# Symbiotic Nitrogen Fixation Technology

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# Collection, Maintenance, and Cultivation of Azolla

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## I. INTRODUCTION

The aquatic fern azolla has a high relative growth rate (RGR) and a symbiotic relationship with a nitrogen-fixing endophytic cyanobacteria called *Anabaena azollae*. This symbiotic relationship gives azolla a competitive advantage over other floating hydrophytes in environments such as rice fields which are relatively low in available nitrogen. The symbiosis is of interest to agriculturalists since incorporation and decomposition of an azolla crop in a rice field soil can result in an increase of available nitrogen to a companion or succeeding rice crop.

Azolla can accumulate more than 10 kg N/ha per day (25 tons/ha of azolla × 6% dry weight × 4% N × RGR 0.17 ton/ton per day), and thus has the potential of supplying the entire nitrogen requirement for a high-yielding rice crop within a few weeks. Under proper management and environmental conditions, this rate can be sustained because azolla reproduces by fragmentation and is not restrained by soil as are terrestrial plants. Azolla can also be used to suppress paddy weeds and is an excellent food for fish, pigs, and ducks (Lumpkin and Plucknett, 1982).

In the 1960s and 1970s, azolla was regularly cultivated on a large scale only in southeastern China (Beijing Agricultural University 1979) and northern Vietnam (Vo and Tran, 1970). In these areas, azolla was used primarily as a green manure for rice and was viewed as a crop of rather low economic value. Only short periods, before or with the spring rice crop were allocated for its cultivation. Azolla would make use of short cool periods in the cropping sequence because some varieties had both (a) the ability to sustain a relatively high growth rate under cool temperatures (10 to 20°C) and (b) a potential for doubling their biomass in as little as 2 days (0.35 g/g per day) under more optimal conditions (Peters et al., 1980; Watanabe and Berja, 1983; Lumpkin and Bartholomew, 1986).

Tolerance of cool weather was the key to the success of azolla in parts of Vietnam and China. A high growth rate in cool seasons made azolla an economic competitor against alternative crops grown for food and a good biological competitor against other hydrophytes and algae. In addition, cultivation during cool seasons allowed azolla to avoid devastation by its thermophyllic insect pests.

The cool season (winter-spring) cultivation of *Azolla pinnata* var. *imbricata* in Vietnam's Red River Delta and irrigation system probably reached an area exceeding 500,000 ha during the late 1960s and has continued to be an important crop through the 1970s and 1980s because of a lack of chemical nitrogen fertilizers in Vietnam. In China, cool season cultivation of *A. pinnata* var. *imbricata* and *Azolla filiculoides* peaked in the late 1970s at over 1,000,000 ha and has since declined rapidly in the face of improved supplies of nitrogen fertilizer, low economic return, and the elimination of commune and research support systems through decentralization.

Outside China and Vietnam, azolla has met with scant success, primarily because of a lack of knowledge and problems with insects and diseases. However, the general level of interest and understanding about the agricultural uses of azolla has been enhanced by descriptive reviews and handbooks designed for scientists and researchers in developing countries (Peters, 1977; FAO, 1978; IRRI, 1979; Watanabe et al., 1981; Lumpkin and Plucknett, 1980, 1982; National Azolla Action Program, 1982; Van Hove et al., 1983; Lumpkin, 1984; Watanabe, 1984). These publications cover topics such as management practices, environmental requirements, identification of species, sexual and vegetative reproduction, alternative uses, and insect and disease pests.

For azolla to become an economically viable crop in rice-producing countries, numerous efforts and breakthroughs in azolla research must be made, especially in collection of germplasm with desirable traits, procedures to reduce or eliminate maintenance requirements, and a reduction in the labor and water requirements for cultivation. The intent of this chapter is to provide a short review of the current level of understanding about azolla and to comment on aspects constraining wider use.

## II. COLLECTION

### A. Identification

The name *Azolla* is derived from the Greek *azo*, to dry, and *ollyo*, to kill (Jaeger, 1978) and is a genus of heterosporous leptosporangiate ferns from aquatic and semiaquatic habitats. These habitats include ponds, ditches, canals, and paddy fields from temperate to tropical regions. Azolla is also a frequent pioneer in aquatic areas disturbed by man or animals. Countless places where agricultural runoff or urban effluent accumulate are seasonally covered by a thick mat of azolla, usually in conjunction or succession with other free-floating aquatics, such as *Lemna, Pistia, Salvinia, Spirodela, Ricciocarpus*, and *Riccia*.

#### *Taxonomy*

The genus *Azolla* was established by Lamarck in 1783 and is grouped with the genus *Salvinia* in the order Salvinales, but is separated into the monotypic family Azollaceae (Reed, 1954). Part of the reason for grouping *Azolla* with *Salvinia* is that both genera produce two distinct types of spores (heterospory), in contrast to most other ferns, which produce only one type of spore. In addition, the spores of *Azolla* and *Salvinia* are borne on special stalks (columnella) and are contained in special capsules called sporocarps.

The seven extant species of *Azolla* are shown in Figure 1. The conspectus of classification (Table 1) is consistent with recent reports (Moore, 1969; Fowler and Stennett-Willson, 1978; Lumpkin and Plucknett, 1980), except that *A. rubra* R. Brown has been retained in preference to the *A. filiculoides* var. *rubra* of Strasburger (1873) in

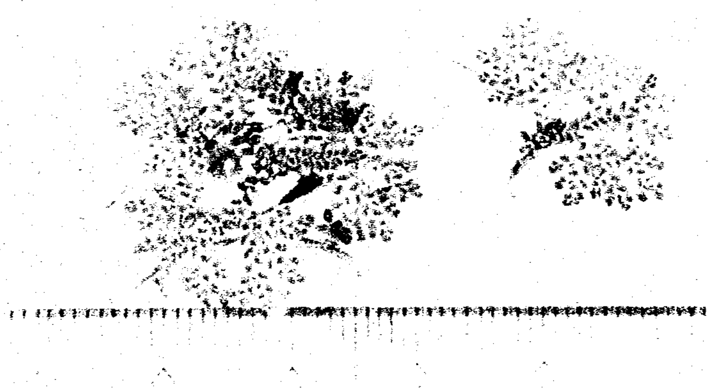
*Azolla caroliniana*

*Azolla nilotica*

*Azolla filiculoides*

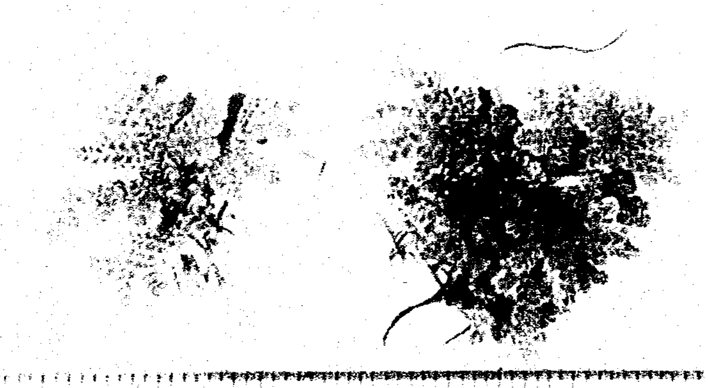
*Azolla pinnata var. pinnata*

*Azolla mexicana*



*Azolla pinnata var. imbricata*

*Azolla microphylla*



*Azolla rubra*

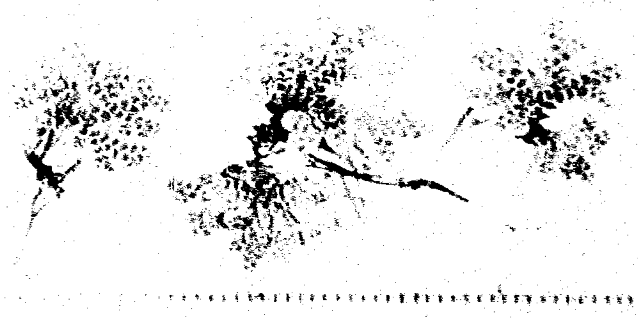


**Table 1** Conspectus of Classification

| | | |
|---|---|---|
| Division | Pteridophyta | |
| Class | Filicopsida | |
| Order | Salviniales | |
| Family | Azollaceae | |
| Genus | *Azolla* | |
| Sections | *Azolla* | *Rhizosperma* |
| Species | *A. caroliniana* | *A. nilotica* |
| | *A. filiculoides* | *A. pinnata* |
| | *A. mexicana* | |
| | *A. microphylla* | |
| | *A. rubra* | |
| Varieties | | *A. pinnata* var. *imbricata* |
| | | *A. pinnata* var. *pinnata* |

light of the reports by Fowler and Stennett-Willson (1978), and Lumpkin and Plucknett (1982), and SEM studies of megaspores (Perkins et al., 1985). Unfortunately, several of the type specimens for species in the section *Azolla* do not represent the current description for those species (K. Fowler, personal communication). Several of the type specimens may be of the same species.

*Morphology*

The diploid spore-producing generation of azolla is called the sporophyte and consists of a horizontal to vertical main rhizome. Leaves on the rhizome are bilobed with alternate pinnation. Rhizomes also bear individual roots or root bundles at branch points. The main rhizome, when mature, may range in size form 0.5 to 7 cm in diameter with individual roots 1 to 5 cm long, except *A. nilotica* (Demalsy, 1953; Lumpkin, 1981), which can produce a trailing rhizome up to 40 cm or more long with root bundles up to 15 cm or more.

The main rhizome is usually achlorophyllous and has alternating branches with several orders of lateral branches. Species that can mature into a nearly vertical morphology when crowded, such as *A. filiculoides* and *A. nilotica*, have a more highly developed vascular

**Figure 1** The seven species of the genus *Azolla*, including two varieties of *A. pinnata*. The plants as shown are nearly fully developed in size and are approximately life size, except *A. nilotica*, which should be 3.3 times larger for comparison. Note the millimeter scale below each plant for reference.

system, appearing like a amphiphloic siphonostele in mature plants. Well-developed plants of *A. nilotica* have lateral branches which often lack their own roots and are thus dependent on the root system of the main rhizome. Rhizomes lacks stomata, but occasionally have chloroplasts near the shoot tips. In section *Rhizosperma*, the rhizome has trichomes either on the ventral surface or on both surfaces, while plants in section *Azolla* lack trichomes on their rhizomes.

Roots are initiated at branch points along the rhizome and bear root hairs up to 1 cm long, which emerge from under a root cap. Root initials eventually give rise to a single-layered sheath over a two-layered cap, followed by a epidermal layer and two layers of cortical cells containing chloroplasts (Kawamatu, 1963). A rich microflora is usually found in association with the roots and have been partially described by Roger and Reynaud (1979).

