

The Biology and Laboratory Host Range of the Weevil Listronotus Marginicollis (Hustache) (Coleoptera: Curculionidae), a Natural Enemy of the Invasive Aquatic Weed Myriophyllum Aquaticum (Velloso) Verde (Haloragaceae) (Parrot's Feather)

Report to the Water Research Commission

by

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Dr Hill² has consolidated the research and prepared this report as a tribute to Messrs Oberholzer and Mafokoaone¹, who died tragically in a motor accident in South America during the course of the project.

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EXECUTIVE SUMMARY

Myriophyllum aquaticum (Velloso) Verde (Haloragaceae), parrot's feather, is considered to be the second most damaging aquatic weed of waterways in South Africa after water hyacinth. It has a wide distribution throughout the country and impacts all aspects of water utilisation. Different control options have been available for some time, but more often than not, these control options have not rendered satisfactory results. Chemical control and mechanical control are expensive and very labour intensive, without providing sustainable control. The leaf-feeding beetle *Lysathia* sp. (Chrysomelidae) was introduced into quarantine from South America in 1991, tested and released in 1994. This beetle defoliates the emergent growth, but the plant coppices from the submerged stems.

The stem-boring weevil *Listronotus marginicollis* (Hustache) was initially collected in Brazil and imported into South African quarantine in April 2002. Another culture was collected and imported from Argentina in 2006. This weevil completes its life cycle on the plant. The eggs are laid on or close to the shoot tip, just below the epidermis of the stem. The larvae mine the inside of the stem. On the way down the larvae exit the stem and then re-enter, leaving small emergence and entrance holes. The stem starts wilting from the shoot tip. The mature larvae bore down the stem below the water surface to pupate. The duration of development from egg to adult is approximately 30 days. This beetle is damaging to the plant and will complement the damage caused by *Lysathia* sp.

Host specificity trials were conducted under quarantine laboratory conditions in South Africa and included adult no-choice trials on 33 plant species in 22 families. Adult feeding, oviposition and subsequent larval development were only recorded on the target plant, parrot's feather. Field evidence from South America supported the laboratory results from South Africa and indicated that *L. marginicollis* is a safe and damaging additional biological control agent for parrot's feather in South Africa.

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We thank Drs Cilliers and Neser of Plant Production Research Iinstitute for collecting the first consignment of the weevil.

INTRODUCTION

Myriophyllum aquaticum (Velloso) Verde (Haloragaceae) (parrot's feather) is a rooted aquatic plant with stems of up to 3 meters long and leafy terminal shoots. The plant roots in shallow water to form a dense tangle of stems and roots. The shoots emerge 200 mm to 500 mm above the water surface (Figure 1). The leaves of the plant are feathery and occur in whorls.

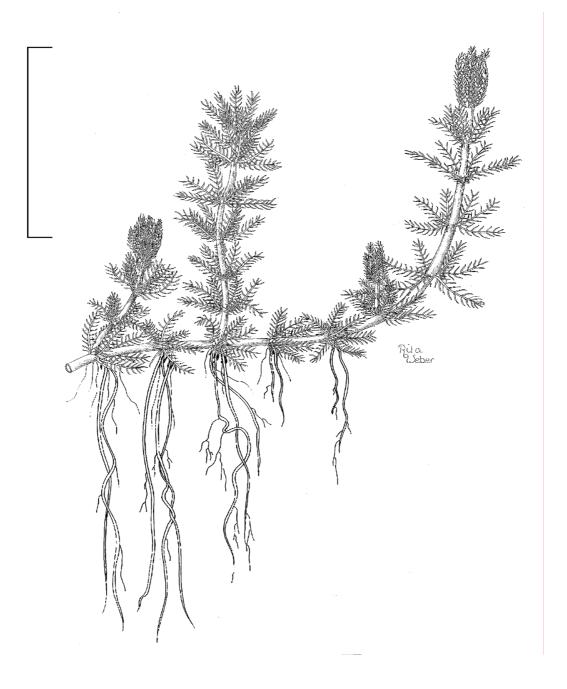


Figure 1. Parrot's feather, Myriophyllum aquaticum. Drawn by R. Weber, SANBI.

