Lumpkin, Thomas Adam

TAXONOMY, PHYSIOLOGY, AND AGRONOMIC POTENTIAL OF AZOLLA SPP.

University of Hawaii PH.D. 1983

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TAXONOMY, PHYSIOLOGY, AND AGRONOMIC POTENTIAL OF *AZOLLA* SPP.

A DISSERTATION SUBMITTED TO THE GRADUATE DIVISION OF
THE UNIVERSITY OF HAWAII IN PARTIAL FULFILLMENT
OF THE REQUIREMENTS FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

IN AGRONOMY AND SOIL SCIENCE

MAY 1983

By

Thomas Adam Lumpkin

Dissertation Committee:

Duane P. Bartholomew, Chairman
Ben B. Bohlool
Douglas J. C. Friend
Richard E. Green
Peter P. Rotar

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In pursuit of this study, numerous individuals and institutions provided invaluable support. In this regard, I wish to express my appreciation to: the University of Hawaii's College of Tropical Agriculture and Human Resources and Department of Agronomy and Soil Science for the patience and freedom afforded me; the U. S. National Academy of Sciences Committee on Scholarly Communication with the People's Republic of China; the P. R. C. Ministry of Agriculture; the Zhejiang Academy of Agricultural Sciences; my Chinese co-workers Li Zhuo-xin, Zu Shou-xian, and Mao Mei-fei who collaborated with me in carrying-out the experiments described in Chapters II and III; the International Rice Research Institute for technical assistance; and the U. S. Agency for International Development for ongoing financial support.

Lastly, I wish to dedicate this dissertation to my mother, Alma Preszler Lumpkin, and the memory of my father, the late Charles Joseph Lumpkin, for giving me spiritual support during the five years it took for completion.

ABSTRACT

The freefloating aquatic fern Azolla pinnata has been grown for centuries in northern Vietnam and isolated areas of southeastern China as a nitrogen-fixing green manure for rice. The area under cultivation was very limited because A. pinnata has several environmental constraints and labor-intensive management practices are necessary to ensure its survival and propagation.

To increase the potential of azolla as a nitrogen source for paddy based crops, the known species of Azolla were collected from their native habitats. Morphology of Azolla caroliniana, A. filiculoides, A. mexicana, A. microphylla, A. nilotica, A. pinnata var. pinnata, A. pinnata var. imbricata, and A. rubra was studied using light and scanning-electron microscopy. An improved identification key was developed based on reproductive and vegetative tissues. Reproductive tissues allow for easy identification of Azolla species, but these tissues are rarely present in most species. Floats of the megaspores can be used for identification to the section of the genus, and the sporoderm of the megaspores and the glochidia of the massulae can be used for identification of the species. Trichomes on the leaf lobes and rhizomes, and growth patterns of crowded plants are important vegetative features for identification.

Adaptation of the known species of Azolla to a wide range of climatic conditions was evaluated by growing cultures on nitrogen-free nutrient solution at a site where air temperatures ranged from 0°C in the winter to 40°C in the summmer. Relative growth rates (RGR) were

calculated from increases in dry weight over growth cycles of one week to one month. The RGRs of individual species were most closely related to minimum water temperature during spring and fall (R^2=0.67 to 0.99); minimum water temperature and daylength for spring plus fall (R^2=0.73 to 0.92); and minimum water temperature, solar radiation, and relative humidity during summer (R^2=0.74 to 0.91).

The agronomic potential of azolla accessions and azolla management treatments were evaluated in a field experiment with spring rice conducted at Hangzhou, China. A. caroliniana, two varieties of A. filiculoides, A. pinnata var. imbricata, and A. rubra were grown as a **monocrop** before rice, as an **intercrop** with rice, and as a monocrop and intercrop. A. microphylla was grown as an intercrop only. Grain yields averaged across species increased in the order: combination > monocrop > intercrop. Grain yields were correlated with accumulated N within azolla accessions across management treatments (r=0.94 to 0.99). However, yields were poorly or negatively correlated across accessions within a management treatment (monocrop, r=-0.27; intercrop, r=0.20; combination, r=-0.61). During the initial weeks of intercropping, the intercrop management treatment resulted in a rice and soil nitrogen content less than that of the zero nitrogen control, suggesting that intercropped azolla was competing with the rice for nutrients. Nitrogen accumulated by azolla accessions averaged across management treatments increased in the order: A. filiculoides > A. caroliniana = A. microphylla > A. pinnata = A. rubra.

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REVIEW OF LITERATURE

A general review of azolla literature for this dissertation was completed and published in 1980 (Lumpkin and Plucknett, 1980). A copy of this article is included as appendix A.

Articles appropriate to the research topics of the dissertation, which were published before and after the general review, are reviewed and cited in the introductions to the three chapters. The complete citations for these articles and other articles cited in the text of the chapters are listed in the Literature Cited section at the end of the dissertation.

CHAPTER I

KEY FOR IDENTIFICATION OF AZOLLA SPECIES

INTRODUCTION

Taxonomic literature concerning the genus Azolla is incomplete and unreliable for identifying the species (Clausen, 1940; Svenson, 1944; Godfrey et al., 1961). Indeed, many specimens of azolla stored in the worlds major herbariums, are incorrectly classified because an identification key describing all species of the genus did not exist. Existing keys only described up to four species and were primarily based on reproductive tissue which is rarely present (Moore, 1969). Thus a study to describe all of the species and develop criteria for their differentiation was undertaken. This report of that study begins with a review of criteria for classifying the genus and species, and is followed by sections on phytogeography and comparative descriptions of the morphology of reproductive and vegetative tissues. These comparisons are summarized in a key for identifying the species.

Azolla -azo (to dry) and ollyo (to kill)- is a genus of heterosporous leptosporangiate ferns from aquatic and semiaquatic habitats. The genus was established by Lamarck in 1783 and is grouped with the genus Salvinia in the order Salviniales, but is separated into the monotypic family Azollaceae (Reed, 1954).

Genera of the order Salviniales, Azolla and Salvinia, are aquatic ferns which produce two distinct types of spores (heterospory). The spores of aquatic ferns are borne on special stalks (columella) and are contained in special capsules called sporocarps. Most ferns produce

only one type of spore which is not enclosed in a sporocarp.

Characteristics of some genera of the clubmoss orders suggest a superficial relationship with Azolla. These include an imbricate dorsiventral leaf arrangement and adventitious roots. In addition, clubmoss orders share other less obvious features with Salviniales; all are heterosporous and have a protostele, endosporic gametophytes, and megaspores which store food. Food reserves of the heterosporous ferns are more complex than reserves found in the spores of homosporous ferns and are akin to those found in heterosporous lycopsids and the seeds of many angiosperms and gymnosperms (Lucas and Duckett, 1980).

However, taxonomists have divided living ferns into two distinct series based on whether the sporogenous tissue develops from a single initial cell (leptosporangiate) or from more than one initial cell (eusporangiate). Of the ferns, only the order Salviniales, to which Azolla belongs, and the order Marsileales are leptosporangiate. This difference in the initiation of sporogeonous tissue is the primary feature that separates Salviniales from the clubmoss orders, Lycopodiales and Selaginellales, that contain the superficially similar genera Lycopodium and Selaginella.

Besides differences in the initiation of sporogenous tissue, there are other important features which separate the lycopsids from Azolla. For example, features found in Selaginella but not in Azolla include: ligules, rhizophores, very large chloroplasts, flagellate sperm, eusporangiate sporangia initiation, and strobili. Features found in Azolla but not in Selaginella include: leptosporangiate soral sporangia as in the division Pteridophyta, and sori covered by a protective

indusia as found in the order Filicales. Thus, the features which Azolla shares with Selaginella and other heterosporous lycopsids probably result from convergent evolution rather than from a phylogenetic relationship.

Bower (1935) and Wagner (1969) suggested a phylogenetic relationship between the order Salviniales and the family Hymenophyllaceae (filmy ferns) of the order Filicales because of their soral features and aquatic habitat, with the former evolving from the latter. Both the Salviniales and Hymenophyllaceae have a protective cap (indusium) over the sporangium, columella on which the sporangia are borne, and a gradate basipetal receptacle to which the sporangia are attached. However, the Salviniales differ from the Hymenophyllaceae in that they are heterosporous, endosporic, and leptosporangiate.

The genus Azolla is subdivided into the sections Azolla Meyen (three floats per megaspore) and Rhizosperma Sadeb. (nine floats per megaspore). Glochidia (appendages on microspore packets) of species belonging to the section Azolla are septate, arrow-like, and cover the entire surface of the massula (microspore packet). This section includes the species A. caroliniana, A. filiculoides, A. mexicana, A. microphylla and A. rubra. Glochidia of species belonging to the section Rhizosperma are either absent (A. nilotica), or are simple, or occasionally branched and cover only part of the massula surface (A. pinnata). Further discussion of these features is provided later in this chapter.

Septa in the glochidia were the primary feature used in keys for differentiating species in section Azolla. Their use was questioned by

Godfrey et al. (1961) because of morphological variation within a given species. Variation in the presence or absence of septa lead to contradictory reports about the same species (Clausen, 1940; Svenson, 1944; Hills and Gopal, 1967; Seto and Nasu, 1975). The inadequacy of available keys to the genus Azolla (Svenson, 1944; Moore, 1969) made identification of specimens collected in nature difficult or impossible. The objectives of the study reported here were to study phytogeography and morphology of reproductive and vegetative tissues of the known Azolla species so that an adequate key to the species could be developed.

MATERIALS AND METHODS

Approximately forty accessions representing all known species of the genus Azolla were obtained from collections at research institutions and from native sites. Native sites were located from herbarium sheets of azolla specimens deposited in the Smithsonian Institution, Kew Herbarium, Museum of Natural History-Paris, East African Herbarium and from botanical literature. After collection, accessions were maintained in pot culture at the University of Hawaii, Honolulu, Hawaii. Sporocarps were obtained at collections sites in the field and from plants which became fertile in pot culture. Fresh, dried, and fixed specimens were observed and photographed via light microscopy and scanning electron microscopy (SEM). Samples for the SEM were fixed with 3% glutaraldehyde in 0.2 m phosphate buffer at pH 7.4, post-fixed in 1% osmium tetroxide, and dehydrated in a graded acetone series. Fixed samples were dried by the critical point method in liquid carbon dioxide

and examined by SEM.

RESULTS AND DISCUSSION

The species of Azolla(Figure 1, Tables 1 and 2) can be differentiated by phytogeography, and the morphology of reproductive and vegetative tissues. Phytogeography gives the researcher a clue to identification by virtue of collection site. Features of the reproductive tissue, if available, can then be used to determine the taxonomic section, and species in the section Rhizosperma. When reproductive structures are not present, vegetative tissue must be used for identification of the species.

Phytogeography

The genus Azolla is distributed from near sea level to elevations of 5,000 meters in equatorial regions, as far north as Denmark and Alaska(about 55°N lat), and as far south as the Tierra del Fuego(about 55°S lat). The distribution of individual species has been reviewed previously by Svenson (1944), Sculthorpe (1967), Moore (1969), Ott and Petrik-Ott (1973), and Lumpkin and Plucknett (1980). Sweet and Hills (1971) reported the distribution of the two varieties of A. pinnata used in this study.

The native habitat of the seven species has been confirmed through collection or by noting collection sites given on herbarium sheets (Figure 2). Usually only one of these species is found at any location. However, when two or more species are found in the same vicinity they usually occupy different parts of the environmental range. A.

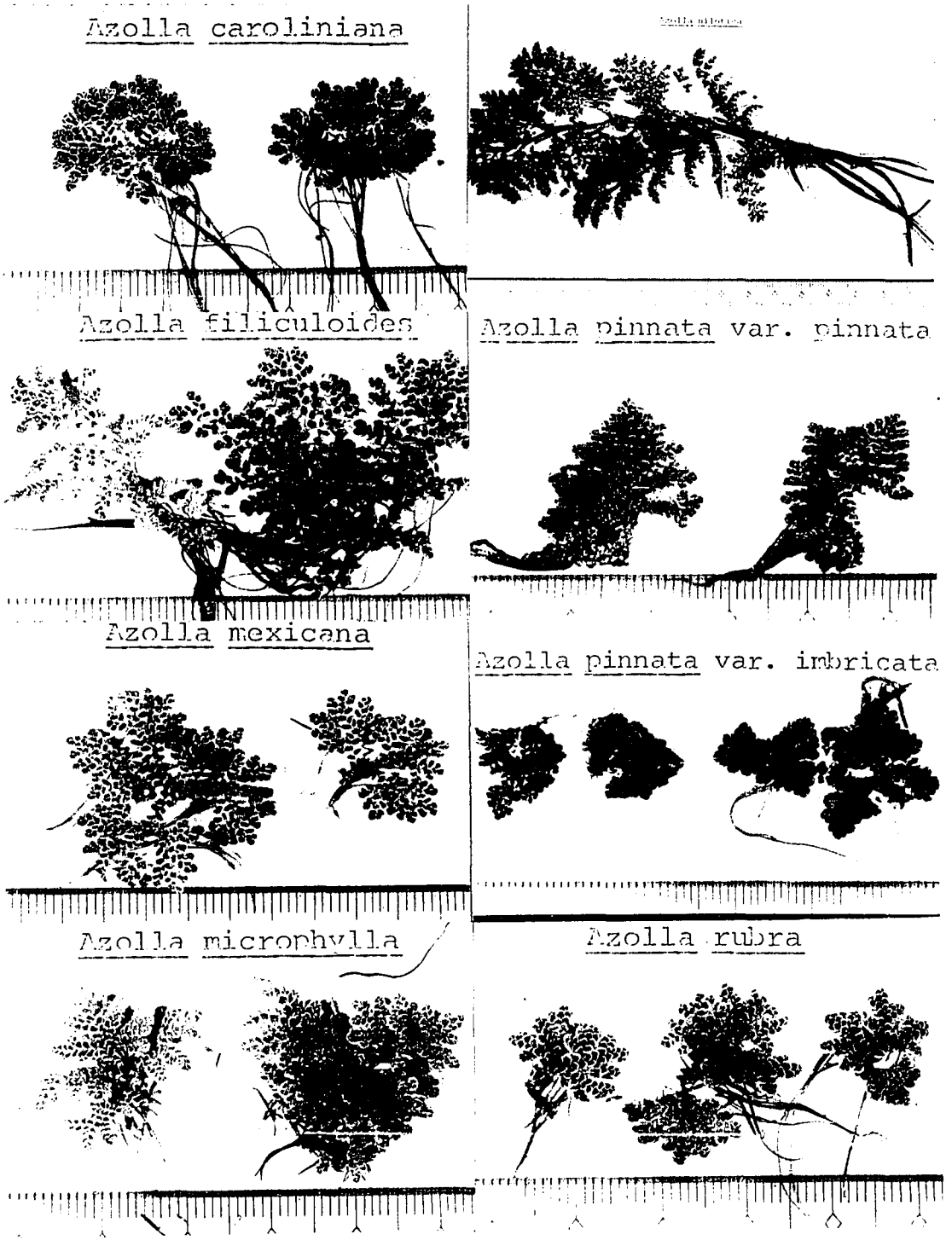

FIGURE 1. The seven species of Azolla, including two varieties of A. pinnata. The fronds shown are approximately life size, except for the young frond of A. nilotica, top right.

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*

TABLE 2. Index Azollaceae

A. caroliniana Willdenow, Sp. Pl. 5:541. 1810.

A. filiculoides Lamarck, Encycl. 1:343. 1783.

A. mexicana Presl, Abh. Bohm. Ges. Wiss. V. 3:150. 1845.

A. microphylla Kaulfuss, Enum. Fil., 273. 1824.

A. nilotica Decaisne, Mett., Pl. Tinn., p. 54, t.25. 1867.

A. pinnata R. Brown, Prodr. Fl. N. Holl., 167. 1810. var. *pinnata*

A. pinnata R. Br. var. *imbricata* (Roxburgh) Bonaparte, Notes Pterid. 7:130. 1881.

A. rubra R. Brown, Prodr. Fl. N. Holl., 167. 1810.

FIGURE 2. The distribution of Azolla species.

caroliniana is distributed in eastern South America. A. filiculoides is found in the Rocky Mountain states of the western U.S. and Canada, through Central America and most of South America. A. mexicana is found from the west coast of the U.S., east to the Mississippi River and South into Mexico and Central America. Its occurrence has been reported but not confirmed in the eastern half of South America. Very few reports or collections of A. microphylla have been made. Only the population found in the Galapagos Islands (Morton and Wiggins, 1971) has been confirmed. A. nilotica occurs only in Africa and has been reported as far south as Mozambique, north through the upper reaches of the Nile River drainage basin to Kosti in the Sudan, and from the east coast through the drainage basin of the Congo River to the southwest coast. A. pinnata (syn. A. imbricata or A. africana) is found in East and South Asia through equatorial Asia to northern Australia, and in equatorial and southern Africa including Madagascar. A. rubra (syn. A. japonica or A. filiculoides var. rubra) is found only in higher latitudes of the western Pacific, i.e. 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 significant. Man has introduced A. filiculoides from the U.S. into Europe, South Africa and China; A. caroliniana into Europe; and A. pinnata into New Zealand. Collections of all species of Azolla are being maintained at various institutes around the world and some of these species may eventually become established on all of the continents. A factor contributing to dispersal is the important role azolla plays in many ecosystems as a source of food for insects, herbivorous fish, waterfowl,

and even tortoises, water rats (Woollard et al., 1978), and manatees. Amphibians, rodents, and waterfowl frequently transport fragments of azolla on their bodies as they move from place to place. Fragments and especially spores of azolla can be carried long distances by waterfowl. This dispersion of species calls into question identification of a species purely on the basis of location and requires examination of plant tissues.

Morphology of Reproductive Tissue

The sporophyte (diploid phase in ferns) of azolla reproduces by vegetative fragmentation via an abscission layer that forms at the base of each branch. Secondary branches extending from older lateral branches bend the lateral branch and put pressure on the abscission layer, contributing to branch separation. Lateral branches then drift away from their parent, becoming independent.

In most species, initiation of the gametophytic cycle (Figure 3) seems to be stimulated by a combination of environmental factors and is most often associated with the beginning or end of a period of stress. Environmental factors also may affect the ratio of microsporocarps to megasporocarps. Study of this variation is difficult because many species rarely or never become fertile, especially when cultured outside of their native habitat, and the gametophytic cycle has not been artificially induced in culture. Accessions of A. filiculoides, A. mexicana, and A. nilotica are consistently fertile in Hawaii. The species A. filiculoides, A. microphylla, and A. nilotica, only become fertile after attaining a mature morphology. In the first two species,

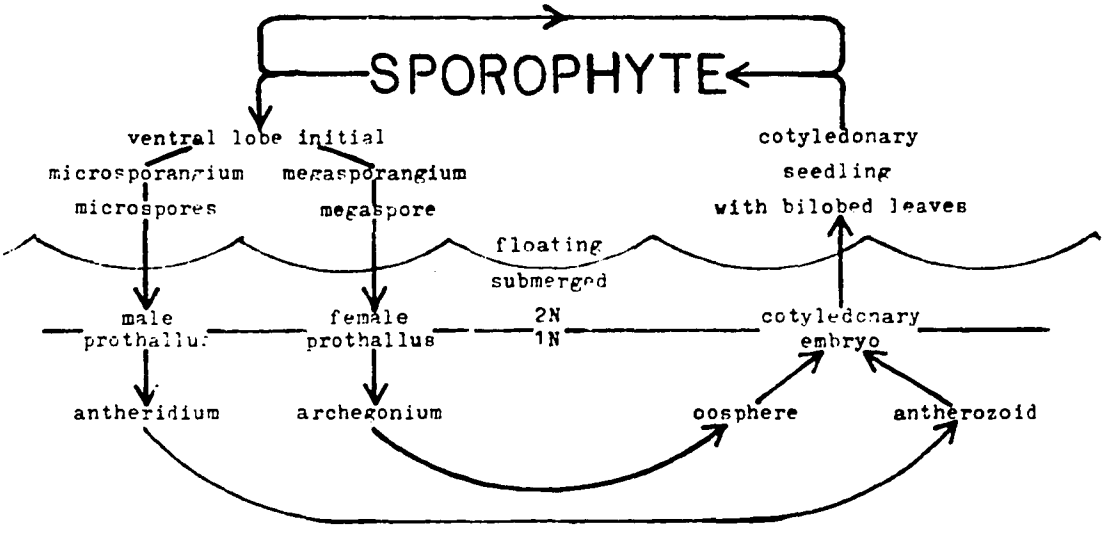

FIGURE 3. The sporophytic and gametophytic life cycles of azolla. The sporophyte reproduces by fragmentation.

mature morphology only occurs when plants are crowded. Mature morphology is characterized by internode elongation into large, nearly vertical fronds and is a precondition for, but not necessarily concurrent with fertility in A. filiculoides and A. microphylla. A. rubra also develops a mature morphology but it is not a precondition for fertility. In certain situations, initiation of the mature morphology may also be environmentally dependent. A variety of A. filiculoides introduced from Washington State, U.S.A. (47°N), to Hangzhou, China (30°N) failed to initiate mature morphology during any period of the wide environmental range at the new site. When crowded, the introduced variety only produced multiple layers of horizontal fronds, similar to a thick mat of A. caroliniana.

When the gametophytic cycle is initiated, sporocarps are formed in pairs (tetrads on A. nilotica) from division of a fertile ventral lobe initial on the first leaf of a branch (Figure 4). The pair (or tetrad) of sporocarps may be all of one sex or any mix (Figure 5).

From initiation, a sporocarp matures on the plant in a week or more depending upon temperature and other growth conditions. A sporocarp has a dark cone (indusium) at the top of a spherical megaspore 0.4 to 0.6 mm in diameter (Figure 6). The indusium overlies an algal colony and the characteristic floats, 3 floats in section Azolla and 9 floats in section Rhizosperma. After maturation, the megasporocarp dehisces and the megasporangial wall disintegrates exposing the sporoderm (perispore) which is usually covered with rubber-like or resin-like hairs (filosum) (see Brederoo et al., 1976). These hairs are formed on the megaspores and function to entangle appendages (glochidia) attached to microspore

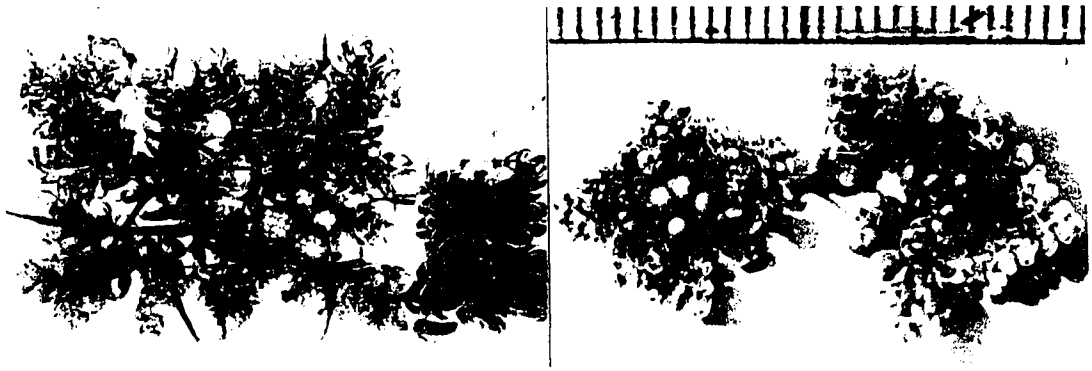

FIGURE 4. Fertile fronds of A. caroliniana (left) and A. pinnata (right) were inverted to show the sporocarps. Only the microsporocarps are visible. Their size can be compared to the millimeter scale in each photo.

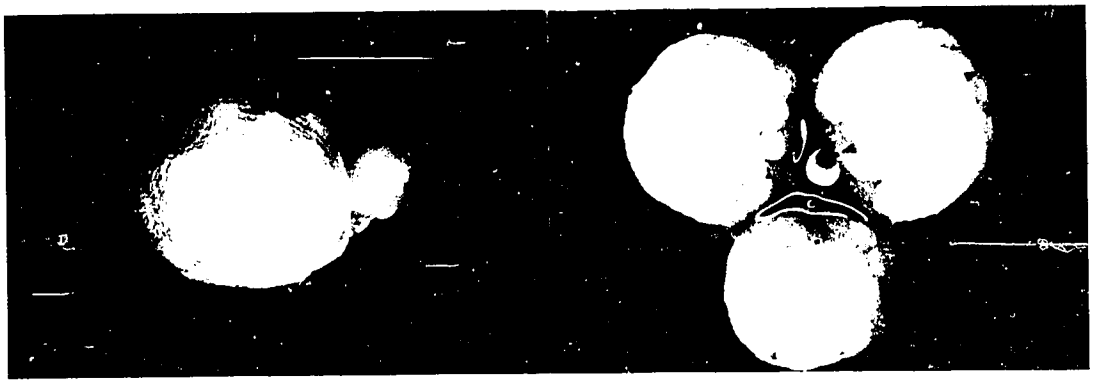

FIGURE 5. Combinations of azolla sporocarps. Sporocarps occur in pairs for all species (left), except for A. nilotica (right) which occur in tetrads. Note the dark indusia (arrows) atop both micro and megasporocarps, and the clusters of microsporangia within the microsporocarps.

