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Environmental requirements for successful *Azolla* growth

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Azolla is traditionally grown under cool, wet conditions. The plant prefers a placid water surface, temperatures between 20 and 35 °C, water pH of 4-7 and rich in all essential plant nutrients except N, solution salt content <0.3%, exposure to <25% full sunlight, long daylength, and freedom from competitors, insects, and diseases. Efforts to expand its use in the humid tropics have met with limited success and a host of environmental problems. High temperatures and humidity stimulate insects and diseases that attack *Azolla*. Algal blooms compete for nutrients and cause a change in pH and poor water circulation. Areas dependent on monsoon rains rarely have water for multiplying *Azolla* before the rice growing season and usually suffer from intermittent droughts, which desiccate intercropped *Azolla*. Excessive rainfall and typhoons can cause flooding, which can wash away the entire *Azolla* crop. Water is usually deficient in P and the applications of phosphate fertilizer and possibly other nutrients such as Fe, Mo, and K are required for the *Azolla* crop.

Azolla is an aquatic fern which has potential for expanded use as a green manure for rice. In placid water where available N is low, *Azolla* has a competitive advantage over other floating plants because of its symbiotic relationship with N-fixing endophytic cyanobacteria called *Anabaena azollae*. The *Anabaena* can supply the entire N requirement of the *Azolla* even at growth rates of about 35%/d. In turn, under suitable environmental conditions, *Azolla* can supply the entire N requirement for a high-yielding rice crop (50-100 kg N) in 10-20 d. For example, an *Azolla* mat weighing 10 t/ha and increasing its biomass at 20%/d, (relative growth rate [RGR] of 0.2) could fix up to 4.8 kg N/d, assuming a 6% dry weight and a N content of 4% dry weight, i.e., 10,000 kg fresh wt × 0.2 RGR × 0.06 dry wt × 0.04 N dry wt.

Azolla is also more cold tolerant than most other aquatic plants. This tolerance allows *Azolla* to flourish during cool seasons with little competition from other plants and damage from pests. Chinese (30) and Vietnamese (46) farmers recognized *Azolla*'s potential and have utilized it for centuries as a cool-season green manure. In China, *Azolla* is grown in the south during winter and cultivation advances north to central China with the coming of

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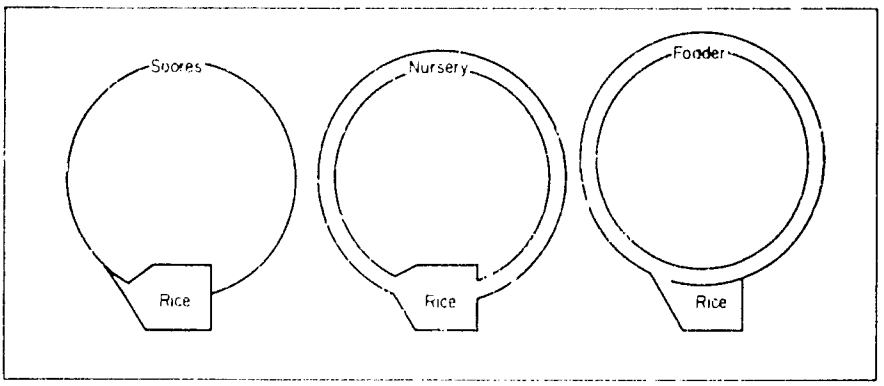
spring to cover an area estimated to be more than 680,000 ha. In northern Vietnam, *Azolla* is primarily grown during winter and was reported to cover an area of 700,000 in 1978 (7).

Outside of China and Vietnam, very few rice producing areas in the world have a wet-cool season for growing *Azolla* before the rice season. Usually water is not available or temperatures are very high before rice is transplanted, such as in the months before the monsoon in South and Southeast Asia.

Even if environmental conditions are favorable, *Azolla* is usually difficult to grow. For field multiplication of *Azolla*, farmers will need 1-5 t of *Azolla*/ha as planting material. This massive amount of plant material will be expensive and must come from one of three basic sources or combinations of these sources as illustrated in Figure 1. Because large-scale use of spores (12, 13, 14, 18, 47, 56) is not yet possible, farmers must either keep a nursery throughout the off-season or buy *Azolla* plant material from people who grow *Azolla* for sale. People who produce *Azolla* planting material for sale may also grow *Azolla* throughout the year as fodder for pigs, ducks, or fish. This combined type of system may be most practical for the tropics and should be encouraged by government programs. Fodder producers can multiply their *Azolla* stocks and sell to farmers as the rice-*Azolla* season approaches.