The leaf consists of two lobes: a thick aerial dorsal lobe and a thin ventral lobe occasionally of a slightly larger size (Figure 2). The dorsal lobe is chlorophyllous, except in the transparent margin, and contains a colony of *Anabaena* (Figures 3 and 4) within a basal cavity connected to the atmosphere by a pore on the adaxial side. The surface of the dorsal lobe has an epidermis covered with vertical rows of single-celled stomata and trichomes of one or more cells. Dorsal lobe tissue consists of a single layer of elongate palisade parenchyma with prominent intercellular spaces, one or more layers of sparsely branched spongy mesophyll, and a single concentric vascular bundle.

The thin ventral lobe is nearly achlorophyllous with few stomata and trichomes and several chambers. A single vascular bundle consists of several tracheary and sieve elements, but lacks parenchyma cells. The ventral lobe probably provides bouyancy as a result of its convex surface touching the water. It may also function in absorption, since azolla plants are known to survive without roots.

All dorsal lobes produce a cavity for housing the *Anabaena* symbiont. As the leaf primordium differentiates at the growing point, a slight depression is formed near the base on the adaxial side of the dorsal lobe. A portion of the *Anabaena* colony generating at the shoot apex above the dorsal lobe primordia is scooped into the enlarging depression by a glove-shaped transfer hair (Calvert and Peters, 1981). This colony becomes entrapped in the cavity by a ring of meristematic epidermal cells originating around the circumference of the depression. The cells grow inward to cover the depression, leaving a pore over the center where they meet. The inside of the cavity is lined by a porous envelope which develops only if the symbiont is present (Peters et al., 1978).

*Life Cycle*

Although azolla normally reproduces vegetatively by fragmentation via an abscission layer that forms at the base of each branch, some species

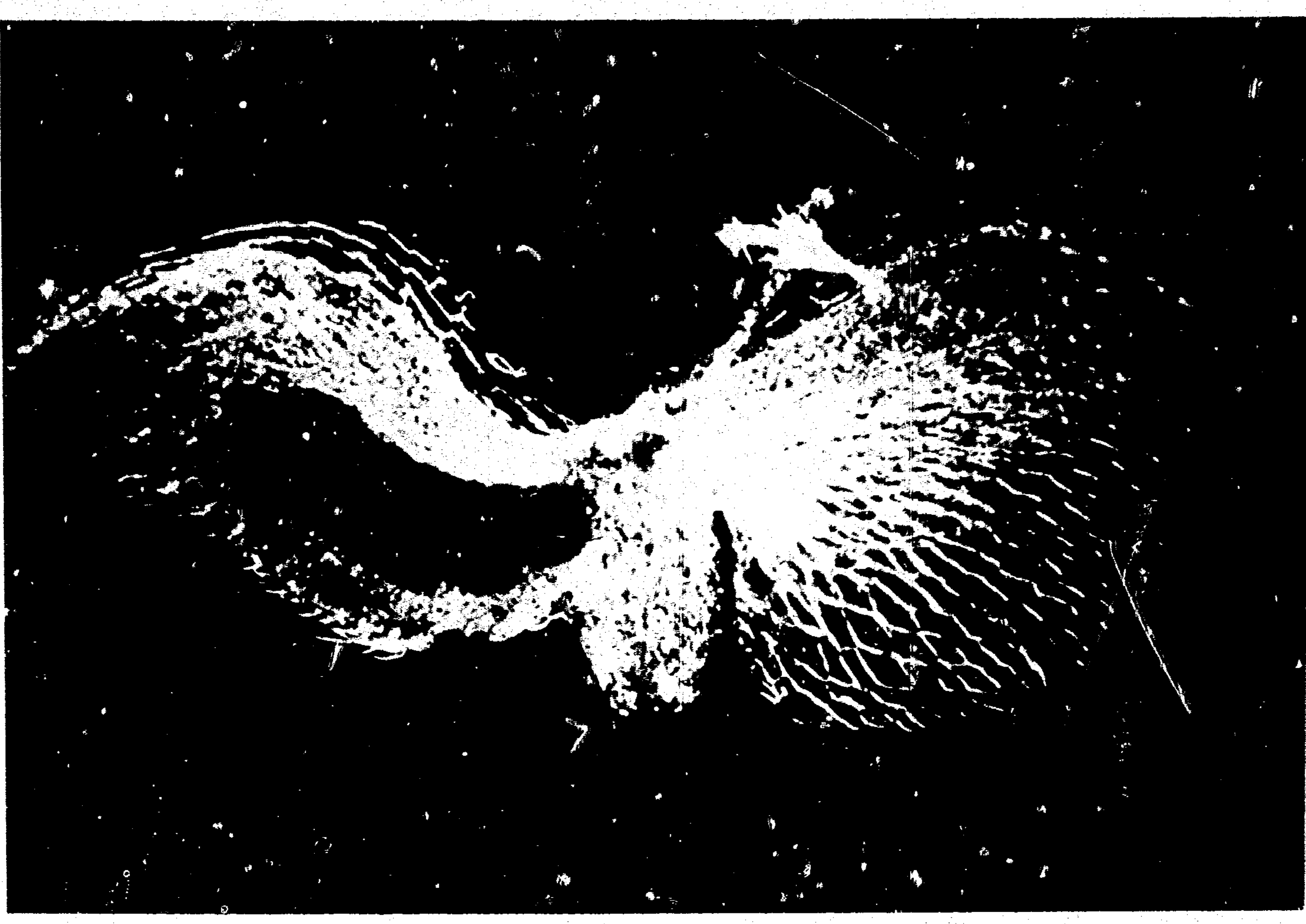


Figure 2 Complete leaf of *A. nilotica*, with the two lobes flattened for illustration. The smaller dorsal lobe contains the dark-colored cavity where the *Anabaena azollae* symbiont resides. The larger ventral lobe is normally boat shaped and often touches the surface of the water when the plants are not crowded.

occasionally express a gametophytic cycle (Lumpkin and Plucknett, 1982). The gametophytic cycle is usually absent in most situations for most species. Initiation of the cycle seems to be stimulated by a combination of environmental factors (Ashton, 1977), and is most often associated with the beginning or end of a period of environmental stress. Environmental factors also may affect the ratio of microsporocarps to megasporocarps.

Varieties of certain species (e.g., *A. filiculoides* and *A. nilotica*) become fertile only after attaining a mature morphology. Mature morphology is a precondition but not necessarily concurrent with fertility in these species. Like fertility, initiation of the mature morphology is also environmentally dependent. The failure to initiate mature morphology has a positive agronomic value because multilayered immature fronds have a higher nitrogen content and lower lignin content, and thus decompose more easily than do mature fronds.

Figure 3 SEM photo (×300) showing the upper end of the dorsal lobe cavity of an *A. pinnata* leaf. The filamentous cyanobacteria and the larger fingerlike transfer hairs are clearly visible in the cavity.

When the gametophytic cycle is initiated. sporocarps are formed in pairs (tetrads on *A. nilotica*) after a few divisions by a fertile ventral lobe initial of the first leaf of a branch (Bonnet, 1957). The dorsal lobe of the fertile leaf retains its normal shape and function. The sporangial initials develop in a leptosporangiate fashion (Pfeiffer, 1907) and eventually give rise to two megasporangia, each containing 32 megaspores initials produced by meiotic division. If a megaspore is to develop within the megasporangium, all but one of the 32 megaspores initials abort. If all of the megaspore initials abort, microsporangia initials arise from basal outgrowths on the stalk of the megasporangium. The resulting pair (or tetrad) of sporocarps may be all of one sex or any mix (Figure 5).

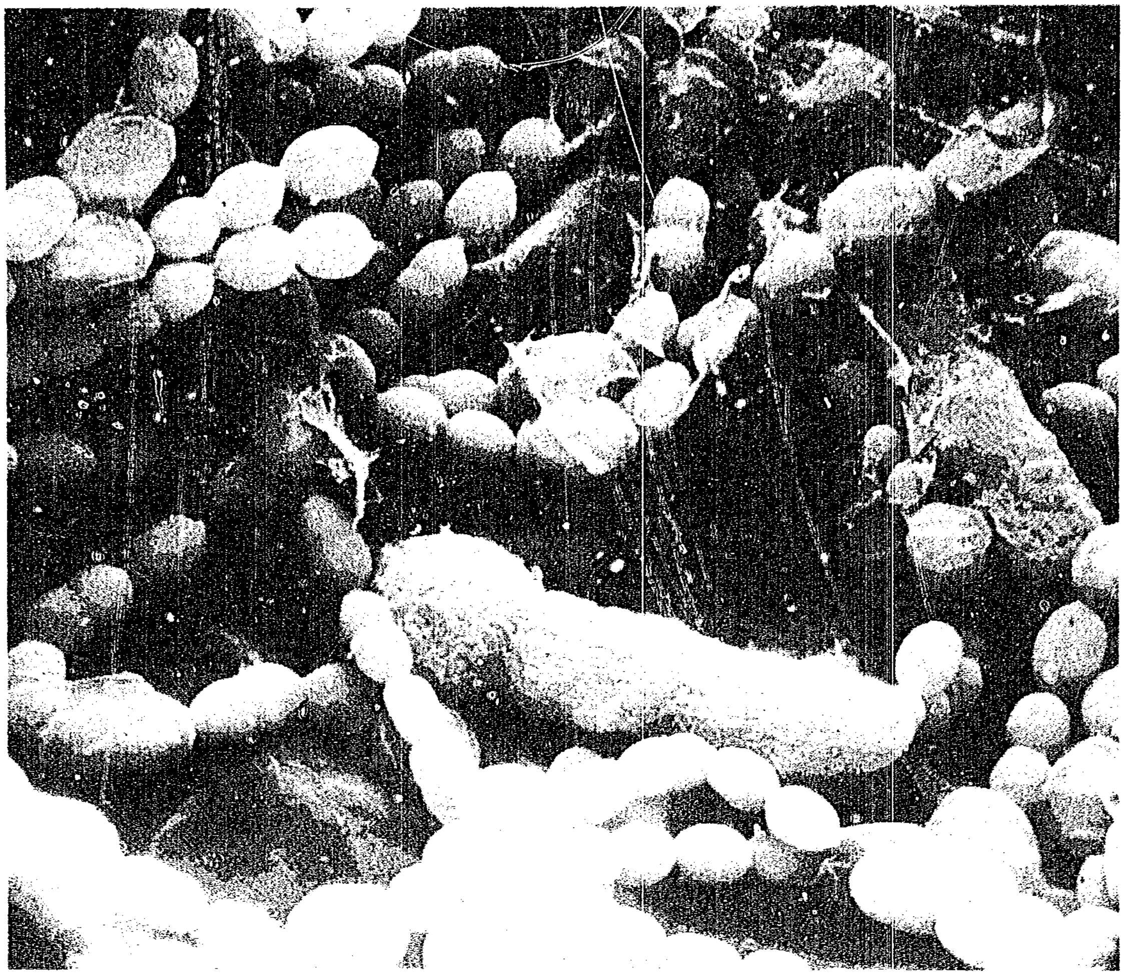


Figure 4 SEM photo (×1500) inside the leaf cavity of *A. pinnata*. *Anabaena azollae* filaments and two azolla transfer hairs are visible. The *Anabaena* is composed of numerous small vegetative cells, some in the process of division, and larger heterocyst cells, the site of nitrogen fixation.