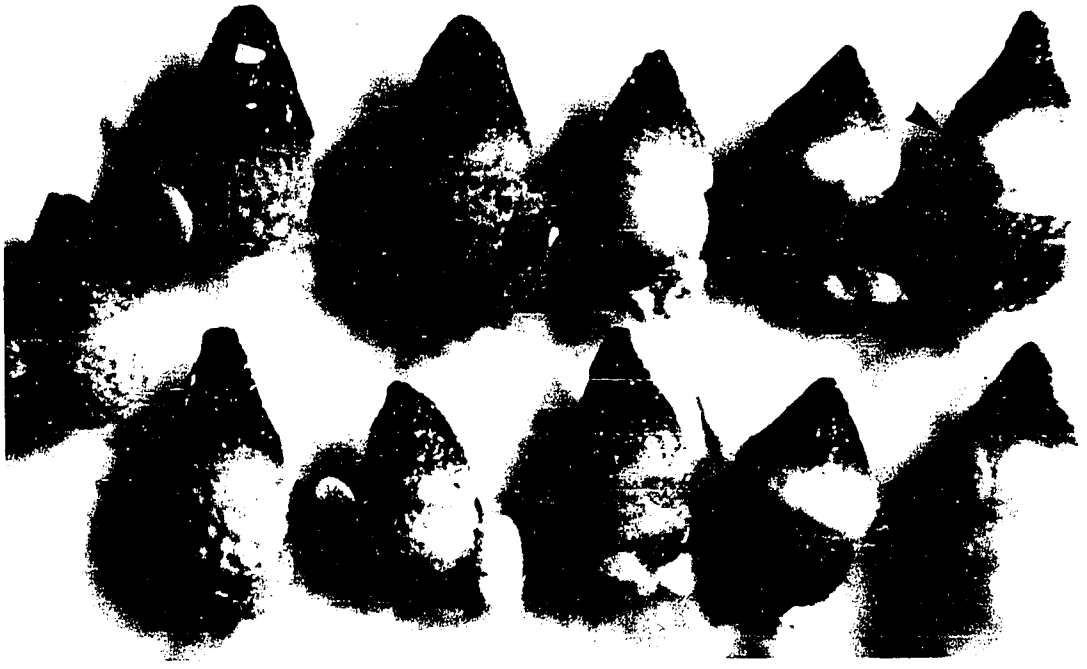

FIGURE 6. Paired megaspores and attached massulae of six Azolla species (x20). From left to right: A. caroliniana (single megaspore), A. filiculoides, A. mexicana, A. microphylla, A. nilotica, and A. pinnata. Note the tier of six floats (arrows) below the indusia of the latter two species. These species in the section Rhizosperma have nine floats on their megaspores; a tier of three floats above the tier of six floats. The species in section Azolla only have three floats on their megaspore.

packets (massulae) of all species except A. nilotica, which does not have glochidia. The appearance of the mat formed by sporoderm hairs is very useful for differentiating species and is described in the identification key.

A mature microsporocarp is globular in shape and is easily visible, measuring about 2 mm tall by 1.5 to 1.75 mm in diameter (Figure 6). A microsporocarp may completely conceal a megasporocarp when they occur on a frond as a mixed pair. Microsporocarps contain up to 130 stalked microsporangia which appear like a cluster of balloons enclosed in the sporocarp (Figure 7, left). Each microsporangium contains 32 or 64 microspores divided into aggregates of 3-10 alveolar massulae (Figure 8). Massulae may be bald (A. nilotica), partially covered with simple branched or unbranched glochidia (A. pinnata), or totally covered with arrow-like glochidia (section Azolla, Figure 7, right). After dehiscence, the wall of the microsporocarp disintegrates, releasing the microsporangia, which in turn disintegrate, releasing the massulae.

Morphology of Vegetative Tissue

The azolla sporophyte consists of a horizontal to vertical main rhizome with individual roots or root bundles at branch nodes (Lumpkin and Plucknett, 1980). The bilobed leaves are alternately arranged and contain the endophytic cyanobacterium Anabaena azollae. With the exception of A. nilotica, fully developed fronds of Azolla species range in length from 0.5 to 7 cm with individual roots 1 to 5 cm long. A. nilotica can produce a trailing rhizome up to 40 cm long with root bundles up to 15 cm in length (Figure 9).

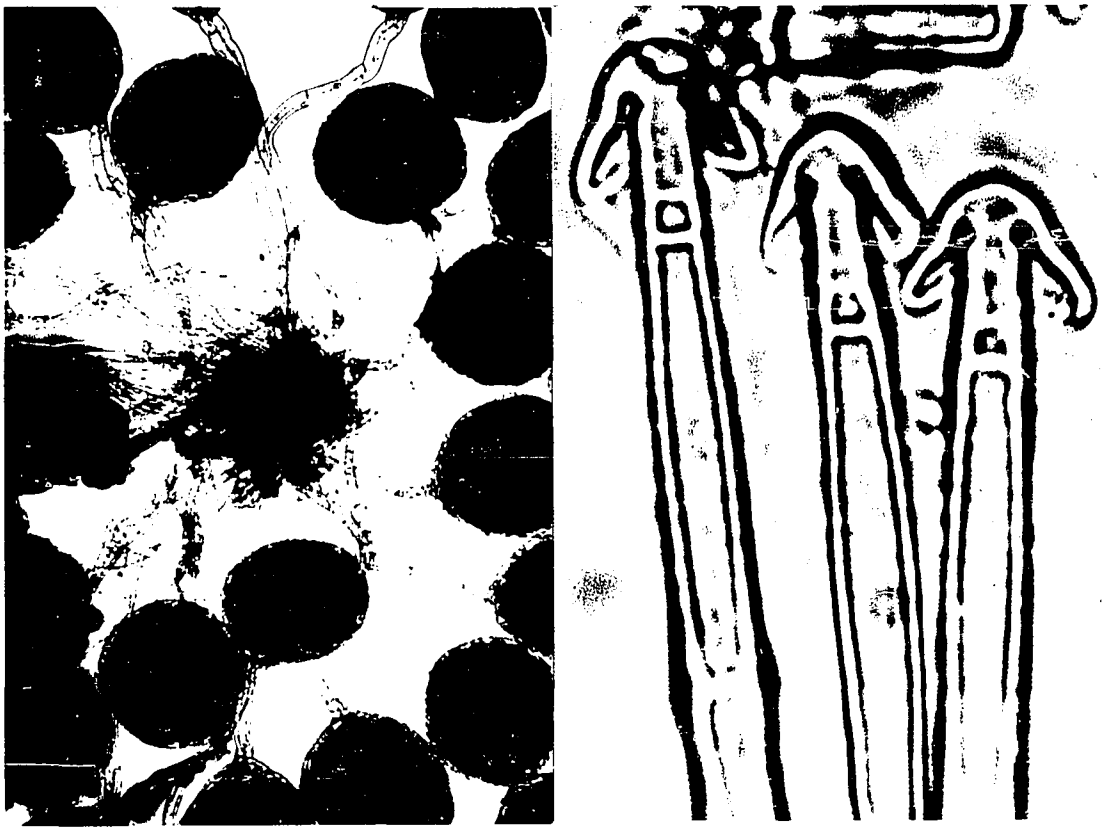

FIGURE 7. Microsporangia (left) and glochidia (right) of A. filiculoides are representative of all species in the section Azolla. Septa bridging the interior of the glochidia are unreliable for differentiating species within the section.

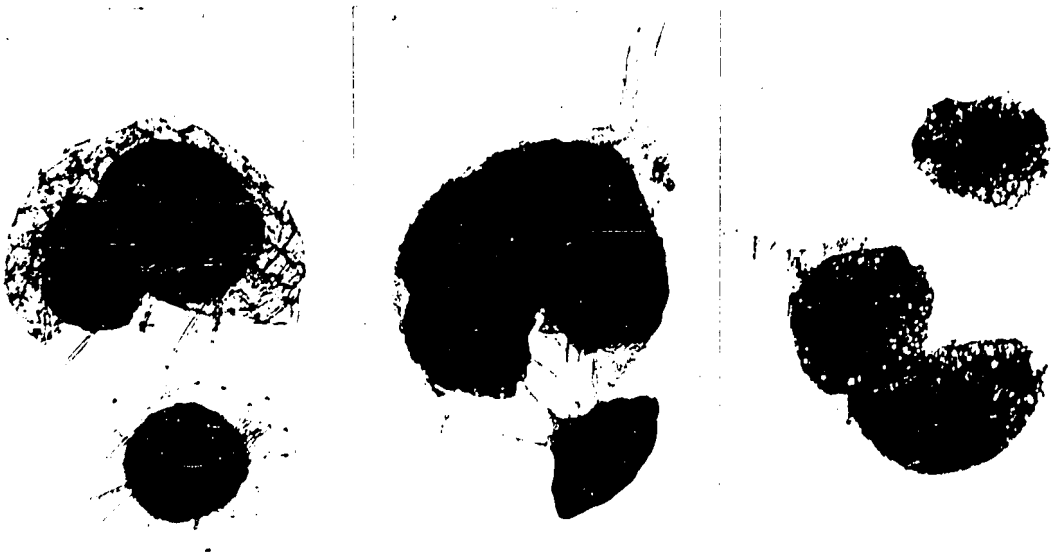

FIGURE 8. The three types of azolla microsporangia, massulae, and glochidia. The type common to all species in the section Azolla are on the left. Note the arrow-like glochidia covering the entire surface of the massulae. The type common to varieties of A. pinnata are in the center. Glochidia cover only part of the surface and may be branched or unbranched. A microsporangium and massulae of A. nilotica are on the right. Glochidia are absent from this species.

FIGURE 9. Root bundles emerging near the shoot apex of A. nilotica in the mature stage of growth. Note the long trichomes on the stem and lack of roots on lateral branches.

The Rhizome.

The usually achlorophyllous main rhizome bears alternate branches having several orders of lateral branches. Species in the section Rhizosperma have trichomes on the ventral surface or on both surfaces of the rhizome, while section Azolla lacks trichomes on the rhizome.

In comparison to other species, the rhizomes of A. filiculoides, A. microphylla, A. nilotica, and A. rubra can undergo a maturation process in which they develop a more distinguishable vascular system in conjunction with internode elongation. When mature, the primary vascular system of A. nilotica resembles amphiphloic siphonostele and produces rootless lateral branches (Figure 9).

The Leaf.

The leaf consists of two lobes, a thick aerial dorsal lobe and a thin ventral lobe which is usually larger than the dorsal lobe (Figure 10). The dorsal lobe is chlorophyllous, except for a transparent margin, and contains the Anabaena colony within a basal cavity connected to the atmosphere by a pore on the adaxial side. The dorsal lobe has an epidermis covered with rows of single-celled stomata and trichomes of one or more cells. The thin ventral lobe is nearly achlorophyllous with several chambers and few stomata and trichomes. The main function of the ventral lobe is probably to provide buoyancy as a result of a pontoon that is formed by ventral lobes which become imbricate when in contact with the water surface. It may also function in absorption, since azolla plants survive with roots removed.

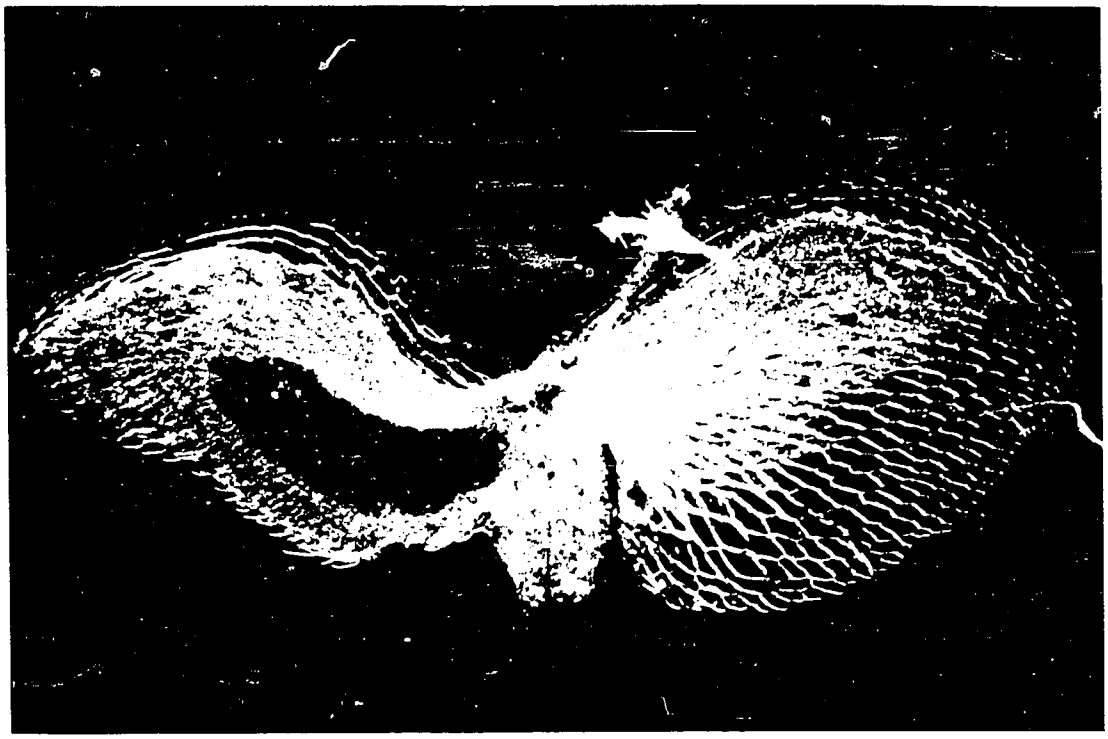

FIGURE 10. A complete leaf of A. nilotica. The dorsal lobe of the leaf, with a clear margin and dark algal cavity, is on the leaf. The partially chlorophyllous ventral lobe is on the right. Several artifacts lie around the periphery of the ventral lobe.

Trichomes.

Although the presence of trichomes has been noted (Lumpkin and Plucknett, 1980), they have not been previously used for the identification of the species. Single or multiple-celled trichomes (Greek: trichoma = a growth of hair) are found on the epidermis of all species of Azolla (Figure 11). The characteristics of these trichomes, when viewed through the light microscope, are very useful for grouping and differentiating the species. Epidermal trichomes are found only on the dorsal surface of both the ventral and dorsal leaf lobes of all species and additionally on the rhizomes of A. pinnata and A. nilotica. Trichomes are erect on the rhizome and at the base of the leaf lobes, and then progress to nearly prostrate near the lobe tips. Epidermal trichomes should not be confused with the hair-like transfer cells found within the dorsal lobe cavity or the trichomes (filaments) of Anabaena azollae cells.

The number of cells composing a leaf trichome can be used to group the Azolla species. A. filiculoides (Figures 11C and 11D) and A. rubra (Figures 11O and 11P) have only single-celled trichomes; A. caroliniana (Figures 11A and 11B) and A. microphylla (Figures 11G and 11H) have single and double-celled trichomes; A. mexicana (Figures 11E and 11F) and occasionally A. microphylla have single to triple-celled trichomes; A. pinnata (Figures 11K to 11N) has double-celled trichomes; and A. nilotica (Figures 11I and 11J) has two to five or more cells per trichome.

Other morphological characteristics of the trichomes are also useful in grouping and distinguishing the species. Trichomes of A.

FIGURE 11. Trichomes (leaf and rhizome epidermal hairs) of the respective Azolla species. Their shape and location are useful in identification. A. caroliniana: (**A**) leaf x200, (**B**) leaf x500; A. filiculoides: (**C**) leaf x200, (**D**) leaf x125; A. mexicana: (**E**) leaf x600, (**F**) leaf x250; A. microphylla: (**G**) leaves x70, (**H**) leaf x500;

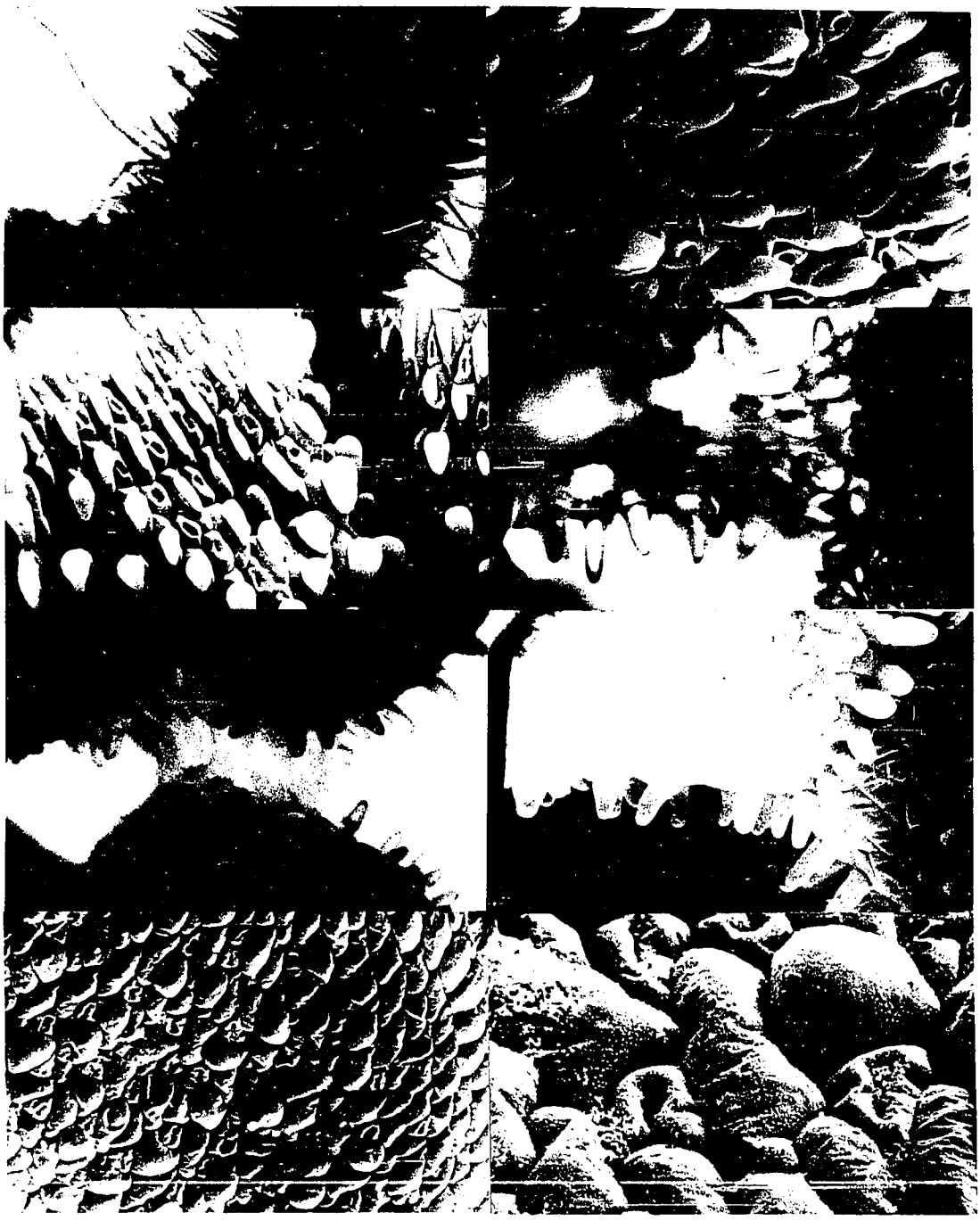

FIGURE 11. Continued.

A. nilotica: (**I**) young stem x125, (**J**) leaf x220; A. pinnata var. pinnata: (**K**) leaf x100, (**L**) stem x125; A. pinnata var. imbricata: (**M**) leaves and stem x50, (**N**) stem x125; A. rubra: (**O**) leaf surface x100, (**P**) leaf surface x400.

caroliniana (Figures 11A and 11B), A. mexicana (Figures 11E and 11F) and A. microphylla (Figures 11G and 11H) are typically erect and are two or three-celled at the base of the lobe progressing to prostrate and single-celled at the lobe tip. A. mexicana differs from the two other species in that it commonly has three-celled trichomes at the lobe base. These three-celled trichomes are composed of a mound-shaped basal cell, a cylindrical extension cell and a teardrop-shaped apical cell which is attached obliquely to the extension cell.

The single-celled trichomes of A. rubra (Figures 11O and 11P) barely protrude above the epidermal surface, while trichomes of A. filiculoides (Figures 11C and 11D) form a sawtooth pattern and protrude well above the epidermal surface.

Trichomes of species in the section Rhizosperma have a nearly flat basal cell and one (A. pinnata, Figures 11K-11N) or, one or more (A. nilotica, Figures 11I and 11J) apical or extension cells. Trichomes cover the ventral rhizome surface of A. pinnata except between the dorsal lobes. Trichomes cover the entire rhizome surface of A. nilotica near the shoot tip and wither away as the rhizome matures and internodes elongate. The apical cells of trichomes on the rhizome are tapered on A. nilotica and A. pinnata var. imbricata but are knob-like on A. pinnata var. pinnata.

Identification Key

This study sought to clarify the taxonomy of the species via a comparative presentation of phytogeography and morphological features. Specimens of azolla were collected from every continent and their

distribution was discussed in the section on phytogeography. These accessions formed the first cultured collection of all known Azolla species and allowed the study to be based on the simultaneous observation of all species through various stages of growth often including heterosporous reproduction. Although the description of many features found in this study are supported by other reports cited in the text, the descriptions of A. nilotica, A. microphylla and A. rubra may not be definitive since their germplasm base was limited to a few accessions and supported by few reports.

Clear evidence was found for identification and classification of the seven Azolla species maintained in the culture collection and this information was summarized in a key for identification of the species. Major morphological differences exist between the species and allow for simple identification of fresh vegetative material, particularly when mat development can be observed over time. For differentiation from vegetative tissue, observing mat development may be necessary for positive identification of species in the section Azolla, especially if the plants were collected in the immature state when species are small and grow horizontally.

The morphological features of reproductive and vegetative tissues, discussed in this study, were used to develop the identification key listed below. This key is an improvement over existing keys in that it describes all species of the genus Azolla.

KEY FOR THE IDENTIFICATION OF AZOLLA SPECIES

Heterosporous leptosprangiate plants found floating in placid water or growing on soft mud. Dorsiventral bilobed leaf arrangement with lobes 1-3 mm wide. Anabaena cyanobacterium colony found on apical meristem, under sporangial indusium, and in ovoid cavity on the adaxial side at the base of each dorsal leaf lobe.

A. Echinate trichomes especially on basal portion of dorsal lobe extending onto internode. Immature fronds angular 1-3 cm in length, growing horizontally, composed of one or occasionally more main rhizomes with lateral branches. Megaspore with 9 floats. Massula without glochidia or with simple glochidia, branched and unbranched, on portion of massula surface.....section Rhizosperma

  B. Plants of immature and mature morphology with trichomes up to 5 or more cells long on the rhizome near the shoot tip. Internodes on mature plants up to 10 mm or more often on brown rhizome up to 40 cm long with senescent trichomes, root bundles at each node, and usually rootless lateral branches. Immature stage upto 3 cm long has a chlorophyllous rhizome near the shoot tip with trichomes on emerging roots, and on entire surface of rhizome. Usually fertile in mature stage with sporocarps in tetrads. Megasporoderm uniformly scrobiculate, dark red with net-like pattern of small spines, hair nearly absent.....
  .....A. nilotica

  B. Uncrowded plants deltoid with one main rhizome up to 3 cm long. Double celled trichomes found on leaf lobes and on ventral surface of rhzome, not found on emerging roots. Megasporoderm with irregular prostrate rods, hair on collar only.....
  .....A. pinnata

    C. Main rhizome dominant with laterals generally unbranched except at decaying end of main rhizome. Usually single main rhizome, deltoid or bullet shaped, especially when growing under stressed conditions .....A. pinnata var. pinnata

    C. Main rhizome not dominant but with dichotomous branching. Fronds often rounded, deltoid or trapezoidal in shape
    .....A. pinnata var. imbricata

A. Trichomes on leaf lobes only. Immature fronds 1-3 cm in diameter growing horizontally, composed of two or more flabelliform main rhizomes with lateral branches. Megaspore with 3 floats. Entire massula surface covered with arrow-like glochidia.....
  .....section Azolla

  B. Plants with two growth stages: immature plants horizontal, 1-3 cm in diameter; mature plants nearly vertical up to 5 cm or more above the water surface. Main rhizome with elongate internodes up to 5 mm long. Fertile in mature stage only.

C. Trichomes with one pedicel cell, an occasional extension cell, and one apical cell. More yellowish than other species, pink in leaf and margins but frond never red. Megasporoderm scrobiculate but with smooth appearance caused by even cover of hair .....A. microphylla

C. Trichomes single celled, similar to A. rubra, but clearly discernable from epidermal layer. Plants often produce dark red pigmentation under stress. Megasporoderm of wart-like excrescence each covered with a weft of hair. Megaspore collar glabrous in comparison to A. rubra ....A. filiculoides

C. Trichomes unicellular only, same as A. filiculoides, but barely discernable from epidermal layer. Leaves most imbricate of all species and apex of dorsal lobe folded parallel to rhizome. Some red pigmentation usually present. Similar in appearance to A. mexicana, but rarely fertile. Megasporoderm superficially similar to A. filiculoides but with more numerous and taller pads and megaspore collar covered with hair .....A. rubra

B. Plants horizontal, mature up to 4 cm in diameter, growing in multiple nearly horizontal layers when crowded.

C. Trichomes of two or more cells: a broad pedicel cell often half or more of the trichome height, an apical cell perpendicular to lobe at base of lobe to nearly parallel at lobe apex. Megasporoderm foveolate.

D. Trichomes occasionally of 3 cells. Fronds gray-green to dark red; some red usually present. Layered mat to 2.5 cm thick. Usually fertile. Megasporoderm with large foveae especially near collar ....A. mexicana

D. Trichomes never more than 2 cells. Fronds dark green or with margins of bright crimson to dark red. Layered mat to 4 cm thick. Individual immature fronds easily mistaken for A. filiculoides. Rarely fertile. Megasporoderm foveae partially masked by a thin weft of hair .....A. caroliniana

CONCLUSIONS

Several important conclusions were reached from data obtained in this study and supporting literature. The fern order Salviniales which consists of the two leptosporangiate heterosporous genera Salvinia and Azolla, has no clear evolutionary or fossil connection with members of the Pterophyta (Smith, 1955; Follieri, 1977) though it does share certain features with the family Hymenophyllaceae in the order Filicales (Wagner, 1969) and with species in the orders Lycopodiales and Selaginellales. This lack of a clear connection had lead previous authors to label the Salvinales as an order of fern allies rather than as true ferns (Correll, 1956). In this study, no additional evidence was found to justify a change from this loose association.

Reproductive tissue is rarely available for use in identification. This has been the major weakness of the key for section Azolla developed by Svenson (1944). The lack of a key based on vegetative characteristics and the similarity of species within section Azolla calls into question the classification of most specimens of this section.

