was very poorly developed. It is likely that, as with the boll weevil (*Anthonomis grandis grandis* Boheman) (Grodowitz & Brewer 1987) and the two water hyacinth weevils (*Neochetina eichhorniae* Hustache and *N. bruchi* Warner) (Buckingham & Passoa 1985), wing muscle development only occurs when the insects are under food and population stress. During times of little stress, the wing muscles are atrophied and energy is directed into gonad development.

Host specificity of *Stenopelmus rufinasus*

Host specificity of *S. rufinasus* was determined on 26 species of plant in 15 families (Table 3.2). For the majority of plant species tested, the adults walked off the plants and no feeding or oviposition were recorded. It is significant that *Salvinia molesta*, which is closely related to the genus *Azolla*, did not supply the necessary ovipositional cues, nor was any feeding damage recorded on this species. *S. rufinasus* adults were able to feed on all species of *Azolla* tested and oviposition and larval development occurred. However, it is obvious that *A. filiculoides* is the most suitable host for this weevil as significantly more larvae were able to develop on it than on the other species (Table 3.3). The results obtained thus far for *A. nilotica* and the results for *A. pinnata* (Namibia) indicate that these two species are poor hosts for *S. rufinasus* and they would not be capable of supporting a field population of this weevil. However, the apparent suitability of *A. pinnata* (natal) is cause for concern.

Larval development time on a plant species is usually a good indication of that plant's suitability as a host (Harris 1973). The mean duration of development of *S. rufinasus* larvae differed significantly between *A. filiculoides*, *A. pinnata* (Natal), *A. pinnata* (Namibia) and *A. nilotica*, development was significantly faster on *A. filiculoides* than the other three species (Table 3.3). This is a further indication that *A. pinnata* (Natal), *A. pinnata* (Namibia) and *A. nilotica* are inferior hosts for *S. rufinasus*.

Table 3.2. Initial results of the no-choice, adult feeding and oviposition trials involving *Stenopelmus rufinasus* a new potential natural enemy for *Azolla filiculoides* in South Africa.

Plant species	Common name	F*	O*
Ricciaceae			
Ricciocarpos natans (L.) Corda		0	0
Thelypteriaceae			
Thelypteris confluens (Thunb.) Morton		0	0
Marsileaceae			
Marsilea capensis A. Braun	common fern	0	0
Marsilea sp.		0	0
Salviniaceae			
Salvinia molesta D.S. Mitch.	kariba weed	0	0
Azollaceae			
Azolla filiculoides Lam.	red water fern	+	+
Azolla pinnata (Namibia)		+	+
Azolla pinnata (Natal)		+	+
Azolla nilotica DeCaisne ex Mett.		+?	+?
Lemnaceae			
Lemna gibba L.	duck weed	0	0
Wolffia globosa (Roxb.) Hartog & Plas		0	0
Alismataceae			
Alisma plantago-aquaticum L.	water alisma	0	0
Poaceae			
Zea mays L.	maize	0	0
Araceae			
Colocasia esculenta L. Schott.	taro	0	0
Alliaceae			
Allium cepa L.	onion	0	0
Nymphaceae			
Nymphaea capensis var. *caerulea* (Sav.) Verdc.	blue water lily	0	0
Brassicaceae			
Brassica oleracea L.	cabbage	0	0
Brassica napus L.	spinach	0	0
Apiaceae			
Daucus carota var. *sativa* L.	carrot	0	0
Hydrocotyle americana L.		0	0
Solanaceae			
Solanum melongena var. *sativus* L.	eggplant	0	0
Solanum tuberosum L.	potato	0	0
Lycopersicon esculentum (L.)	tomato	0	0
Capsicum annuum L.	pepper	0	0
Cucurbitaceae			
Cucurbita pepo L.	marrow	0	0
Asteraceae			
Lactuca sativa var. *sativa* L.	lettuce	0	0

* F - Feeding, O - Oviposition

Table 3.3. Development of *Stenopelmus rufinasus* on species of *Azolla*.

Host species	n	Mean no. of adults/replicate[a,c]	n	Mean duration of development (days)[b,c]
Azolla filiculoides	10	80.00 (9.83)a	710	21.72 (5.23)a
Azolla pinnata (Natal)	10	38.20(18.76)b	363	27.87 (7.63)b
Azolla pinnata (Namibia)	10	9.22 (7.21)bc	83	29.33 (10.23)bc
Azolla nilotica	3	2.67 (2.52)c	8	30.45 (13.33)c

[a]Figures in parentheses represent the standard deviation
[b]Development time from oviposition to adult eclosion.
[c]Means in columns not followed by the same letter differ significantly at the 5% level (Kruskal Wallis test followed by Dunn's multiple range test).

