# Environmental conditions and biological control in the Top End: experiments suggesting new directions

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**Summary** Biological control can be a lottery where many agents are released in the hope of finding one that works. This paper describes experiments that investigate why two biological control agents that are extremely effective in some situations have little impact under certain conditions in the Top End of Australia. We found that epidemics of the Noogoora burr rust are restricted by low humidity and high temperatures in the Top End. Effects of the salvinia weevil appear to be reduced when salvinia is not floating on water. We then suggest how this information can be used to focus future biocontrol projects that use these agents, rather than introducing new agents in the hope that one will be effective.

**Keywords** Noogoora burr, salvinia, agent selection, climate matching.

## INTRODUCTION

Weed biological control has been compared with a lottery in which many agents are released and only a few are effective (McEvoy in press). Unless the agent is successful within a few years, efforts are concentrated on finding and releasing the next agent. We propose a new approach – when an agent is effective in some regions but not others, and we know why its effective range is limited, then the agent could be selectively bred in the laboratory to increase the area in which it is likely to be effective.

Biological control of Noogoora burr (*Xanthium occidentale* Bertol.) using the rust fungus *Puccinia xanthii* Schw., and of salvinia (*Salvinia molesta* D.S.Mitchell) with the weevil *Cyrtobagous salviniae* Calder and Sands are good examples of where this approach may be useful in the 'Top End' of the Northern Territory.

Noogoora burr is a widespread weed of riparian zones throughout much of northern Australia. It can form dense thickets that limit animal and human access to water. A rust fungus that is specific to Noogoora burr was first discovered in Australia in 1975. Impact of the rust on Noogoora burr varies considerably within the weed's geographic range, possibly because it requires temperatures below 25°C combined with high humidity to infect leaves (Morin *et al.* 1992). The rust is very successful in eastern Australia, but although present, has no noticeable impact on plants in the Northern Territory (NT) or Kimberley region of Western Australia.

Salvinia is a free-floating aquatic fern that is able to form thick mats that smother waterways throughout much of Australia. The weevil *C. salviniae* was released in Australia as a biological control agent in 1980 and in the NT in 1981 (Miller and Wilson 1989). Overall the weevil has reduced the salvinia problem in the Top End, especially in deep, permanent waterways, but has had little effect on salvinia growing on shallow floodplains (Miller and Wilson 1989).

Three theories have been proposed to explain the poor performance of the weevil in shallow waterways. One theory is based on water temperature, as shallow water can get very hot. Skeat (1990) suggested that water may often be too hot for weevils to thrive, and weevil eggs will not hatch above 37°C (Forno *et al.* 1983). Another theory relates to the ability of the weed to sink in shallow water. Weevil larvae tunnel into buds and rhizomes, which destroys the plant's buoyancy, causing it to sink and drown, increasing the effect of weevil feeding. The weevil's effectiveness may therefore be limited if salvinia cannot sink below the water surface. The third theory involves the fact that floodplains often dry up in the dry season. A few salvinia ramets may survive in damp pockets, which may not favour weevil survival (Storrs and Julien 1996). Salvinia can recolonise these waterways very rapidly in the wet season.

One approach to make biocontrol less of a lottery and more of a science could involve selecting agents in the laboratory to tolerate environmental conditions that currently limit their populations in the field. Such an approach requires knowledge of the conditions that limit agent populations. This paper summarises two experiments that investigate environmental conditions that limit these promising biocontrol agents in the Top End, and suggests how these limitations may be overcome.

## MATERIALS AND METHODS

Experiments were conducted in the field at Berrimah Agricultural Research Centre, Darwin. The first experiment investigated effects of increased humidity and decreased temperature on the Noogoora burr rust. The second experiment investigated how the weevils affected salvinia under different temperature and water depth treatments.

**Noogoora burr** A study of the spread of the rust was conducted during the 2002–03 wet season. Four treatments were imposed: plots were shaded (2 m × 2 m shadecloth above the plants); watered (misters running for five minutes every hour); watered and shaded; or had no shade or water (control). Each replicate plot consisted of 10 Noogoora burr plants in a circle around a heavily rust-infected inoculum plant. Each treatment had five replicate plots.

Temperature and humidity probes measured conditions in one randomly selected plot from each treatment. The number of periods suitable for rust infection was calculated based on results of Morin *et al.* (1992). The time elapsed until half the plants in each plot were infected was estimated and compared between treatments. These methods are described in more detail in Edgar and Grace (2004).

**Salvinia** A study of the effects of the weevil on salvinia commenced 3 February 2004. We used 54 L plastic tubs (25 cm deep), with a gauze cover to prevent weevils moving between tubs. Each tub contained 100 g (fresh weight) of salvinia, and was fertilised with 0.1 g $L^{-1}$ Thrive™ and 1 mg N $L^{-1}$, as $KNO_3$. Salvinia was collected from the Howard and Finniss rivers (near Darwin) and was treated twice with $CO_2$ for two hours, to kill any weevils or larvae present.