Sporocarps mature on the plant in a week or more depending on enviornmental conditions. Megasporocarps are 0.4 to 0.6 mm in diameter and give the appearance of a dark cone (indusium) resting atop a sphere. The indusium overlies the alagal colony and the characteristic floats: three floats in section *Azolla* and nine floats in section *Rhizosperma*. After maturation, the megasporocarp becomes dehiscent and the megasporangial wall disintegrates, exposing the sporoderm (perispore), which is usually covered with rubberlike or resinlike hairs (filosum) (Brederoo et al., 1976). These hairs seem to function as entanglements for appendages (glochidia) attached to packets (massulae) of microspores, except for *A. nilotica*, which does not produce glochidia.

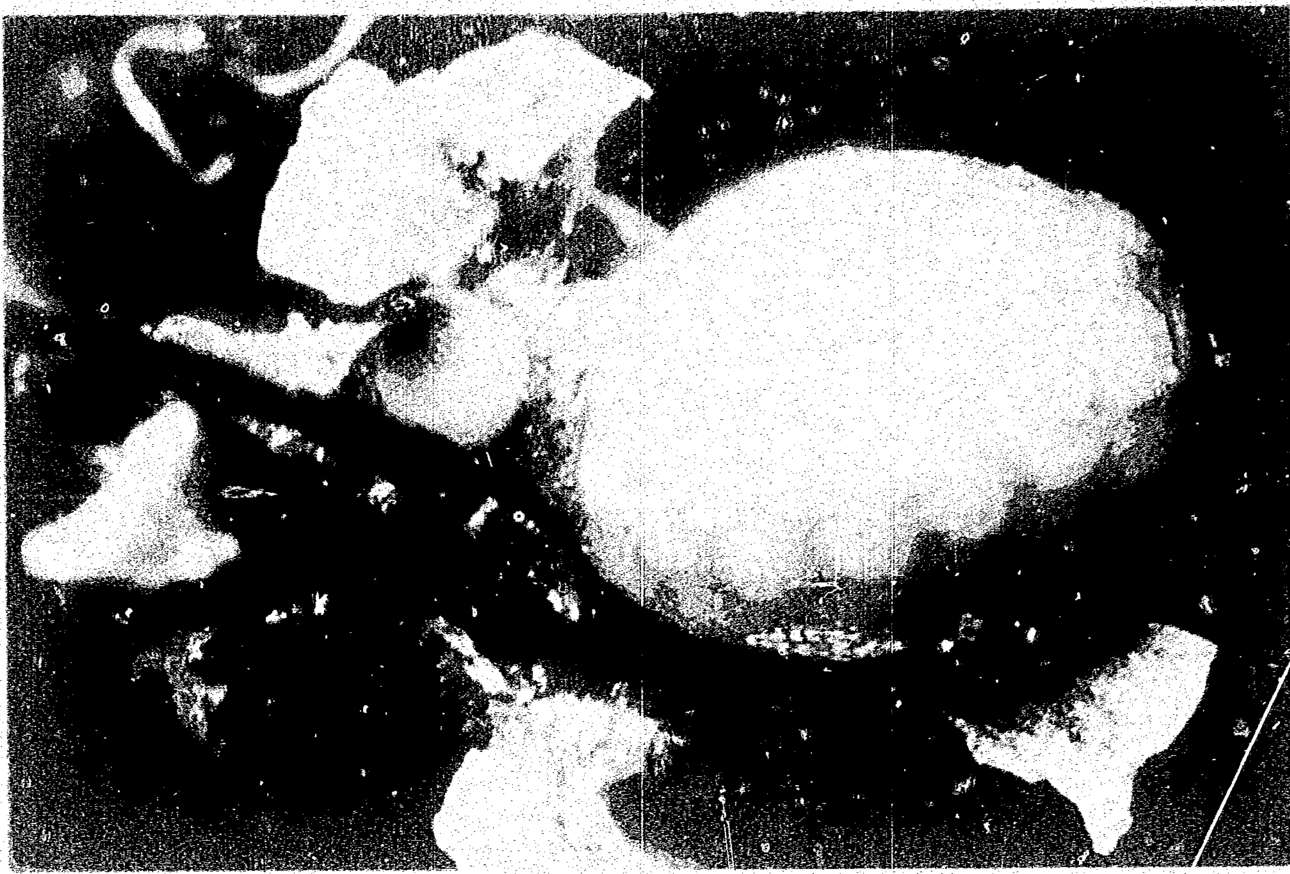


Figure 5. Pair of sporocarps (× 10) on a rhizome of *Azolla filiculoides*. [illegible]

[illegible]

zoids which escape through the gelatinous massula to fertilize an archegonium produced by the megaspore gametophyte. The tipping of the indusium is the first visible sign of fertilization and is caused by the emerging alga-free cotyledon (Figure 6). This can occur within 5 days or more after initial contact of mature gametophytes, depending on the water temperature. A portion of the generative *Anabaena* colony surviving under the indusium becomes entrapped in the shoot apex and succeeding true leaves. As the root appears and the first or second true leaf emerges through the cotyledon, the seedling floats to the surface, beginning sporophytic reproduction (Cheng et al., 1980; He and Lin, 1981; He et al., 1982; Lumpkin, 1985).

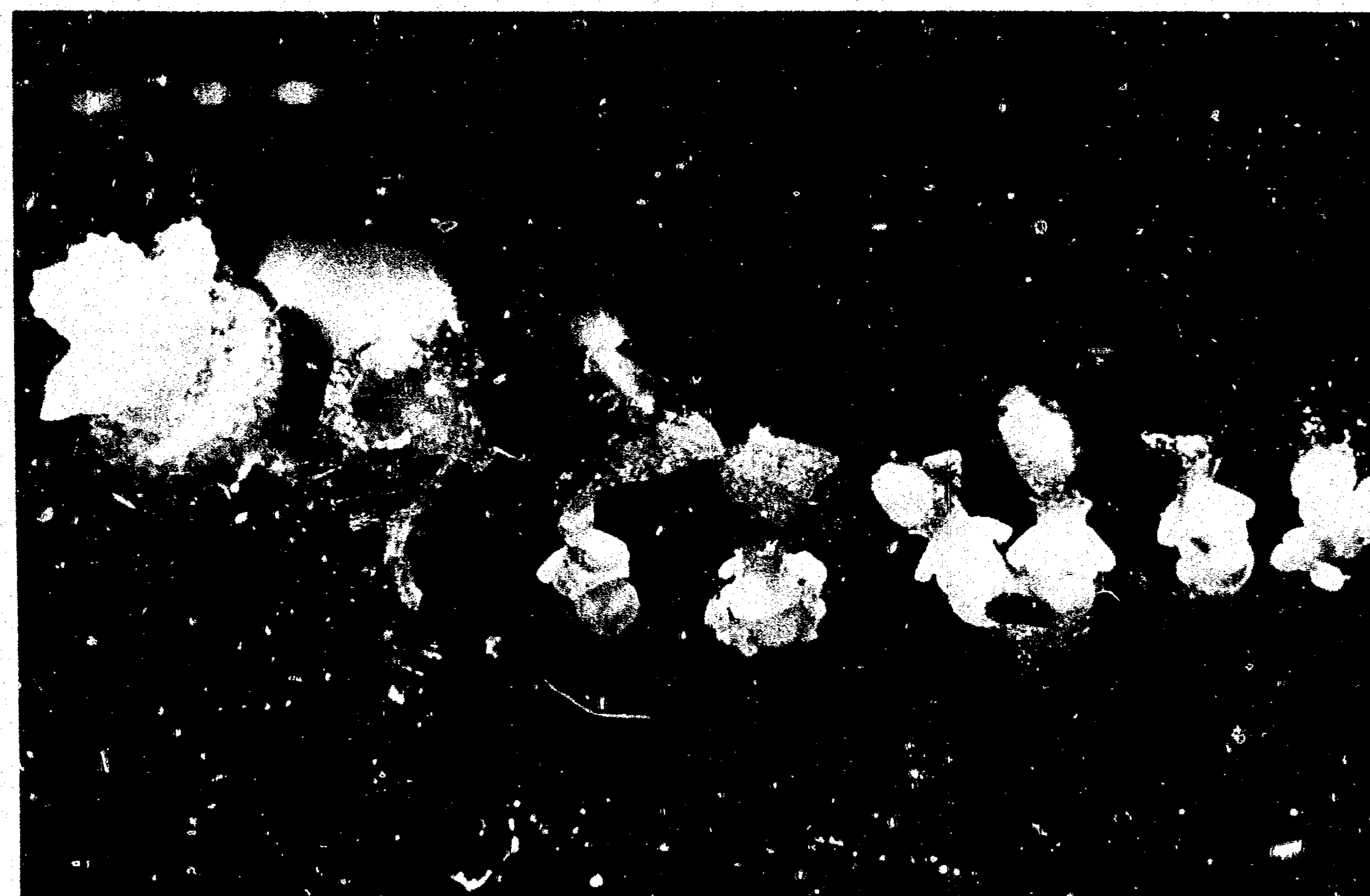


Figure 6 From right to left, stages in the development of azolla sporelings. Massulae containing microspores are attached to the base of the megaspore on the right. The first visible sign of fertilization and germination is the tipping of the indusium caused by emergence of the cotyledon through the columella, second from right. Later, to the left, the growing point emerges through the cotyledon and he root and root hairs develop.

## B. Distribution

The distribution of *Azolla* species has been reviewed by Svenson (1944), Sculthorpe (1967), Moore (1969), Ott and Petrick-Ott (1973), and Lumpkin and Plucknett (1980). Sweet and Hills (1971) discussed the distribution of *A. pinnata* var. *pinnata* and *A. pinnata* var. *imbricata*.

