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Life history and laboratory host range of *Stenopelmus rufinasus*, a natural enemy for *Azolla filiculoides* in South Africa

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Abstract. The frond-feeding weevil, *Stenopelmus rufinasus* Gyllenhal, was imported into quarantine for testing as a potential natural enemy for the invasive fern *Azolla filiculoides* Lamarck in South Africa. Adult *S. rufinasus* lived for approximately 55 days during which the females produced on average 325 offspring. The developmental period for the immature stages (egg, three larval instars and pupation) was about 20 days indicating the potential for several overlapping generations per year. Both the adults and the larvae caused severe damage to *A. filiculoides* in the laboratory. Host specificity of this insect was determined by adult no-choice oviposition and larval starvation tests on 31 plant species in 19 families. Adult feeding, oviposition and larval development was only recorded on the *Azolla* species tested (*A. filiculoides*, *A. pinnata* subsp. poss. *asiatica* R.K.M. Saunders and K. Fowler, *A. pinnata* subsp. *africana* (Desv.) R.K.M. Saunders and K. Fowler and *A. nilotica* De Caisne Ex Mett.). *A. filiculoides* proved to be significantly the most suitable host for the weevil. The low adult emergence from *A. nilotica* and *A. pinnata* subsp. *africana* would most probably prevent the weevil from establishing on them in the field. *A. pinnata* subsp. poss. *asiatica* which supported greater development, is thought to be introduced and has a weedy phenology in South Africa and is thus of low conservation value. Therefore, any damage inflicted on this plant in the field may be an acceptable trade-off for the predicted impact of *S. rufinasus* on the aggressive exotic weed, *A. filiculoides*.

Key words: biological control, host specificity

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).

Azolla filiculoides Lamarck (Azollaceae) (red water fern) was first recorded in the South Africa in 1948 (Oosthuizen and Walters, 1961). Its method of introduction to the country is unknown, but was probably as an aquarium plant poured into a river (Jacot Guillarmod, 1979). The fern was confined to small streams and farm dams in a localised area of the country for many years. However, presence of enriched waters, the lack of natural enemies and frequency of movement between water bodies by man and waterfowl resulted in its inevitable spread and increase in abundance. The weed's 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 herbicide control make it a suitable candidate for biological control.

Two species of *Azolla* are recorded as being native to southern Africa: *A. nilotica* and *A. pinnata* R. Brown, with two subspecies of *A. pinnata* having been recorded in the same region: *A. pinnata* subsp. *africana* from several localities in northern Namibia, Botswana, Malawi and southern Zambia (Ashton and Walmsley, 1984; Schelpe and Anthony, 1986) and *A. pinnata* subsp. poss. *asiatica* from three localities in the KwaZulu-Natal Province of South Africa where it has a weedy phenology and is thought to be introduced. The origin and mode of introduction of *A. pinnata* subsp. poss. *asiatica* are unknown.

The pre-introductory survey of the fauna associated with *A. filiculoides* in South Africa (Hill, 1997), and host records from elsewhere in the world revealed that the genus *Azolla* is attacked by generalist herbivorous insects and that very few specialist insect species have evolved on these plants (Gomez, 1978; Lumpkin and Plucknett, 1982). However, two beetle species, the weevil, *Stenopelmus rufinasus* Gyllenhal (Coleoptera: Curculionidae) and the flea beetle, *Pseudolampsis guttata* (LeConte) appear to have specialised on the genus *Azolla* (Richerson and Grigarick, 1967; Habeck, 1979; Buckingham and Buckingham, 1981) and have been identified as potential natural enemies 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 *A. caroliniana* and *A. filiculoides* (Richerson and Grigarick, 1967), but it has also been collected from *A. filiculoides* in Argentina and Paraguay (S. Neser, PPRI, South Africa, 1995, 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 then 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 host genus. This species was redescribed as *Degorsia champenoisi* by Bedel (1901) who mistakenly thought it indigenous to France.

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

Materials and methods

All studies were conducted in a quarantine glasshouse with fluctuating temperatures of 27 $\pm$ 2 °C (day) and 20 $\pm$ 2 °C (night) under natural light conditions, with a photoperiod of about 16 hours in summer and 12 hours in winter. Biological observations were conducted on whole, actively growing plants floating in gauze-covered glass aquaria (300 mm $\times$ 250 mm $\times$ 250 mm). Fresh plant material was added as required. Adult longevity and fertility was determined by placing a recently eclosed female with two recently eclosed males in a plastic, gauze-covered pill vial (diameter 55 mm, depth 50 mm) filled with water and *A. filiculoides* plants. The weevils were placed in new vials every three days until they died. All adults emerging from the vials were collected daily to indicate number of offspring produced by each female. There were 30 replicates.