The identification of species used in this study differed in two cases from the indexes of Azolla listed by Svenson (1944) and Reed (1954). Reed recognized the species A. rubra but called A. microphylla synonymous with A. caroliniana. Moore (1969) and Svenson (1944) recognized A. microphylla but called A. rubra a variety of A. filiculoides. Evidence provided in this chapter supports the opinion that A. rubra R. Brown is a distinct species (Reed, 1954) rather than a variety of A. filiculoides as reported by Strasburger (1873; also Nakai, 1925) and that A. microphylla is distinct from A. caroliniana.

CHAPTER II

THE GROWTH RESPONSE OF EIGHT AZOLLA ACCESSIONS TO CLIMATIC VARIABLES

INTRODUCTION

The nitrogen-fixing fern-alga symbiosis, Azolla-Anabaena, is used in certain parts of the world as an aquatic green manure and fodder (Lumpkin and Plucknett, 1982). Two of the seven known species of Azolla, A. pinnata var. imbricata and A. filiculoides, are presently cultivated on over 2 million hectares a in Vietnam and China. Azolla is primarily grown as a winter/spring green manure crop because heat and insect intolerance reduce productivity during summer and early autumn. Control procedures are available to overcome insect problems, but the lack of heat tolerant cultivars and effective heat avoidance measures results in low productivity or death during the summer, particularly when water temperature exceeds 40°C.

Azolla plants must be maintained over the summer and winter to reestablish the crop in paddy fields at the start of the growing season. This is because no large scale methods are known for using azolla spores as seeding material. The maintenance of vegetative material during the off-season is a significant cost in azolla production. If varieties tolerant to the annual range of climatic conditions prevailing in temperate and tropical regions could be identified, production costs could be reduced.

The climatic limitations of present cultivars has induced scientists to investigate the environmental range of other species in

the genus. In growth chamber and glasshouse studies, Peters et al. (1980) found that the relative tolerance of four species to a constant high temperature of 40°C was A. mexicana >A. pinnata >A. caroliniana >A. filiculoides and that their maximum growth rates occurred at either 25°C or 30°C and a photon flux density of 400 u mol $m^{-2} \cdot s^{-1}$. In phytotron studies, Watanabe et al. (1981) reported that under 30 Klux light for 12 hours per day, maximum growth rates occurred at 37/29°C (day/night temperature) for these same species, except A. filiculoides which could not survive at such a high temperature. In a review of the literature, Becking (1979) found that relative growth rates ($mg \cdot g^{-1} \cdot day^{-1}$) ranged as high as 413 for A. caroliniana, 239 for A. filiculoides, 137 for A. mexicana and 334 for A. pinnata var. imbricata.

Growth rates for the species A. microphylla, A. nilotica and A. rubra have never been reported, nor have all of the species been compared simultaneously. This lack of information stimulated research to simultaneously compare the growth of all known species to a wide variation in climatic conditions. Data generated from this research were used to develop predictive models of relative growth rate in response to climate.

MATERIAL AND METHODS

Plant Material

Eight accessions of azolla ,including all seven species of the genus, were used for the study (Table 3). A. pinnata was represented by two of its recognized varieties.

TABLE 3. Sources of *Azolla* accessions used for the growth study.

SECTION SPECIES VARIETY	COLLECTION SITE	LAT.	LONG.	SOURCE
Azolla				
A. caroliniana	Yellows Springs, Ohio, U.S.A.	39.47N	83.54W	G.A. Peters
A. filiculoides	East Germany	52.30N	13.30E	Li Z.X.
A. mexicana	Sutter Basin, Ca, U.S.A.	38.20N	121.30W	S.N. Talley
A. microphylla	Galapagos Islands, Ecuador	0.38S	90.20W	T.A. Lumpkin
A. rubra	Japan	34°N	136°E	K. Seto
Rhizosperma				
A. nilotica	Kosti, Sudan	13.09N	32.39E	T.A. Lumpkin
A. pinnata				
var. *pinnata*	Ivory Coast	8°N	6°W	C. VanHove
var. *imbricata*	Hangzhou, China	30.19N	120.12E	Li Z.X.

Cultivation Site

The eight accessions were grown in pot culture at the Zhejiang Academy of Agricultural Sciences in Hangzhou, China (30.19N, 120.12E). The site is located on an alluvial plain at sea level, and has a continental climate characterized by hot summers and cold winters (Figure 12). Mean temperatures at Hangzhou are approximately 27.9°C in July and 4.1°C in January and extremes of 42.1°C and -10.1°C have been recorded (Co-ching Chu, 1937).

Culture Conditions

The accessions (treatments) were grown in 6.6 liter glazed porcelain pots (20 cm diameter, 21 cm high) for periods ranging from one week during summer to one month during winter (Figure 13). Each treatment was replicated six times in separate pots in a completely randomized design. The pots were placed under a wall-less structure with a glass roof approximately 2.5 meters high, to allow for near ambient light and air conditions but to exclude precipitation.

Each pot was inoculated with five grams of fresh azolla, an amount equivalent to 160 $g \cdot m^{-2}$ or 1.6 $t \cdot ha^{-1}$ over the 314 cm^2 pot surface. Biomass did not exceed 25.1 g fresh weight per pot during any of the growth periods. This biomass is equivalent to 800 $g \cdot m^{-2}$ or 8 $t \cdot ha^{-1}$, and is less than one third of the maximum mat fresh weight attained by species which only form thin mats. Thus it was assumed that growth of the accessions was not limited by competition. Because of the lack of crowding, all *Azolla* species capable of two stages of growth remained in the immature stage. After measuring fresh weight at the end of each

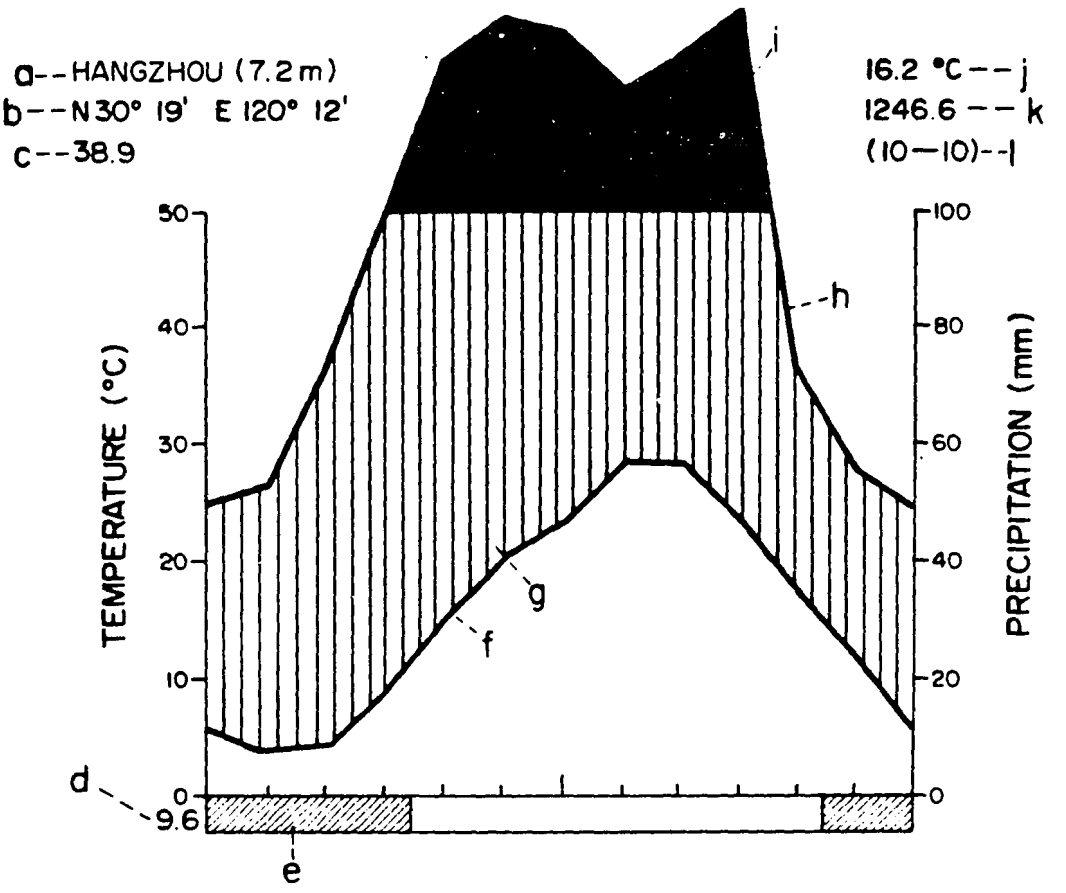

FIGURE 12. Climate-diagram for Hangzhou, China (Wu Zheng-yi, 1980). Temperature during 1980 was similar to this climate-diagram except for short periods of variation below 0°C and above 30°C. The symbols and figures on the diagram have the following meanings: **a**, weather station name and height above sea level; **b**, coordinates for Hangzhou; **c**, absolute maximum temperature; **d**, absolute minimum temperature; **e**, months with absolute minimum temperature below 0°C; **f**, curve of monthly mean temperature; **g**, humid period; **h**, curve showing mean monthly precipitation; **i**, mean monthly precipitation exceeding 100 mm (scale reduced to 10^{-1}, black area; **j**, mean annual temperature; **k**, mean annual precipitation; **l**, years of observed temperature and years of observed rainfall.

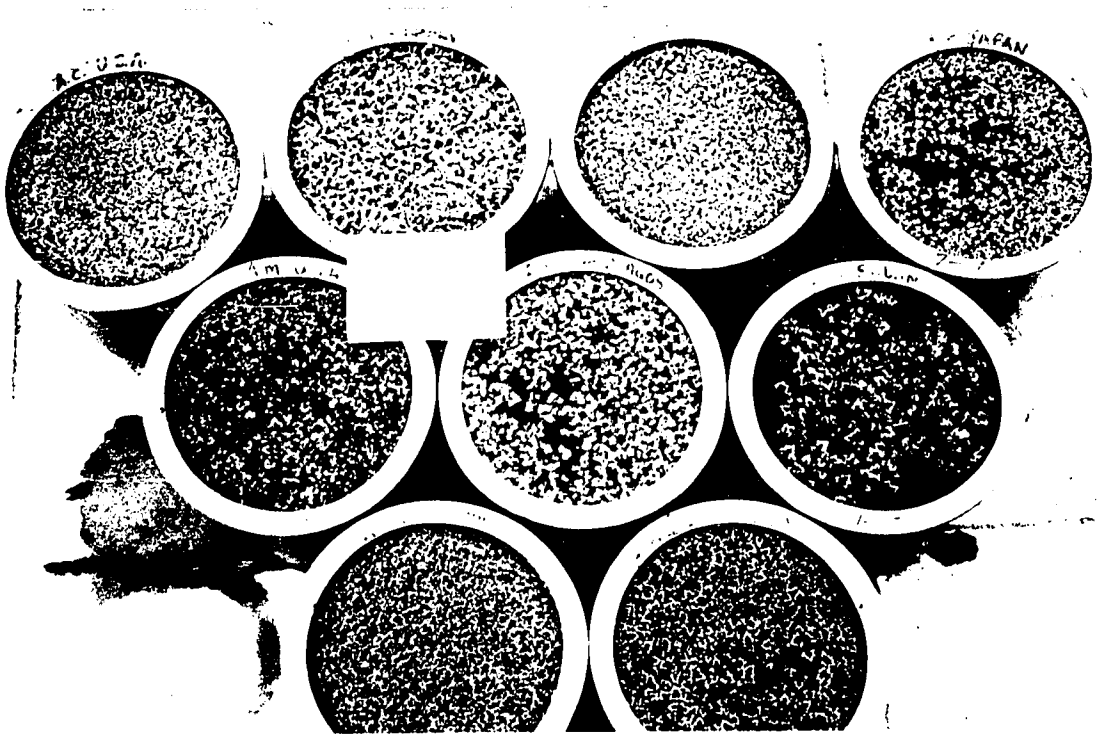

FIGURE 13. Open-air pot culture of azolla. Five grams, fresh weight, of each azolla accession were placed in 6.6 liter pots and grown for periods of one week to one month throughout the year, to measure the influence of climate on relative growth rate.

growth period, some of the material was used as inoculum for the next growth cycle to eliminate the need for pre-conditioning of inoculum and repeated sampling for dry weight. A nitrogen-free nutrient solution including molybdenum, cobalt, and sodium, with a pH range of 5-6, was formulated to supply nutrients to the azolla and its cyanobacterium (Table 4). Six liters of fresh nutrient solution were used at the beginning of each cycle.

The concentration of elements in Table 4 are minimum levels, particularly for Ca and Mg, since only ground water was available for final dilutions of the nutrient solution.

Measurements

The fresh weight of azolla in each pot was measured at the end of each growth cycle. The azolla was prepared for weighing by removing it from the pots in the morning and placing it under shade between two thick layers of blotting paper for approximately one hour. After blotting, fresh weights were measured and five gram samples were taken to re-inoculate the pots for the next growth cycle. Relative growth rate (RGR in $mg \cdot g^{-1} \cdot day^{-1}$) was then calculated by the following formula: $RGR=(\ln X_2-\ln X_1) \cdot t^{-1} \cdot 10^3$ where X_1=initial weight, X_2=final weight, and t=number of days of growth. Relative growth rate is preferred for describing the growth of azolla and other aquatic plants (Mitchell, 1974) since their growth is more similar to single-celled organisms than terrestrial plants.

Air temperature and percent relative humidity were constantly monitored with a hygrothermograph and maximum and minimum pot water

TABLE 4. Formula and concentration of nitrogen-free nutrient solution used to grow azolla.

FORMULA	MACRONUTRIENTS ($mg \cdot l^{-1}$)	CONCENTRATION (ppm)
$NaH_2PO_4.2H_2O$	50.3	P = 10.0 Na = 7.4
$CaCl_2.2H_2O$	27.6	Ca = 7.5
KCl	13.4	K = 7.0 Cl = 33.0
$MgSO_4.7H_2O$	41.2	Mg = 4.0 S = 5.4
Fe sequestrene 138	7.8	Fe = 1.3
	MICRONUTRIENTS	
H_3BO_3	0.56	B = 0.1
$Na_2MoO_4.2H_2O$	0.25	Mo = 0.1
$MnCl_2.4H_2O$	0.36	Mn = 0.1
$ZnSO_4.7H_2O$	0.44	Zn = 0.1
$CoCl_2.6H_2O$	0.04	Co = 0.01
$CuSO_4.5H_2O$	0.04	Cu = 0.01

temperatures were recorded. Light intensities were measured with a Shanghai lux meter and cloud cover was estimated at 9 a.m., noon, and 3 p.m. daily. Daily solar radiation was calculated by methods proposed by Thompson (1976), from clear sky solar radiation for 30°N latitude and the measured light intensities and estimated cloud cover. Daily precipitation data were obtained from the Zhejiang Province Meterological Station at Hangzhou.

RESULTS AND DISCUSSION

Weather During the Study Period

The study period extended from December 1979 through November 1980. January 1980 was the coldest month with a mean air temperature of 4.4°C and a low extreme of -7.3°C. The mean July 1980 air temperature fell exactly on the 10 year mean of 27.9°C and had a high extreme of 37.7°C (Figure 14). Surface water temperatures followed a similar pattern (Figure 14).

Clear sky solar radiation ($cal \cdot cm^{-2} \cdot day^{-1}$) at 30°N has a calculated range of approximately 377 in December to 739 in June (Appendix B), but estimated solar radiation at the site ranged from 280 to 600 (Figure 14). Mean relative humdiity ranged from 55 to 83% (Figure 15). The site had 207 days with precipitation totaling 1534 mm during the study period (Figure 15).

Growth of Azolla species as Affected by Climatic Variables

Relative growth rates of the eight azolla accessions were measured during the period from December 1979 to November 1980. RGR was poorly

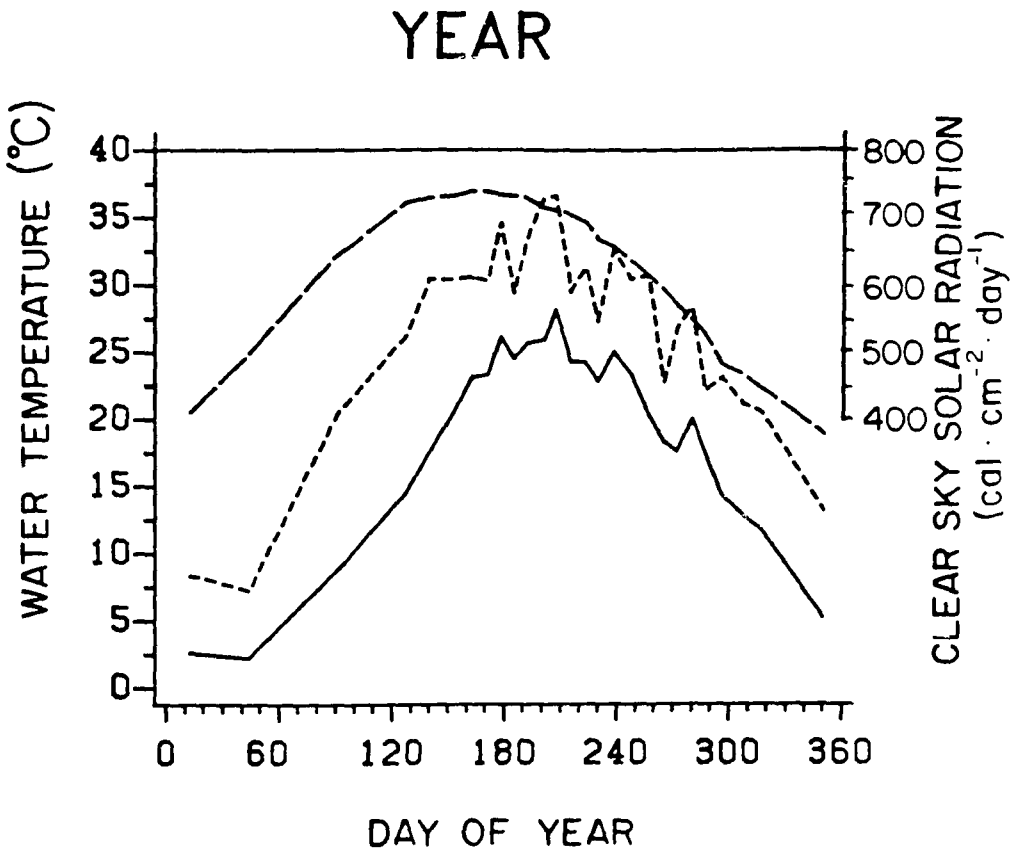

FIGURE 14. Minimum surface water temperature (solid line), maximum surface water temperature (short dashes), and solar radiation above the atmosphere (long dashes) for 1980 at Hangzhou, China. Lines connect averages from each growth period.

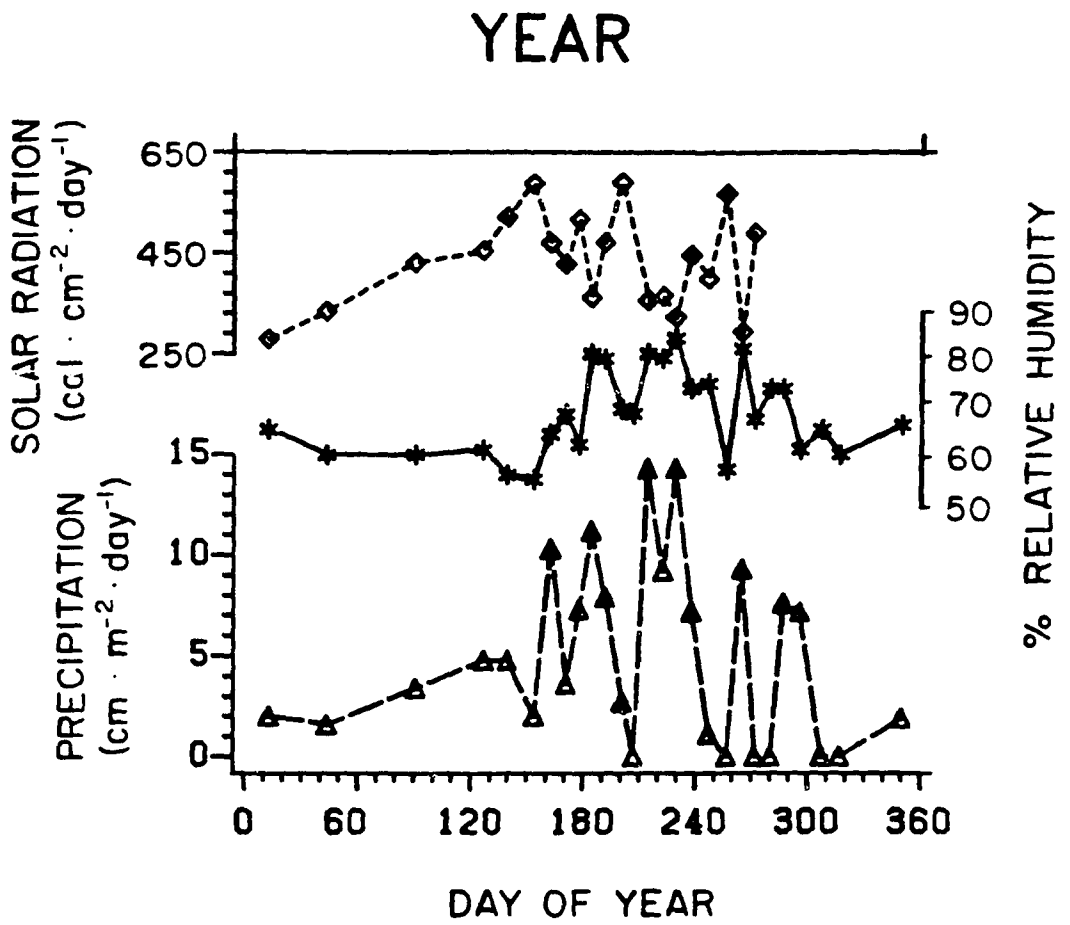

FIGURE 15. Precipitation (△), relative humidity (✱), and estimated surface solar radiation (◇) for 1980 at Hangzhou, China. Points are averages from each growth period.

correlated with any single climatic parameter or combination of them over the entire one year study period (Table 5). In order to determine which climatic variables had the greatest effect on RGR, it was necessary to divide the study period into three seasons. These were defined as a season of increasing temperature and increasing RGR (Spring) from March through June, a season of high temperatures and fluctuating RGR (Summer) from July through September and a season of decreasing temperature and decreasing RGR (Fall) from October through December. Additionally spring and fall observations were combined (Cool season). The spring and fall seasons do not include the overwintering period from January through February. Raw data for all variables are presented in Appendix B.

No single climatic factor accounted for greater than 37% of variation in the RGR of all azolla accessions during the course of the whole year (Table 5). If the observations are subdivided into the seasons described above, most of the variation in RGR during Spring was accounted for by the temperature variable. The R^2 for the RGR-temperature relationship was low when Spring and Fall were combined (Cool) and for Summer.

RGR Over the One Year Study Period

The curves of RGR over time were of similar shape for the eight azolla accessions over the one year growth period. Although some variation among accessions was observed (Figures 16 to 19), the RGR of all accessions showed two peaks during the year. Seven of the eight accessions showed these peaks during the June 27th and September 29th

TABLE 5. Coefficients of determination (R^2) for a linear model (Y = a + bX) of the combined relative growth rates of all accessions and climatic variables[a].

VARIABLE	YEAR[b]	SPRING	FALL	COOL	SUMMER
Maximum air temperature	0.34	0.52	0.60	0.35	0.02
Minimum air temperature	0.32	0.48	0.77	0.33	0.20
Mean air temperature	0.34	0.52	0.76	0.35	0.09
Maximum water temperature	0.31	0.41	0.60	0.23	0.03
Minimum water temperature	0.37	0.52	0.73	0.42	0.19
Mean water temperature	0.35	0.49	0.69	0.33	0.10
Percent relative humidity	0.10	0.09	0.50	0.18	0.07
Mid-day klux	0.08[c]	0.04	-	-	0.10
Mean precipitation	0.01	0.02	0.02	0.01	0.11
Actual solar radiation	0.03[d]	0.03	-	-	0.10
Clear sky solar radiation	0.08	0.20	0.73	0.04	0.06
Minutes of daylight	0.08	0.34	0.69	0.05	0.12
Number of Samples	177	52	42	94	75

[a] R^2 values were calculated for the whole Year, Spring, Fall, a combination of Spring and Fall (Cool), and Summer. Mean observations from azolla growth cycles were used in the calculations.

[b] These time periods include the following months: Year (January through December), Spring (April through June), Fall (October through December), Summer (July through September).

[c] n=146

[d] n=141

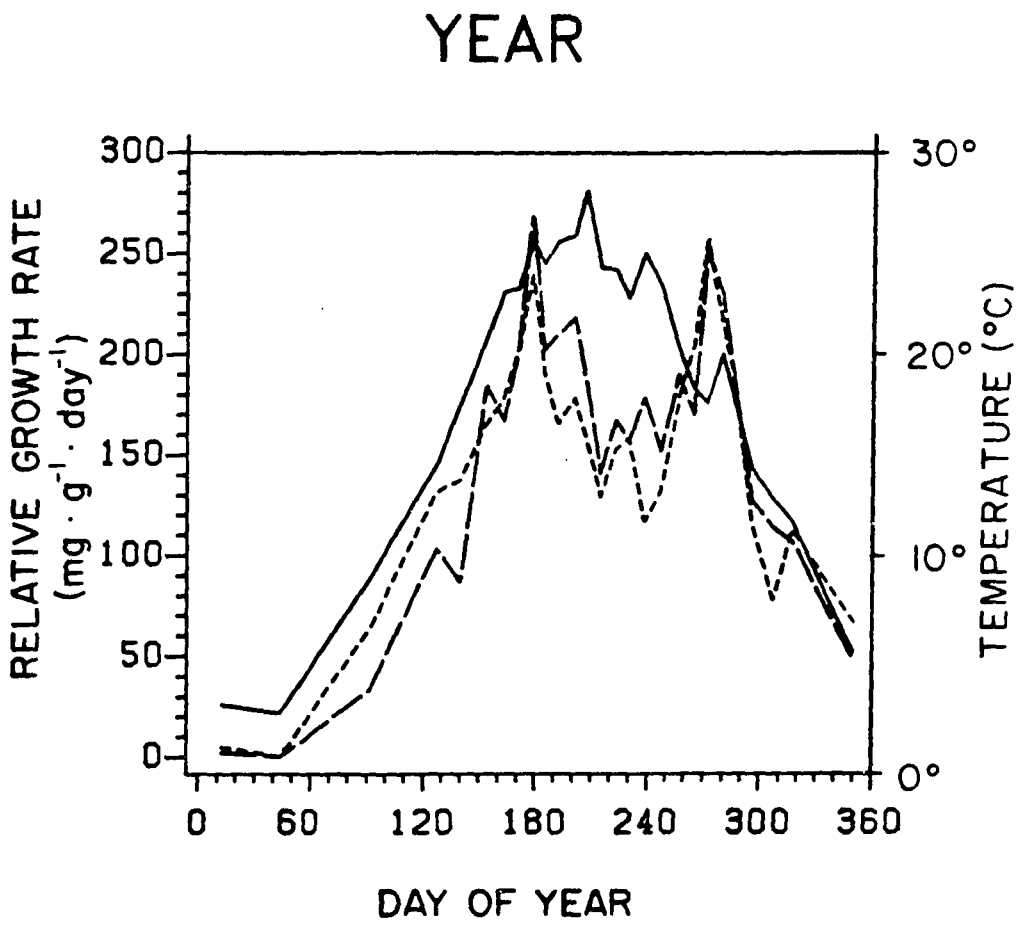

FIGURE 16. Minimum water temperature (solid line) and relative growth rates of A. caroliniana (short dashes) and A. pinnata var. imbricata (long dashes) during 1980 at Hangzhou, China. Lines connect averages from each growth period.