The native distribution of *Azolla* species has been confirmed by the author through collection or by observation of herbarium specimens (Figure 7). The species *A. caroliniana* is distributed in the eastern half of the United States, through Central American, and in South America east of the Andes, but is often confused with immature fronds of *A. filiculoides*. *A. filiculoides* is found in the Rocky Mountain and Pacific coast states of the western United States and Canada, through Central America and most of South America, and may also be native to Japan. *A. mexicana* is found from the west coast of the United States east to the Mississippi River and into Mexico and Central America. Its occurrence has been reported but not confirmed in the northern half of South America. Very few confirmed reports of *A. microphylla* have been made due to problems with identification. Only the population found in the Galapagos Islands (Morton and Wiggins, 1971) has been confirmed, but accessions collected in Paraguay have similar morphology. *A. nilotica* occurs only in Africa as far south as Mozambique, north through the upper reaches of the Nile River drainage basin to Kosti in the Sudan, and from Kenya south along the east coast and west through the drainage basin of the Congo River to the southern west coast. *A. pinnata* is found in East and South Asia through equatorial Asia to northern Australia, and equatorial and southern Africa including Madagascar. *A. rubra* (*A. japonica*) is found only in higher latitudes of the western Pacific, exclusively in Japan, Korea, Australia, and New Zealand.

*Azolla* species have been dispersed throughout the world by a variety of mechanisms, of which man has become the most important. *A. filiculoides* and *A. caroliniana* were introduced into western Europe in the nineteenth century as ornamentals and have since spread eastward as far as the eastern USSR. *A. filiculoides* has also been introduced into South Africa (Ashton and Walmsley, 1984) and China, and *A. pinnata* into New Zealand. Accessions of all species have recently been introduced to research stations on all continents and are undergoing field evaluations which may result in establishment of adapted species.

## C. Preparation and Shipment of Germplasm

Traditional methods and safeguards for preparation and shipment of seeds, plants, or cutting are not directly applicable to azolla since it is an aquatic plant and rarely produces spores. The only requirement for the import of azolla into the United States under a U.S. Department

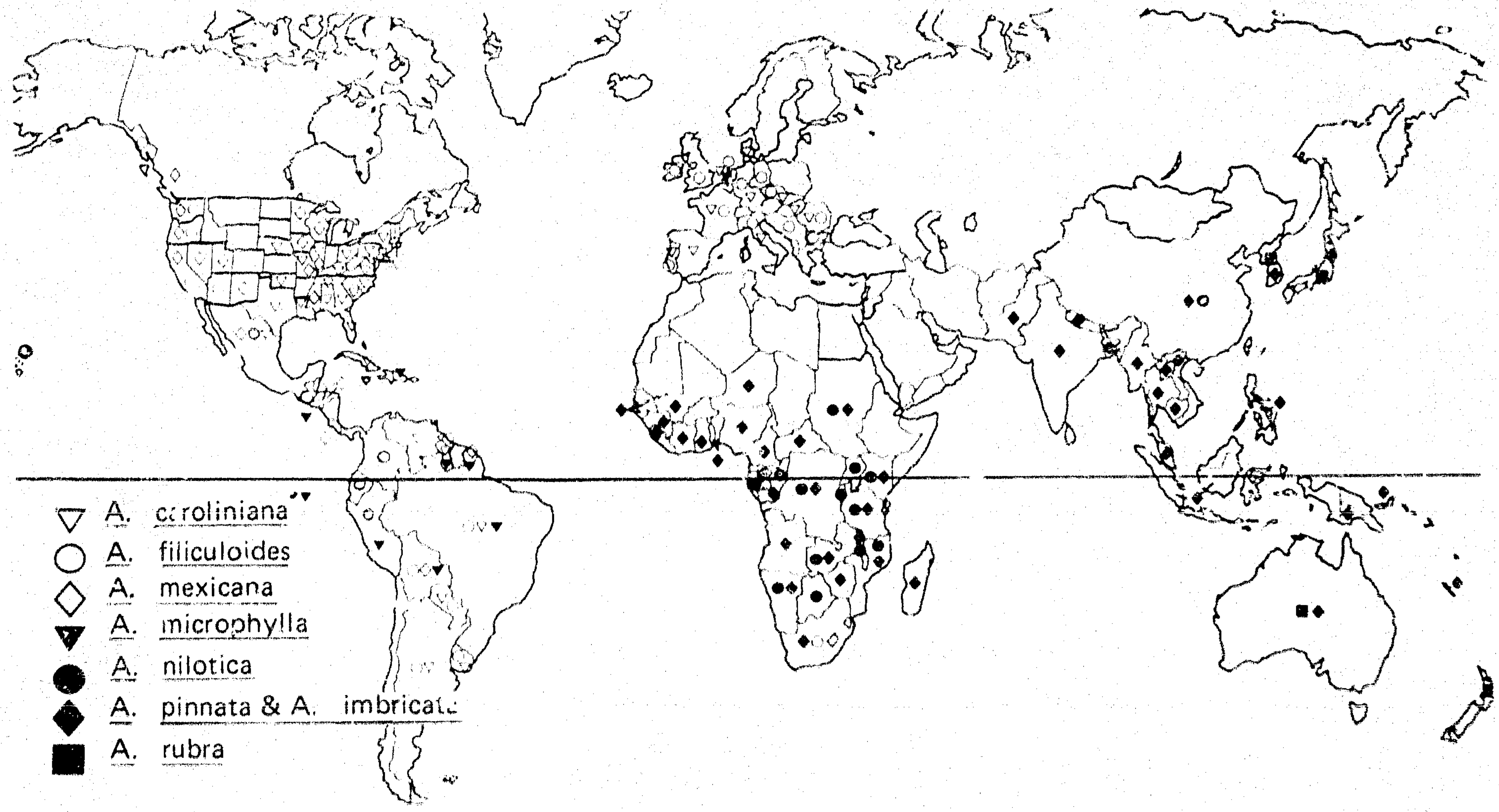



**Figure 7** Global distribution of *Azolla* species. Identification and distribution of species in Central and South America is somewhat tentative since they are largely based on nonfertile herbarium specimens. All species in Europe are introduced.

of Agriculture (USDA) permit is that the plants be free of insects and soil. Methods and safeguards discussed by azolla researchers for shipment are fairly primitive, since the intensive study of azolla in the West is a fairly recent phenomenon.

In the transport of azolla, there is always the possibility that other organisms will be brought along as uninvited guests. Certainly many organisms have been carried along with samples of azolla which have been transported and introduced to new areas of the world, especially rice-growing areas. However, because of a lack of investigation, there has never been a report of any damage being done by the introduction of organisms carried with azolla, but the likelihood and possibility of damage are real, especially for pests of rice.

Ideally, only aseptic specimens of azolla should be transported between different environments, but this is probably unrealistic considering the difficulty of producing aseptic plants and the lax nature of most plant inspection and quarantine measures. Responsible preparation before shipment of azolla should include the application of insecticides and fungicides at both the point of departure and arrival, and culture in isolation at the point of arrival. Insecticides such as carbofuran and lindane, and fungicides such as pentachloronitrobenzene (PCNB) and benomyl have been used (Lumpkin and Plucknett, 1982).

In addition to weeds, weed seeds, insects, nematodes, and microbes, the transport of snails and leeches with azolla germplasm is a real concern. Some of these organisms may be destructive or may host dangerous pathogens such as schistosomes. The culture of azolla on nutrient solution in quarantine is an important step that should be taken whenever azolla is collected outside the laboratory, to reduce the possibility of an unwanted introduction. Observation, pesticide application, and re-isolation of individual plants during a quarantine period can help to prevent the introduction of any unwanted organisms.

Small quantities of azolla can be prepared for shipment in various ways, but all methods must take into consideration the environments to which the azolla will be exposed during shipment. Regardless of the duration of shipment, azolla must not be allowed to desiccate and must not be exposed to temperatures below 0°C or above 35°C. Azolla should not be transported in water since turbulence resulting from transport will cause excessive fragmentation and often death.

Some of the simplest ways to package and maintain small quantities of azolla for shipment include the following:

1. *Bulked azolla.* Azolla can be placed directly in a plastic bag or other container without water or other amendments. This method seems to be the most effective. For periods of transport longer than 1 day, an ice chest or portable refrigerator should be used to maintain a temperature of 5 to 10°C. Under

this cool condition, it may be possible to keep the plants alive up to a month or more, but with reduced viability over time.

2. *Azolla in gel.* Surface-sterilized azolla can be placed on a liquid medium which is then stabilized with a gelling agent. This method was tried for preservation of azolla germplasm (Bai et al., 1981) but has proved troublesome. However, transport on gel can be effective, especially when ice or refrigeration is not available.
3. *Spores.* If fertile azolla is found, whole plants bearing sporocarps can be air dried and the dried material can be shipped. After arrival, the dried material can be placed in pure water in indirect sunlight to induce germination of the spores, fertilization, and development of seedlings. A few days after the dried material is placed in the water, the container should be lightly agitated to cause the male and female spores to make contact. Within 2 to 3 weeks small seedlings should be present and can be moved to nutrient solution.

## III. MAINTENANCE

At present the sexual cycle is insufficiently understood to make possible the use of spores as planting material or for germplasm maintenance. Since only vegetative material can be used for these purposes, a certain amount of azolla must be cultured throughout the year. This requirement significantly increases the cost of using azolla as a green manure, and the cost of maintaining a germplasm collection.

### A. Germplasm Collections

Germplasm collections with accessions representing all known species are currently maintained at the International Rice Research Institute in the Philippines and with their collaborators in England, at Washington State University, the University of California at Davis, the Catholic University of Louvain-la-Neuve in Belgium, and at the Azolla Research Center in Fuzhou (Figure 8) and other places in China.

All of these institutions use culture on nitrient solution as their primary method for germplasm maintenance. Some institutes maintain surface-sterilized azolla on nutrient solution under aseptic conditions in flasks. Nickell (1961) produced *Anabaena*-free azolla by using antibiotics and maintained them under aseptic conditions on agar, but this method is currently unacceptable for germplasm collections since the *Anabaena* are an essential part of the collections. As mentioned above, Bai et al. (1981) tested this method for preservation of surface-

Figure 8 Azolla germplasm collection at the National Azolla Research Institute, Fuzhou, China. Accessions are maintained on nutrient solution culture as shown, and aseptically on nutrient solution in ventilated flasks.

sterilized germplasm, and although it was successful for short- and medium-term preservation, it proved more troublesome than culture on nutrient solution for long-term preservation.

Van Hove (personal communication) at the Catholic University of Louvain-la-Neuve in Belgium also uses nutrient solution maintenance, but has developed a partially automated continuous-circulation system for controlling solution level and pH, filtering recycled solution, and reducing biomass. Other systems have been suggested but have never been studied, including cryopreservation and culture under low light/low temperature.