Laboratory host range of *S. rufinasus* was determined by adult no-choice oviposition and larval starvation trials on a series of test plants selected on relatedness to *A. filiculoides*, habitat 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 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

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

Table 1. Size and duration of immature and adult stages of *Stenopelmus rufinasus* on *Azolla filiculoides* in the laboratory

Stage	N	B.L. (±SD) (mm)	H.C.W. (±SD) (mm)	Duration (±SD) in days
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[a]	118	2.10 (0.24)	1.12 (0.09)	4.82 (1.72)
Adult (Male)	60	1.64 (0.12)	0.52 (0.01)	56.88 (10.16)
Adult (Female)	30	1.70 (0.14)	0.57 (0.03)	59.20 (8.77)

B.L., body length; H.C.W., head capsule width.
Developmental time in days for each stage.
[a]For pupal measurements B.L. = length of pupal cell, H.C.W. = width of pupal cell.

frass. The eggs are 0.3 ± 0.1 mm (n = 48) in length and 0.2 ± 0.1 mm (n = 48) in width. The mean incubation period of the eggs kept at a constant 25 °C was 4.2 ± 1.4 days (n = 65).

Stenopelmus rufinasus has 3 larval instars, all of which feed voraciously on the fronds of *A. filiculoides*. The larvae are 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 and a divided prothoracic shield is present behind the head. The larvae range in size from about 1 mm in the neonates to 3.6 mm in the mature third instar larvae (Table 1). Duration of each instar was 2 to 3 days.

The first instar larvae mined the upper lobes of the fronds. Those in the second and third instars fed externally and were far more conspicuous. Third instar larvae 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.

Pupation occurred in a black, ovoid chamber measuring about 2.1×1.1 mm constructed in an *A. filiculoides* plant above the surface of the water. The larva selected a pupation site on the leaf surface and prepared 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 pupal period was 4 to 6 days (Table 1). The duration of immature stages, egg to adult eclosion, ranged between 16 and 23 days.

The adults are small, about 1.7 mm in length. The females were slightly larger than the males (Table 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 may occur immediately after eclosion. The females had a pre-oviposition period of 1 to 2 days (mean = 1.4 ± 0.7 days (n = 30)) after which they laid eggs frequently, up to 10 per day. Both males and females were long-lived (55–60 days, Table 1) and the females produced a mean of 325 ± 102 offspring per female (n = 30, range 128–474 offspring per female). The sex ratio of 9711 emerging adults was 1:0.98 (males:females).

Laboratory host range of Stenopelmus rufinasus

Host range of *S. rufinasus* was determined on 31 species of plants in 19 families (Table 2). For all of the genera apart from *Azolla*, the adults walked off the plants with no feeding or oviposition. *S. rufinasus* adults fed on all species of *Azolla* tested and oviposition and larval development occurred. However, significantly more adults were reared from *A. filiculoides* than on the other species (Table 3). Despite supporting some adult oviposition and larval development, *A. pinnata* subsp. poss. *asiatica*, *A. pinnata* subsp. *africana* and *A. nilotica* were inferior hosts for the weevil.

The mean duration of development of *S. rufinasus* larvae differed significantly between *A. filiculoides*, *A. pinnata* subsp. poss. *asiatica*, *A. pinnata* subsp. *africana* and *A. nilotica*; development was significantly faster on *A. filiculoides* than on the other three species (Table 3). This was a further indication that the two subspecies of *A. pinnata* and *A. nilotica* were inferior hosts for *S. rufinasus*. The differences in larval development time between the *A. pinnata* spp. and *A. nilotica* were small, despite being significantly different, and were therefore of little biological significance.

Discussion

The biological characteristics of *S. rufinasus* indicate that it has good potential as a natural enemy for *A. filiculoides* in South Africa. The females are long-lived and produce many offspring which are very damaging to mats of *A. filiculoides* in the laboratory, the larval development period is short and they would be capable of several overlapping generations per year (possibly as many as 10, Richerson and Grigarick (1967) reported 4–6 generations per year in California). 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.