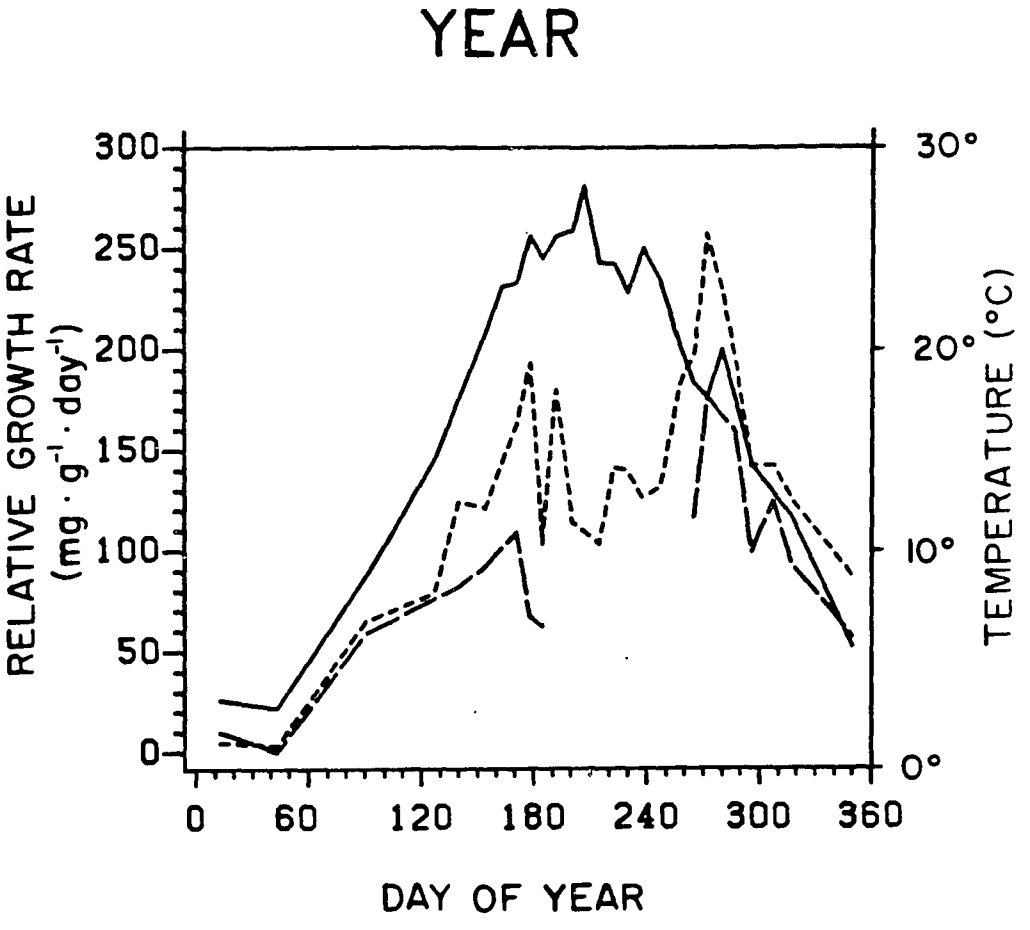

FIGURE 17. Minimum water temperature (solid line) and relative growth rates of A. filiculoides (short dashes) and A. rubra (long dashes) during 1980 at Hangzhou, China. Lines connect averages from each growth period.

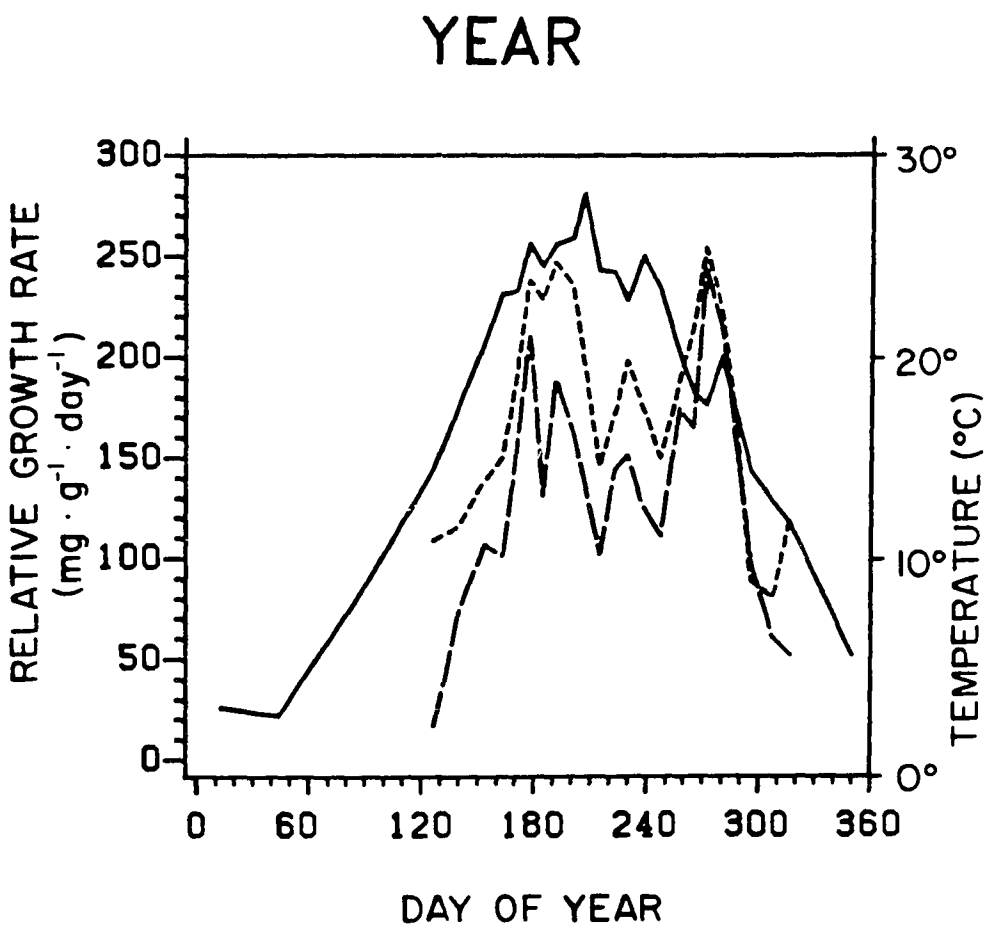

FIGURE 18. Minimum water temperature (solid line) and relative growth rates of A. microphylla (short dashes) and A. mexicana (long dashes) during 1980 at Hangzhou, China. Lines connect averages from each growth period.

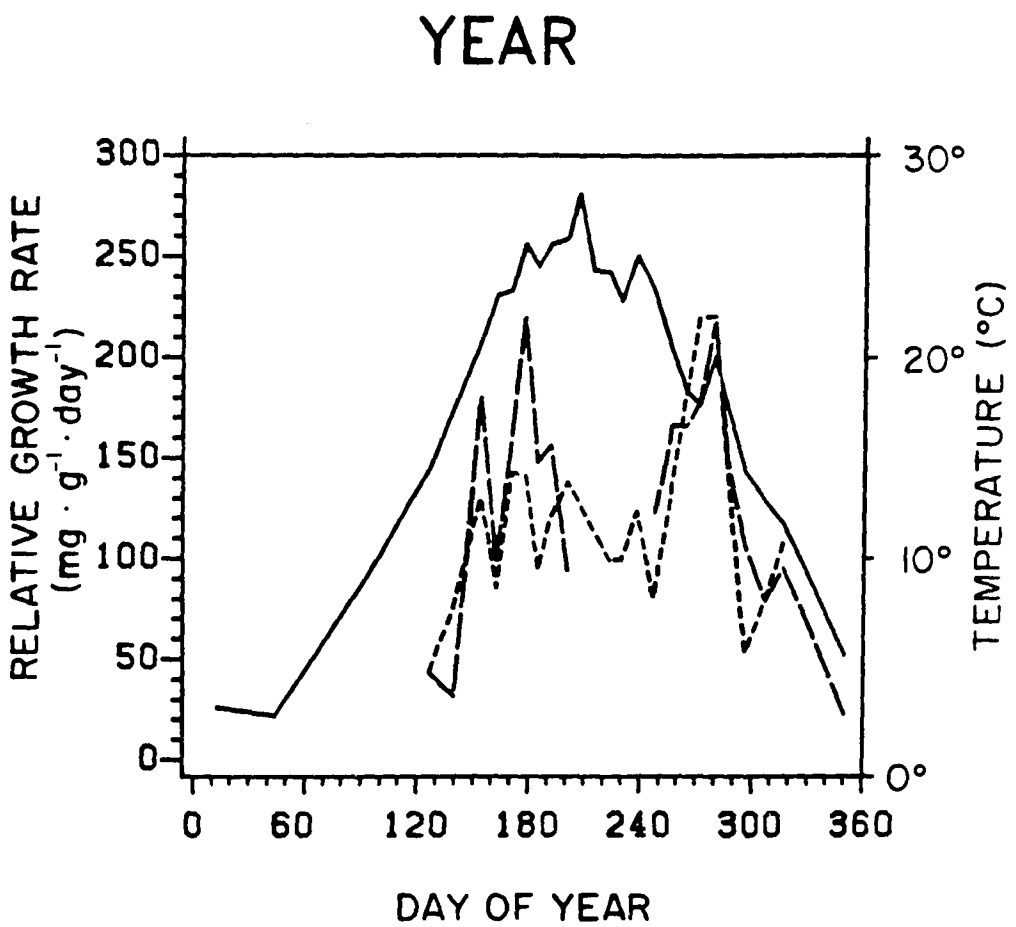

FIGURE 19. Minimum water temperature (solid line) and relative growth rates of A. nilotica (short dashes) and A. pinnata var. pinnata (long dashes) during 1980 at Hangzhou, China. Lines connect averages from each growth period.

growth cycles.

Three accessions (A. caroliniana, A. filiculoides, A. pinnata var. imbricata) had sufficiently wide climatic adaptation to survive most of the year (Figures 16 and 17). The remaining accessions either did not survive the colder months (Figures 18 and 19), or were heat intolerant (A. rubra, Figure 16).

No combination of up to three independent variables and their interactions could satisfactorily account for the variability in the combined RGRs of the eight accessions over the one year study period. When RGR was regressed on temperature for individual accessions, only models for the Hangzhou accession, A. pinnata var. imbricata, and to a lesser extent two American accessions, A. caroliniana and A. filiculoides, approached significance (Table 6). Best fit linear regression models of up to three climatic variables could explain 82, 62 and 59% respectively of the RGR varieties of these three accessions. However, even these models for annual data were unacceptable because they could not adequately describe the variations in RGR which occurred during the summer period when RGRs were high. Most of the observations used in development of the annual models occurred during cool-cold weather when RGR's were positively correlated with minimum water temperature. The annual models could not adequately describe variations which occurred during the summer period because summer RGR's of most accessions were negatively correlated with minimum water temperature (Figures 16 to 19, Table 6).

TABLE 6. Coefficients of determination (R^2) for a linear model of azolla relative growth rates (Y) and mean minimum water temperatures (X) during 1980. The calculations are based on the mean of six replications of each accession from each growth cycle.

AZOLLA ACCESSION	YEAR		SPRING		FALL		COOL		SUMMER	
	R^2	n[a]	R^2	n	R^2	n	R^2	n	R^2	n
A. caroliniana	0.58	26	0.95	7	0.80	6	0.86	13	0.49	11
A. filiculoides	0.33	26	0.90	7	0.93	6	0.44	13	0.65	11
A. mexicana	0.23	22	0.89	6	0.99	5	0.51	11	0.33	11
A. microphylla	0.39	22	0.80	6	0.78	5	0.63	11	0.04	11
A. nilotica	0.03	21	0.69	6	0.67	5	0.24	11	0.56	10
A. pinnata var. pinnata	0.34	19	0.69	6	0.92	6	0.52	12	0.52	7
A. pinnata var. imbricata	0.72	26	0.89	7	0.95	6	0.79	13	0.05	11
A. rubra	0.27	17	0.94[b]	6	0.83	5	0.24[b]	11	-	-

[a] number of samples.

[b] observation of June 27 deleted from model.

RGR During Spring, Fall, and Cool Seasons

Spring Season.

The spring climate in Hangzhou (March through June) was characterized by increasing air and water temperatures, solar radiation, and the RGR of the eight azolla accessions also increased. Spring RGR was linearly related to and highly correlated with temperature (Tables 5 and 6, Figures 16 to 19). Spring RGR (Y) of azolla can be predicted by the equation $Y=a+bX$, where Y = $mg \cdot g^{-1} \cdot day^{-1}$, a = the intercept, b = the coefficient or slope, and X = minimum water temperature in ^{o}C. The equation explains up to 80% of the variation in RGR's of the accessions (Figures 20 and 21). In general, each one degree increase in minimum water temperature (X) will result in an RGR (Y) increase of 3 to 15 $mg \cdot g^{-1} \cdot day^{-1}$.

The spring season includes the period when applied azolla management activities of nursery multiplication and field cultivation are occurring. The most important criteria of economic consideration in the spring linear models are those which affect these management activities. These criteria include the parameters of the models, the intercepts, and the coefficients (slopes) of the lines. Using these criteria, RGR data for spring can be used to divide the eight azolla accessions into two groups on the basis of tolerance to low water temperature. The accessions A. caroliniana, A. filiculoides, A. pinnata var. pinnata, and A. rubra grew during periods when minimum water temperatures dropped to $9^{o}C$. This is reflected by the Y axis intercepts (a) (Figures 20 and 21) which gives an indication of the cold tolerance of these four accessions. In general, an accession with greater cold

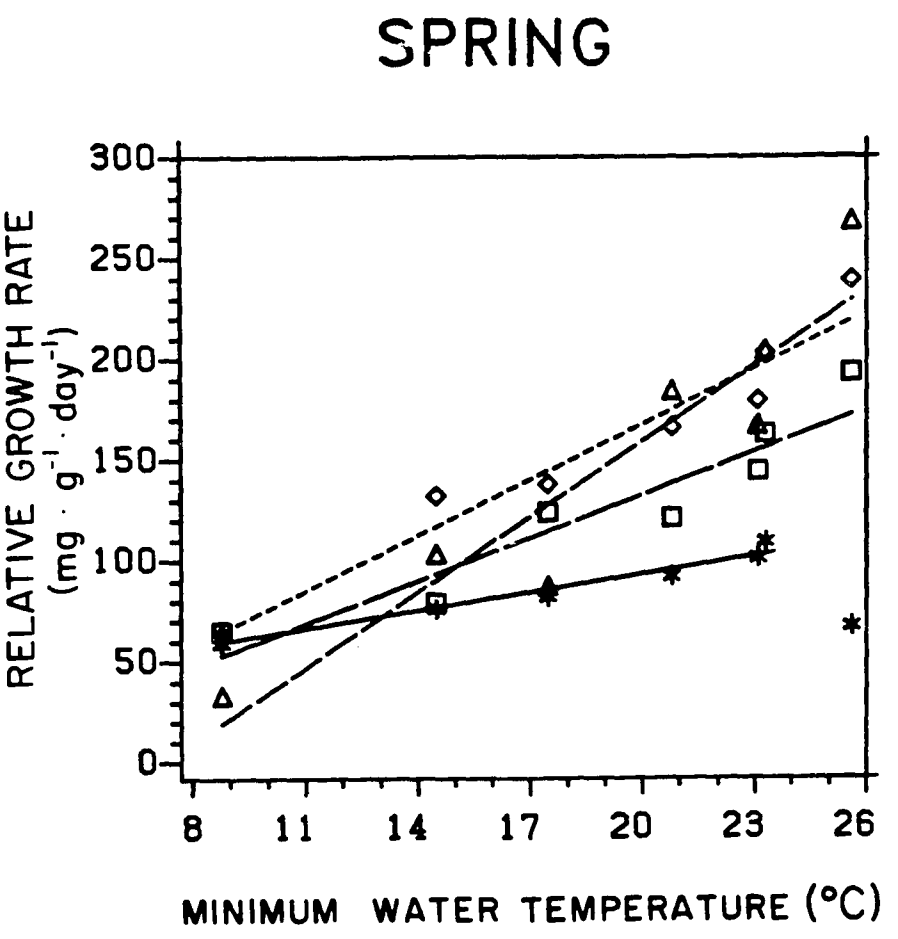

FIGURE 20. Relative growth rates of four azolla accessions during spring as a function of minimum water temperature. Symbols represent observed RGRs and lines represent linear regression of observed RGRs. Azolla accessions are represented by the following symbols and lines (the equation for each line is also given): A. caroliniana, ◇ and short dashes (y=-16.4 + 9.2x); A. filiculoides, □ and long dashes (y=-9.6 + 7.1x); A. pinnata var. imbricata, △ and medium dashes (y=-90.9 + 12.5x); A. rubra, * and solid line (y=33.2 + 3.0x).

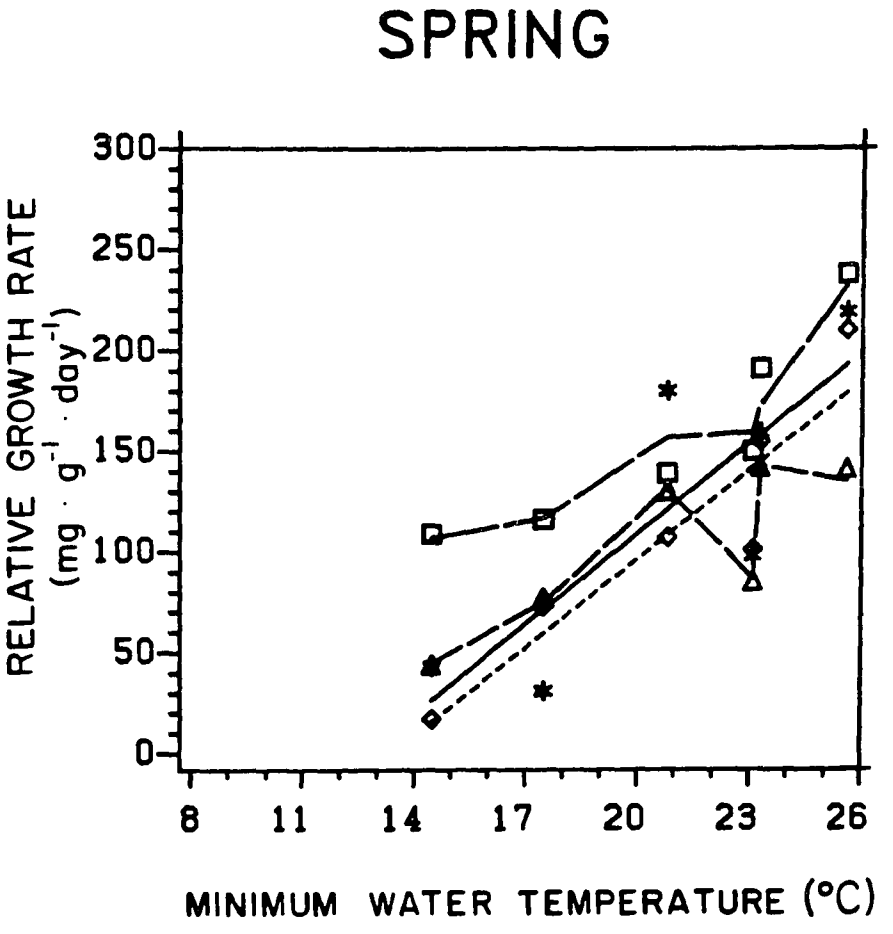

FIGURE 21. Relative growth rates of four azolla accessions during spring as a function of minimum water temperature. Symbols represent observed RGRs and lines represent linear regression of observed RGRs. Azolla accessions are represented by the following symbols and lines (the equation for each line is also given): A. mexicana, ◇ and short dashes (y=-197.4 + 14.7x); A. microphylla, □ and long dashes (for y see table 7); A. nilotica, △ and medium dashes (for y see table 7); A. pinnata var. pinnata, ✱ and solid line (y=-190.9 + 15.0x).

tolerance would be expected to have greater initial biomass and be more productive at the start of the spring season than accessions with less cold tolerance.

The magnitude of RGR change with change in temperature is an important characteristic of production and is indicated by the slope of the RGR-temperature response curve. Accessions with a high RGR at low temperature and a steep slope generally have the highest productivity. A. caroliniana has one of the highest RGRs at the start of spring and its RGR increases more rapidly with increasing temperature than do most of the other species. The linear model for A. caroliniana would predict a doubling time ($\ln 2 \cdot RGR^{-1} \cdot 1000$) of 11 days at the beginning of the spring season and 2.9 days at the end of the season. It is the best of the four cold tolerant accessions for spring multiplication (Figure 20).

The importance of cold tolerance makes A. filiculoides appear to be the second choice for overwintering in Hangzhou. A. pinnata var. imbricata would be one of the two best choices in areas with milder winters because of its steep increase in RGR with increasing temperature. A. rubra was very cold tolerant and the shallow slope of the RGR/temperature curve indicates that it is only suitable for regions colder than Hangzhou where it is too cold for other accessions to grow. Of the remaining four accessions (Figure 21), only A. microphylla was sufficiently responsive to increasing temperature to be considered for spring cultivation, but only in regions with slightly milder winters than Hangzhou.

Fresh weight doubles in one week at a RGR of about 100 $mg \cdot g^{-1} \cdot day$. This doubling time was reached by most of the cold tolerant accessions

(Figure 20) and A. microphylla during the spring at minimum water temperature of about 13 to 15°C. This RGR was not reached by the cold intolerant accessions (Figure 21) until temperatures ranged from 19 to 21°C.

Fall Season.

During fall temperature and RGR of azolla declined. The RGR of azolla during the fall season was highly dependent upon temperature, as during the spring season (Tables 5 and 6) and the RGR-temperature relationship can be explained by the same simple linear model (Figures 22 and 23). However, the the models for fall indicate that the accessions were generally more cold tolerant than the same accessions described by the spring models.

Many factors affect the ranking of accessions for fall cultivation. Productivity in the field during fall first depends upon how much azolla is available after a hot season, and since this cannot be inferred from the RGRs, survival and growth during summer must be considered (Figures 16 to 19). Accessions which survive the summer season and attain a relatively high RGR at temperatures above 25°C before the fall season would be able to produce more inoculum for fall cultivation. Accessions which have a high and slowly declining RGR during the declining temperatures of fall could produce more biomass and have greater overwintering tolerance. In consideration of these criteria, A. rubra, A. nilotica and A. pinnata var. pinnata are unsuitable for cultivation in the fall because of death and low RGRs during summer. The remaining accessions, ranked on the basis of high(intercept) and slowly

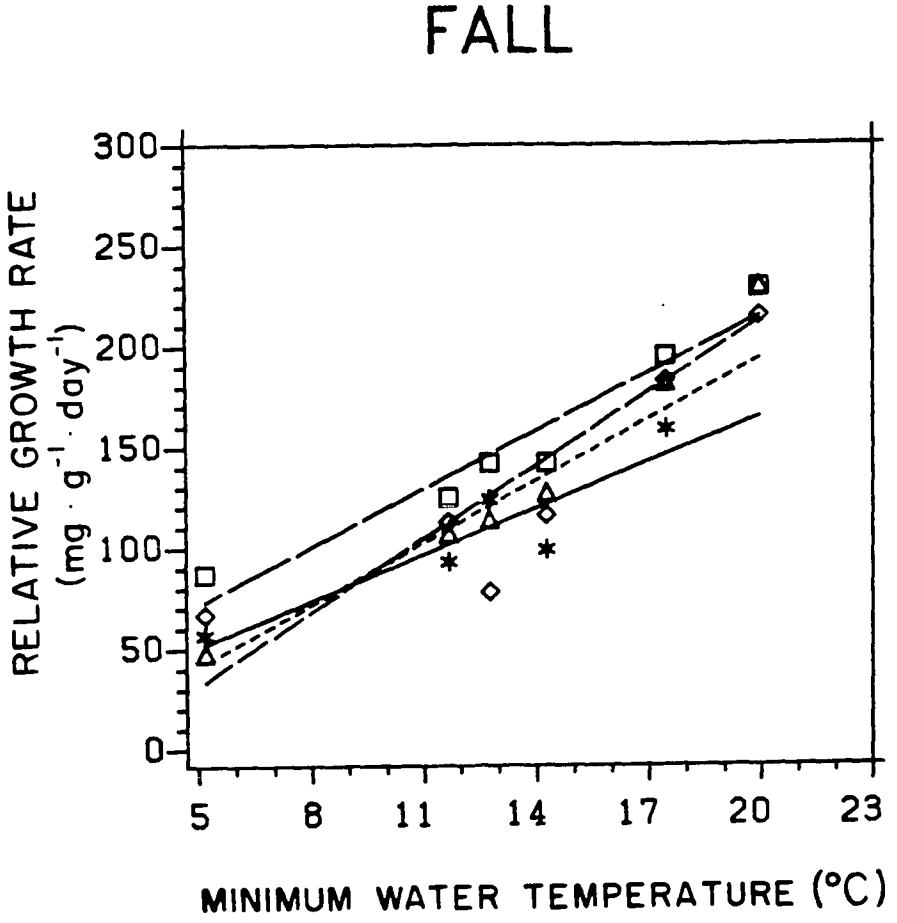

FIGURE 22. Relative growth rates of four azolla accessions during fall as a function of minimum water temperature. Symbols represent observed RGRs and lines represent linear regression of observed RGRs. Azolla accessions are represented by the following symbols and lines (the equation for each line is also given): A. caroliniana, ◇ and short dashes (y=-10.2 + 10.2x); A. filiculoides, □ and long dashes (y=23.3 + 9.6x); A. pinnata var. imbricata, △ and medium dashes (y=-29.2 + 12.1x); A. rubra, * and solid line (y=12.5 + 7.6x).