## B. Nurseries for Cropping Systems

Perhaps the greatest limitation to the wider use of azolla as a green manure for rice is the need to maintain a certain quantity of azolla in nurseries during the off-seasons. This maintenance requirement might be avoided if azolla could be propagated from spores (Figure 9). Azolla is capable of producing spores, but no methods are known for inducing spore formation, large-scale harvesting, or for utilizing them

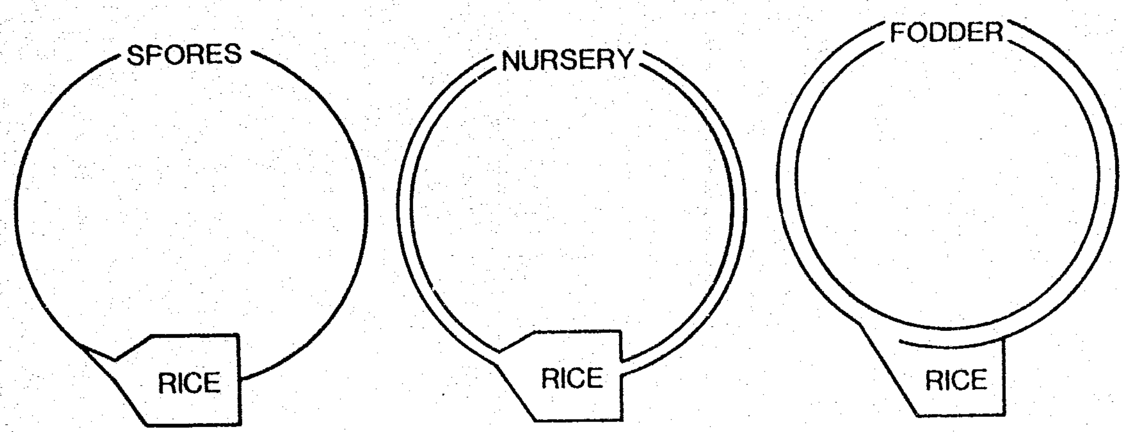



**Figure 9** Three basic systems for maintaining azolla planting material during the off-season. The use of spores is currently not practical, but the other two systems can be combined in application. If the use of spores becomes practical, 2 to 4 weeks must be allowed for germination and the development of full-sized plants.

in cropping systems, although Chinese scientists have been studying these problems (Ke et al., 1981; Wang et al., 1981; Lumpkin, 1985). Methods for inducing spore formation must be developed because few of the accessions that are promising for cultivation in the tropics produce spores or produce them at the time when needed.

To maintain azolla during the off-season, special nurseries must usually be constructed. In China the construction of nurseries is integrated into a year-round process which divides the year into several phases designated as follows: overwintering, spring multiplication, mass cultivation in large fields, oversummering, and autumn multiplication. The Azolla must be protected from death or injury caused by extremes of cold or heat during overwintering and oversummering, and must also be multiplied in sufficient quantities in time for wide-scale monocropping in the field or for direct inoculation as an intercrop with the rice. Careful planning and management are required to satisfy these objectives. Maintenance nurseries must meet the specific needs of particular environments in regard to azolla, climate, available construction materials, and farming systems.

### *Overwintering Nurseries*

At high latitudes or elevation it is often necessary to protect Azolla from cold or frost damage to ensure a sufficient supply for multiplication in the spring. In general, cold-tolerant varieties of azolla die when temperatures drop below -5°C for more than a few hours. Cold-tolerant varieties generally belong to the species *A. caroliniana*, *A. filiculoides*, *A. pinnata*, and *A. rubra*.

Where winters are severe, hot spring water or warm water from factories is useful for the overwintering of azolla (Ye and Wu, 1964).

Where warm water is not available, azolla can be grown in glass or plastic houses. Where winters are mild, azolla can be overwintered in the field or in small field structures. In areas where water does not freeze, ponds, lakes, canals, and large flooded rice fields are sometimes used for overwintering since the temperature in deep bodies of water fluctuates less than shallow water (Yuan, 1964).

The quantity of azolla kept for overwintering varies according to the area for cultivation, the season and duration of azolla field cultivation, the cropping system, and the particular method for overwintering. One hectare of spring-transplanted rice usually requires the overwintering of 400 to 600 kg of azolla, since little time is available for nursery multiplication. A crop of summer-transplanted rice requires only 7.5 to 15 kg of azolla for overwintering since the azolla can be multiplied during the spring for a longer period before large-scale field cultivation.

In most situations where overwintering is necessary some type of protective structure must be constructed. Azolla is moved into the protective structure when night temperatures begin to drop below 10°C. In these structures, azolla is maintained at a density of 0.75 to 1.0 kg/m$^2$ and water in the beds is kept at 6 to 7 cm deep. The beds are fertilized with superphosphate, ammonium sulfate, and straw ash.

In China, arch-shaped plastic greenhouses, lean-to plastic- or glass-roofed greenhouses, or lean-to plaster-roofed greenhouses were often used during winter. In some cold regions, the azolla is taken out of the water and stored under a thick mat of rice straw (Anwei Academy, 1977). Azolla was stored in a loosely piled stack 50 cm high. The pile was covered with 5 to 10 cm of straw ash and then routinely sprinkled with water to keep the azolla from drying out. A removable frame and thick insulating mat of straw were placed over the stack. During the first 10 days of storage the temperature inside the azolla stack was closely monitored to ensure that composting was not taking place. The temperature was maintained at around 5 to 8°C or lower, but not less than 0°C. Azolla was usually stored in these stacks during the coldest 50 to 60 days of winter. After this period 50 to 80% of the azolla was still alive. In late winter or early spring the azolla was moved into wet nursery beds for propagation. This was done before warm weather could cause the stored azolla to rot. Pits (Li, 1977) and plastic bags (Wu, 1978) were also used for out-of-winter storage.

*Oversummering Nurseries*

Oversummering techniques are used in locations where field temperatures are too high for azolla cultivation, or where azolla must be preserved through a hot season for later use (Lu et al., 1965; Feng, 1977). For example, when the azolla is cultivated as a green manure for the autumn rice crop, it is maintained in oversummering sites until it can be cultivated in the field.

Characteristics of a good oversummering site include good ventilation, direct sunlight, and cool flowing water. Ponds, canals, and the edges of placid rivers with low fertility and few fish can be used as maintenance sites. Floating and submerged weeds and algae must be removed. The water surface must be subdivided into small areas for management of azolla and to prevent winds from blowing azolla away or into a pile along the bank. Living fences can be used to subdivide the azolla and are prepared by tying large floating aquatic plants such as *Alternanthera*, *Eichhornia*, of *Pistia* together with a plastic rope. These floating fences are anchored by stone weights to the bottom or tied to stakes on the shore. Extra-long anchor ropes are used so that the floating fences can adjust to changes in the water level.

If azolla is oversummered in paddies, the water should be drained or cold water should be added when the paddy water temperature begins to exceed 35°C. The occurrence of insects must be closely monitored during summer because of their rapid reproductive rates at high temperatures. Azolla can also be oversummered between shading bands of rice or in the shade of tall herbaceous plants such as *Sesbania* that are planted along the edges of rivers, ditches, and ponds. *Alternanthera philoxeroides*, *Eichhornia crassipes*, and *Pistia stratiotes* are sometimes intercropped with azolla to provide shade.

## IV. CULTIVATION

The cultivation of azolla is generally thought of as the production of biomass for use as a green manure, and in this section we discuss only the production of biomass. However, in essence, the purpose for cultivating azolla is to fix atmospheric nitrogen and make it available to rice and or other crops. Farmers usually feel that they are accomplishing this purpose by producing biomass, but the quantity of biomass does not indicate how much nitrogen has been fixed and how much of the azolla nitrogen has become available to the rice crop. In the cultivation of azolla, total nitrogen accumulation, portion derived from fixation, and decomposition rate must be considered in analyzing the value of biomass. If the azolla scavenges most of its nitrogen from its aquatic environment and decomposes very slowly, it may be of little value to a farmer. Nitrogen fixation by azolla and its decomposition in soil can best be studied through the use of $^{15}N$ (IAEA, 1985).

The decomposition rate of azolla in soil and the mineralization of its nitrogen have been studied somewhat in China. The decomposition of species such as *A. filiculoides* and *A. nilotica* which have more lignin in their mature stages is much slower than with species that can develop only overlapping horizontal layers, such as *A. caroliniana*, *A. mexicana*, and *A. pinnata*. Shi et al. (1980) labeled a species in the former group (*A. filiculoides*) with $^{15}N$ and used it to fertilize rice. After the incorporation of azolla, the first rice crop recovered only about 15 to 20% of the azolla nitrogen, and a second rice crop recovered only about 1 to 5% of the initial application.

## A. Nursery and Field Multiplication

The goal of multiplication is to produce azolla as early and as fast as possible, so that the largest possible area of paddy fields can be inoculated with sufficient azolla. To meet this goal, intensive management and close coordination are essential.

In south China, spring-type nursery multiplication takes place during the winter, and from mid-March or mid-April to mid-June along the Yangtze River (Figure 10). When the average temperature rises to 14 to 20°C, azolla can double every 3 to 5 days. The azolla mat is continually subdivided to prevent crowding and consequent slowing of its growth rate. Whole nurseries are subdivided to provide inoculum for adjoining fields. Any extra azolla is used as a fodder or is composted for upland crops. Every available area is used for the extension of the nursery, and can include canals, ditches, ponds, and other bodies of water.

A few weeks before azolla inoculum is needed on a large scale to plant in rice production fields, it is often necessary to use large fields as multiplication nurseries. Rice fields must be used for this last stage of multiplication and are usually chosen because of close proximity to assured water supplies and suitability for growing azolla. As with maintenance nurseries, multiplication nurseries and fields must be partitioned to prevent wind and wave damage. Floating barriers, temporary low bunds, or rows of rice stubble can be used.

In an article on multiplication methods, the Subei Agricultural College (1971) has generalized the approaches taken for the multiplication of azolla: "first smaller land area, then larger land area; first shallow water, then deeper water; first nutrient-rich water, then nutrient poor water; first still water, then running water; first in water without fish, later in water with fish; and first nitrogen plus phosphorus fertilizer, later phosphorus only."

## B. Field Cultivation and Incorporation

There are three different systems used for the cultivation of azolla as a green manure for rice: azolla is grown (a) as a monocrop and in-

Figure 10 Field-level multiplication nursery in Guangdong Province, China. The rice field is subdivided with temporary bunds to ease application of inputs and harvest of azolla and to reduce piling by the wind. The photo was taken from the edge of a canal, through which excess azolla was transported by barge to other fields.

corporated into the paddy mud as a basal green manure before the rice is transplanted; (b) as an intercrop with rice (Figure 11) and either incorporated as a topdressing manure or allowed to die naturally without incorporation; or (c) as both a monocrop and an intercrop.