A planting level of 1-5 t/ha is necessary to adequately exploit the available water and sunlight. From this level, the *Azolla* is multiplied in the field as a monocrop or intercrop to attain a biomass of 10-80 t. From this level a single or multiple soil incorporations are carried out so that *Azolla* N can become available to a following or standing rice crop. The level of *Azolla* biomass varies according to cropping intensity and environment. Biomass is kept at a lower density under intense cropping to keep *Azolla* at its highest rate of productivity on a unit area basis (10). Only lower levels of biomass (10-30 t/ha) are currently possible under hot humid conditions because of insect and disease problems.



1. The three basic methods of maintaining *Azolla* planting material during off-seasons. These methods can be combined. About 3 wk must be allowed for germination of spores and development to dividing plants.

International (15) and national institutions are currently selecting varieties and developing management practices that will allow *Azolla* to become an important green manure for rice farming systems in the tropics. *Azolla* cultivation in China and Vietnam is generally limited to cool seasons because hot humid weather brings a host of environmental problems to an *Azolla* crop (26). These problems are so devastating that most farmers will not attempt summer cultivation of *Azolla* in paddy fields. I describe the environmental requirements for cultivation in hot humid conditions and review research articles about environmental constraints. I will expand on previous reviews of *Azolla* literature (4, 27, 30, 33).

ENVIRONMENTAL REQUIREMENTS

Climate, especially the climatic variables that affect *Azolla* growth, is difficult to manipulate. To determine the climatic variables and interactions that most influence *Azolla* growth, climatic variables were regressed on the RGR of *Azolla* species (28) that had been grown in a year-round pot experiment (2). The correlation analysis of data recorded during hot humid weather indicated that temperature and humidity had a negative effect on growth rate and solar radiation had a positive influence.

Although it may be possible to develop management practices that give some degree of control over these variables (52), the environment in which *Azolla* lives encompasses many other interacting factors. For example, *Azolla*'s susceptibility to fungal pathogens probably interacts with high temperature and humidity. Algal blooms not only compete with *Azolla* for nutrients but also cause a change of pH in the paddy water which can result in loss of nutrients from solution. They also restrict water movement which can result in high water temperatures lethal to *Azolla*. A true picture of *Azolla*'s environmental requirements cannot be given by describing individual requirements in isolation. Often the interactions or indirect effects of factors are more important than the direct effect. Unfortunately very little research has been conducted on the interactions of environmental factors.

Environmental factors

Water. The lack of good control and availability of water in rice growing areas is the primary constraint to the spread of *Azolla* use. As a delicate aquatic plant, *Azolla* can survive only for a few minutes on a dry surface under the tropical sun, and for a few days on paddy soil that dries during intermittent rains. Some varieties can survive indefinitely on moist, shaded mud, but will not multiply to any useful extent without a water surface on which to spread. Thus, without good water control and availability, *Azolla* multiplication may not succeed.

The need for water extends beyond the growing season and remains important to a limited extent throughout the year. A small amount of water must be available to maintain nursery stocks of *Azolla* plants during the

off-seasons. A larger amount of water will be needed for the multiplication of this nursery before wide-scale field multiplication begins as the rice season approaches. Unfortunately, a large quantity of water is usually not available for growing *Azolla* just before the rice crop is planted.

The source for water is also important. Like rice, *Azolla* grows better during dry seasons when irrigation water is available. If water comes from precipitation, additional problems may occur. A rice farmer on the east coast of India, Vietnam, and China, or anywhere in the Philippines can have his entire *Azolla* crop washed away by a typhoon. Farmers in a monsoon area can suffer the same fate from excessive rainfall, which causes flooding. With the exception of a few areas such as South Cotabato in the Philippines (48), most farmers will need to invest considerable effort to produce an *Azolla* crop. This investment can be completely lost due to flooding or a mild drought.

Light. The growth rate of *Azolla* has been reported to saturate at 25-50% of full sunlight (2, 44), and is not inhibited by full sunlight as long as other factors are not limiting (3). As an intercrop under rice, the growth rate of *Azolla* will begin to decline as the developing rice canopy reduces light quantity and quality below that necessary to saturate growth rate. The rice canopy will start influencing growth about 2-3 wk after transplanting and will stop growth in most *Azolla* species at 45 d after transplanting, depending on such factors as rice maturation period, leaf area index, weather, paddy water fertility, etc.

