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NATIVE NORTH AMERICAN *AZOLLA* WEEVIL, *STENOPELMUS RUFINASUS* (COLEOPTERA: CURCULIONIDAE), USES THE INVASIVE OLD WORLD *AZOLLA PINNATA* AS A HOST PLANT

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Azolla pinnata R. Br., an Old World mosquito fern, was found to be naturalized in waterways of southern Florida near Jupiter in May 2007 (Bodle 2008). This tiny aquatic fern is native to a large area of the tropics, subtropics, and warm temperate regions of Africa, Asia, and Australia (Sweet & Hills 1971; PIER 2007). *Azolla pinnata* has fame as a promoted component of rice culture in China because of the nitrogen fixing symbiotic bacteria that live in its leaves (Moore 1969). The fern is a Federal Noxious Weed (Kay & Hoyle 2000) that was found to be naturalized in North Carolina in 1999, where it continues to have a presence (Bodle 2008). *Azolla pinnata* has also naturalized in New Zealand where it displaced the native *A. rubra* R.Br. in much of the country (PIER 2007). Another mosquito fern, the western North American native *A. filiculoides* Lamarck (Lumkin 1993), has invaded Europe and South Africa, where it became an important weed (Hill 1998). The source of the naturalized *A. pinnata* in Florida is unknown. The discovery of plants at an aquarium and water garden shop in North Carolina (Kay & Hoyle 2000) indicates either purposeful or accidental introduction through aquatic plant commerce as a likely source.

Because of the problems that the invasive *A. pinnata and A. filiculoides* have caused elsewhere, and the problems caused by many other aquatic weeds, there was immediate concern about the potential weediness of *A. pinnata* in Florida when it was found. In the waterways where it was detected, it dominated the habitats (see photos in Bodle 2008). This invasive *Azolla* could diminish or displace the Florida native *A. caroliniana* Wild. (considered to be *A. filiculoides* by Evrard & van Hove 2004) and other valued native plants.

An intensive eradication effort with herbicides began soon after the plant was detected and continues, led by the second author. Eradication, despite being the appropriate control approach for such a geographically limited invader, may prove difficult for a number of reasons. Some plants and their spore may have dispersed beyond the infested sites before treatment was started**.** Plants and probably spore (borne in hard sporocarps) may escape treatment and generate new plants at the infested sites. Furthermore, there may be undetected *A. pinnata* plants at other locations in the region.

Because of the difficulty in eradicating *A. pinnata*, it is prudent to consider other potential control approaches. Biological control appears feasible for a number of reasons. There are two insect herbivores that are *Azolla* specialists in North America (Center et al. 2002). One is a flea beetle, *Pseudolampsis guttata* (LeConte), which occurs in the eastern US, where it uses *A. caroliniana* as its host plant (Buckingham & Buckingham 1981). The other is a weevil, *Stenopelmus rufinasus* Gyllenhal, which occurs in both the western and southern U.S. (Richerson & Grigarick 1967). The weevil uses *A. filiculoides* in the west and *A. caroliniana* in the south, the native *Azolla* species occurring in these respective areas of the U.S. (Lumpkin 1993). Both beetles are known to devastate the mats of these *Azolla* species (Center et al. 2002), and both occur in Florida (Buckingham & Buckingham 1981; Hill 1998).

Stenopelmus rufinasus was introduced from Florida into South Africa in a biological control effort against the introduced weed *A. filiculoides* (McConnachie et al. 2002). The weevil completely controlled the weed at most sites in a matter of months (McConnachie et al. 2002), making it one of the most successful biological control agents ever employed. During the host specificity testing of the *S. rufinasus* weevil in South Africa prior to its release, 2 forms of *A. pinnata* were exposed to it (Hill 1998). One was the indigenous South African *A. pinnata* (subspecies *africana*) and the other apparently an introduced form from Asia (possibly subspecies *asiatica*). The performance of *S. rufinasus* on these *A. pinnata* forms was poorer than on the target weed *A. filiculoides*, which as mentioned above is one the weevil's host plants in North America. Although the weevil completed its development on *A. pinnata*, Hill (1998) stated that *A. pinnata* "is an inferior host and unlikely to support field populations of the weevil in the absence of *A. filiculoides*".

To detect the possible occurrence of both the flea beetle and the weevil on the newly naturalized *A. pinnata* in Florida, plants were obtained from the main infested site prior to herbicidal