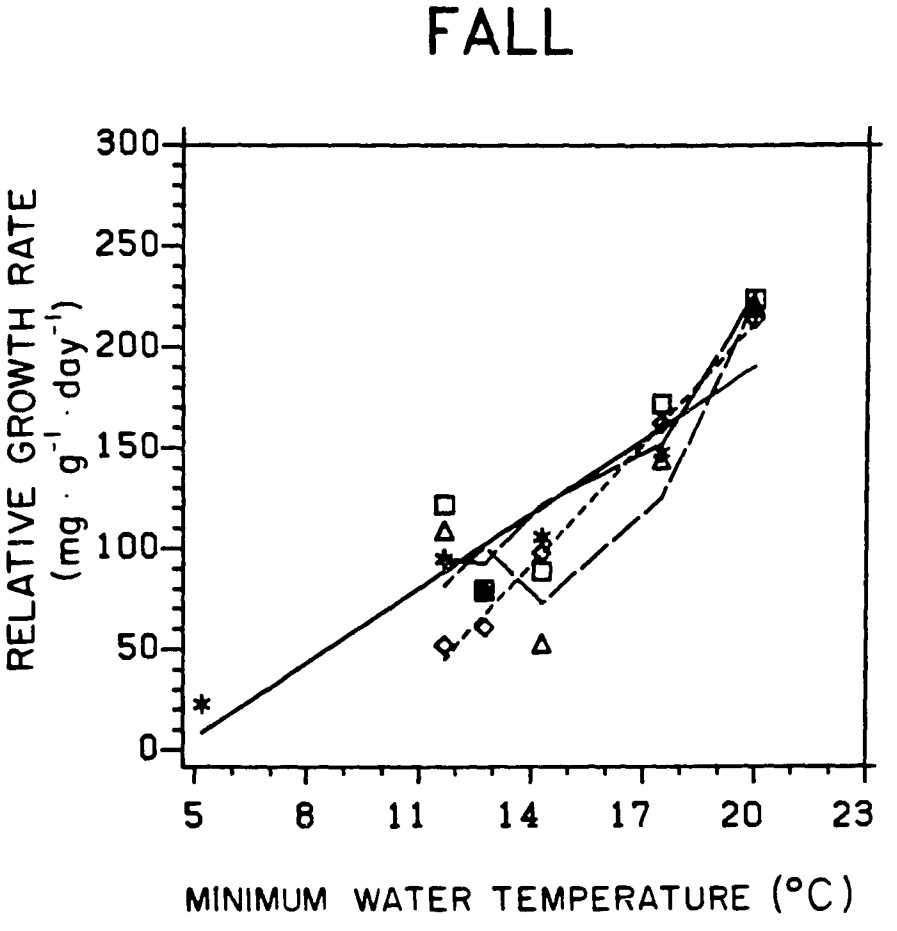

FIGURE 23. Relative growth rates of four azolla accessions during fall as a function of minimum water temperature. Symbols represent observed RGRs and lines represent linear regression of observed RGRs. Azolla accessions are represented by the following symbols and lines (the equation for each line is also given): A. mexicana, ◇ and short dashes (y=-190 + 20.1x); A. microphylla, □ and long dashes (for y see table 7); A. nilotica, △ and medium dashes (for y see table 7); A. pinnata var. pinnata, * and solid line (y=-55.4 + 12.3x).

declining (slope) RGR, are A. filiculoides >A. pinnata var. imbricata >A. caroliniana >A. microphylla >A. mexicana.

A. filiculoides began the fall season with an RGR of 230 $mg \cdot g^{-1} \cdot day^{-1}$ (3 days doubling time) and maintained an RGR greater than 100 until minimum water temperature reached about 8°C. Based on regressions of RGR on temperature, the other accessions would be expected to drop below RGR of 100 at minimum water temperature of 10°C or higher. During the fall, a RGR of 100 occurs at a minimum water temperature which is about 6°C cooler than during the spring. This differential response to temperature is described in the Cool Seasons section below.

The coefficients of determination (R^2) for the simple linear models of RGR on minimum water temperature for the spring and fall seasons were fairly high for most of the accessions. However the R^2 values for A. microphylla and A. nilotica were somewhat low, indicating that other variables significantly influenced RGR. The addition of clear sky solar radiation to the spring and fall models of A. microphylla, increased the R^2 value from 80 to 94 and 78 to 84% respectively (Table 6). The addition of centimeters of precipitation into the spring and fall models of A. nilotica, increased the R^2 value from 69 to 99 and 67 to 88% respectively (Table 7). Since the accessions were protected from precipitation by a glass roof, A. nilotica must have responded to other factors associated with precipitation.

Cool Seasons

Combining the results for the spring and the fall seasons allowed for analysis of a larger population. Equations developed from regressing RGR on minimum water temperature during the cool season could

TABLE 7. Multiple regression equations for RGR (Y) during the spring and fall seasons including mean minimum water temperature (X_1) and their coefficients of determination. (The inclusion of clear sky solar radiation for latitude 30°N (X_2) for A. microphylla and centimeters of precipitation for A. nilotica improved coefficients of determination.)

		X_1 + X_2		LINEAR MODELS
	n	R^2	R^2	$Y = b + a_1X_1 + a_2X_2$
A. microphylla				
spring	6	0.80	0.94	$Y = 3555 + 16.8X_1 + (-)5.12X_2$
fall	5	0.78	0.84	$Y = 1013 + 60.7X_1 + (-)3.65X_2$
A. nilotica				
spring	6	0.69	0.99	$Y = (-)60.4 + 9.97X_1 + (-)8.17X_2$
fall	5	0.67	0.88	$Y = (-)118 + 17.1X_1 + (-)7.42X_2$

not adequately fit the observed RGRs of any accession except A. caroliniana and A. pinnata var. imbricata (Table 6). However, when minutes of daylight was added to the temperature model, high R^2 values were obtained for most accessions (Table 8). Since the RGRs of most accessions were positively correlated with daylength during spring and fall (Table 5), the merging of spring and fall RGRs should include adjustments for the daylength which coincides with a given temperature. However, the RGR of A. caroliniana did not require adjustment of temperature to daylength and appeared to be relatively insensitive to daylength (Table 8). As in their equations for spring and fall, the equations for A. microphylla and A. nilotica required the inclusion of clear sky solar radiation and centimeters of precipitation, respectively, to adequately fit their observed RGRs.

In addition to the analysis of variables affecting the combined RGRs for spring and fall, winter RGRs can be compared with the combined spring and fall RGRs to determine which accessions were most cold tolerant. This is important because azolla plants must be maintained during off-seasons to provide a source of inoculum at the start of the next growing season. Those which are more tolerant of off-season stresses will be cheaper to maintain and more productive. Protective management procedures can either be reduced or eliminated and the azolla cropping seasons before and after the stress-period can be extended. As seen in Figure 24, the RGRs of the four cold tolerant accessions, A. caroliniana, A. filiculoides, A. pinnata var. imbricata, A. rubra, were higher at mean minimum water temperatures above 9°C during the fall than during the spring. Minimum water temperature of 2.0°C appear to be near

TABLE 8. Multiple regression equations for the RGR (Y) during the cool seasons. The model includes mean minimum water temperature (X_1) and minutes of daylight (X_2). Equations for *A. microphylla* and *A. nilotica* include clear sky solar radiation (X_3) and centimeters of precipitation (X_3) respectively.

AZOLLA ACCESSIONS	n	X_1 + X_2 + X_3 R^2	R^2	R^2	$Y=a+b_1X_1+b_2X_2(+b_3X_3)$
A. caroliniana	13	0.86	0.89	-	$Y=97.5+10.5X_1+(-)0.168X_2$
A. filiculoides	13	0.44	0.91	-	$Y=374+11.2X_1+(-)0.561X_2$
A. mexicana	11	0.56	0.88	-	$Y=298+19.4X_1+(-)0.709X_2$
A. microphylla	11	0.63	0.77	0.86	$Y=1035+17.0X_1+(-)2.88X_2+1.61X_3$
A. nilotica	11	0.24	0.59	0.82	$Y=241+14.0X_1+(-)0.465X_2+(-)7.64X_3$
A. pinnata var. *pinnata*	12	0.52	0.79	-	$Y=325+16.7X_1+(-)0.661X_2$
A. pinnata var. *imbricata*	13	0.79	0.92	-	$Y=229+15.0X_1+(-)0.446X_2$
A. rubra	11	0.24	0.73	-	$Y=259+6.89X_1+(-)0.362X_2$

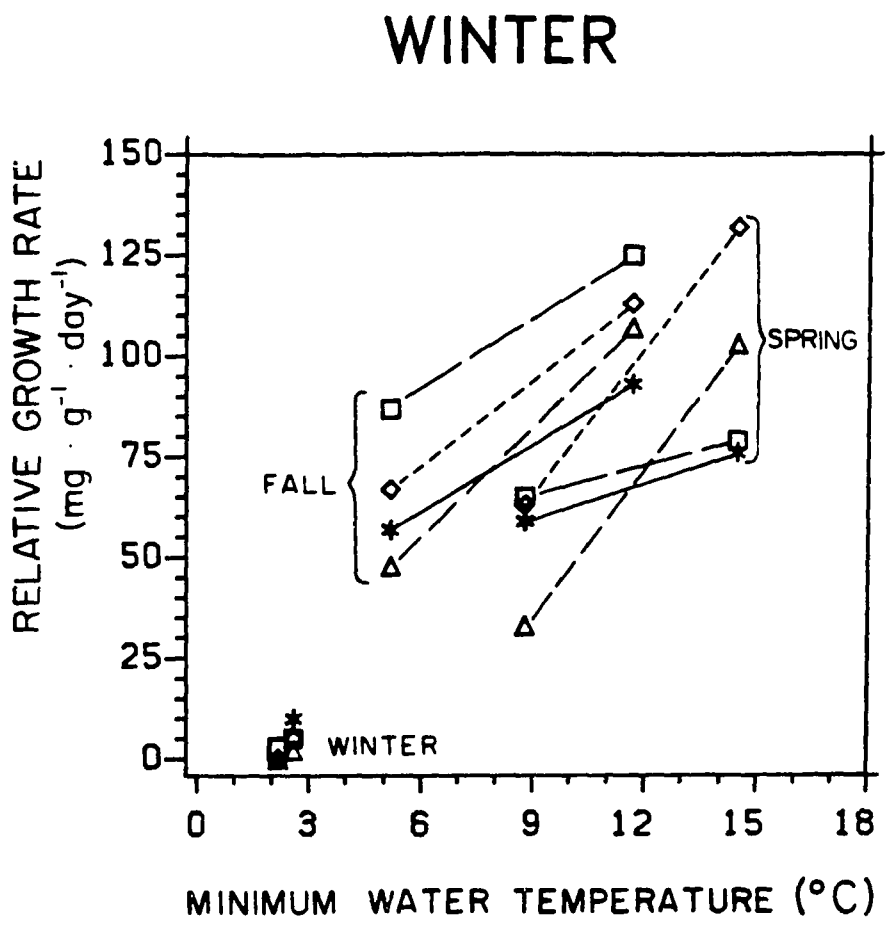

FIGURE 24. The relationship of early spring and late fall RGRs to the RGRs of the two winter growth cycles, as a function of minimum water temperature. Symbols represent observed RGRs. Azolla accessions are represented by the following symbols: A. caroliniana, ◇ ; A. filiculoides, □ ; A. pinnata var. imbricata, △ ; A. rubra, * .

the threshold for growth.

The ranking of cold tolerant accessions for the combined spring and fall season was A. filiculoides >A. caroliniana >A. pinnata var. imbricata >A. rubra and was similar though less different than the separate spring and fall rankings. A. filiculoides and A. caroliniana were ranked first and second because their productivity at the beginning of spring and at the end of fall exceeded that of A. pinnata var. imbricata and their productivity over the remaining parts of the two seasons far exceeded that of A. rubra (Figures 20 to 23).

RGR During the Summer Season

Relative growth rates during the summer season were not linearly related to one or a combination of two climatic variables as was the case during the cool season. Plots of RGRs for the year (Figures 16 to 19) show that summer season RGRs were characterized by peaks and valleys that were unrelated to individual climatic variables.

In the spring, the RGR of azolla was positively correlated with increasing minimum temperature until the high temperatures of June began affecting growth. From this point, the RGRs of most accessions were negatively correlated with minimum temperature and remained so throughout the summer. The exact temperature at which the correlation changes from positive to negative probably varies according to the genotypic and phenotypic character of the accession. The RGRs observed in summer were highly variable due to the hot and humid conditions during July which were conducive to growth of a Rhizoctonia sp., a fungal pathogen. In addition, all eight accessions either died or

stopped growing at the end of the July 20th growth cycle because of a one week period when high water temperatures averaged 36.6°C with a one day extreme of 39.7°C and low water temperatures averaged 28.1°C. The high temperature effect on the accessions was probably exacerbated by the fungal attack.

Although Rhizoctonia sp. has been found with azolla everywhere in the world under hot humid conditions, fungicides were not used in this study since they add to the cost of azolla production and pose a serious environmental threat to fish if used on a large scale under azolla's aquatic growth conditions.

The pattern of fungal attack on an azolla mat was random rather than uniform. Susceptible accessions showed a large standard deviation of RGR (six replications) during the summer, while resistant accessions had a small standard deviation. During the four growth cycles from 20 July to 3 August when fungal attack was most common, accessions with the lowest standard deviations of RGR ($mg \cdot g^{-1} \cdot day^{-1}$) were A. microphylla (9), A. pinnata var. imbricata (12), A. mexicana (19) and A. caroliniana (22).

The variation in RGR at high temperature resulted in a poor correlation between RGR and any single climatic variable during the summer. Correlation analysis was then used to show the relationship of these climatic variables to the RGRs of individual accessions and to each other (Table 9). The RGR's of the six accessions were negatively correlated with minimum water temperature and relative humidity, and positively correlated with solar radiation. Solar radiation and relative humidity were highly and negatively correlated but the

TABLE 9. Simple correlation coefficients(r) between the RGRs of azolla accessions and several climatic variables occurring during the summer season.

	MINIMUM WATER TEMPERATURE	SOLAR RADIATION	RELATIVE HUMIDITY	MAXIMUM WATER TEMPERATURE
A. caroliniana	-0.70	0.20	-0.27	-0.43
A. filiculoides	-0.81	0.19	-0.36	-0.46
A. mexicana	-0.58	0.43	-0.40	-0.17
A. microphylla	-0.21	0.34	-0.09	-0.01
A. nilotica	-0.75	0.26	-0.36	-0.41
A. pinnata var. *imbricata*	-0.23	0.62	-0.46	0.18
Minimum water temperature	-	0.08	0.31	0.79
Solar radiation	0.08	-	-0.84	0.65
Relative humidity	0.31	-0.84	-	-0.29

correlation between minimum water temperature and solar radiation was poor. However solar radiation was positively correlated with maximum air and water temperatures (0.71 and 0.62) and thus represented them through interaction in the summer model. When the product of minimum water temperature times solar radiation was added to the model as a fourth variable, it increased the R^2 values by an average of 0.05. The Y intercepts, R^2 values and number of observations of each model describing the RGR of each accession are presented in Table 10 and are plotted in Figures 25 and 26.

The failure of simple regression analysis to account for the variability of RGRs during summer, prompted an analysis by multiple linear regression (Tables 5 and 6). All possible combinations of up to four independent variables were evaluated with the RSQUARE procedure of the Statistical Analysis System Institute (Helwig and Council, 1979). A. pinnata var. pinnata and A. rubra were not included in this evaluation because of an insufficient number of observations. High R^2 values were obtained for every accession with a model containing minimum water temperature, solar radiation, and relative humidity.

Disease and heat stress during the summer increased the variability of the RGRs and thus must be considered in addition to productivity. Because of this, accessions grown during the summer were ranked on the basis mean RGR's and standard deviations across the 11 summer growth cycles times 6 replications (Table 11), rather than on the basis of coefficients from linear equations. Mean RGRs give an indication of productivity while the standard deviation gives a measure of variability.

TABLE 10. A multiple linear model and equations for predicting RGR ($mg \cdot g^{-1} \cdot day^{-1}$) of six azolla accessions during the summer. The model uses the following climatic variables: minimum water temperature (X_1), solar radiation (X_2), relative humidity (X_3), and X_1 times X_2 (X_4).

ACCESSIONS	$Y=a+b_1X_1+b_2X_2+b_3X_3+b_4X_4$	R^2	n
A. caroliniana	$Y=(-)569+(-)4.45X_1+1.31X_2+7.83X_3+(-)0.0305X_4$	0.80	11
A. filiculoides	$Y=(-)571+(-)2.37X_1+1.54X_2+7.16X_3+(-)0.0420X_4$	0.85	11
A. mexicana	$Y=(-)1002+3.72X_1+1.97X_2+9.74X_3+(-)0.0502X_4$	0.91	11
A. microphylla	$Y=(-)912+(-)3.05X_1+1.40X_2+11.0X_3+(-)0.0257X_4$	0.74	11
A. nilotica	$Y=23.9+(-)21.6X_1+0.254X_2+5.17X_3+0.0108X_4$	0.86	10
A. pinnata var. imbricata	$Y=(-)762+5.83X_1+1.52X_2+7.13X_3+(-)0.0370X_4$	0.74	11

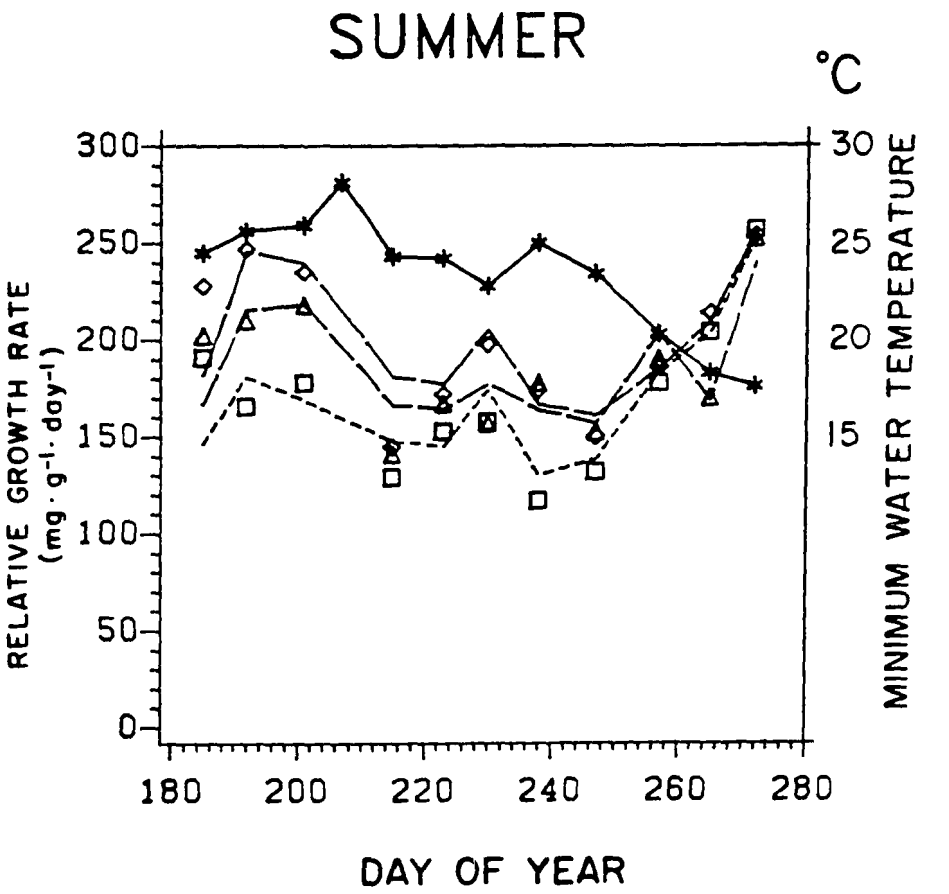

FIGURE 25. Minimum water temperature (✱), observed relative growth rates ($mg \cdot g^{-1} \cdot day^{-1}$) of A. caroliniana, □ ; A. microphylla, ◇ ; and A. pinnata var. imbricata, △ , during summer, and regressions of their RGRs based on models incorporating minimum water temperature, solar radiation, and relative humidity.

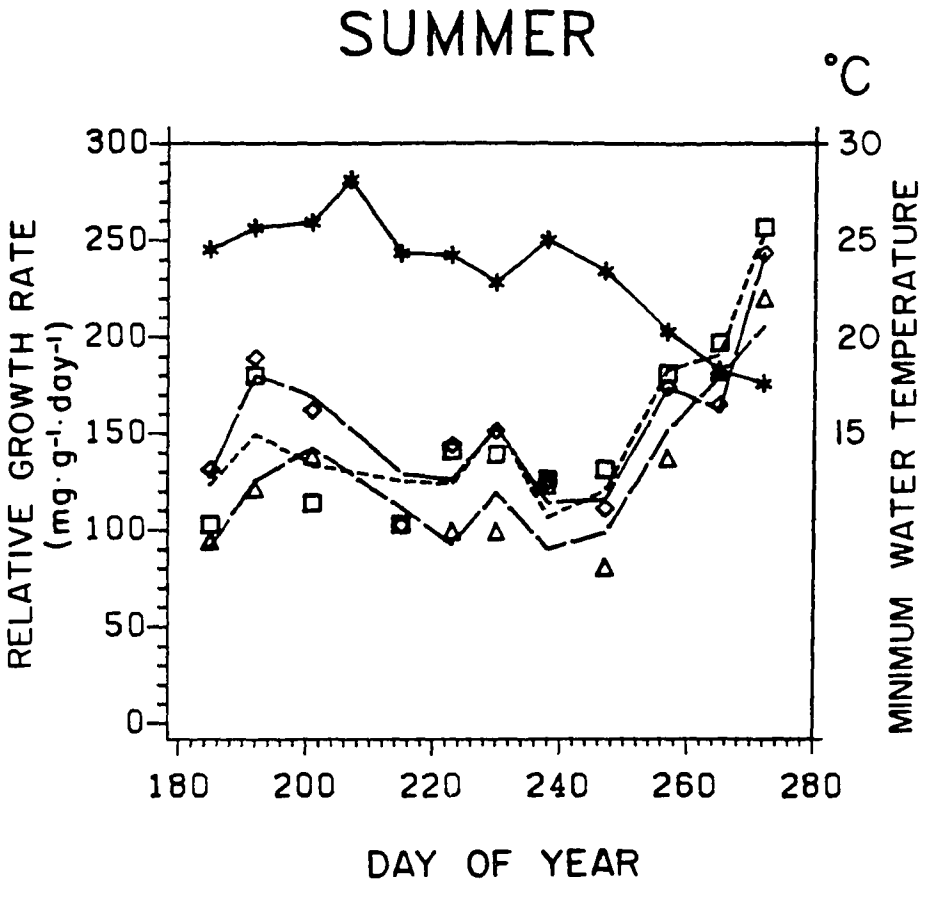

FIGURE 26. Minimum water temperature (✱), observed relative growth rates ($mg \cdot g^{-1} \cdot day^{-1}$) of A. filiculoides, □ ; A. mexicana, ◇ ; and A. nilotica, △ , during summer, and regressions of their RGRs based on models incorporating minimum water temperature, solar radiation, and relative humidity.

TABLE 11. Mean relative growth rates ($mg.g^{-1}.day^{-1}$), standard deviations, and ranges of six accessions grown during the summer of 1980 at Hangzhou, China. These values were derived from mean RGRs of growth cycles and do not include variation within growth cycle replicates.

AZOLLA ACCESSIONS	n	MEAN	STD. DEV.	RANGE	
				MINIMUM	MAXIMUM
		$mg \cdot g^{-1} \cdot day^{-1}$			
A. caroliniana	11	169	40	117	257
A. filiculoides	11	152	47	103	257
A. mexicana	11	154	40	102	243
A. microphylla	11	200	38	145	254
A. nilotica	10	129	43	80	220
A. pinnata var. imbricata	11	185	33	141	252

A. microphylla and A. pinnata var. imbricata had the highest mean RGRs and lowest standard deviations (Table 11). These two accessions were superior because they were relatively disease free and tolerated the high water temperature of summer. The RGRs of both A. microphylla and A. pinnata var. imbricata were poorly correlated with mean minimum and maximum water temperatures, which may indicate heat tolerance. A. microphylla was also more resistant to fungal attack. This may be indicated by its poor correlation with relative humidity, since fungal attack is always associated with high relative humidity. The RGR of A. pinnata var. imbricata had the strongest correlation with solar radiation, which may indicate its ability to utilize solar radiation and/or increase its RGR with high temperature. Although A. mexicana had a relatively low mean RGR, correlation coefficients for summer were similar to those of A. microphylla and A. pinnata var. imbricata. Its lower mean RGR may be partially explained by its continuous production of spores, which consumes photosynthate but does not add to RGR.

The lower productivity of other accessions was assumed to be caused by the high temperatures which prevailed during summer. This is substantiated by the negative r values between RGR and water temperature (Table 9).