An aspect of shading which is of possible interest but has not been studied is the intracrop shading of *Azolla* as it becomes crowded. As in other crops, crowding probably results in competition for light, nutrients, etc. It is difficult to take measurements within the *Azolla* canopy because of the smallness of *Azolla*, but it is not impossible. Competition can be measured indirectly through RGR. Further research could help optimize productivity of biomass and accumulated N if more information was available about intracrop competition.

Daylength is another important aspect of light. Growth rate has been shown to positively correlate to daylength (28) and continues to increase up to continuous illumination (37). Higher latitudes, such as central China, will have a longer daylength during the late spring and early summer *Azolla*-growing season than the tropics which have an almost uniform daylength all year-round. *Azolla* growth rate is higher in higher latitudes than in the tropics.

Temperature. Temperature is probably the most important limiting environmental factor in *Azolla* cultivation. It also is very difficult to manipulate. Its direct effects are not as serious as its indirect influences. For example, certain *Azolla* varieties can grow at temperatures of 40 °C or higher (22, 49) and some management practices can prevent paddy water temperatures from exceeding 40 °C in most cases if water is available.

The most serious problem with temperature is its stimulating effect on *Azolla* pests, e.g., insects, pathogenic fungi, and free-living algae. The optimum temperature for most *Azolla* species is within the range of 20-35 °C. At higher temperatures in this range and above, the generation time for

insects (57) and the growth rate of fungi greatly increase. Insects, particularly lepidoptera (32) and dipte (43), can destroy an *Azolla* crop if pesticides such as carbofuran or BHC are not used (30). Often, insects will sample a small part of a plant and then move on to another. The resulting injury makes the plant highly susceptible to various fungi which attack *Azolla* (1, 41, 45) and can greatly magnify the insect damage under hot-humid conditions.

Because high temperatures are not a direct limitation, *Azolla* has an excellent potential for successful cultivation in irrigated deserts where humidity is relatively low and alternate host plants for insects are limited. *Azolla* does very well on the northern border of Senegal in West Africa (Van Hove, pers. comm.) and can probably do well in the traditional rice areas of Mali with good water control.

Mineral nutrition. Because *Azolla* is an aquatic plant, essential elements must be available in the water for *Azolla* to survive. *Azolla* requires all essential plant elements plus Mo or Co for N fixation (4, 29, 51). However, most paddy water does not contain an adequate balance of essential elements for successful *Azolla* cultivation.

Phosphorus has been the most common limiting element for *Azolla* growth (31). The threshold concentration of P in *Azolla* tissue is probably about 0.2-0.3% on a dry wt basis (42; Sombath Somphone, pers. comm.). P stressed plants are usually smaller, pink to red, less vigorous, and have a low concentration of total N. Under severe stress, the plants become highly compact and dark red, and often develop very long curled roots.

P deficiency can be overcome by applying P fertilizer. Highly soluble forms such as triple superphosphate or phosphoric acid are effective. In some situations, deficient plants may be able to extract sufficient P from the paddy soil if the water level can be reduced to the point where *Azolla* roots can touch the soil.

Perhaps the most efficient way to use P fertilizer is to take advantage of *Azolla*'s ability to use up P luxuriously. *Azolla* requires a minimum concentration of about 0.2-0.3% P on a dry wt basis for a normal N concentration of 3.0-4.0%. This equals a N:P weight ratio of 10-20 N for each P. Thus, 1 unit of P as fertilizer could result in 10-20 units of plant N at 100% uptake efficiency. But 100% uptake efficiency is impossible in the field because of losses of P from fixation, leaching, runoff, erosion, etc. Uptake efficiency can be significantly improved by preloading *Azolla* in phosphorus-rich nursery beds. Preloaded *Azolla* should be able to accumulate P up to its maximum luxury consumption level of 1.0-1.6% (30). After preloading, the plants can be placed in P-deficient fields for further multiplication. Even if no P is available in the paddy water, the *Azolla* plants should be able to increase their biomass 3-8 times (1.0/0.3 to 1.6/0.2) on stored P before reaching their threshold level for P. Plants should not be allowed to drop below the threshold level of P or their N yield will be reduced.

Other essential elements have been reported to be absent in paddy water. Applications of K have been found to be necessary on light soils in Vietnam and China (24, 25, 46). Liu et al (23) compared the K absorption ability of

Azolla and rice. They believe that *Azolla* is more efficient at K uptake and can be a K source for rice in K-deficient soils.

