EXPERIMENTAL ASSESSMENT OF THE BENEFITS TO THE ALGA *ANABAENA AZOLLAE* IN ITS SYMBIOSIS WITH THE FERN *AZOLLA MICROPHYLLA*

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Abstract : The alga *Anabaena azollae* and the fern *Azolla microphylla* are symbionts, with *A. azollae* receiving nutritional benefits. To identify additional benefits to the alga, we compared algal abundance under three experimentally-altered conditions: herbivore density, water temperature and prolonged *A. microphylla* submersion. *A. azollae* in the submerged treatment declined in abundance, indicating that *A. azollae* may receive a benefit from the *A. azollae- A. microphylla* symbiosis that is compromised by *A. microphylla* submersion.

Key Words: environmental stressors

INTRODUCTION

Anabaena azollae, a nitrogen-fixing cyanobacterium, is symbiotic with the water fern *Azolla microphylla*. *A. azollae* lives on the top surface of floating *A. microphylla* leaves. *A. azollae* is not motile, but can, by expending energy, control its buoyancy in the water column. In the symbiosis, *A. microphylla* receives nitrogen and *A. azollae* receives photosynthetic assimilates (Dawar and Singh 2003). Little else is known about benefits to the alga.

A. microphylla and *A. azollae* are capable of existing independently, but are usually found in symbiosis. High phosphorus addition causes *A. azollae* to leave *A. microphylla* (Dawar and Singh 2003).

To determine if environmental stressors other than nutrient limitation could disrupt the *A. azollae-A. microphylla* symbiosis, we designed three treatments simulating commonly-occurring variations in the environment of symbiotic *A. azollae*: increased herbivore density, increased water temperature, and prolonged *A. microphylla* submersion in the water column. We predicted that these treatments would disrupt the symbiosis and cause *A. azollae* to separate from *A. microphylla*, suggesting that *A. azollae* receives other benefits from *A. microphylla* that were compromised by the changed environmental conditions.

METHODS

Our experiments were conducted in the Rio Tempisque marshland at Palo Verde National Park, Costa Rica on 13 and 14 January 2005. The marsh floods annually during May - October and dries up when the Rio Tempisque recedes in February - April. We constructed opaque water columns 30 cm tall that contained 1 L of marsh water collected under patches of *A. microphylla*. Our columns were held in a large plastic container placed in the marsh, to prevent any disturbance from wind or waves. Each column contained ten *A. microphylla* plants from the same patch. Col-

umns were placed at 0800 and removed at 1400, when *A. azollae* abundance on *A. microphylla* was measured.

In our first experiment, we examined the effects of herbivory by creating two levels of herbivore densities with four replicates of each. The low density treatment had no herbivores, while the high density treatment had 5x the average natural herbivore density per volume of marsh water.

In our second experiment, we examined the effect of increased water temperature, as may occur late in the dry season as the water level drops. As a control, four 30 cm water columns were placed in a large plastic container in the marsh. By placing the columns in a large container of water in the marsh, the column temperatures were maintained at the marsh temperature of 28.9 °C at 1200. Four smaller 10 cm columns were placed in open air for the high temperature treatment. They reached 34.5 °C at 1200.

For our third experiment, we simulated prolonged submersion of *A. microphylla*, which commonly occurs due to wind and waves. We used four 30 cm water columns with a 0.4 cm mesh exclusion that restricted *A. microphylla* to the bottom third of the water column, but allowed uninhibited *A. azollae* vertical migration. For a control, we used four 30 cm water columns, without a mesh exclusion, that allowed *A. microphylla* to remain at the surface.

We quantified *A. azollae* abundance on *A. microphylla* by haphazardly removing five plants from each column and scraping *A. azollae* off two haphazardly-chosen leaves from each plant. We counted the number of *A. azollae* colonies on each leaf using 50x light microscopy. We used a one-way ANOVA on JMP 5.0.1 to analyze the differences between treatments in the herbivore density, water temperature and submersion experiments. A two-way nested crossed ANOVA examined the effects of time, treatment and interactions in the submersion experiment.