Four environmental treatments were applied: 1) tubs were shaded, similarly to the Noogoora burr experiment; 2) mesh was placed 2.5 cm below the water to prevent salvinia from sinking; 3) salvinia was not in contact with free water (placed on a cotton tea towel floating on foam above the water, so that the tea towel was always damp); and 4) tubs were unshaded and salvinia was growing in full depth water (control). Each environmental treatment was replicated twelve times, six times with weevils and six times without.

Twice a week we visually estimated the proportion of leaf material eaten by weevils. After 32 days the salvinia was removed from each tub and thoroughly dried on paper towel, weighed (fresh weight), placed in Berlese funnels to extract weevils, oven dried and then weighed again (dry weight). We recorded the number of weevils extracted. Surface water temperature was measured every 15 minutes in one randomly selected plot from each treatment.

Weevil damage to leaves was square root transformed, then analysed using a repeated measures linear model, with each tub treated as a random variable. Weights of salvinia were analysed using ANOVA, with appropriate orthogonal contrasts to compare individual treatments. The number of weevils removed at the end of the experiment was compared using a generalised linear model, with Poisson error distribution.

## RESULTS

Impacts of both biocontrol agents were strongly affected by the experimental treatments.

**Noogoora burr** The rust spread most quickly in plots with additional water and no artificial shade (Table 1). Plastic shade actually slowed the spread of the rust, despite decreasing daytime temperatures, and watering increased the rate of spread of the rust.

The heaviest rust infestation occurred in plots without shade that received water, but overall infection was light. Few plants had more than 5% and no plants more than 20% of leaf area infected. Even with artificially cool and humid conditions, no plants were infected heavily enough to kill them. Further details are given in Edgar and Grace (2004).

Artificial shade decreased daily maximum temperatures and had little effect on minimum (night time) temperatures. Watering maintained higher humidity throughout the experimental period.

**Salvinia** Treatment had a large impact on leaf damage caused by weevils (Figure 1). There were significant differences between treatments (P = 0.0003), and treatment:time interaction was also significant (P = 0.007). The tea towel treatment, where salvinia was not in contact with free water, had much less leaf damage than other treatments. Leaf damage was not significantly different between the other three treatments. Shading had little effect on water temperature (Table 2).

A mean of 4.2 weevils was recovered from each tub of salvinia. This corresponds to 84 weevils per kg of fresh weight of salvinia. No weevils were recovered

**Table 1.** Suitability of experimental environmental conditions for Noogoora burr rust and estimates of how quickly epidemics spread through Noogoora burr populations (from Edgar and Grace 2004).

| Treatment | Number of 2 h periods suitable for rust infection | Days until 50% of plants infected |
|---|---|---|
| No shade, no water | 38 | 11 |
| Shade, no water | 20 | 50 |
| No shade, water | 52 | 7 |
| Shade, water | 53 | 14 |

**Table 2.** Water surface temperatures throughout the salvinia experiment (°C).

| Treatment | Mean | Mean daily minimum | Mean daily maximum |
|---|---|---|---|
| Shaded | 29.9 | 27.0 | 34.0 |
| No sinking | 30.1 | 25.3 | 40.7 |
| Tea towel | 30.7 | 26.5 | 40.0 |
| Control | 30.1 | 27.2 | 34.4 |

from tubs without added weevils. The number of weevils extracted at the end of the experiment from tubs with added weevils did not vary between treatments (P = 0.5).

Adding weevils resulted in lower fresh weight of salvinia overall (Figure 2, P = 0.001). There were significant differences in fresh weight between environmental treatments (P <0.001). Results in Figure 2 suggest that weevils had slightly less effect when salvinia was not in contact with free water, although weevil effect was constant across all treatments (P = 0.1 for the treatment:weevil interaction). Salvinia grew poorly in all treatments. Fresh weight actually decreased over time even in tubs without added weevils. Dry weight of salvinia was not affected by addition of weevils overall (P = 0.4).

Allowing salvinia to sink did not affect salvinia biomass. Biomass in the two treatments where salvinia could not sink was not significantly different to the other two treatments (P = 0.1).

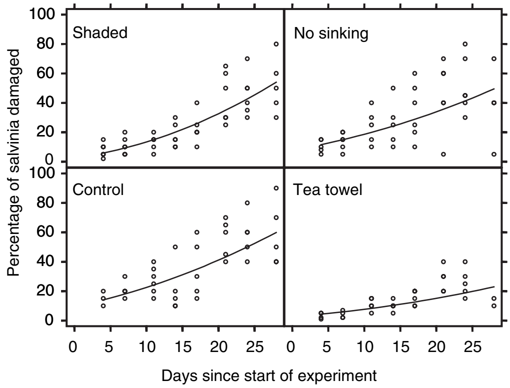


**Figure 1.** Percentage of salvinia leaf material eaten by weevils in four treatments. Circles show raw data and lines show back transformed data predicted by linear mixed effects model.