CONCLUSIONS

Studies have been carried out on the effects of single climatic variables, usually temperature, on the relative growth rate of azolla (Lu et al., 1963; Tran and Dao, 1973; Ashton, 1974; Peters et al., 1980; Talley and Rains, 1980), but no reports have been found of studies

combining numerous climatic variables with the relative growth rates of all known Azolla species.

The year-round pot experiment carried out at Hangzhou was of sufficient magnitude and breadth to allow a statistical assessment of the effect of climatic variables on the relative growth rate (RGR) of all known Azolla species. Equations developed to predict the RGR of the species during particular seasons probably have little application outside of Hangzhou. However, models for these equations may have broader application. Testing of a model at any particular location and comparing it to other models is essential. This is illustrated by the fact that the cool season model for A. filiculoides developed by Talley and Rains (1980), involving the log of maximum air temperature, proved helpful though inferior to the models reported herein for all accessions in all seasons because it was based on a narrow climatic range.

Before testing, an appropriate model should be selected on the basis of season and location. Use of the spring and fall RGR model involving mean minimum water temperature, will probably be limited to certain seasons such as spring and fall in temperate locations when daylength is either constant, decreasing, or increasing. In subtropical locations, such as northern Vietnam, where the winter cool season is associated with a reversal of daylength, the cool season RGR model involving temperature and daylength could be tested. The summer RGR model involving temperature, solar radiation, and relative humidity may be applicable during most of the year in tropical locations and during summer in temperate and subtropical locations. Of the three models the applicability of the summer model is probably the most controversial and

possibly of the most importance because of the present unpredictability of azolla RGR during hot weather. The major weakness of the summer model, is that it cannot predict heat induced death of azolla.

The selection of an azolla cultivar involves many characteristics other than just RGR performance. These may include morphology, C:N ratio, and overwintering and oversummering tolerance. However RGR performance is probably the foremost consideration since time is often the major consideration in azolla cultivation because of tight cropping schedules. In this regard the relative performance of the individual accessions during appropriate seasons should be considered. However, although their performance may indicate some differences between the species, varietial variation should be investigated before selecting a cultivar.

CHAPTER III

THE EFFECT OF SIX AZOLLA ACCESSIONS UNDER THREE MANAGEMENT TREATMENTS ON THE YIELD OF PADDY RICE

INTRODUCTION

Azolla is a delicate freefloating fern living in symbiosis with a nitrogen-fixing blue-green alga, Anabaena azollae. Farmers in China and Vietnam have exploited Azolla pinnata var. imbricata for centuries as a green manure crop for rice (Lumpkin and Plucknett, 1980; Zhejiang Acad. Agr. Sci., 1975).

The genus Azolla is composed of seven known species that demonstrate considerable physiological and morphological variation. These species have never been simultaneously compared as a green manure crop for rice. A. filiculoides and A. mexicana have been studied in field experiments at the University of California, Davis (Talley and Rains, 1980) and A. filiculoides and A. pinnata var. imbricata have been studied at the International Rice Research Institute in the Philippines and in China. The objective of this experiment was to compare accessions of as many species as possible as green manures under existing azolla management practices.

MATERIALS AND METHODS

The presently known species of Azolla were collected in various parts of the world in the spring of 1979. Representative accessions of these species were taken to the Zhejiang Academy of Agricultural Sciences in the People's Republic of China to evaluate their agronomic

potential as green manures for paddy rice.

All accessions were evaluated for cold tolerance during the winter of 1979-80 in China. Five cold-tolerant accessions and one moderately cold-tolerant accession were selected for use as green manures for the spring "Early Rice" crop (late April-early August) in Hangzhou (N30° 19', E120° 12'). Of these six accessions, A. filiculoides (V1), A. microphylla, and A. rubra were introduced into China by the author; A. caroliniana was obtained via the International Rice Research Institute from G.A. Peters of the Kettering Lab., A. filiculoides (V2) was obtained via a botanical exchange between China and East Germany, and A. pinnata var. imbricata was native to Hangzhou.

The azolla accessions were compared under the following management practices which are similar to those presently used in China, i.e.:

MONO - Azolla was grown as a **monoculture** green manure crop (Figure 27) and was incorporated into the soil before the rice was transplanted (Figure 28).

INTER - Azolla was grown as an **intercrop** green manure with rice (Figure 29) and incorporated by hand into the soil (Figure 30).

COMBI - MONO plus INTER.

A randomized complete block design with 18 treatments and 6 replications was used to study the effects of azolla as a green manure on rice (Table 12). The orientation of the block design was based on an unfertilized winter barley crop that was grown and harvested to measure soil heterogeneity (Gomez, 1972). Among the treatments was a zero N control and a basal application of N as NH_4SO_4 at 60 kg N ha^{-1}. The azolla treatments included MONO, COMBI, and INTER with five, five, and

FIGURE 27. Monocropped azolla (MONO & COMBI) being sampled for mat weight. A one meter square area was sampled.

FIGURE 28. Hand incorporation of monocropped azolla into paddy mud. Spiked field hoes were used to turn the azolla under and hand planes were used to level the soil surface before reflooding.

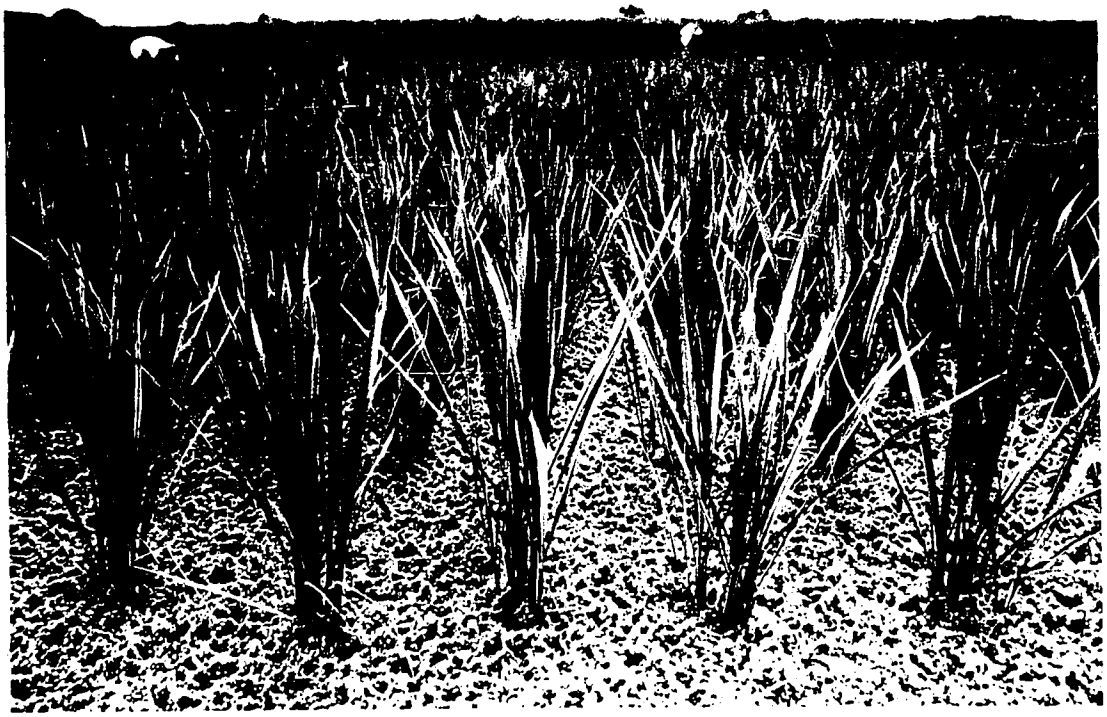

FIGURE 29. Azolla being grown as an intercrop (**INTER**) with rice on 5 to 10 cm of standing water. The mat completely covered the paddy and controlled most weeds.

FIGURE 30. Hand incorporation of intercropped azolla into paddy mud (the traditional method for intercropped azolla). While this method of incorporation is rather time consuming, the weed control effect of an azolla mat reduces or eliminates the time required for weeding.

TABLE 12. Eighteen treatments used in the azolla-rice field experiment, conducted at Hangzhou, China, in 1980. Each treatment was replicated six times.

TREATMENT	CHARACTERISTICS
1	zero N control: no azolla, no nitrogen fertilizer
2	60 kg N: 60 kg $N \cdot ha^{-1}$ as NH_4SO_4
3	monocrop (MONO): *A. caroliniana*
4	" *A. filiculoides* (V1)
5	" *A. filiculoides* (V2)
6	" *A. pinnata* var. *imbricata*
7	" *A. rubra*
8	intercrop (INTER): *A. caroliniana*
9	" *A. filiculoides* (V1)
10	" *A. filiculoides* (V2)
11	" *A. pinnata* var. *imbricata*
12	" *A. rubra*
13	" *A. microphylla*
14	MONO + INTER (COMBI): *A. caroliniana*
15	" *A. filiculoides* (V1)
16	" *A. filiculoides* (V2)
17	" *A. pinnata* var. *imbricata*
18	" *A. rubra*

six accessions respectively. Each plot was 26 m^2 and was irrigated and drained independently (Figure 31).

In early spring, the azolla accessions were moved to the field and propagated in small plastic covered nurseries and then later in larger field nurseries. The growth of Azolla microphylla was slow in the early spring because it was only moderately adapted to the cold water and air temperatures. As a result, insufficient inoculum of A. microphylla was available for use under all management practices in all replications, and was only grown under the INTER treatment.

Twenty eight days before transplanting the rice, the five cold-tolerant azolla accessions were inoculated into the MONO and COMBI plots at the rate of 500 $g \cdot m^{-2}$ fresh weight (determined by draining the inoculum overnight). The developing azolla mats were partially incorporated into the paddy soil with spiked field hoes after 13 days of growth and were totally incorporated after 23 days (Figure 28). The incorporated azolla was allowed to decompose for five days before transplanting "Makuang Lu-ai No. 4" rice seedlings into the test plots, using the spacing shown in figure 31.

Azolla for cultivation as an intercrop under the INTER and COMBI treatments was inoculated into the test plots at the rate of 500 $g \cdot m^{-2}$ fresh weight on the day before the rice seedlings were transplanted (Figure 32). Approximately half of the intercropped azolla mat was incorporated into the soil by hand 12 days after inoculation (Figure 30). Unincorporated azolla was allowed to grow for an additional 8 days. This final mat was first hand incorporated as much as possible, and then all plots were drained temporarily to kill any azolla remaining

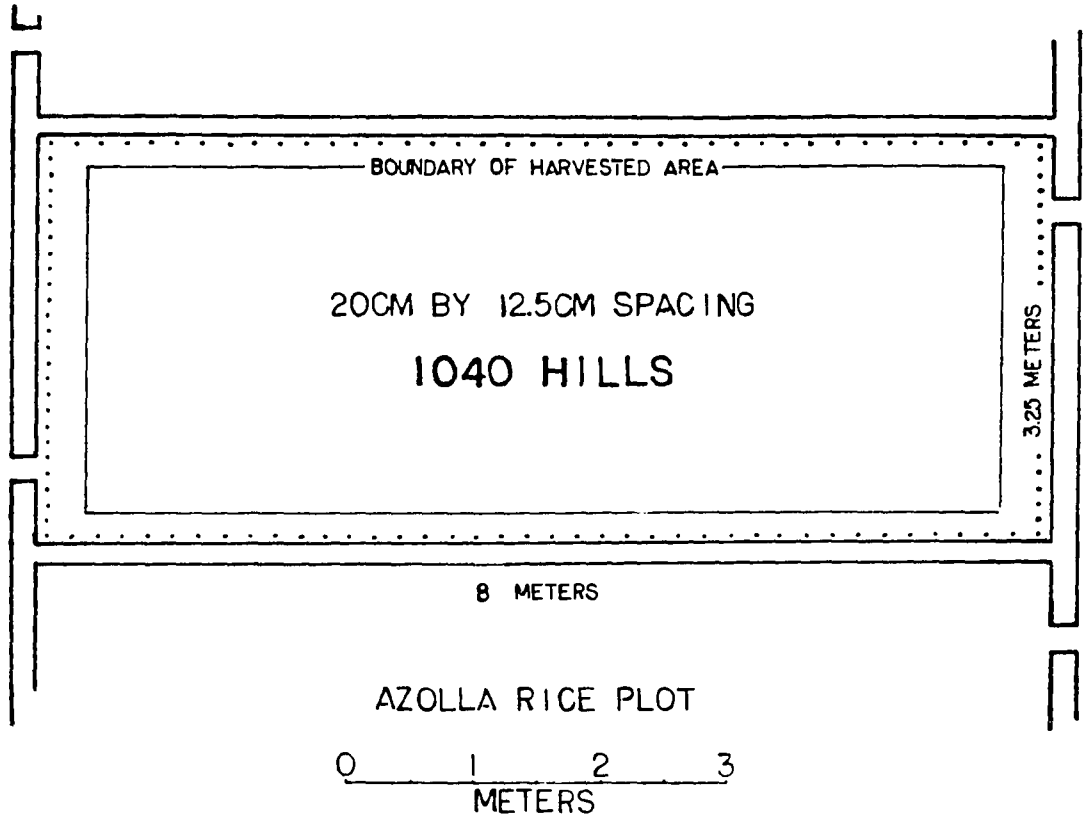

FIGURE 31. Dimensions of the field plots and spacing of rice seedling hills. Two rows of rice hills around the boundary of each plot were excluded from experimental results to reduce the influence of boundary effects.

FIGURE 32. Transplanting of rice seedlings after the azolla intercrop was placed into the plots. Azolla was uniformly dispersed across the water surface one day prior to transplanting the rice seedlings.

in the INTER and COMBI plots. All plots were then reflooded and kept stagnant.

Rice plants of Block V were destructively sampled five times for total nitrogen by micro-Kjeldahl analysis. Soil in the plow layer of Block V was sampled ten times and analyzed for ammonia by colormetric determination in complex with phenol. Azolla mat weight was measured about every five days during the experiment by collecting azolla from within a 1 m^2 bamboo frame (Figure 27). Small samples of fresh azolla were retained after fresh weight measurements for determination of oven dry weight and elemental composition. Oven dry weight was used for calculation of relative growth rate ($mg \cdot g^{-1} \cdot day^{-1}$).

RESULTS AND DISCUSSION

Effect of Azolla Management Treatments on the Yield of Paddy Rice

The differences in rice yields of all 18 treatments were highly significant (Table 13), though the range of yields was narrow. The small variation in yields was due to a high level of residual nitrogen in the field and unusually cool, wet, cloudy weather during ripening which suppressed yields of the high azolla biomass treatments in ways discussed below.

There was sufficient residual soil nitrogen in the test plots to produce average rice yields in the zero N plots of 4.7 $t \cdot ha^{-1}$, even after the growth and removal of an unfertilized winter barley crop. Inclement weather, which occurred throughout northeastern Asia, resulted in some of the lowest rice yields recently recorded in the region. Except for six days of clear, hot weather at the flowering stage, the

TABLE 13. Significance probability values (PR > F) for F values of yield and its components. (ANOVA was calculated using five blocks times three management treatments times five azolla accessions).

	GRAIN YIELD	PANICLE/HILL	SPIKELETS/PANICLE	% UNFILLED GRAINS	100 GRAIN WEIGHT
BLOCK	0.0204	0.3872	0.1316	0.1177	0.4054
TREATMENT(t)	0.0001	0.0001	0.0004	0.0001	0.0418
ACCESSION(a)	0.0008	0.4591	0.7325	0.0718	0.3918
t x a	0.5611	0.0149	0.8824	0.5091	0.8800

June mean air temperature was 3°C cooler than normal under generally cloudy conditions. Weather affected the experiment in four ways: (1) incorporated azolla decomposed at a slower rate because of lower temperatures; (2) the rice was unable to fully utilize the high levels of nitrogen that were available from the high azolla biomass plots; (3) some of these high azolla biomass plots became infected with Sheath Blight; and (4) the rice in many COMBI plots lodged and their grain began to germinate. A combination of these factors limited the high azolla biomass COMBI plots to an average rice yield of 5.9 $t \cdot ha^{-1}$. However, even with these problems the experiment produced rice yields consistent with normal wet season rice yields recorded by IRRI in the Philippines (DeDatta et al., 1974).

Mean rice yields of the three azolla management treatments were COMBI > MONO > INTER (Figures 33 and 34); in addition the COMBI treatment out-yielded the 60 kg N treatment by an average of 300 kg/ha. The MONO treatment produced a yield higher though not significantly different from the 60 kg N treatment (Appendix D). Mean straw yields and the components of yield for the three management treatments generally followed textbook trends (IRRI, 1965). Straw yields increased as the supply of azolla nitrogen increased, except for the COMBI Azolla filiculoides (V1). Straw yield indices (straw yield·grain $yield^{-1}$) were highest for practices including a topdressing of azolla. Treatments were ranked in the order: COMBI (2.09) > INTER (2.07) > MONO (2.02) > 60 kg N (1.73).

The effect of treatment on grain yield can be evaluated from components of yield for the rice crop. Grain yield can be integrated

COMPONENTS OF YIELD

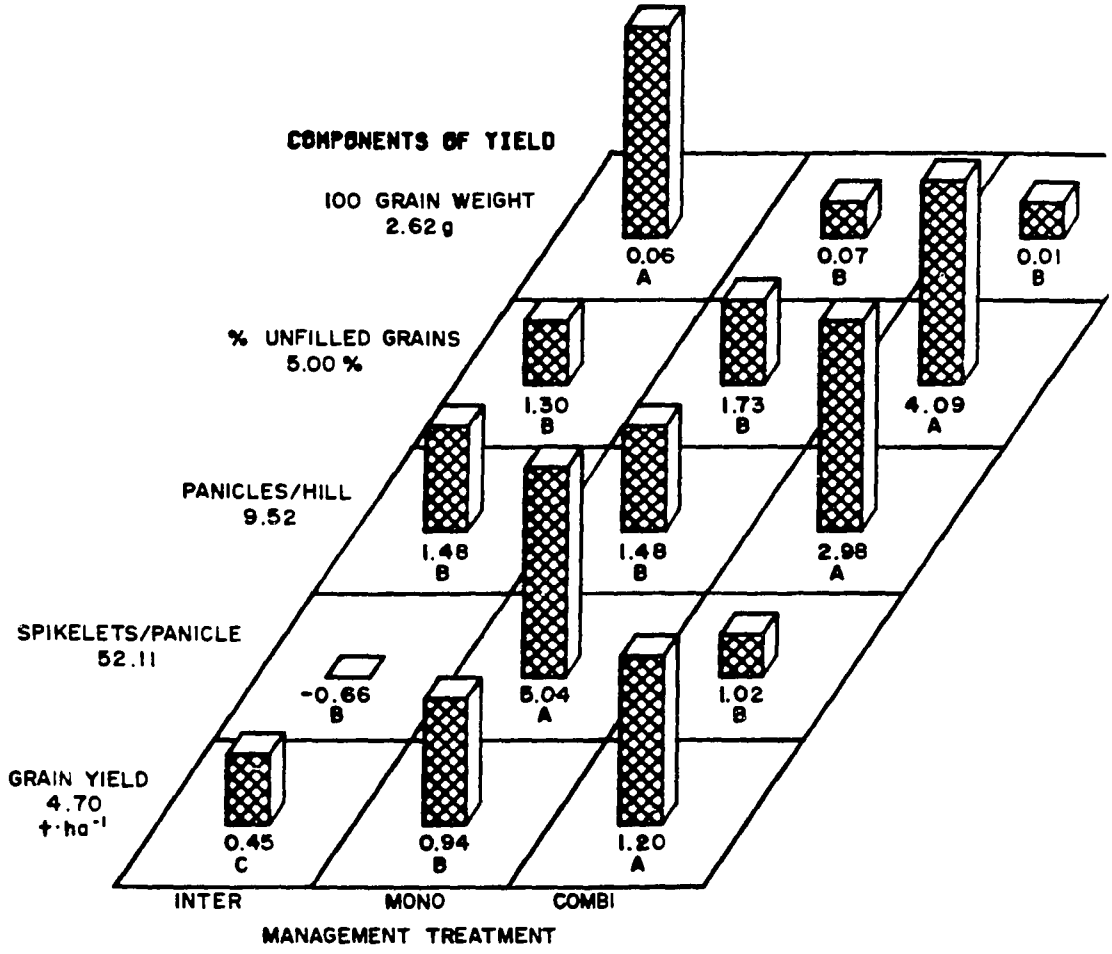

FIGURE 33. Influence of azolla management treatments on rice yield and the components of yield. Mean values from the zero N control plots were subtracted from the mean values of management treatments to show trends more clearly. Observed values for each management treatment can be obtained by adding the value below each label to the values in the squares to their right. DMRT letter groupings were calculated from observed values and apply across rows only.

FIGURE 34. Visible variation in the appearance of plots during the heading stage of rice due to different azolla management treatments. The dark color of the plot with the COMBI management treatment is an indication of delayed maturity caused by a high level of nitrogen available to the rice.

from components of yield by the following formula: grain yield (t/ha) = (hills/m^2) x (panicles/hill) x (spikelets/panicle) x (% filled grains) x (weight of 100 grains) x 10^{-6} (Matsushima, 1980). The variation of each yield component as influenced by azolla management treatment was evaluated by averaging all accessions within each management treatment and by subtracting the value of the zero N control to accentuate the differences (Table 13, Figure 33). The effect of the three management treatments on variation in any yield component can usually be explained by the development stage of the rice (Table 14) at which azolla nitrogen becomes available and the magnitude of competition for nitrogen and other nutrients during intercropping of the azolla and rice.

In the short duration rice variety used for this experiment individual components of yield were fixed in the following sequence: spikelets per panicle first but partially overlapping panicles per hill, followed by percent filled grains and weight of 100 grains. The number of spikelets per panicle (Figures 33 and 35) was was reduced by both the INTER and COMBI treatments when compared to the MONO treatment. This was probably due to competition for nitrogen and other nutrients between the young rice seedlings growing at the soil surface and the azolla intercrop of the INTER and COMBI treatments.

The number of panicles per hill was dependent on the number of tillers and the percentage of tillers that become fertile. The INTER and MONO treatments resulted in an identical number of panicles per hill (Figures 33 and 36). The MONO treatment produced a large number of tillers per hill but a low percentage of those tillers were fertile. The high tiller number resulted from a high level of azolla nitrogen

TABLE 14. Calendar of events for the experiment of azolla with the 110 day "Early Rice" crop at Hangzhou, April through August 1980.

--DATE--	--DAYS--		STAGE
April 21	2[a]	-34[b]	pre-germinated rice seed sown in nursery; start of vegetative stage of rice.
24	5	-31	inoculated field plots where azolla was grown as a monocrop, i.e. MONO and COMBI plots.
May 9	20	-16	partially incorporated azolla mat into soil.
19	30	-6	totally incorporated all monocropped azolla.
24	35	-1	inoculated field plots where azolla was grown as an intercrop, i.e. COMBI and INTER plots.
25	36	0	transplanted rice seedlings into field plots.
June 5	47	11	partially incorporated intercropped azolla.
9	51	15	maximum tillering stage of rice.
13	55	19	totally incorporated intercropped azolla.
19	61	25	panicle initiation stage; end of vegetative stage and beginning of reproductive stage.
July 8	80	44	heading stage.
11	83	47	flowering stage; end of reproductive stage and beginning of grain filling and maturation.
Aug 7	110	74	rice harvest.

[a] from the time of soaking the rice seed.

[b] from time of transplanting the rice seedings.

SPIKELETS PER PANICLE MINUS CONTROL

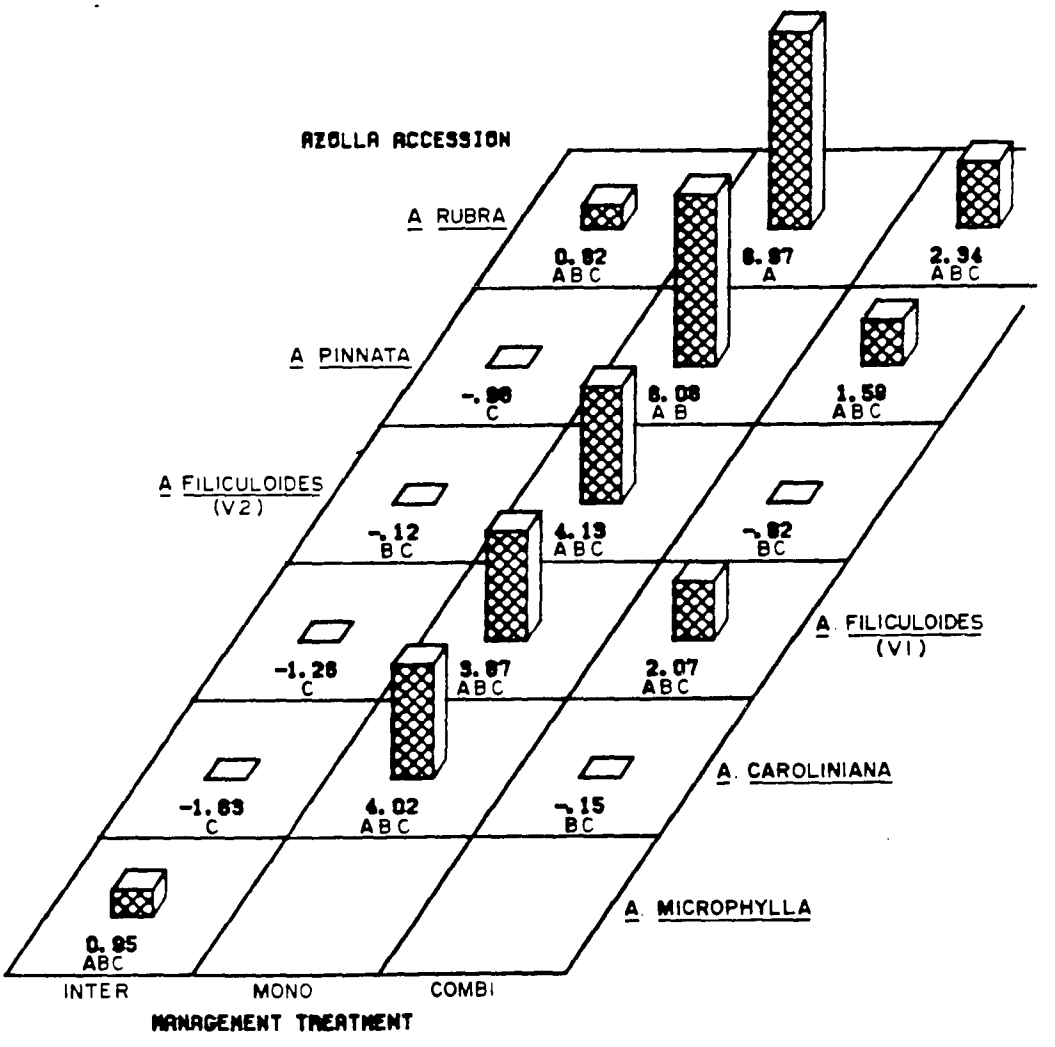

FIGURE 35. Influence of azolla accessions and management treatments on spikelets per panicle. The mean value from zero N control plots (52.11) was subtracted from observed values of plots fertilized with azolla to show trends across management treatments more clearly. Observed values for spikelets per panicle can be obtained by adding the control value to the values in individual squares. DMRT letter groupings were calculated from observed values and apply to all combinations.