Some micronutrients have been reported to be deficient in paddy water. Adding small amounts of Mo salts stimulated growth in paddy fields in Korea and Vietnam (19, 46). Fe was reported to be deficient in California (39) and Australia (34). Adding 1.5 kg Fe/ha eliminated the deficiency in California.

Nitrogen can also be necessary to maintain *Azolla* under stressed conditions. Zhang (54) and Chen and Li (6) found that N fertilizer along with P and K were effective in maintaining *Azolla* during summer. Zhang and others (16, 20, 36, 53) found that combined N can adversely affect *Azolla* under good growing conditions. This adverse effect probably results from the beneficial effect of combined N on nonnitrogen-fixing organisms which compete with *Azolla*, such as weeds and algae.

Water quality. Several other aspects of water quality have been studied. Pollution from sewage (40) and herbicides (30) is of little concern to developing countries, but may be important as *Azolla* cultivation is attempted. Other factors are known to limit *Azolla* use. pH is perhaps the most important in developing countries. Optimum growth of *Azolla* in culture solution is in pH range of 4.5-7, but *Azolla* can survive in pH 3.5-10 (2, 35, 37, 50) if all essential elements are available.

The most important concern about pH is the availability of essential elements in the paddy water. The pH of most acid and alkaline soils changes toward a range of 6-7 a few weeks after flooding (38). However, some acid soils — acid sulfate and Histosols — which are low in organic matter or active Fe or high in sulfate, may not reach a pH of 6.0 even after months of submergence (8). These acid soils can create toxic levels of Al and Fe, and P deficiencies in the paddy water. Saline soils often have a pH greater than 7 and may be deficient in P, Zn, and Cu.

Chinese researchers (9, 17) recommended that water for *Azolla* cultivation should contain no more than 0.3% salt. Higher salt concentrations decreased plant N (9), and more salt increased plant N (17). Haller et al (11) found that *A. caroliniana* growth ceased when the concentration reached 1.3%. Salinity or alkalinity may be a problem in ricefields located along the coast (55) and in poorly drained irrigated deserts, such as in areas of Pakistan (5).

CONCLUSIONS

Azolla can be found throughout the world growing under a wide range of environmental conditions. However, conditions for survival in nature can be much more variable than those necessary for successful field cultivation, where growth rate and N accumulation are important. In China and Vietnam, wide-scale field cultivation normally occurs during winter and spring when conditions are cool and wet. *Azolla* cultivation is rarely attempted during hot-humid conditions because 1) more important crops occupy the paddy

fields, 2) insects and diseases are very difficult to control, and 3) paddy water must be managed to prevent temperatures of 45 °C or higher. These same problems exist in the tropics and are often compounded by monsoon weather of extreme wet and dry seasons.

The following questions about cultivation should be considered before *Azolla* is recommended to farmers:

- Does the cropping system allow the growing of *Azolla* as a monocrop or intercrop, and is water readily available?
- How can some *Azolla* be maintained during off-season and how can it be multiplied and distributed before large-scale field cultivation?
- Is the water rich in essential plant elements, especially P, to support rapid growth of *Azolla*?
- Is the water neutral to mildly acidic in pH and does it contain less than 0.3% salt?
- Will it be necessary to frequently use pesticides for insect control? and
- Will *Azolla* cultivation improve the economic situation of the farmer?

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DISCUSSION

CRASWELL: Are data available on the total area of *Azolla* used in farmer fields in various rice-growing countries? If not, some effort should be made to collect such data so that the trends in *Azolla* use in various countries can be monitored.

LUMPKIN: I have not seen precise data on the area of *Azolla* cultivation in any country. Reports from both China and Vietnam are highly variable. Perhaps remote sensing could be used for regions where *Azolla* use is concentrated.

MOCHIDA: Is it possible to use *Azolla* as feed for snails, which are used as food of humans? How shall we resolve the schistosomiasis disease where *Azolla* is used in rice fields, for example, in Leyte or South Cotabato, in the Philippines? Do you have any comments?

LUMPKIN: I have not seen any literature reporting the acceptability of *Azolla* to snails which are used as human food. Perhaps a coordinated snail eradication program with clonitralide could be effective. A concentration of 0.5 ppm in paddy water solution has been reported to be effective. All efforts should be used to eliminate detrimental environmental consequences of such a program.

DE WAHA: What method do you use to establish equations permitting correlation between productivity and climatic data?

LUMPKIN: We use a main frame computer software called SAS. Within SAS we use SYSREG and STEPWISE programs.