The azolla cropping system used in any particular location may vary somewhat each year depending on the weather, the rice production system being used, tradition, and other crops in the cropping system. When azolla is monocropped in China and Vietnam, it is usually grown in the fields for 20 to 30 days before the rice and incorporated into the paddy mud once or twice during that period by plowing or harrowing. As an intercrop it grows beneath the rice in the inter-row areas until shaded out by the developing rice canopy; usually

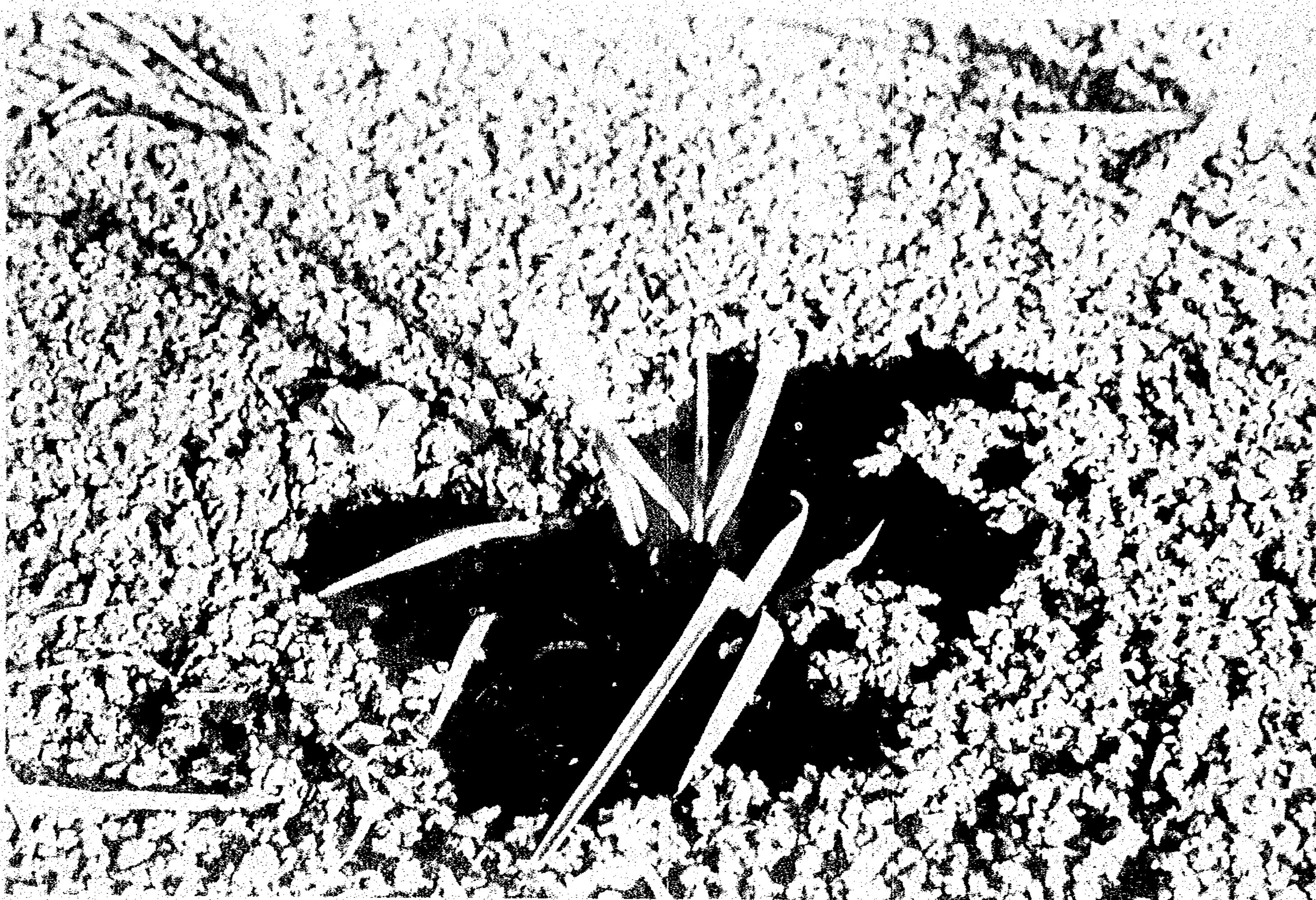


Figure 11 Azolla growing as an intercrop with rice at a research station in Thailand. Azolla was removed from around the central hill of rice seedlings. Azolla at this biomass density should be partially incorporated into the soil to reduce its inhibition of rice tiller emergence.

within 20 to 40 days. After transplanting the rice, the azolla intercrop is partially incorporated once or twice during hand weeding operations, or, in a few locations, with a modified rice weeder. The last azolla mat that develops before the rice canopy completely closes is either allowed to die naturally due to fungal rot and light starvation, or is incorporated by hand before the maximum tillering stage of the rice.

*Environmental Factors Affecting Cultivation*

Environment is the primary influence on the use and productivity of azolla in rice production. For example, when irrigation water is available, relative growth rate, total biomass accumulation, and nitrogen concentration are higher in cool dry environments. This higher productivity of azolla usually promotes a greater increase in rice yields when compared to the wet monsoon season. Rice is usually more responsive to any fertilizer during dry weather, but this response is increased by higher azolla productivity. The reliability of azolla productivity during dry weather is also higher because of fewer insect and disease problems. Improved dry season rice yields resulting from azolla use were 5 to 69% greater than comparative zero nitrogen control plots in reports from Brazil, India, Pakistan, and Thailand (Mandal and Bharati, 1983; Barthakur and Talukdar, 1983; Kannaiyan et al., 1983; Subudhi and Singh, 1983; Haq and Rosh, 1984; Fiore, 1984). Wet-season rice yields have nearly the same range of yield improvement (-1 to 56%), but tend to have a lower average in reports from Burma, India, Sri Lanka, and Thailand (Swatdee and Seetanum, 1979; Kulasooriya et al., 1980; Kaushik and Venkataraman, 1981; Partohardjono et al., 1983; Subudhi and Singh, 1983; Win, 1984). Individual environmental factors have been studied for their influence on azolla and include water, temperature, humidity, light, and plant nutrition.

Water: The primary constraint to the use of azolla is its requirement for an aquatic habitat. An individual plant can survive for only a few moments on a dry surface under a tropical sun. It can survive for only a few days or a week on paddy soil that dries during intermissions between rains. Some varieties can survive indefinitely on moist shaded mud, but these will not multiply to any useful extent.

In places where rice is grown in paddies without water control (i.e., where rainfed bunded rice is grown) azolla would probably be an unreliable option as a green manure since a continuous supply of water from rain cannot be assured. With water control, azolla can usually be grown in nurseries and fields whenever desired during the cropping season, and the timeliness of inoculum needed for production fields can be assured.

The fact that azolla is produced vegetatively from whole plants compounds the problem with water. Some azolla must be maintained all year round and multiplied in nurseries prior to large-scale field cultivation. With the present system, a certain amount of water must be available all year for maintenance and multiplication. Water availability for these nurseries is especially critical if azolla is to be cultivated for rice at the start of a monsoon season. For cultivation as an intercrop, an area of azolla equal to 20% of the rice area has to be produced before the rice is transplanted.

Water control is also necessary for avoiding injury due to high or low temperatures. If air temperatures are too low or too high, water can be made deeper to provide a larger mass that will moderate changes in water temperature. If water temperatures are too high, cooler water can be introduced into the field to provide cooling, or water levels can be dropped to allow the azolla to come into contact with the relatively cooler soil. When temperatures are moderate, a fairly shallow depth of water, 2.5 to 5 cm, is most suitable for azolla growth.

Once an azolla crop is grown as a green manure, it must be incorporated into the soil in order to recover a significant portion of its nitrogen. Since the rhizome and leaves of azolla are hydrophobic, water must be completely drained from the field or azolla will be washed aside in the effort to incorporate. Farmers are reluctant to drain rainfed and even irrigated rice fields in order to incorporate an azolla crop. Incorporation of an azolla monocrop is possible with existing implements, but water is not usually available for monoculture. Incorporation of an intercrop is presently impractical because no efficient methods or implements are known.

Temperature and humidity: Although the need for water control greatly reduces the area under which azolla can be cultivated, the indirect effects of a humid tropical climate further reduce the possibility of successful cultivation. Many researchers suggest that high water or air temperatures are the most important factor preventing azolla use. Growth rates are depressed at temperatures above 35°C and even the most heat-tolerant varieties are killed at prolonged temperatures above 45°C. The temperature of shallow water standing over dark soil may exceed 40°C in hot weather. However, it is also possible to avoid high temperatures by draining, shading, or flushing. Thus high temperature will not necessarily prevent cultivation, although it certainly makes it more difficult.

*Azolla* species and varieties have different tolerances to temperature extremes. Some can survive a very wide temperature range (i.e., -5 to 35°C), whereas others can survive only within a very narrow temperature range. *A. filiculoides* and *A. rubra* (*japonica*) are quite tolerant of low temperatures, while *A. mexicana, A. microphylla, A. caroliniana, A. nilotica*, and certain varieties of *A. pinnata* possess greater tolerance to higher summer temperatures (Peters et al., 1980; Watanabe and Berja, 1983; Lumpkin and Bartholomew, 1986).

The worst threat from the climate comes from the stimulating effects of high temperature and humidity on the population dynamics of insects and fungi that attack azolla (Figures 12 and 13). Insects and fungi can be controlled by a comprehensive program of azolla management and frequent pesticide applications, but the environmental and economic consequences of frequent pesticide applications would make azolla cultivation unrealistic by any method of cost accounting.

Light: Most azolla species do not need full sunlight for optimum growth (Talley and Rains, 1980), except that full sunlight is helpful at high latitudes during the somewhat lower light intensities of the spring, autumn, and winter. For oversummering nurseries 25 to 35% of full sunlight is sufficient.

Figure 12 Larva of *Nymphula* spp. build cocoons of azolla, from which they move through a mat of azolla feeding on the buds. This individual abandoned its cocoon after being placed on a leaf of *Marsilea*. Besides species in the order Lepidoptera, azolla is known to be attacked by species in the Chironomidae, Coleoptera, Orthoptera, Homoptera, and Arrenurae.

Azolla grown in combination with rice will suffer and may die from insufficient light during the tillering stage of the rice plants. *A. caroliniana* seems to be somewhat tolerant of lower light intensities and fungal attack, and a small amount may survive under a full stand of rice through crop maturation. This may be an important characteristic for its use in dual rice/azolla production systems, or when it is desired to grow azolla after a single crop of summer rice.

Plant nutrition: Plant nutrition is very important for successful cultivation of azolla (Becking, 1979; Chen and Li, 1980; Yatazawa et al., 1980; Tang et al., 1984). Since azolla is an aquatic plant, it must obtain most required nutrients from the water. Water that flows from residential areas or from fertilizer-enriched agricultural lands will often contain sufficient nutrients to ensure good azolla growth. However, most sources of water do not provide sufficient nutrients or nutrients in the right combination to meet the nutritional needs of azolla.