PANICLES PER HILL MINUS CONTROL

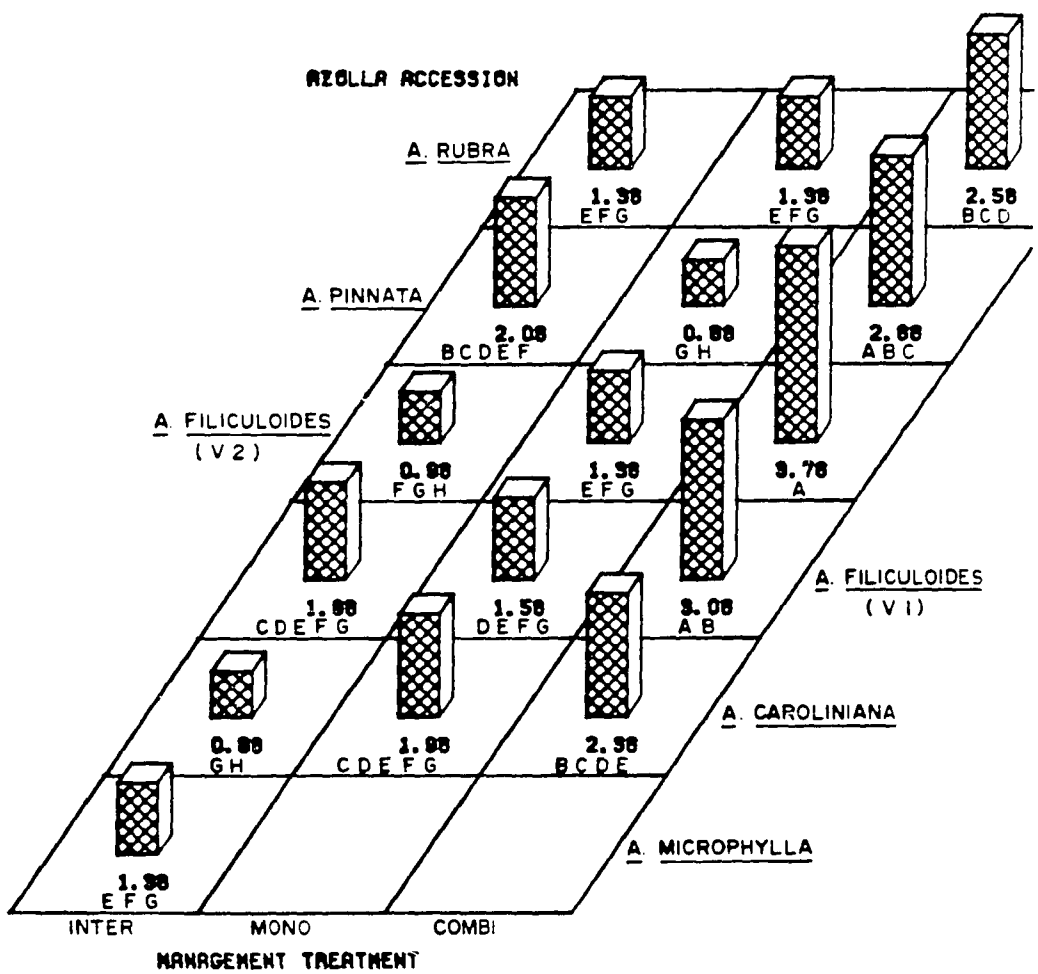

FIGURE 36. Influence of azolla accessions and management treatments on panicles per hill. The mean value from zero N control plots (9.52) was subtracted from observed values of plots fertilized with azolla to show trends across management treatments more clearly. Observed values for panicles per hill can be obtained by adding the control value to the values in individual squares. DMRT letter groupings were calculated from observed values and apply to all combinations.

available at the time of transplanting. However the availability of N per tiller was reduced before the time of panicle initiation and thus reduced the percentage of fertile tillers. The INTER treatment had few tillers per hill because intercropped azolla competed with the rice for N immediately after transplanting. However azolla nitrogen released after incorporation of the azolla intercrop produced a high percentage of fertile tillers. The COMBI practice had both a high tiller number and high percentage of fertile tillers due to a relatively high and stable nitrogen supply.

The percentage of unfilled grains was determined before, at, and after heading (near 80 days from seeding) and was directly related to nitrogen availability and rice yield (Figures 33 and 37). Thus the COMBI treatment had both the highest percentage of unfilled grains and grain yield, followed by the MONO and INTER treatments. A higher percentage of unfilled grains results from high nitrogen levels because of mutual shading and lodging (DeDatta, 1981).

Grain weight is genetically controlled and thus shows little response to nutrient status (Yoshida, 1981). However average grain weight for the INTER treatment was higher than the weight for the other two practices. This higher weight may have resulted from a higher level of N available for the relatively fewer rice grains per unit area during ripening of the rice in the INTER treatment (Figures 33 and 38).

The Relationship of Six Azolla Accessions to the Yield of Paddy Rice

Although the management treatment by azolla accession interaction for grain yield was not significant by ANOVA (Table 13), some of the

PERCENT UNFILLED GRAINS MINUS CONTROL

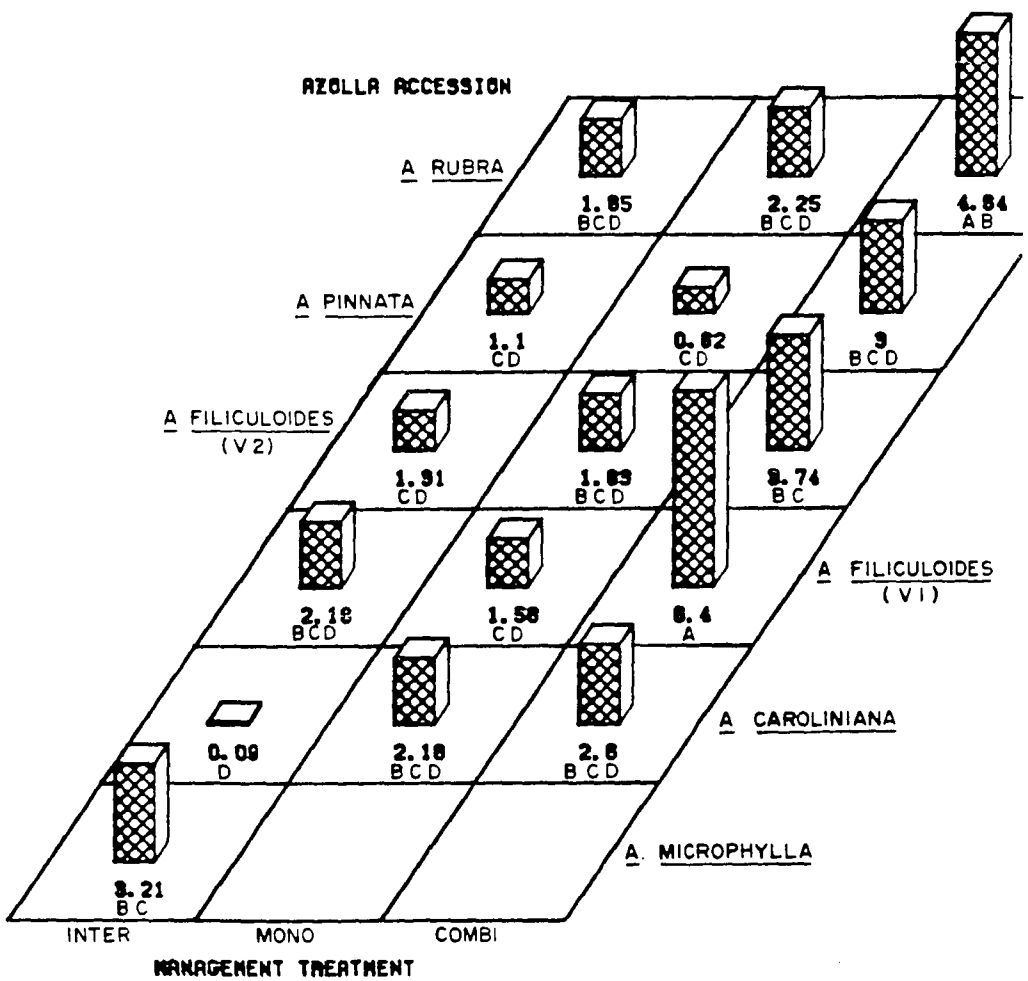

FIGURE 37. Influence of azolla accessions and management treatments on the percentage of unfilled grains. The mean value from zero N control plots (5.00%) was subtracted from observed values of plots fertilized with azolla to show trends across management treatments more clearly. Observed values for the percentage of unfilled grains can be obtained by adding the control value to the values in individual squares. DMRT letter groupings were calculated from observed values and apply to all combinations.

WEIGHT OF ONE HUNDRED GRAINS MINUS CONTROL

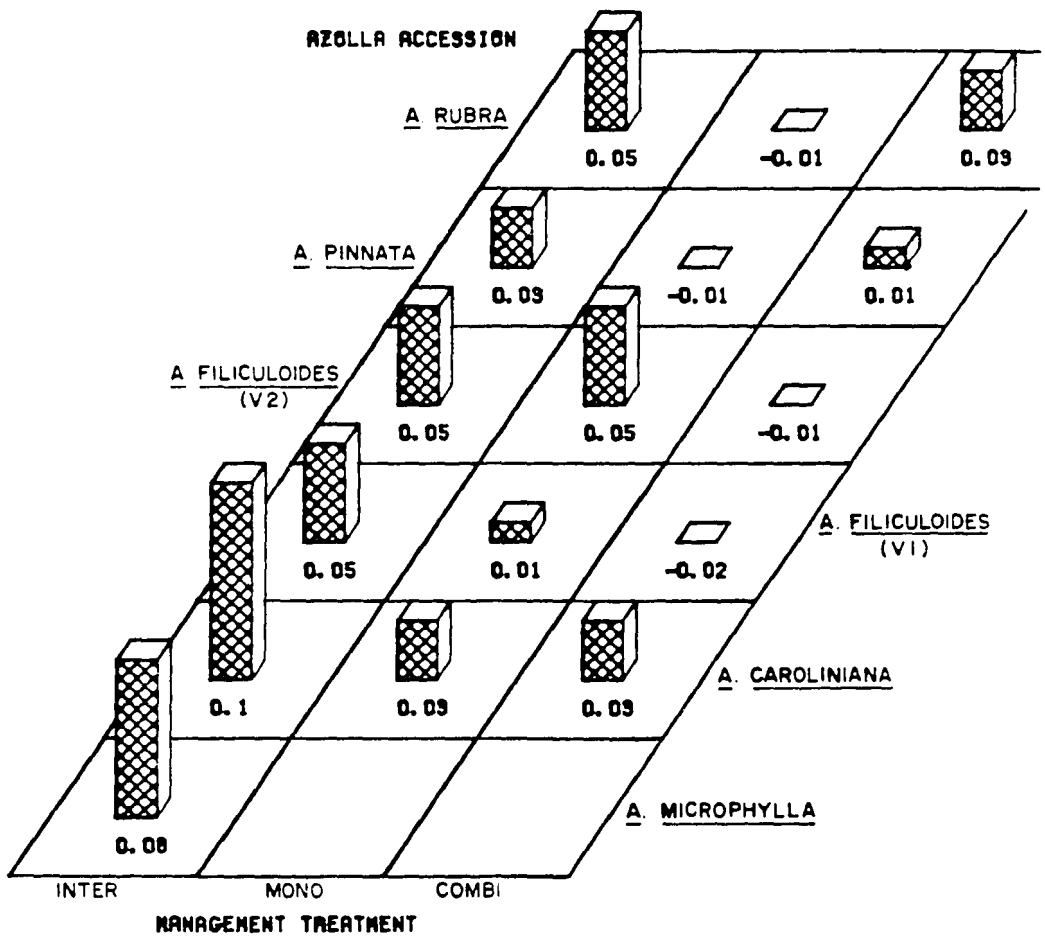

FIGURE 38. Influence of azolla accessions and management treatments on the weight of one hundred grains. The mean value from zero N control plots (2.62g) was subtracted from observed values of plots fertilized with azolla to show trends across management treatments more clearly. Observed values for the weight of one hundred grains can be obtained by adding the control value to the values in individual squares. DMRT letter groupings were calculated from observed values and apply to all combinations.

yield responses to accessions, between management treatments, were significantly different using Duncan's Multiple Range Test (Figure 39). For example, A. filiculoides (V1) under the MONO treatment produced a mean rice yield that was not different from yields produced by azolla accessions in the COMBI treatment.

Differences in rice yield between accessions were assumed to be due primarily to different rates of decomposition for individual accessions. Variable rates of decomposition are indicated by the observation that V1 and V2 of A. filiculoides, which are morphologically distinct, accumulated nearly identical amounts of nitrogen under the MONO treatment (Figure 40) yet produced significantly different rice yields (Figure 39). Also, while A. pinnata var. imbricata yields for the COMBI treatment produced the highest mean rice yield of the experiment, it accumulated the lowest level of nitrogen within that management treatment (Figures 39 and 40). This accession has been reported to have a faster rate of decomposition after soil incorporation than A. filiculoides (Shi Su-lian et al., 1980).

Rice yields were correlated with accumulated nitrogen within azolla accessions across management treatments (r=0.94 to 0.99). However yields were poorly or negatively correlated with accumulated nitrogen across accessions within a management treatment (MONO, r=-0.27; INTER, r=0.20; COMBI, r=-0.61). For example, the four accessions with the lowest rice yields in the MONO treatment had levels of accumulated nitrogen which varied by as much as 50%, yet rice yields varied by only 4%. The inconsistency between rice yields and azolla nitrogen was also apparent within the INTER treatment. Azolla filiculoides V1 accumulated

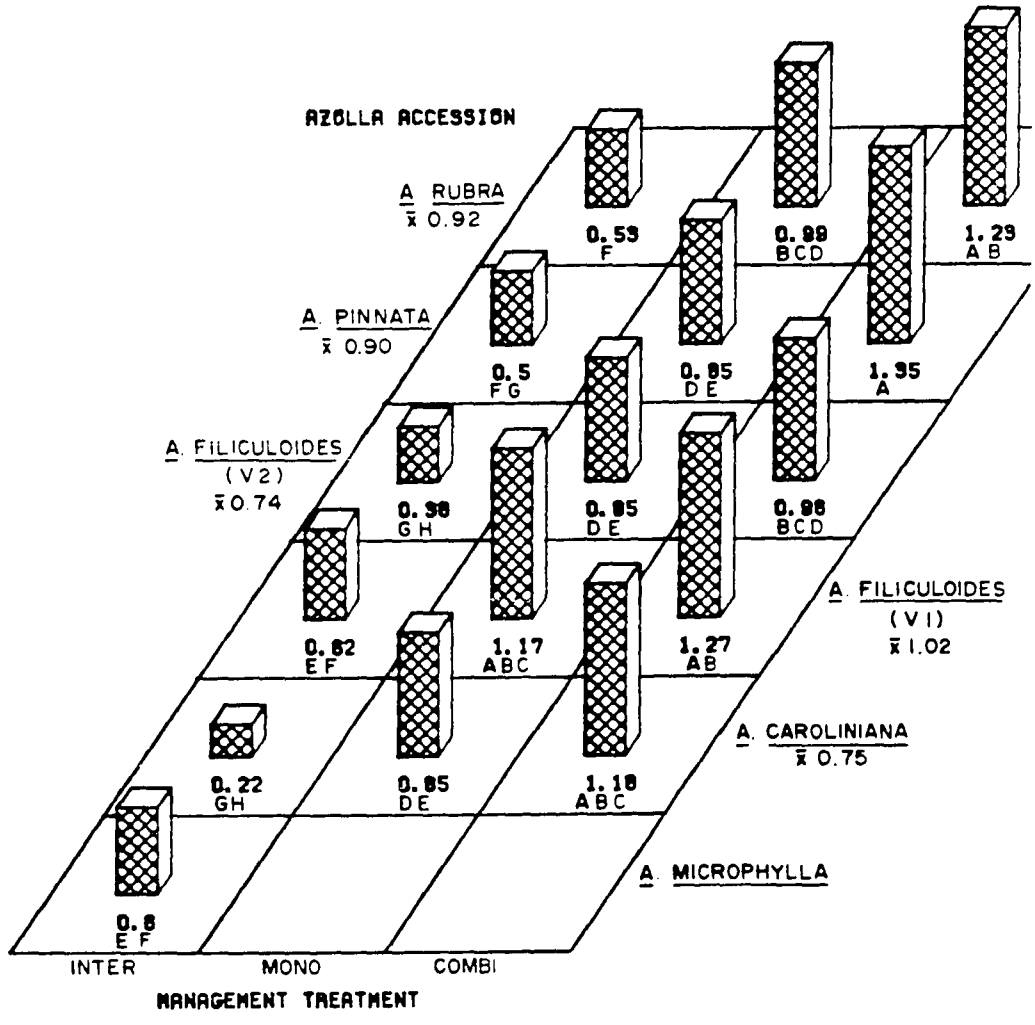

FIGURE 39. Influence of azolla accessions and management treatments on grain yield of rice. The mean value from zero N control plots (4.70 t/ha) was subtracted from observed values of plots fertilized with azolla to show trends across management treatments more clearly. The mean value for each accession across management treatments appears below the accession name. Observed values for grain yield can be obtained by adding the control value to the values in individual squares. DMRT letter groupings were calculated from observed values and apply to all combinations.

ACCUMULATED NITROGEN

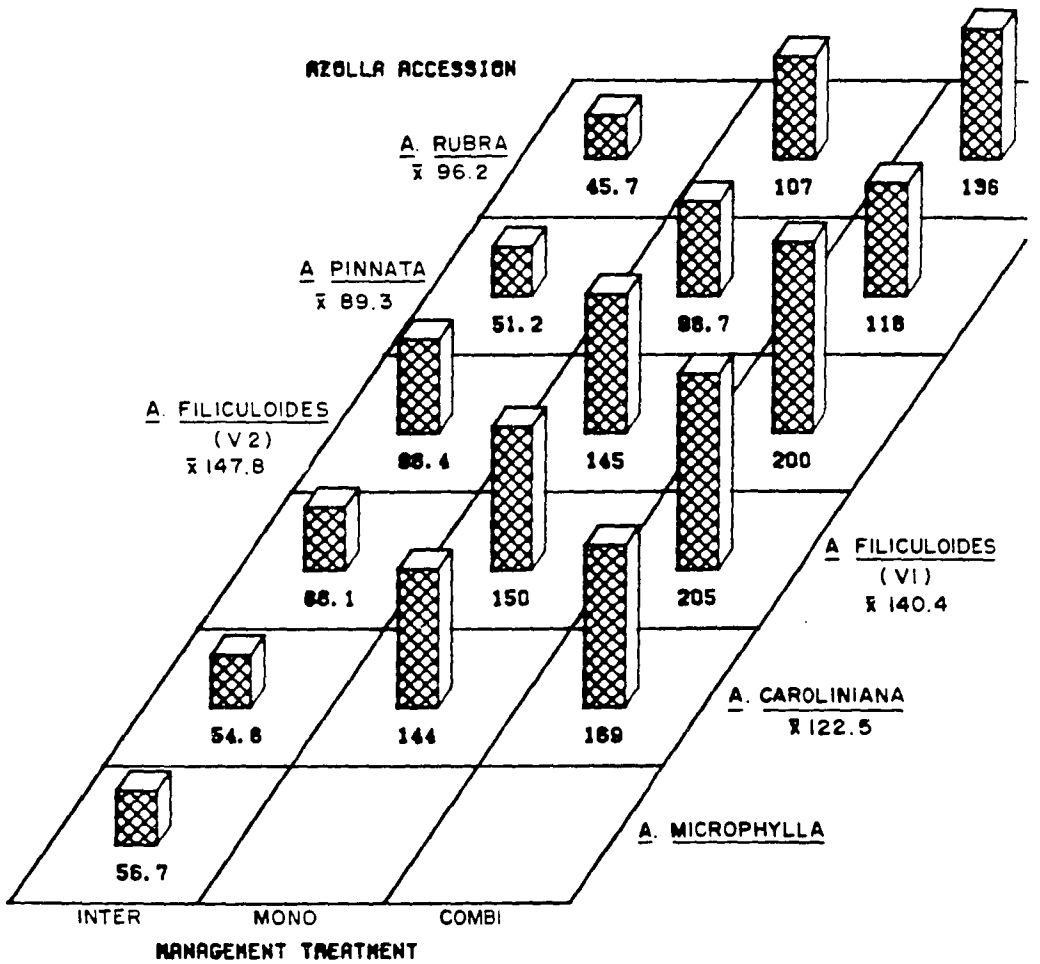

FIGURE 40. Kilograms of nitrogen per hectare accumulated by azolla accessions as a function of azolla management treatment. No statistical analysis was done since blocks were combined for Kjeldahl analysis.

nearly twice as much nitrogen as other accessions, yet had the second lowest rice yield (Figures 39 and 40). It is assumed that the differential response was due to variable rates of decomposition and mineralization among the accessions.

Effects of Azolla Treatments on Rice and Soil Nitrogen Content

The nitrogen content of rice plants was closely related to the timing and quantity of incorporated azolla (Figures 41 and 42). Nitrogen content followed the typical pattern of increasing rapidly to a peak between 2 to 4 weeks after transplanting and then slowly declining during the remainder of the growth period (Figure 41; IRRI, 1965). The rice seedlings contained 1.74% nitrogen at the time of transplanting. However, within 12 days after transplanting, rice plants under the different management treatments had nitrogen contents ranging from two to three percent on a dry weight basis (Figure 41).

The lowest N content of rice was measured when azolla was grown only as an intercrop (INTER). The young rice seedlings were noticeably chlorotic during the first weeks of intercropping. The depression of N in the plant below that in the control may have been the result of nitrogen competition between the young seedlings and intercropped azolla. Immobilization of soil N was probably not an important factor in the depression of N because the depression occurred before the first soil incorporation of the intercropped azolla.

Although intercropping with azolla initially resulted in a reduction in rice nitrogen content relative to the zero N control, after the first soil incorporation at 11 days, the rice nitrogen content in

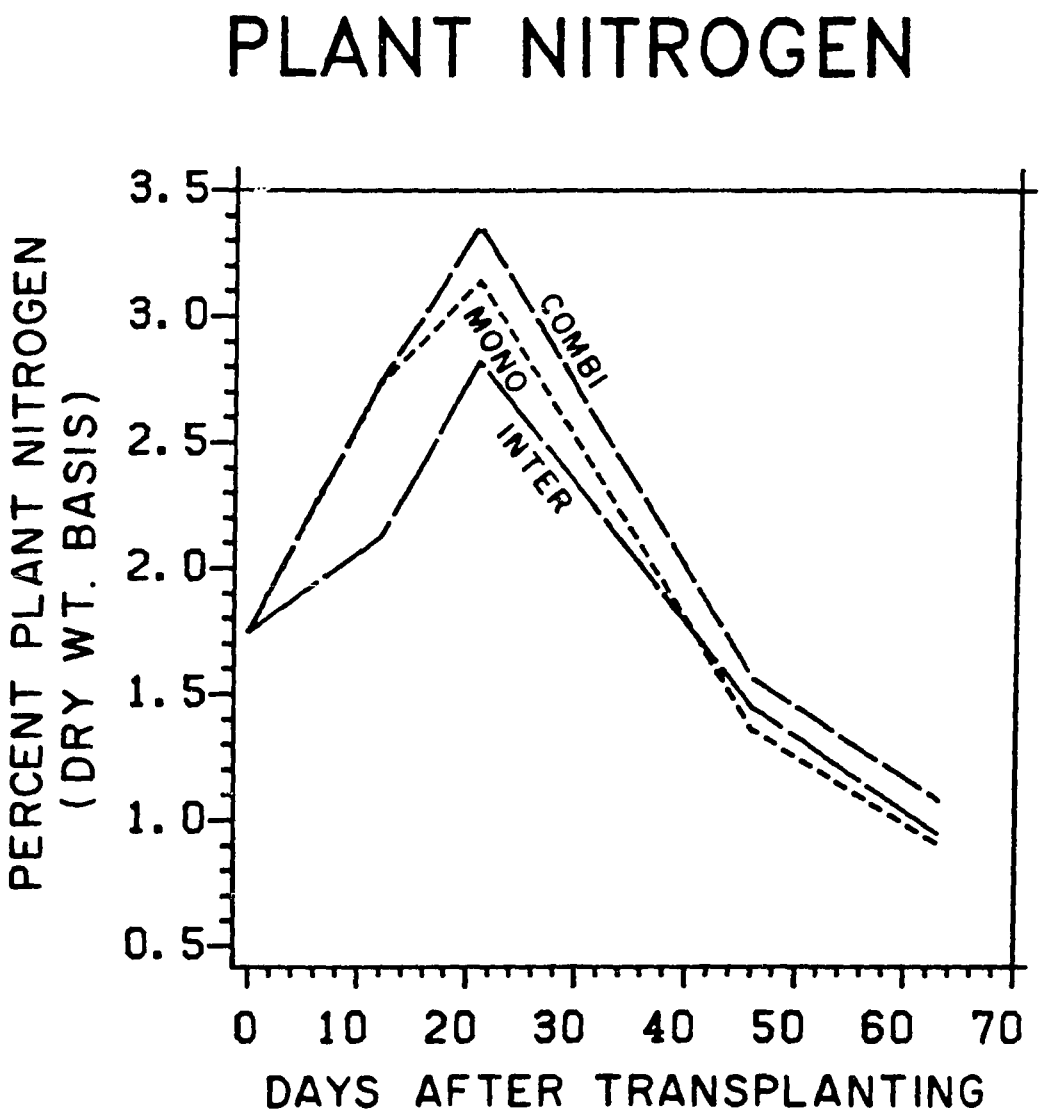

FIGURE 41. Nitrogen content of rice plants during the first 65 days after transplanting as influenced by azolla management treatments.

