

Azolla is an aquatic fern that floats atop water by means of overlapping scale-like leaves that extend several centimeters across the water surface. There are at least six species of *Azolla* worldwide, including *A. filiculoides*, *A. Mexicana*, *A. microphylla*, *A. pinnata*, *A. rubra* and *A. caroliniana*. The *Azolla caroliniana*, *A. filiculoides* and *A. Mexicana* are native to the US, with their distribution dependent primarily on the temperature tolerance of a particular species (Watanabe and Berja 1983)

Azolla reproduces sexually and asexually by the breakage of side branches (Lumpkin and Plucknett, 1980). A study conducted on six different species listed above found *A. microphylla* to have the greatest biomass production rate followed next by *A. caroliniana* (Arora and Singh 2003).

Azolla is a shade plant, needing only 25 – 50 % of full sun for normal growth. Growth optimization tests on newly bred strains of *Azolla* for the Controlled Ecological Life Supports (CELSS) program indicated that these plants were able to grow normally under relatively low light conditions (i.e. 3200 LUX) (Liu *et al* 2008). Its optimum photoperiod is 20 hours (Laurinavichene *et al* 1990).

The optimum water temperature for *A. pinnata*, *A. mexicana*, and *A. caroliniana* is approximately 30C (Watanabe and Berja 1983). The growth rate is greatly reduced above 35C and at temperatures above 45C the plant quickly dies. Although the plant can survive in a pH range of 3.5 to 10, its nitrogen fixation rate appears to be dependent on both the water temperature and pH level. (Ashton and Waimsley 1976). Greenhouse experiments with both *A. pinnata* and *A. filiculoides* showed maximum growth at pH values of 5-7 and a water temperature of 25C (Cary and Weerts 1992). The optimum relative humidity for *Azolla* is about 85-90% (Watanabe 1983). At levels under 60% the plant becomes dry and fragile (Hamdi 1982).

Optimum *Azolla* growth with full nitrogenase activity occurs when the concentration levels for P, K, Mg and Ca nutrients are 0.03, 0.6, 0.5, and 0.5 mmol l⁻¹, respectively (Lumpkin 1987) (Yatazawa *et al* 1980). If phosphorus levels are too low, the plant becomes stressed and develops into smaller plants, often turning pink to deep red with longer than normal roots. The micronutrients Fe, Mn, Mo, and B concentration levels should be a least 50, 20, 0.3, and 30 ug l⁻¹, respectively. In addition, Co is required for the symbiotic growth of the cyanobacterium within the host plant (Johnson *et al* 2001). Iron is an essential element for nitrogenase activity (Newton and Herman 1979). Plants deficient in Fe become yellow due to the depletion of chlorophyll.

Although *Azolla* can grow in a nitrogen free solution, the nitrogen concentration level in water does affect growth and nitrogen-fixation rates. Growth rates of *A. caroliniana* and *A. pinnata* were highest at nitrate concentration levels of 5 mM (Singh *et al* 1992). At greater nitrate concentration levels the growth rate declines. The source of nitrogen is shown to affect the growth of *A. caroliniana* (Hechler and Dawson 1995). Nitrogen as nitrate increased growth rate, but the same concentration of ammonium-nitrogen decreased growth rate. Urea had no effect.

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