Parrot's feather is native to South America but now occurs throughout the world including countries such as Japan, Australia, New Zealand, Malaysia, North America and east and southern Africa. It has been spread mainly by the aquarium trade (Jacot Guillarmod 1979) but becomes abundant in freshwater systems that are eutrophic. Parrot's feather was first recorded in South Africa from the Berg River near Paarl in the Western Cape Province in 1918 (Jacot Guillarmod 1979). Since then it has spread throughout the country in all major waterways, in streams, impoundments and even marshy areas. *Myriophyllum aquaticum* occurs widespread in the Eastern Cape, Western Cape, North West and Mpumalanga provinces. It also occurs, throughout the country in smaller rivers and in smaller quantities in the Limpopo, KwaZulu-Natal and Gauteng provinces (Figure 2). A disturbing fact contributing to the spread of the weed is that it occurs widely in gardens and nurseries despite the fact that is has been declared a category 1 invader plant (Henderson 2001).

There are two species of *Myriophyllum* that are declared weeds in South Africa, the other being *M. spicatum* L. (spiked water milfoil) but this species is not as invasive in South Africa as *M. aquaticum* (Henderson *et al.* 1987). In South Africa, propagation of *M. aquaticum* is vegetatively as there are no male plants in the country and no seeds are produced (Henderson 2001). The plants are perennial in sub-tropical regions and annual in elevated areas (above 1000 m) as frost kills the shoots above the water surface. The parts that die off in winter provide shelter for new shoots that sprout when conditions become favourable in spring (Cilliers 1999).

The plants pose a threat to South African water supply as the stems in the water column reduce stream flow, increasing siltation. At one stage, the Berg River, was completely covered by the weed for more than 25 km (Jacot Guillarmod 1979). The blockage of water-supply inlets and the interference with infrastructure and recreational activities have been recorded, further it causes flooding and drainage problems (Jacot Guillarmod 1979). A reduction of water quality was also noted by Cilliers (1999) where tobacco farmers along the Mokolo River in the Vaalwater district reported a reduction in crop value of up to 50% because of the red discolouration of the water extracted for irrigation from an infested site.

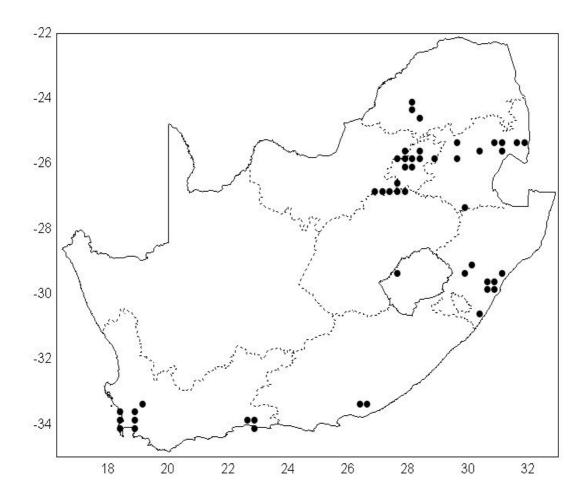


Figure 2. Distribution of *Myriophyllum aquaticum* in South Africa (Map drawn by L. Henderson, PPRI, SAPIA Database).

Due to the growth pattern of the weed, infestations are difficult to contain. No herbicides have been registered to control the weed, primarily because the stems are rooted in the substrate and the herbicides do not translocate down the stem. Where the plant grows in a thick intertwined mat, mechanical removal is very difficult and has proved to be unsuccessful. Because of these methods proving to be unsuccessful, the Plant Protection Research Institute (PPRI) of the Agricultural Research Council (ARC) initiated a biological control programme against this weed in 1991. The leaf-feeding beetle *Lysathia* sp. (Coleoptera: Chrysomelidae) was imported into quarantine in 1991 and the population was supplemented in 1993 with another culture of the beetles. The insect underwent host specificity testing in quarantine and was found to be host specific (Cilliers 1998).

First releases of the beetle were made in 1994. Cilliers (1999) undertook a quantitative post-release evaluation of *Lysathia* and showed that in some instances the presence of the beetle caused a reduction of 20% to 50% of the total water surface covered by *M. aquaticum*, over a period of 3 years. Plants that were severely damaged by the beetle feeding re-sprout after 3 weeks and show total recovery after about 6 weeks. This caused a cyclical abundance of both the beetles and the plants. It was therefore recognized that *Lysathia* sp. was not achieving sufficient levels of control and the stem boring weevil *Listronotus marginicollis* (Hustache) (Coleoptera: Curculionidae) was considered as the most promising additional biological control agent for the control of *M. aquaticum* in southern Africa (Cilliers 1999).