RESULTS

There was no significant difference in algal colonial abundance between reduced and increased herbivory treatments ($F = 0.08$, $df = 1, 78$, $P = 0.78$) nor between 29.7 °C and 33.3 °C water patches at 1400 ($F = 0.13$, $df = 1, 78$, $P = 0.72$). In the sub-

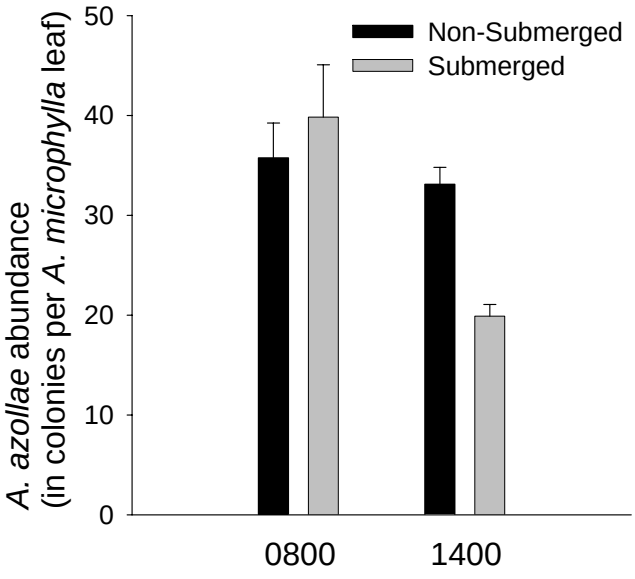

Figure 1. Decline of *Anabaena azollae* colony abundance on *Azolla microphylla* after *A. microphylla* submersion in the Rio Tempisque marsh, Palo Verde. *A. microphylla* was submerged for 6 hours in four 30 cm columns.

mersion experiment, we found a significant interaction in a model of time, treatment, and time x treatment interaction, with plant as a non-random effect ($F = 13.26$, $df = 1$, $P = 0.002$; Fig. 1). *A. azollae* colony abundance decreased with time ($F = 22.64$, $df = 1$, $P = 0.0003$), while treatment, and plant as a non-random effect, were both insignificant ($F < 1.99$, $df = 1$, $P > 0.11$). In the submerged treatment, mean algal abundance decreased from 40 to 20 colonies per leaf between 0800 and 1400 ($F = 22.10$, $df = 1, 14$, $P = 0.0003$). Algal abundance in the non-submerged treatment did not significantly change from 0800 to 1400 ($F = 0.60$, $df = 1, 12$, $P = 0.45$).

DISCUSSION

Our hypothesis that altering environmental conditions could cause *A. azollae* to leave its *A. microphylla* host was confirmed. Cyanobacteria are not preferred food particles for copepods, the primary herbivores found in the marsh, thus it is not surprising that changing herbivore density had little effect on *A. azollae*. The minimal temperature interval (3.6 °C) between treatments at 1400 may explain the lack of a significant temperature effect. It remains possible that *A. azollae* could leave *A. microphylla* if temperature rises higher than 34.5 °C.

The lower algal abundance on submerged *A. microphylla* may be due to reduced *A. azollae* photosynthesis at depth, due to light attenuation. This would imply a benefit to *A. azollae* using *A. microphylla* as a substrate. Submerged *A. azollae* could face a tradeoff between the nutritional benefits from *A. microphylla* symbiosis and the necessity for photosynthesis.

We assume that decreased *A. azollae* abundance on *A. microphylla* was due to *A. azollae* actively separating from *A. microphylla*. It was possibly due to the death of *A. azollae*; however, we do not think this is likely and were unable to examine the water columns for dead algal cells. If *A. azollae* died, they would probably remain on top of the *A. microphylla* leaves while submerged, because they are negatively buoyant (Karlsson-Elfgren and Brunberg 2004).

LITERATURE CITED

Dawar, S. and P. K. Singh. 2003. Disappearance of the symbiont *Anabaena azollae* in *Azolla* subjected to high phosphorus. International Rice Research Notes, 28 (1): 53-54.

Karlsson-Elfgren, I. and A. Brunberg. 2004. The importance of shallow sediments in the recruitment of Anabaena and Aphanizomenon (Cyanophyceae). Journal of Phycology, 40: 831-836.