Table 2. Results of the no-choice, adult feeding and oviposition trials with *Stenopelmus rufinasus*, a new potential natural enemy for *Azolla filiculoides* in South Africa

Plant species	Common name	F	O
BRYOPHYTA			
Ricciaceae			
Ricciocarpos natans (L.) Corda		0	0
Sphagnaceae			
Sphagnum sp.		0	0
PTERIDOPHYTA			
Isoetaceae			
Isoetes transvaalensis Jermy & Schelpe		0	0
Marsileaceae			
Marsilea capensis A. Braun	common fern	0	0
Marsilea sp.		0	0
Azollaceae			
Azolla filiculoides Lam.	red water fern	+	+
Azolla pinnata subsp. *africana*		+	+
Azolla pinnata subsp. poss. *asiatica*		+	+
Azolla nilotica DeCaisne ex Mett.		+	+
Salviniaceae			
Salvinia molesta D.S. Mitch.	kariba weed	0	0
Salvinia hastata Desv.		0	0
Thelypteriaceae			
Thelypteris confluens (Thunb.) Morton		0	0
ANGIOSPERMAE			
MONOCOTYLEDONAE			
Alismataceae			
Alisma plantago-aquaticum L.	water alisma	0	0
Lemnaceae			
Lemna sp.		0	0
Wolffia globosa (Roxb.) Hartog & Plas	duck weed	0	0
Poaceae			
Zea mays L.	maize	0	0

Table 2. Continued

Plant species	Common name	F*	O*
Araceae			
Colocasia esculenta L. Schott.	taro	0	0
Alliaceae			
Allium cepa L.	onion	0	0
DICOTYLEDONAE			
Nymphaeaceae			
Nymphaea nouchali var. *caerulea* (Sav.) Verdc.	blue water lily	0	0
Brassicaceae			
Brassica oleracea var. *capitata* L.	cabbage	0	0
Brassica oleracea var. *italica* L.	broccoli	0	0
Brassica oleracea var. *botrytis* L.	cauliflower	0	0
Chenopodiaceae			
Spinacia oleracea 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.

The results of the host specificity trials strongly indicate that none of the plant species in genera outside of *Azolla* are in any way under threat from the weevil, *S. rufinasus*. Although it is widely accepted that insect species can often show unusually wide host ranges under restricted cage conditions (Harris and Zwölfer, 1968; Zwölfer and Harris, 1971; Wapshere, 1974, 1989)

Table 3. Mean number of adult *Stenopelmus rufinasus* reared on species of *Azolla* during adult no choice experiments in which ten males and ten females were confined to each *Azolla* species for a period of 7 days

Host species	n	Mean no. of adults/replicate[a,c]	n	Mean duration of immature development in days[b,c]
Azolla filiculoides	10	80.00 (9.83)a	710	21.72 (5.23)a
Azolla pinnata				
subsp. poss. *asiatica*	10	38.20 (18.76)b	363	27.87 (7.63)b
subsp. *africana*	10	9.00 (6.83)bc	90	29.33 (10.23)bc
Azolla nilotica	10	1.60 (1.84)c	16	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).

the results here show that the weevil has very specific host requirements, which are present only within the genus *Azolla*. Furthermore, host records show the weevil to have a restricted host range (Richerson and Grigarick, 1967; Lumpkin and Plucknett, 1982; Center et al., 1992) and the fact that it has not been recorded as a pest of any economically important species in its region of origin (Hayward, 1958; Costa-Lima, 1968) further support its host specificity to *A. filiculoides*.

Azolla is phylogenetically isolated, and although related to *Salvinia* and *Marsilea* (evidenced by the various placing of *Azolla* in the Salviniaceae and Marsileaceae) the exact phylogenetic affinity to these groups is uncertain. Furthermore, *S. rufinasus* is the only member of the tribe Stenopelmi (LeConte, 1876) and this indicates a possible long association between the insect and its host.

Stenopelmus rufinasus, under the laboratory conditions described here, was able to feed and oviposit, and the larvae were able to develop, on the non-target *Azolla* species tested. However, extremely poor adult emergence was recorded on the two southern Africa species, indicating that they would probably not support field populations of the insect. *A. pinnata* subsp. poss. *asiatica* supported the highest level of adult emergence of the non-target species tested. Although adult emergence on this plant was much lower than on *A. filiculoides*, indicating that it is an inferior host and unlikely to support field populations of the weevil in the absence of *A. filiculoides*, the emergence of nearly 40 adults per replicate might be cause for concern. However, *A. pinnata* subsp. poss. *asiatica* is introduced and restricted to the coastal areas of the KwaZulu-Natal Province of South Africa where it has a weedy phenology and invasive status and is thus of no conservation value. Therefore, any damage inflicted on this plant in the field may be an acceptable trade-off for the predicted impact of *S. rufinasus* on *A. filiculoides*.

The results presented here show that a release of the weevil *S. rufinasus* on *A. filiculoides* in South Africa poses no threat to native non-target plant species whilst it has the potential to contribute to the control of the weed.

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