One kilogram of phosphorus applied to azolla can result in the fixation of about 5 to 10 kg of nitrogen by azolla. However, if 100% of the applied phosphorus could be absorbed by the azolla and the phosphorus content in the azolla was kept at a minimum for maximum nitrogen (about 0.25% P for 4% N on a dry weight basis), 1 kg of phosphorus would be sufficient for 16 kg of nitrogen (1:16 ratio). For some countries that have local phosphate deposits, supplying phosphorus to azolla to obtain nitrogen for the rice crop may be an economical proposition. For example, Vietnam is deficient in petroleum products for the production of nitrogen fertilizer, but has sufficient phosphate deposits to fuel its miniature "azolla nitrogen factories."

Azolla should be fertilized with a soluble phosphate source such as phosphoric acid or triple superphosphate. The dripping of a phosphorus solution into irrigation water or the use of small rafts of superphosphate would minimize losses of phosphorus through fixation by soil. Also, a 1 to 2% solution can be sprayed on azolla in areas where fixation and leaching are severe. The most phosphorus-efficient way to utilize phosphorus fertilizers is to multiply azolla in nurseries ponds that are lined with plastic. Through luxury consumption, azolla can be loaded with over five times its minimum phosphorus requirement. This stored phosphorus can be utilized by the plants for growth during cultivation in the paddy fields.

In certain depleted soils, azolla responds to the application of other nutrients, such as potassium and lime. In some deficient soils, the addition of a small amount of molybdenum and/or iron (Rains and Talley, 1979) has proved useful in increasing the rate of nitrogen fixation of azolla.

### *Management Factors Affecting Azolla*

A number of special management steps are required for the successful cultivation of azolla for rice. Some of these are related to the requirements of the azolla. Others are related more to the needs of the rice crop.

Inoculation: To begin cultivation, the fields must be inoculated with azolla. Inoculation usually consists of broadcasting azolla plants from the nursery onto the water surface of a well-prepared field. The planting rate can vary according to the time of year, the amount of field space available, the time available until the planting of the rice crop, and the soil and water fertility conditions. For example, during cool weather in areas with a long fallow season, fields can be inoculated with a low level of azolla (>25 g/m$^2$) when there is sufficient time for development of a thick mat of azolla before the rice crop is planted. However, when azolla needs to be fitted into a very short fallow period between crops, much higher levels of inoculum (300 to 800 g/m$^2$) will be required to produce sufficient green manure.

To be used as an inoculum, azolla can either be removed from the nurseries (Figure 14) and carried to the field by hand, animal, or machine, or with proper planning, can be moved from the nursery through the irrigation system by laminar flow to the field, without being removed from the water. Researchers at the University of California at Davis, where low inoculum densities were used during the spring, experimented with the use of airplanes to broadcast the azolla inoculum. For upland transportation, azolla inoculum can be efficiently collected by first concentrating it by wind, water flow, or pushing, and then harvesting with a net or by developing a floating mechanized harvester. The same harvester could be designed to spread the azolla in a fashion similar to a mechanized manure spreader (see Talley and Rains in Lumpkin and Plucknett, 1982).

For guidance in selecting an inoculum application rate, the following points should be considered:

1. If azolla and land are scarce but labor is plentiful, inoculate at the rate of 0.5 to 0.8 kg/m$^2$ and subdivide or partially incorporate every 2 to 4 days. This system produces the highest yield in the shortest time, since azolla is kept in a linear growth phase. However, this

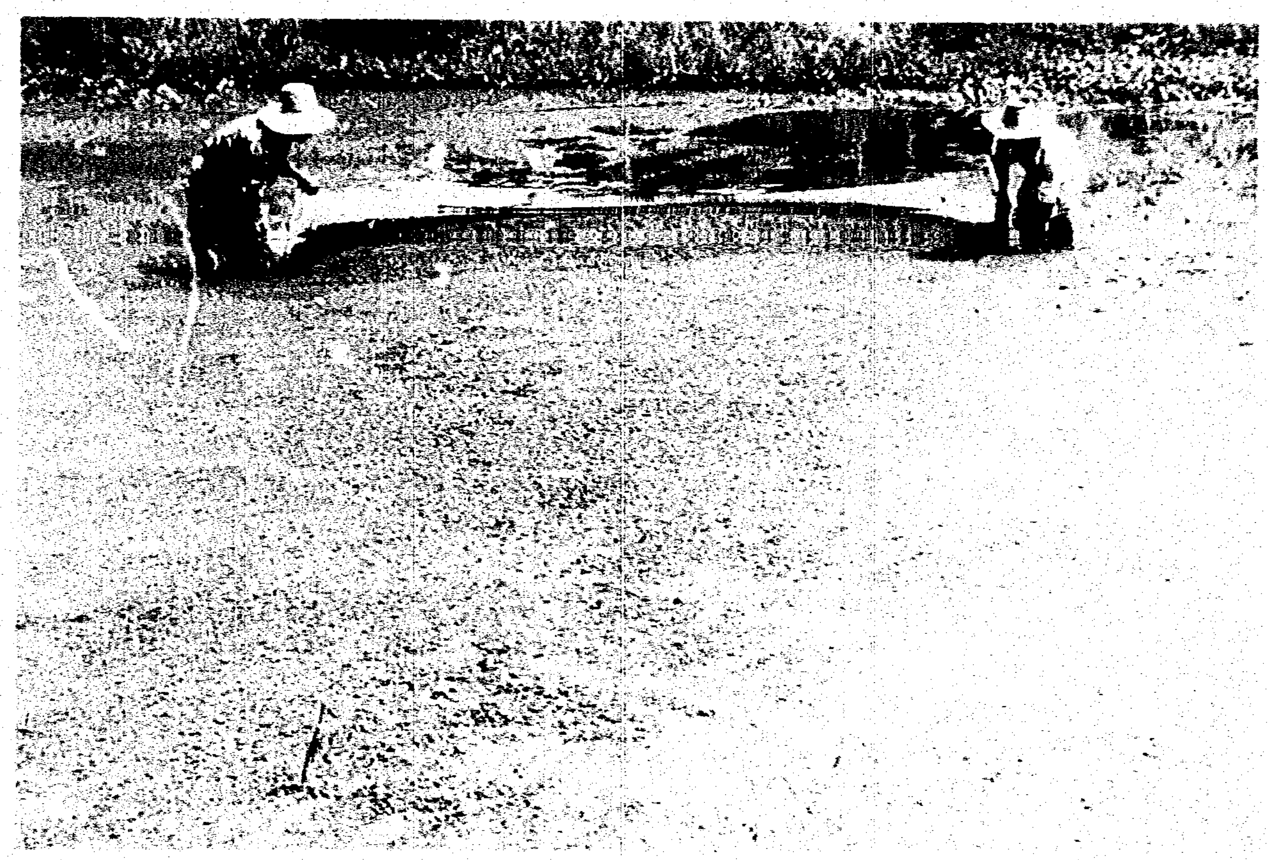


Figure 14 The use of plastic mosquito netting is one of the most effective ways to collect azolla from nurseries under a labor-intensive system, as shown here in Thailand. The water level must be raised so that a portion of the net can slip under the roots of azolla and avoid collecting mud.

high level of inoculation means that a higher labor requirement will be necessary for more frequent subdivision or incorporation.

2. If azolla is plentiful but labor and land area are scarce, inoculate at 0.3 to 0.5 kg/m$^2$ and subdivide or partially incorporate every 5 to 10 days. This is the best system for producing azolla for a large area. This moderate inoculation rate ensures better utilization of sunlight, field area, and water, and should bring about the best economic return in developing countries.

3. If azolla and land area are plentiful but labor is scarce, inoculate at the rate of 0.1 to 0.3 kg/m$^2$ and subdivide or harvest every 10 to 14 days. This system is used when azolla is grown on a large scale for soil incorporation as a basal manure. This low level of inoculation will save labor but may result in more insect damage since the prolonged growth of azolla allows for the population of insects to increase.

Monoculture: When azolla is monocropped in large fields, provisions should be made to prevent the wind from blowing the floating azolla inoculum from one side of the field to the other. Strong winds can result in piles of azolla being formed along the paddy bunds, and these will rot if not quickly dispersed. Azolla grows best if the plants are kept evenly spread over the water surface. To do this the Chinese often used single or double rows of rice about 1 to 2 m or so apart to provide bands of water surface for the azolla. Also, azolla can be grown in rice stubble, which serves to anchor the fronds. In Vietnam, windbreaks of sesbania or dense rows of rice were planted to protect azolla from wind damage. Where water is flowing from one field to another, it is necessary to place barriers made of reeds, twigs, netting, or other screening materials that will prevent the azolla from being washed out of the field.

Azolla grown as a monocrop or as an intercrop with rice should be incorporated into the soil before its maximum growth density (i.e., while it is still in the linear growth phase). Monocropped azolla can be incorporated into the soil with regular tillage equipment before the rice is planted or transplanted (Figure 15). This feature makes the use of monocropped azolla very feasible for all types of rice cultivation, whether transplanted, broadcast, or drill sown, and in temperate regions as well as the tropics. The system usually requires draining of the field to ensure contact of the azolla with the soil, and to prevent wave action caused by the machinery from moving the azolla around and causing incomplete and uneven incorporation patterns. Should it be desired to grow a second or third mat of azolla as a monocrop or intercrop with rice, enough azolla can be left in the field in puddles and low spots to ensure sufficient inoculum to initiate the next mat.