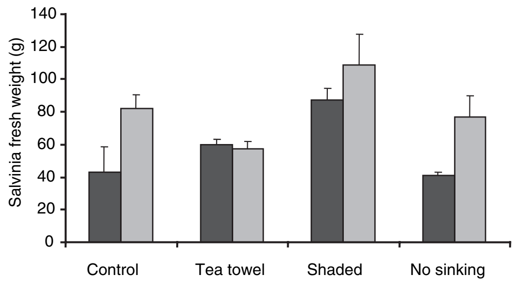


**Figure 2.** Fresh weights of salvinia at the conclusion of the experiment. Dark bars show means of tubs with added weevils and the light bars had no added weevils. Error bars show standard errors.

## DISCUSSION

These experiments suggest that both biocontrol agents are limited by environmental conditions in the field. The rust is limited by too few periods with low temperatures and high humidity. Weevil damage was reduced when salvinia was in contact with free water.

**Noogoora burr** Our results support the hypothesis that the Noogoora burr rust is limited by high temperatures and low humidity in the Northern Territory. The rust spread faster and infected plants more heavily when conditions were more humid. Rust infection was too light to cause significant damage to the plant, even in artificial conditions, during the cooler, humid wet season.

Rust spread slowly through plots with artificial shade, even though shade lowered daytime temperatures. Daytime temperatures under the shade were still too high (>25°C) for basidiospores to penetrate. In northern Australia temperatures favourable for basidiospore penetration are more likely to occur at night. The plastic mesh appeared to reduce the amount of dew reaching the leaves. In fact, plots with shade and no added water were too dry for many plants to survive.

The rust did not contribute to plant mortality in our experiment. Although the rust spread quickly with artificial watering, no plants had more than 20% of leaf area infected and no stem lesions were observed.

Overall, it appears that a combination of high temperature and low humidity limits the effects of the rust on Noogoora burr in the Top End. A strain of the rust that is better able to cope with warm temperatures might help control Noogoora burr in northern Australia.

**Salvinia** Salvinia leaves were less damaged by weevils when not in contact with free water. On floodplains, salvinia ramets may survive in damp pockets which do not favour weevil survival (Storrs and Julien 1996), either because weevils will only lay eggs on salvinia in contact with water (Forno *et al*. 1983), or because such clumps get hot. Salvinia can recolonise floodplains very rapidly when the wet season arrives. Leaf damage is mainly caused by adult weevils, although larvae also feed on leaves, whereas reduction in biomass of salvinia is most likely to be due to larval feeding (Julien *et al.* 1987). Weevils reduced fresh weight of salvinia in all treatments except the tea towel, suggesting there were fewer larvae feeding in this treatment. Effects of weevils on salvinia biomass were variable and difficult to interpret, especially differences between dry weights and fresh weights, possibly because salvinia growth was poor, most likely due to inadequate nutrients or poor water quality.

Temperatures recorded in our experiment were similar to those that Storrs and Julien (1996) considered ideal for weevil development. Shading made little difference to surface water temperatures. Storrs and Julien (1996) found that leaf, air and water temperatures regularly exceeded 37°C, with no noticeable effect on weevil populations, and noted that weevils may not always persist in shallow floodplains, even when shaded by trees. Preventing salvinia from sinking did not affect salvinia biomass. In fact larval feeding in buds, rhizomes and roots can severely reduce salvinia growth rate even if it does not sink (Julien *et al.* 1987).

**Future directions** Information about why heavy rust epidemics do not occur in the Top End and why the weevils have little effect on floodplains may be used to overcome limitations of these two agents. Given that these two agents are highly effective in other locations, future successful biocontrol is likely to involve these agents, rather than importing new species lottery-style.

One approach could be to return to the agent's native range and select new strains that exist in similar climates or locations. It is expensive to collect new strains of agents, verify their taxonomy and rear these through one generation in quarantine. This has been done for other biocontrol agents, with mixed success. This may not be appropriate for salvinia because *S. molesta* has a restricted native range and the weevil is specific to *S. molesta* (Forno *et al.* 1983).

Another potential approach would involve searching for and utilising any variation found in the agent across Australia. Investigations may commence soon into variation in the Noogoora burr rust across Australia and its native range (R. van Klinken pers. comm.). If agents are adapting to local environmental conditions, then this should be considered when redistributing agents.

We propose an alternative method of selecting agents in the laboratory to tolerate environmental conditions that limit populations in the field. The Noogoora burr rust could be cultured under high temperatures, and the salvinia weevil grown on salvinia that is not in contact with free water. This approach is as yet untried, so the probability of success is unknown, but would be relatively cheap to attempt. Investigations of selection pressure occurring in the field and population genetics models may also be useful in predicting the likely outcomes of such an approach. Mutagens or molecular biology could also be used to speed up selection.

The two studies outlined in this paper provide information that would be required for any of these methods to be effective. The suggested approaches involve using agents already present, rather than creating a lottery of new agents.

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