Discussion

The life history data on *S. rufinasus* obtained during this study compares favourably with that described by Richerson and Grigarick (1967) for the same weevil on *A. filiculoides* in California, USA. This implies that we can be confident with these results and that they are not merely laboratory artifacts. The biological characteristics of *S. rufinasus* indicate that it has great potential as a biological control agent for *A. filiculoides* in South Africa in that it has a high rate of increase, long-lived adults, would be capable of several overlapping generations per year (Richerson and Grigarick (1967) reported 4-6 generations per year in California), and a high per capita feeding rate. In addition, Center *et al.* (1992) report that this insect is capable of devastating mats of *Azolla* in southern USA and along with the flea beetle, *Pseudolampsis guttata* probably reduces the weedy potential of *A. filiculoides* in this region. Richerson and Grigarick (1967) stated that *S. rufinasus* probably overwinters as an adult in California. An effective overwintering or diapause stage would be vital to the success of this insect as a natural enemy for *A. filiculoides* in South Africa as the most severe infestations of the weed occur in regions characterised by cold winters, the Free State and Gauteng.

An efficient dispersal stage is an important criterion for the success of an insect as a natural enemy for a weed species (Harris 1973). As the flight activity of *S. rufinasus* is at this stage unknown, one would have to assume that it might be a poor disperser and inefficient at finding isolated infestations of the weed in closed systems (e.g. farm dams). Dispersal in longitudinal systems (rivers) would probably be efficient, providing the insects were introduced at the most upstream extent of the infestation and could then float downstream with the *Azolla*, infecting infestations along the system. However, it is highly likely that *S. rufinasus* does have a winged phase, probably induced by poor food quality (Buckingham & Passoa 1985; Grodowitz & Brewer 1987), and is capable of dispersing.

Host records (Richerson & Grigarick 1967; Lumpkin & Plucknett 1982; Center *et al.* 1992) and the data presented here indicate that *S. rufinasus* is restricted in it host plant requirements and therefore specific to the genus, and that plants in other genera are not in danger of attack. These results show further that, of the *Azolla* species tested, *A. filiculoides* was a significantly more suitable host for the weevil.

What are the potential threats to the native *Azolla* species? The preliminary results for *A. nilotica* and the results for *A. pinnata* (Namibia) indicate that they would be unlikely to support a field population of *S. rufinasus* in the absence of *A. filiculoides*, and as these species have not been recorded growing in sympatry with *A. filiculoides* the potential threat to them is minimal. However, *A. pinnata* (Natal) might be able to support field populations of the weevil. Available information, based on taxonomic revisions of the genus (see taxonomy section of Chapter 1) and the results of the survey on the insects associated with the plant (Chapter 2) indicate that *A. pinnata* (Natal) that occurs in Kwazulu-Natal is introduced. What if it is native? *S. rufinasus* is not going to drive this species to extinction, even if it does attack it in the field. *A. pinnata* (Natal) has a weedy phenology in South Africa, forming dense mats on water bodies (e.g. Bluff Nature Reserve near Durban), producing the same negative impacts as *A. filiculoides* does on water bodies in other parts of the country. The perception of *A. pinnata* (Natal) is that it could be regarded as a weed (despite its possible native origins) and is thus of low conservation status. In the unlikely event that some damage is inflicted on *A. pinnata* (Natal) in the field, it may be considered as a fair trade-off for the predicted impact on an aggressive exotic weed. The situation can be compared to that of nodding thistle *Carduus nutans* L. (Asteraceae) in Canada and the United States, where the weevil *Rhinocyllus conicus* Froel. was released although it was known to attack native thistles (Harris 1990). This resulted in the successful control of the weed and negligible damage to native thistles. It could be envisaged that, even assuming the worst case scenario, the same (i.e. negligible damage to *A. pinnata* (Natal)) will be true for *S. rufinasus* in

South Africa.

Conclusions

The biological characteristics of *S. rufinasus* suggest that it would be an ideal candidate as a natural enemy for *A. filiculoides* in South Africa. However, it could pose a potential threat to *A. pinnata* (Natal) but this is highly unlikely. The recommendation at this stage is that the testing on *A. nilotica* be completed (once it can be reimported from Malawi) and that the taxonomic status and therefore origins of *A. pinnata* (Natal) be determined before applying for release of this insect.

RECOMMENDATIONS

Biological control remains the only practical and sustainable control method for *A. filiculoides* in South Africa. This study has shown that *S. rufinasus* has all of the biological characteristics required to contribute towards the control of this weed. However, the apparent suitability of the supposed native *Azolla* species, *A. pinnata* (Natal) is cause for concern. It is recommended that this weevil is not released until the taxonomic confusion surrounding *A. pinnata* is resolved. If the Natal variety of *A. pinnata* is shown to be native to South Africa, a decision will need to be made regarding its contribution to the biodiversity of the country (keeping in mind *S. rufinasus* will not drive it to extinction) and the number of native species threatened by increasing populations of *A. filiculoides* (e.g. the near extinction of the fish the Eastern Cape Rocky *Sandelia bainsii* due a dense mat of the weed covering one of its last refugia.

Stenopelmus rufinasus was collected from *A. caroliniana* in Florida, USA. It is quite possible that ecotypes of this insect occur and before considering it for release, an effort should be made to collect it from *A. filiculoides* in areas of South America in the hope that they might prove to be more host specific. In addition to which if an attempt is made to climatically match collection areas with areas of most severe infestations in South Africa, chances of establishment and success of the weevil will be enhanced.

It is also recommended that another natural enemy of *A. filiculoides*, the flea beetle, *Pseudolampsis guttata*, be collected and imported into quarantine for host specificity testing.

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