Before the incorporation of an azolla monocrop, the field should be completely drained for several days before the combined azolla incorporation-puddling operation is carried out. This will ensure that

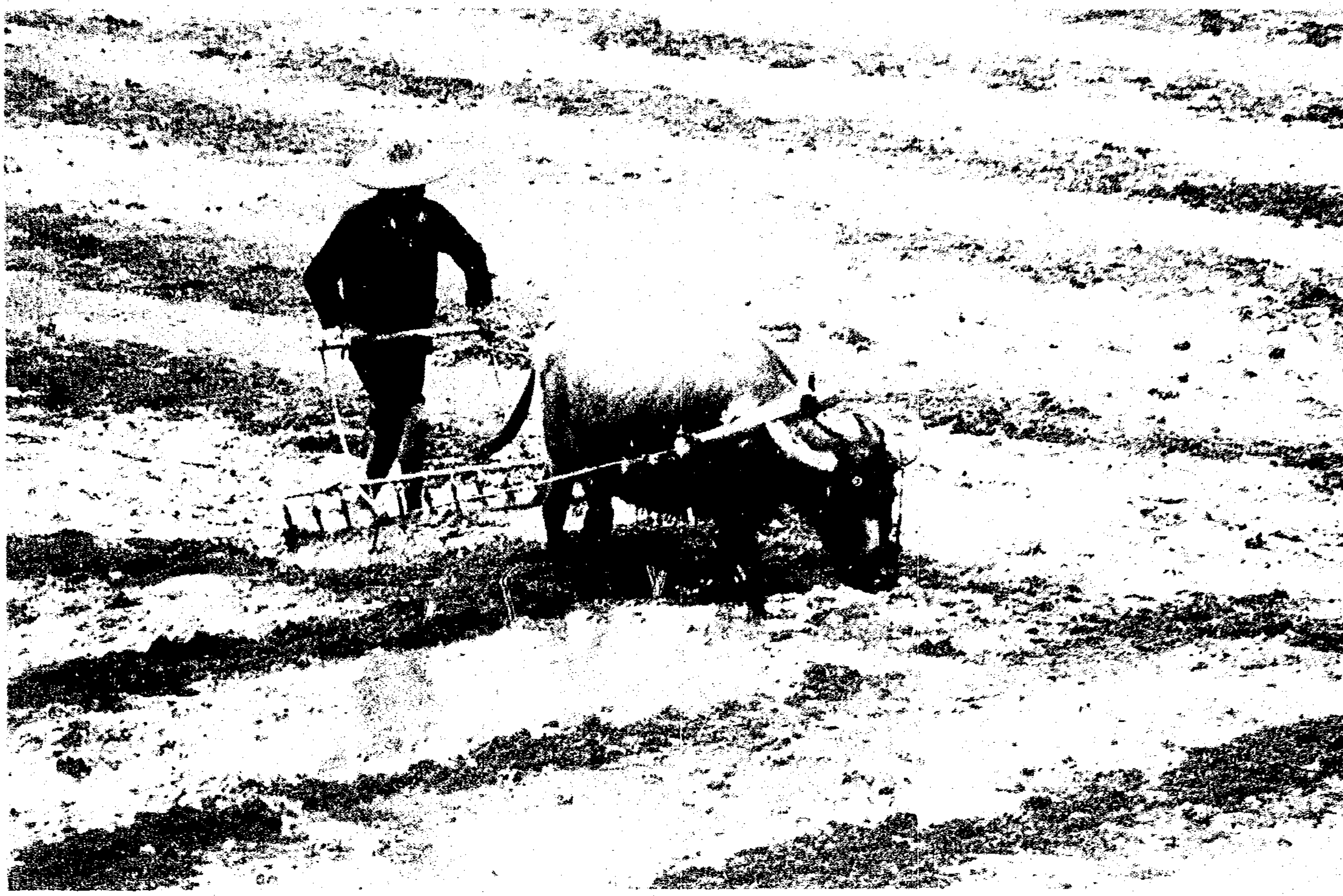


Figure 15 A paddy harrow can be used to incorporate an azolla monocrop into the soil as a basal green manure. As shown here on a commune in Guangdong province, China, a small amount of water is left in the field so that some of the azolla will escape the incorporation process to serve as inoculum for an azolla intercrop with the rice crop.

all or most of the azolla is incorporated. However, if it is desired to grow the azolla as an intercrop in addition to the monocrop, the monocropped azolla mat should be incorporated immediately after draining the paddy while scattered puddles of water remain. In either case, monocropped azolla only or with an intercrop, the rice seedlings should not be transplanted until 4 or 5 days after the final monocrop incorporation. A thick mat of freshly incorporated azolla will aggravate the "transplanting shock" suffered by transplanted rice seedlings and result in a higher incidence of empty hills. The heavier the azolla mat, the longer the fallow period should be. During this period, it is best to keep the paddy drained to speed decomposition, unless an intercrop of azolla is also desired. In the latter case, several shallow irrigations will keep enough azolla alive to grow as an intercrop with the rice.

Interculture: When environmental or cultural limitations do not allow for the growing of monocropped azolla, it may be possible to grow azolla concurrently with rice. Azolla can be grown as an intercrop for use as a green manure, or when grown in early planted rice fields, to produce additional inoculum for late planted rice fields (Agriculture Technical Station, 1964). In fields where azolla is inoculated into transplanted rice to grow as an intercrop, an even distribution of azolla can be difficult. For this situation the Chinese flooded the fields to the point of nearly covering the rice seedlings before broadcasting and dispersing the azolla inoculum.

In China, intercropped azolla was usually incorporated by hand into the soil. This method was used because an effective hand tool for the various types of paddy soils and azolla mat thicknesses had not been developed. Hand incorporation is quite effective, but it is very labor intensive and backbreaking. Attempts at incorporating azolla into a flooded paddy are less effective than incorporating into drained soil, but the former method conserves precious water (Agriculture Bureau, 1964).

A small hand-operated (Figure 16) or gas-powered rotary rice weeder can be used to incorporate a thin mat of azolla into soils of suitable texture. If these weeders are used in both directions in a field (e.g., west to east and north to south), they can do a very complete job of incorporation. However, for this two-way incorporation technique to be used effectively, the rice must be planted equidistant, both in the rows and between the rows, to allow passage of the weeding machine.

The intercrop system brings new factors into the cultivation of both rice and azolla, including that of competition. Despite the fact that azolla floats on the water, whereas rice grows in the soil and in the vertical plane, competition does arise. There are noticeable and measurable effects of competition between an azolla intercrop and rice. Some effects are negative but transitory, while others are more long term (Experiment Station, 1978). First, azolla competes with rice for nutrients, including nitrogen. Azolla is a scavenger for combined nitrogen in the water, and thus reduces available nitrogen for rice. This period of competition lasts for about 10 to 20 days after azolla is planted, usually until after the first azolla incorporation, and was referred to in China as "early inhibition" of rice by azolla. However, this early competition usually disappears after nitrogen from the first incorporation of azolla becomes available to the rice. After an additional incorporation, the end result is usually increased rice yield from more panicles per hill and increased grain weight. The Chinese called this "late promotion" of rice by intercropped azolla; they therefore spoke of "early inhibition and late promotion" as the effects of intercropped azolla on rice.

The decomposition rate of azolla becomes quite important when azolla is intercropped with rice. The rate of decomposition is also affected by the azolla variety, temperature, the quantity of biomass,

Figure 16 The rotary rice weeder can be used to incorporate thin mats of intercropped azolla into the soil, as shown here being done by an

the C:N ratio of the azolla, and the soil environment. Excessive manuring with azolla can "overload" the soil and result in rice seedling fatalities early in the season, and lodging and a higher incidence of disease and insect in the later stages of rice development.

An azolla intercrop affects the water temperature of the rice field. A thick azolla mat does not allow sunlight to penetrate to the water, so the water remains cooler under azolla during the day than when azolla is absent. At night the azolla mat tends to prevent heat loss, whereas an open water surface looses more heat. The net result is that changes in water temperature are much less with an azolla mat than without. In temperate regions, an azolla mat keeps the water cold during the spring and thereby causes a setback in growth of young rice seedlings. This is the case in California where the irrigation water for rice is quite cool. Under such conditions an open water surface that would heat up during the day could be an advantage to the thermophyllic rice plants and may override any advantage of using azolla as an intercrop.

A heavy azolla mat growing under rice can also interfere with tiller emergence and development. Indeed, new tillers can be physically inhibited by a heavy azolla mat caused by excessively high inoculation levels and/or a failure to incorporate part of the azolla mat. However. this is usually not a problem when azolla is incorporated one or more times, since incorporation reduces mat density. Before the widespread use of herbicides, some Japanese rice farmers used to complain about the harm done to tillering by the naturally occurring azolla in their rice fields (Tuzimura et al., 1957). In China, where azolla was grown as an intercrop, peasants would plant up to 10 rice seedlings per hill to overcome the inhibitory effects of azolla.

## V. OTHER USES

Several studies have investigated the weed-suppressing effect of an azolla mat (Satapathy and Singh, 1985). Janiya and Moody (1981) observed that *Monochoria vaginalis*, *Echinochloa glabrescens*, *Cyperus difformis*, and *Paspalum* sp. were suppressed by azolla, but *Scirpus maritinus* and *Echinochloa crus-galli* were unaffected. Srinivasan (1981) found that azolla could suppress *Marsilea quadrifolia*.

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organic farmer in the Philippines. Unfortunately, the usefulness of the rotary weeder is limited because (a) the rice must be planted in rows, which is rarely the case in most developing countries; and (b) the fields must be drained or allowed to dry out so that the azolla does not escape incorporation. A new version has been recently developed by IRRI.

Figure 17 Azolla has long been recognized as a food for domesticated ducks in China and Vietnam, and for wild waterfowl in the United States. In Vietnam, birds are fed 0.1 to 0.3 kg of fresh azolla per day; however, for efficient production, azolla should not contribute more than 25% of the food intake.

Azolla has also been shown to have a beneficial effect on maize yields (Ferrera Cerrato and Miranda, 1982) and on the population of an actinomycetes called "5406" commonly used for agriculture in China (Xiang and Li, 1981). Additional studies on the use of azolla as feed have been conducted with chickens (Castillo et al., 1981), ruminants (Dolberg et al., 1981; Scharpenseel et al., 1982; Waha, 1983), tilapia (Carraro, 1983), and grass carp (Cassani, 1981). Results are generally positive but show that the feeding of azolla must be limited in the diet since its constituents are not well balanced and have undigestible fractions (Figures 17 and 18).

## VI. CONCLUSIONS

The nitrogen-fixing azolla is an excellent green manure, suppressor of certain paddy weeds, and fodder for pigs, ducks, and fish. How-

Figure 18 In China, azolla has been grown for centuries on canals and ponds as a fodder for pigs and is considered to be equivalent to sweet potato tops in feed value. The taste of azolla is not preferred by pigs, so it must be fed before more palatable foods. The addition of a small amount of salt to the azolla can improve its attractiveness.

ever, despite its previous cultivation and use in China and northern Vietnam, it has not been successful in the humid tropics, including southern Vietnam, because of problems with insects and disease, availability of water and phosphorus, and an intensive labor requirement. Its use in China has declined rapidly along with the use of other green manures because they were not economical in light of recent economic reforms.

Azolla cultivation is often promoted as a means of reducing the importation of nitrogen fertilizers or of the fossil fuel needed for their manufacture. However, any savings on nitrogen fertilizers can be quickly negated by imports to support additional pumping of irrigation water and the application of pesticides and phosphorus if these inputs become necessary for azolla's cultivation.

If cultivation is ever to be economically successful on a large scale, a strong research program and a number of scientific breakthroughs are necessary. As a part of a strong program, azolla germplasm must be aggressively collected, maintained, and characterized, and important breakthroughs will have to be made in the use of spores as planting material and the development of efficient implements and fertilizer application methods and formulations. These objectives can be accomplished most effectively by a well-focused and well-financed internationally coordinated research program.

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