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treatment. On May 9, 2008, approximately 25 L of plant material were collected from a dense, apparent monoculture of *A. pinnata* covering the water's surface of a canal at a naturalization site. The site was searched a number of times to try to detect plants of *A. caroliniana* but none were found. The collected *A. pinnata* plants were brought to Ft. Lauderdale the same day. One third of the collection (approximately 8 L) was divided evenly and placed in 6 Berlese funnels to extract insects from the material. Four of the funnels had glass jars containing water and *A. pinnata* plants fitted to the neck of the funnels. The other 2 funnels were fitted with jars containing 70% ethanol. The Berlese funnels were run for 5 d. A smaller quantity of *A. pinnata* plants were floated on the 0.15-m^2 surface of a small aquarium filled with approximately 20 L of water and closed with a fine mesh screen top. The aquarium was placed inside an outdoor screen house, and checked for insects biweekly. The remaining material (approximately 17 L) was placed in an outdoor concrete coffin liner tank (about 2 × 1 m) filled with approximately 200 L of water and covered with a fine mesh cover, and checked weekly for 3 weeks. Small quantities of plants were removed from the concrete tank twice during that period and searched more carefully for insects. On May 20, a second set of Berlese funnel extractions was set up with approximately 8 L of *A. pinnata* plants taken from the concrete tank. The procedure was the same as the first run, except that the funnels were run for 7 d instead of 5. Prior to the placement of the field collected *A. pinnata* plant material in funnels, the aquarium and the concrete tank were examined to detect and remove material of other plant species, especially *A. caroliniana*, and debris that may have harbored insects unassociated with *A. pinnata*.

The Berlese funnels produced no *P. guttata* flea beetles, but 1funnel in the first run did yield a single adult specimen resembling *S. rufinasus*. Various aquatic insects were found but none appeared to be *Azolla* feeders. No flea beetles or weevils were found with the *A. pinnata* plants in the concrete tank. The plants in the tank died after about 3 weeks after being set up after being over fertilized, which may have prevented the full development of any associated beetles. Three weeks after *A. pinnata* plants were set up in the aquarium, 8 adult weevils appearing to be *S. rufinasus* were found feeding and mating on the surface of the plants. These weevils and the single suspect weevil extracted in a Berlese funnel were compared with *S. rufinasus* specimens obtained from and determined by Mike Thomas from the Florida Arthropod Collection of Division of Plant Industry, Gainesville, and confirmed to be *S. rufinasus*. Examination of plants from the aquarium revealed heavy feeding on the leaves, and oviposition tunnels in individual leaves. Oviposition sites were recognized by a black plug inserted at the outside of oviposition tunnel as described by Richerson & Grigarick (1967). Single eggs were found in some of the leaves with plugs. Empty egg chambers within the leaves with plugs indicated hatched eggs. During the first week after adult weevils were seen in the aquarium, at least 17 adult *S. rufinasus* were found in the aquarium.

The discovery of adult *S. rufinasus* actively mating, feeding, and laying eggs 3 weeks after the plants were set up in the aquarium indicates that the adults developed from undetected immature larvae in the aquarium. No adults were detected at the time of the set up of the plants, nor, more importantly, during the inspections of the *A. pinnata* plants in the aquarium for 2 and half weeks following the set up. This indicates that at the time of the collection the *A. pinnata* plants contained the eggs and/or larvae of the weevil, resulting from oviposition events that occurred in the field prior to collection of the plants. This indicates that *S. rufinasus* is breeding on *A. pinnata* in Florida.

That only 1 adult weevil was obtained from the Berlese funnels indicates that the population of *S. rufinasus* was probably low in the field. But the 17 weevil adults emerging from a relatively small amount of *A. pinnata* placed in the aquarium suggests otherwise. It may be that the weevil recently had colonized the plants in the field, had yet to produce large numbers of adults, and that the insect had a patchy and limited occurrence on the *A. pinnata* plants.

The native *A. caroliniana*, the only host of the weevil in Florida prior to the naturalization of *A. pinnata*, had to be the source of the weevils that colonized the *A. pinnata* plants. *Azolla caroliniana* is a common plant in Florida, but as mentioned above, no plants of this species were found at the *A. pinnata* collection site, and none were found in the collected *A. pinnata* material. The absence of *A. caroliniana* from the *A. pinnata* site indicates that the weevil is able to live on *A. pinnata* in the field independently of its normal host *Azolla*. It is not known which of the subspecies of *A. pinnata* the weevil is using or which subspecies has naturalized in Florida. *Stenopelmus rufinasus* may have also adopted *A. pinnata* as host plant in South Africa.

The absence of the *Azolla* flea beetle, *P. guttata,* in the *A. pinnata* samples probably indicates that the flea beetle did not discover and colonize the plants, rather than a lack of suitability. Host range testing of this flea beetle in South Africa demonstrated that the indigenous *A. pinnata* is a good developmental host (Hill & Oberholzer 2002). Concern that the flea beetle could harm *A. pinnata* and another native South African *Azolla* prevented its release as a biological control *A. filiculoides* (Hill & Oberholzer 2002). The rapid near eradication of *A. pinnata* limited the oppor-

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tunity to learn whether the weevil, and the flea beetle, may contribute to the control of *A. pinnata* in Florida. Should the weed escape eradication, the presence and potential of these insects as control agents can be evaluated.

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Summary

The native weevil, *Stenopelmus rufinasus,* a specialist herbivore on North American mosquito ferns (*Azolla* spp.), has adopted *Azolla pinnata,* an incipient invasive weed in Florida from the Old World. This situation, in which a weed has invaded the native area of a successful biological control agent of a congeneric weed in another region, is unique. Should *A. pinnata* escape eradication, *S. rufinasus* may prove useful as a control agent.

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