Cordo and DeLoach (1982) investigated the biology and host range of *L. marginicollis*, in Argentina, for possible introduction to the USA between 1972 and 1975. Host specificity testing was conducted with field-collected insects on 43 plant species (Cordo and DeLoach 1982). This included both plants native to North America and economically important plants of that region. The authors reported that the biology and host range were not sufficiently complete to allow introduction without further tests being conducted. This further testing was never undertaken (H. Cordo pers. comm.).

AIM OF THIS STUDY

The aim of this study was to asses the suitability of *L. marginicollis* for release as a biological control agent for parrot's feather in South Africa.

MATERIALS AND METHODS

Collection of Listronotus marginicollis

Listronotus marginicollis was collected from Brazil, St. Catarina Province, about 6 km South West of Sombrio in April, 2002 by Dr. S Neser and Dr. C.J. Cilliers of PPRI. Sixteen adults were collected by hand and imported into quarantine for the establishment of a population in South Africa. The offspring of the original culture insects were accessioned in the National Collection of Insects (Accession number 2562). Another culture was imported from Argentina, Buenos Aires Province, Otemendi Island (34°10′05.4"S/58°51′48.4"W) in 2006. This collection included 2 adult weevils and some 30 stems infested with larvae. Once in quarantine, the larvae were dissected out of the stems and the plant and packaging material were incinerated.

Rearing in quarantine

The population was established on plants grown in the laboratory at the Rietondale Experimental Farm (ARC -PPRI) in Pretoria. Biological studies were conducted in a quarantine glasshouse with temperatures ranging from a minimum (night) temperature of 20° C to a maximum (day) temperature of 30° C under natural daylight, where the photoperiod ranged form 10 hours in winter to 14 hours in summer.

Adult *L. marganicollis* were collected and placed in a 55cmx40cmx30cm container filled with healthy growing plants in water.

Biology

Aspects of *L. marginicollis* that were studied included the biology and duration of the immature stages and the adult stage. Adults were placed in the same plastic containers as described above. All adults were placed in the same container for a week, then recaptured and moved to another container. Every week a new container was added. Daily observations were made on number of eggs laid, number of larvae tunneling and number of pupae / adults present in each container. Total duration of development was recorded.

Host specificity

A thorough literature survey was conducted on previous work done on determining the host specificity of L. M arginicollis. Furthermore, laboratory host range of L. M arginicollis was determined through adult no-choice oviposition and larval starvation trials on a series of plant species selected on relatedness to M. M aquaticum (Wapshere, 1974), habitat and economic importance (Table 1). A number of the species tested were introduced species (e.g. water hyacinth and water lettuce) and although feeding and development on these species is not of importance to the environment, they were included because they occur in a similar habitat and give an indication of host range.

Two species of within the genus *Listornotus* have been recorded as pests. *Listronotus* bonariensis Kuschel, which originates in Argentina is a pest of pastures, notably ryegrass, *Lolium* spp. in New Zealand (McNiell *et al.* 1998) and *L. oregonensis*

(LeConte) is recorded as a pest of carrot (Boivin 2004). As a result several species of Poaceae and Apiaceae, including carrot were tested in this study.

The test plant species were grown in various glass containers covered with gauze lids. Ten *L. marginicollis* adults (five males and five females), that had recently eclosed (adults were used within a week of eclosion) were placed on each of the test plant species for a period of 24 hours after which they were removed and the test plant species were then monitored for larvae development. There were at least 5 replicates per test species. The number of adults emerging and the duration of development were compared between the plant species using a Kruskal-Wallis single factor analysis of variance by ranks followed by Dunn's multiple range test where applicable (Fowler *et al.* 1998).

RESULTS

Biology

The female weevil lays a single egg or rarely 2-3 eggs per stem. The eggs are yellow and are inserted into the leaf tissue between the upper and lower epidermis. The eggs take 8 - 9 days to hatch.

Newly emerged larvae usually nibble on the leaves before they bore into the stem. In South Africa they usually enter the stems at, or close to the tips, while in South America they are reported to crawl down the stem and enter the stem some distance from the water surface (Cordo and DeLoach 1982). The larvae tunnel downward inside the stem to below the water surface, where they pupate. On the way down they leave 2 - 3 entrance / exit holes as they exit and re-enter the stem. There are three larval instars and time spent inside the stem (the immature stages) for the development through larval and pupal stages requires about 3 weeks (mean = 22.78 days ± 5.21 days, n = 48).

These entrance / exit holes are important, as they are the places where the plants are more vulnerable to pathogen attacks and where they are structurally weakened. The tunnels turn black after feeding, possibly due to a fungus, but this does not kill the plant, though it does stunt the growth of the plant.

The adults are quite large (about 4 cm long and 1.5 cm wide) and they are covered with fine gold and silver hairs. The feed on the leaf and leaf tips of *M. aquaticum* above the water. They often feed on the terminal bud, killing it. The impact of the weevil on the plant can be high and at one site in Argentina up to 80% of the stems were infested by the weevil, causing significant dieback (Cordo and DeLoach 1982).

Host specificity

Literature

In South America the weevil has only been recorded from, *M. aquaticum*. Cordo and DeLoach (1982) tested *L. marginicollis* in no choice trials on leaf discs of 43 test species in 22 families. The insect showed a strong preference for *M. aquaticum*, only nibbling on *Limnobium stoloniferum* (G.F.W. Meyer) Griseb., which is not present in South Africa. In choice trials, where the insects were exposed to leaf discs, whole leaves and small plants some feeding was recorded on 12 of the test plant species. However, this feeding was significantly less than on the target species and the weevil only laid eggs on *M. aquaticum* (Cordo and DeLoach 1982).

Laboratory no-choice trails

The laboratory host range of *L. marginicollis* was determined on 33 plant species in 22 families (Table 1). For all of the test species with the exception of the target species the adults walked off the plants and no adult nibbling was recorded. Parrot's feather was the only plant species to support oviposition and larval development (Table 1). Thus in the trials conducted in this study, *L. marginicollis* is host specific to *M. aquaticum* and is safe for release in South Africa.

DISCUSSION

The decision to release a biological control agent for an invading alien plant species should be based on the answer to three questions (Louda *et al.* 2003). First, is the agent needed? In other words are there not other control options that could control the weed? In this situation, *L. marginicollis* is definitely needed for parrot's feather as mechanical control is not viable, no herbicides have been registered for the weed and *Lysathia* sp. is not providing an acceptable level of control despite having been established in South Africa for over a decade and having been thoroughly evaluated (Cilliers 1999).

Second, is the agent sufficiently damaging? Quantifying the potential damage by an agent based on laboratory studies is notoriously difficult. Some agents that appeared damaging in the laboratory such as the mirid, *Falconia intermedia* (Distant) on *Lantana camara* L. (Baars *et al.* 2003) proved to be disappointing in the field (Heystek 2006)., while other agents such as the weevil, *Stenopelmus rufinasus* Gyllenhal for the control of red water fern, *Azolla filiculoides* L., that did not appear damaging in the laboratory (Hill 1998) proved to be a very effective agent in the field (McConnachie *et al.* 2004). However, Cordo and DeLoach (1982) considered this to be a damaging agent for the weed and showed an 80% dieback of the plant at one locality in Argentina. The main reason for the selection of *L. marginicollis* as an agent was the fact that the larvae burrow down stem, below the surface of the water and would thus compliment the defoliation caused by the *Lysathia* sp. larvae causing more effective control.

The third, and most important consideration is the safety of the agent. The results presented in this paper represent field host records from the region of origin and laboratory host range testing in South Africa. It is clear from the work of Cordo and DeLoach (1982) that they considered this agent to have a very restricted host range. Although they recorded some nibbling on another species (*Limnobium stoloniferum*) this species is not present in South Africa.

The validity of laboratory based host specificity trials has been debated for decades. Cullen (1990) suggested that insects display unnaturally wide host ranges under quarantine conditions that they would not realize one released into the field. Baars (2000) and van Klinken and Heard (2000) argued that laboratory trials measure physiological/fundamental host range and that in the field the insects display their ecological host range that is more restricted. These and several other authors (e.g. Balciunas *et al.* 1996, Clement & Cristofaro 1995, Marohasy 1998) have suggested that laboratory host range studies, in particular those that rely on no-choice trails are conservative and could result in weed biological control practitioners rejecting potentially safe agents. However Louda and Arnett (2000) urge us to believe our laboratory results and that in applying the Cautionary Principle should adopt the conservative approach and rather reject a potentially specific agent rather than release a potentially non-specific one. Fortunately in the case of *L. marginicollis* for the control

of parrot's feather in South Africa, these arguments are not relevant as this agent has been shown to be host specific under the most conservative tests

The results of this study show that L. marginicollis is a potentially damaging and safe agent for release against M. aquaticum in South Africa.

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Table 1. Results of the adult no-choice oviposition and larval survival trials for Listronotus marginicollis. There were 5 replicates for all species tested except *Myriophyllum aquaticum* where there were 23 replicates. For each replicate 5 male and 5 female weevils were introduced for a period of 24 hours and then removed. (Kruskal-Wallis ANOVA, H = 21.179, p = 0.002).

Family	Species	Common name	Mean number	Mean duration
•	•		of adults/rep	of development
			\pm SE	$(days \pm SE)$
Haloragaceae	Myriophyllum aquaticum (Velloso) Verde	Parrot's feather	6.32 ± 3.45	32.46 ± 6.76
	Myriophyllum spicatum L.	Spiked water-milfoil	0	ı
	Gunnera perpensa L.		0	ı
	Laurenbergia ripens Berg subsp. brachypoda		0	ı
Commelinaceae	Berula erecta (Hudson) Cov. subsp. thunbergii (DC) B.L. Burtt		0	ı
	Commelina africana var. africana		0	ı
Onagraceae	Ludwigia stolonifera (Guill & Perr.) Raven	Willow-herb	0	ı
	Oenothera gradiflora Ait.		0	ı
Trapaceae	Trapa natans L. var. pumila Nakano ex Verdc.	Water chestnut	0	ı
Polygonaceae	Polygonum acuminatum H.B. & K. var. capense		0	ı
Nymphaeceae	Nymphaea capensis Brum. F. var. caerulea (Sav.) Verdc.	Blue water lily	0	ı
Potomogetonaceae	Potamogeton pectinatus	Fennel-leaved pondweed	0	1
Aponogetonaceae	Aponogeton distachyos L.	Cape pondweed	0	1
Juncaceae	Juncus lomatophyllus	Leafy juncus	0	ı
Brassicaceae	Nasturtium officinale (R.Br)	Water cress	0	ı
Cyperuceae	Cyperus eragrostis		0	ı
	Schoenoplectus brachyceras	Water reed	0	1
	Schoenoplectus paludicola	Sedge	0	1
Asphodelaceae	Chlorophytum comosum (Thunb.) Jacq.	Hen & chickens	0	•

Family	Species	Common name	Mean number	Mean duration
			of adults/rep ± SE	of development (days ± SE)
Ceratophyllaceae	Ceratophyllum demersum	Water hornwort	0	
Apiaceae	Hydrocotyle bonariensis	Water pennywort	0	ı
	Hydrocotyle ranunculoides L.f.		0	ı
	Daucus carota var. sativa	Carrot	0	ı
Araceae	Pistia stratiotes L.	Water lettuce	0	ı
Azollaceae	Azolla filiculoides Lam.	Red water fern	0	ı
Pontederiaceae	Eichhornia crassipes (Mart.) Solms-Laub.	Water hyacinth	0	ı
	Monochoria africana L.	Monochoria	0	ı
Cannaceae	Canna indica L.	Canna	0	ı
Musaceae	Musa paradisica L.	Banana	0	ı
Hydrocotylaceae	Centella asiatica		0	
Alismataceae	Alisma plantago-aquatica L	Water alisma	0	
	Limnophyton obtusifolium (L.) Miq.		0	
Poaceae	Triticum aesitivum	Wheat	0	
	Sorghum bicolor (L.)	Sorghum	0	ı
Hydrocotylaceae	Centella asiatica		0	
Alismataceae	Alisma plantago-aquatica L	Water alisma	0	ı
	Limnophyton obtusifolium (L.) Miq.		0	ı
Poaceae	Triticum aesitivum	Wheat	0	
	Sorghum bicolor (L.)	Sorghum	0	1