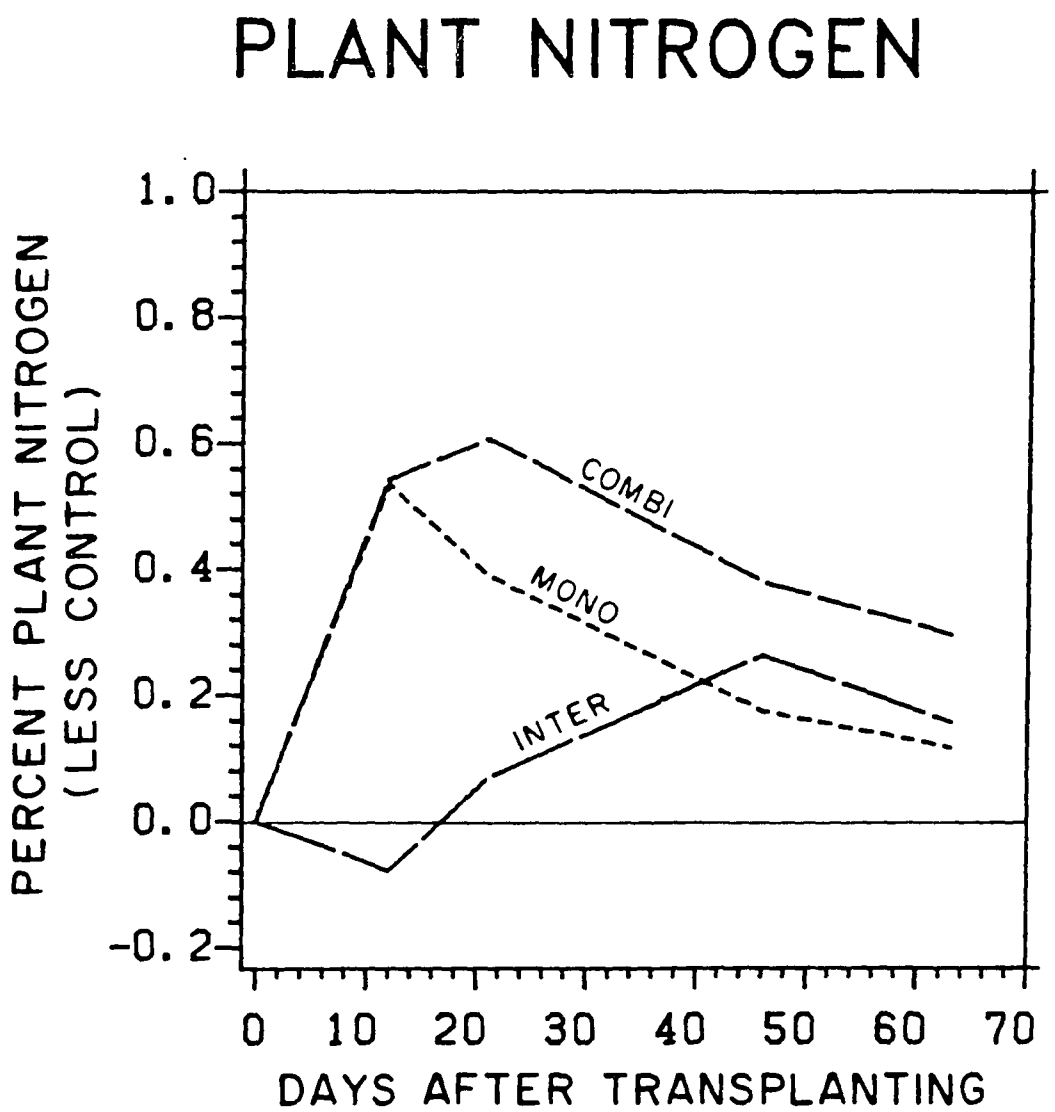

FIGURE 42. Nitrogen content of rice plants (minus control) during the first 65 days after transplanting as influenced by azolla management treatments. The nitrogen content of rice in the control plots was subtracted from mean values for azolla management treatments to show trends of rice nitrogen content as influenced by management treatment.

the INTER treatment surpassed the control and continued to increase throughout the growing season (Figure 42). By harvest time, rice in the INTER treatment had a higher nitrogen content in the grain than rice from the MONO treatment. However, yields were low in the INTER treatment because nitrogen became available too late to produce a high number of spikelets per panicle.

The nitrogen level was initially highest in rice seedlings fertilized with 60 kg N/ha as a basal application of ammonium sulfate. By the third week the N content of these plants leveled off at a concentration parallel with the COMBI and MONO treatments. Surprisingly, the intercropped azolla of the COMBI treatment did not reduce plant nitrogen content during the first weeks after transplanting, as did the INTER treatment. It is assumed that the relatively low nitrogen requirement of the young seedlings during the first weeks after transplanting (DeDatta, 1981) was being met by mineralization of N from the decomposing monocropped azolla of the COMBI treatment.

Soil from each of the 18 treatment plots of Block V was sampled 10 times and analyzed for ammonia content during development of the rice. Wide variation in ammonia content was observed in the soil samples. Actual soil ammonia nitrogen values minus the zero N control (Figure 43) show the influence of azolla management practices on soil ammonia. Management treatments were ranked on the basis of soil ammonia concentration in the order MONO > COMBI > INTER up to 20 days after transplanting. The ammonia in the INTER soil was lower than the zero N control because nitrogen was scavenged by the intercropped azolla

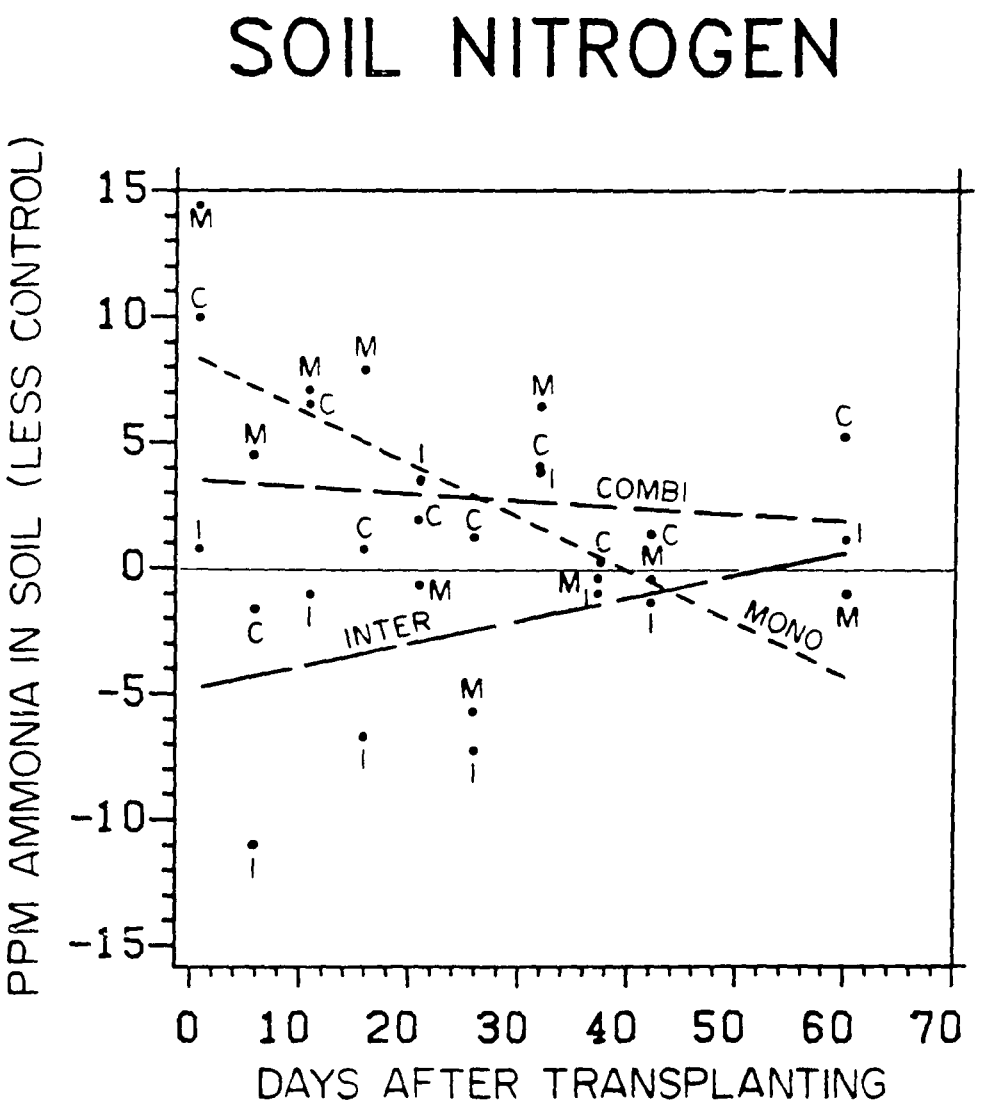

FIGURE 43. Soil ammonia concentration (minus control) during the first 65 days after transplanting rice as influenced by azolla management treatment. Data points represent observed values by management treatment minus the control and lines represent their simple linear regressions.

(Figure 43). The soil ammonia content under the COMBI treatment was initially lower than the MONO treatment, perhaps due to N scavenging by the azolla intercrop in the COMBI treatment. Some soil N may also have been immobilized by microbes during decomposition after the first incorporation of the azolla intercrop.

Observed values of soil ammonia for the three management treatments, less those for the zero N control, and their respective trend lines are plotted in figure 43. The relatively steep decline of soil ammonia in the MONO treatment represents depletion of N during growth and profuse tillering of the rice. The relatively flatter slopes of the INTER and COMBI treatments indicate that the ammonia content of the soil was being buffered by release of ammonia from decomposing intercropped azolla.

Relative Growth Rates of Azolla Grown Under Three Management Treatments

Azolla biomass in each plot was estimated by measuring drained mat weight over 1 m^2 at approximately five day intervals. The area of mat to be measured was delineated by a bamboo frame. The azolla mat was removed, drained, weighed, and then replaced in the frame (Figure 27). A small sample of fresh azolla was retained for determination of oven dry weight and elemental composition. Measurements from this procedure were used to calculate azolla relative growth rates (Figure 44) and the total quantity of nitrogen accumulated by each azolla accession in each plot (Figure 40).

Low temperature (see Chapter 2) and interplant competition were assumed to be the primary factors limiting relative growth rate (RGR) of

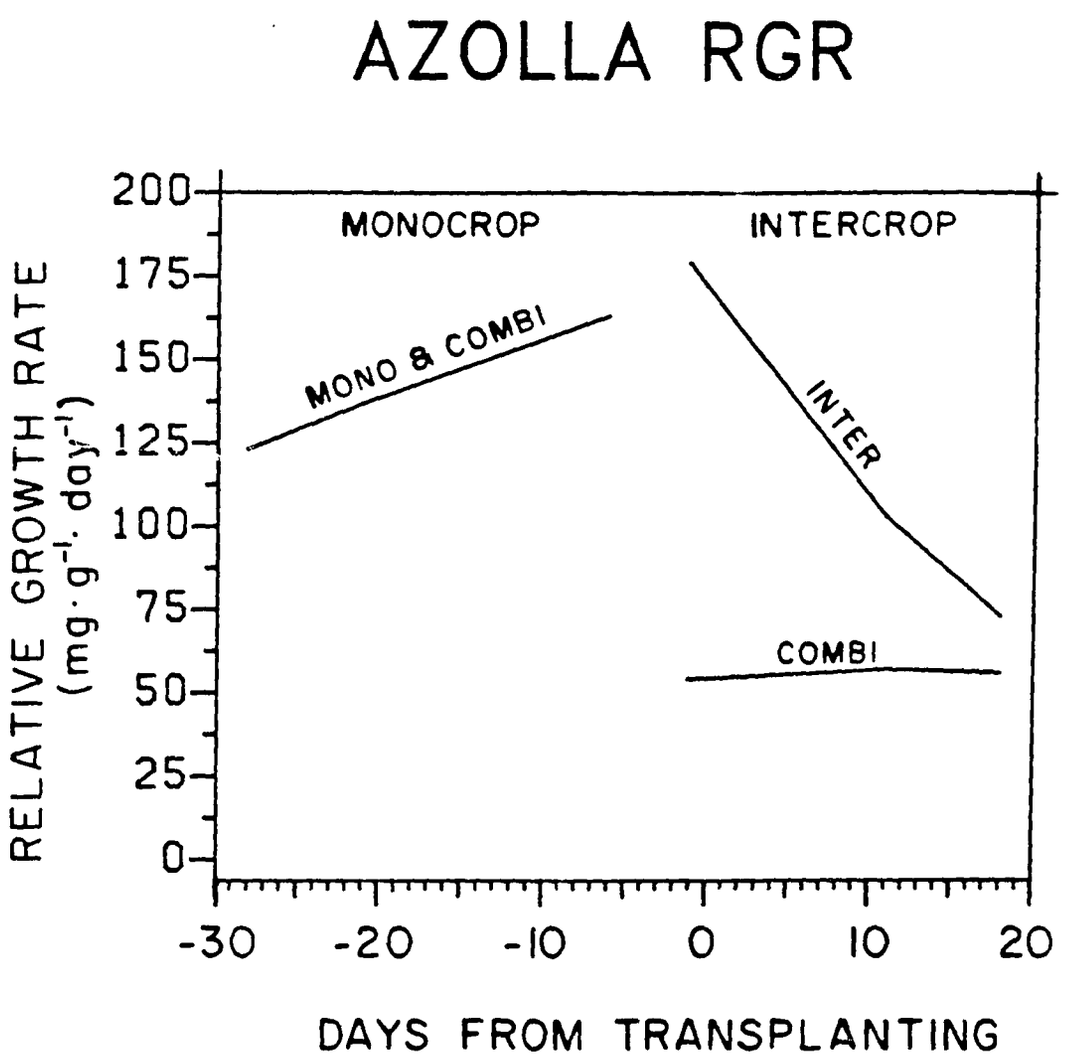

FIGURE 44. Mean relative growth rates of azolla accessions under the three azolla management treatments (MONO, COMBI, INTER) during the time before and after transplanting the rice.

the MONO and COMBI monocropped azolla, since other environmental variables were within the normal range for rapid azolla growth (Lumpkin and Plucknett, 1982), i.e. water pH 6.0 to 7.6, water conductivity 600 to 1700 MHOS, high tissue nutrient content (on a dry weight basis, P was 0.38 to 0.81% and K, was 2.72 to 3.58%), mean % R.H. >49 and <67, and estimated mean daily solar radiation 471 $cal \cdot cm^{-2} \cdot day^{-1}$. Interplant competition may have had some influence on RGR prior to the mid-term and final soil incorporations.

The mass of an azolla mat is an important factor affecting interplant competition. In this experiment, the azolla mats attained weights ranging from 19.85 to 31.85 $t \cdot ha^{-1}$ fresh weight (1.04 to 1.69 $t \cdot ha^{-1}$ oven dry wt.) at midterm and 34.91 to 43.97 $t \cdot ha^{-1}$ (1.52 to 2.52 $t \cdot ha^{-1}$ oven dry wt.) before the final incorporation of the monocropped azolla. Soil incorporations kept mat weights below the maximum mat weights for various species reported by Gopal (1967), Talley et al. (1977) and others (Lumpkin and Plucknett, 1980).

Disturbance of the azolla during collection and transportation for inoculation also may have had some affect on RGR. Mean RGR for all azolla accessions increased from 120 to 160 $mg \cdot g^{-1} \cdot day^{-1}$ as water temperature increased from a weekly mean of 16°C at the start of monocropping, to 24.4°C at the end. This field RGR was higher than the RGR of these same accessions grown simultaneously in pot culture on nitrogen-free nutrient solution at the same site. The mean pot RGR increased form 97 to 112 $mg \cdot g^{-1} \cdot$day during this period. The lower RGR in pot culture was primarily due to the pot water temperature being approximately 2.5°C colder than field water. The pot water was 20 cm

deep compared to about 5 cm in the field and thus required more energy per unit surface area for a similar increase in water temperature.

Mean RGRs of azolla in the INTER and COMBI treatments during intercropping were strikingly different (Figure 44). Temperature during the intercropping period was stable and within the optimum range for most accessions (24.4 to 26.5°C mean water temperature), and thus had little direct influence on RGR. Rapid rice canopy development in the COMBI treatment was probably an important factor affecting azolla RGR. Rice under the COMBI treatment had received basal applications of monocropped azolla which greatly promoted tiller production. Rice under the INTER treatment had not received any form of N fertilization and tiller numbers were much lower.

Another factor appears to have reduced RGR to a low mean of 53 $mg \cdot g^{-1} \cdot day^{-1}$ in the COMBI treatment during intercropping when compared to the RGR of INTER treatment azolla. This factor was probably the adverse effects of decomposing monocropped azolla on the intercropped azolla of the COMBI treatment. The effects of decomposing monocropped azolla on the RGR of intercropped azolla varied with the accession and produced large differences in the RGR between accessions (Table 15, Figure 44). These differences were probably related to differences in the rates of decomposition and N release of individual accessions. It has been repeatedly reported that high levels of available nitrogen result in an unfavorable environment for the growth of azolla (Zhejiang Acad. Ag. Sci., 1975; Singh, 1977).

The low RGR of A. pinnata var. imbricata in the COMBI treatment during intercropping may be due to its relatively rapid rate of

TABLE 15. Relative growth rates ($mg \cdot g^{-1} \cdot day^{-1}$) for intercropped azolla accessions grown under the INTER and COMBI azolla management treatments.

ACCESSIONS	24 APR-30 MAY		7-12 JUNE	
	INTER	COMBI	INTER	COMBI
A. caroliniana	190	47	87	62
A. filiculoides V1	158	57	64	68
A. filiculoides V2	187	90	135	53
A. pinnata var. imbricata	186	24	36	48
A. rubra	144	66	89	51

decomposition and the high RGR of A. filiculoides (V2) in the 24-30 May COMBI and 7-12 June INTER periods may be due to its slow decomposition. Without additional research it can only be speculated that intermediate or final products of azolla decomposition were directly or indirectly detrimental to the growth of intercropped azolla and yet seem to have little detrimental effect on the rice.

The combination of azolla monocropping and azolla intercropping in the COMBI treatment do not produce additive results because of low RGRs during COMBI intercropping. However, figures 40 and 44 show that there is wide varietal variation. For example, in the COMBI treatment, A. pinnata var. imbricata and A. filiculoides (V1) had the lowest and highest intercrop RGRs, incorporated biomass, and total accumulated N but produced the highest and lowest rice yields, respectively.

CONCLUSIONS

In general, the best combination of azolla accession and management treatment produced rice yields as high as any rice yields recorded on other experimental plots at the Zhejiang Academy of Agricultural Sciences and on surrounding communes for the 1980 "Early Rice Crop". Azolla may be able to completely replace chemical nitrogen ferilizer in certain situations.

Selection of the best azolla accession or management practice depends upon the local conditions. An azolla accession which produces the highest level of organic nitrogen fertilizer would normally be preferred. On the other hand, selection of the best management practice would depend more upon cropping and economic conditions, rather than

characteristics of azolla accessions. If labor is a constraint and fallow paddy fields are available, monocropped azolla would probably be preferred, especially since it is more suitable for mechanization. If land, water, or time is a constraint, intercropped azolla would probably be preferred. If neither land, water nor labor are constraints than both monocropping and intercropping may be feasible. However, this combination does not produce additive azolla nitrogen yields because the intercrop has a lower RGR when grown in combination and scavenges N from the decomposing monocrop.

APPENDIX A.

Azolla: Botany, Physiology, and Use as a Green Manure[1]

THOMAS A. LUMPKIN AND DONALD L. PLUCKNETT[2]

This is a comprehensive review of literature pertaining to the aquatic fern Azolla *and its nitrogen-fixing algal symbiont,* Anabaena azollae. *The preceding decade has witnessed an explosive growth in research on* Azolla, *and hopefully this paper will facilitate those efforts.*

The paper is broken into three major categories: botany, physiology and biochemistry, and agriculture. The botany section includes a world distribution map and reference tables for the 6 Azolla *species, and includes the first review of literature on* Anabaena azollae.

The physiology and biochemistry section covers the range of topics from environmental factors to life processes and nitrogen fixation. Tables on the effect of growth regulators and on the rate of nitrogen fixation measured by acetylene reduction are presented.

The agriculture section draws extensively from literature published in the People's Republic of China and in the Democratic Republic of Vietnam. The major focus of this section is on the history and management practices for Azolla *cultivation as a green manure for rice. The effect of weed suppression, use as a fish food and animal fodder, and the insects and diseases of* Azolla *are also discussed.*

Azolla has been of traditional interest to botanists and Asian agriculturists because of its symbiotic association with a nitrogen-fixing blue-green alga. Stimulated by the recent energy crisis, the interests of these two groups have merged, resulting in the publication of numerous articles in popular magazines and extension bulletins. These articles have focused on the green manure, nitrogen fixation, and hydrogen production qualities of *Azolla* (Galston, 1975; Newton, 1976; Brill, 1977; Singh, 1977b).

The intent of this paper is to provide a current and comprehensive survey of all available literature on the *Azolla-Anabaena* symbiosis, essentially up-dating and expanding the excellent review by Moore (1969). Some of the included references were published prior to 1969 but were hitherto unavailable (e.g., those from Vietnam and China).

The most remarkable characteristic of *Azolla* is its symbiotic relationship with the nitrogen-fixing blue-green alga (cyanobacterium), *Anabaena azollae*. The delicate *Azolla* (Fig. 1) provides nutrients and a protective cavity in each leaf (Fig. 2) to *Anabaena* colonies in exchange for fixed atmospheric nitrogen and possibly other growth-promoting substances (Schaede, 1947; Ashton and Walmsley, 1976). The rate of nitrogen fixation in the *Azolla-Anabaena* symbiosis rivals that of the *Rhizobium*-legume symbiosis. Talley et al. (1977) reported a daily fixation rate

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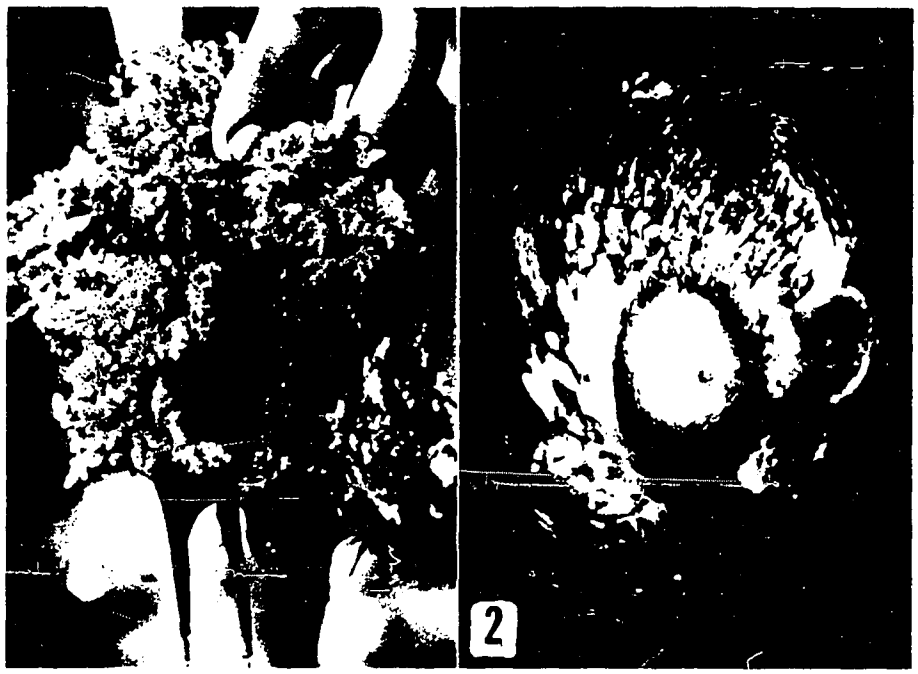

Fig. 1–2. Fig. 1. *Azolla filiculoides* mats, growing in Hawaiian taro fields, contain approximately 70 kg/N/ha. Fig. 2. *Anabaena azollae* filaments are visible within the ovoid leaf cavity of an *Azolla pinnata* dorsal lobe.

of 1.2 kg N/ha and Dao and Tran (1966) reported an annual nitrogen yield of 864 kg N/ha.

BOTANY OF AZOLLA

Taxonomy and stratigraphy

The genus name, *Azolla*, is a conjugation of two Greek words, *Azō* (to dry) and *ollyō* (to kill), suggesting the fern is killed by drought. Some of the fern's vernacular names are: water velvet, mosquito fern (English); Algenfarn (German); Helechito del Agua (Spanish); Lu P'ing, Ho P'ing, Man Chiang hung shu (Chinese); Akaukikusa, Koakaukikusa, Ooakaukikusa (Japanese); Chak pos kra bey, Chak krahan (Khmer); Nae harnghern (Lao); Beo hoa dau, Beo giau (Vietnamese).

Azolla belongs to the Salviniales which is closely related to the Hymenophyllaceae (Copeland, 1947; Bierhorst, 1971). Lamarck established the genus *Azolla* in 1783 after examining specimens brought from Chile (Griffith, 1845). The genus was originally included in the Salviniaceae Sadeb., a family of heterosporous free-floating ferns (Sadebeck, 1902) (Fig. 3), but recently taxonomists have assigned *Azolla* to a monotypic family, Azollaceae C. Chr., separate from the genus *Salvinia* (Christensen, 1938; Reed, 1954; Sculthorpe, 1967; Konar and Kapoor, 1974; Martin, 1976).

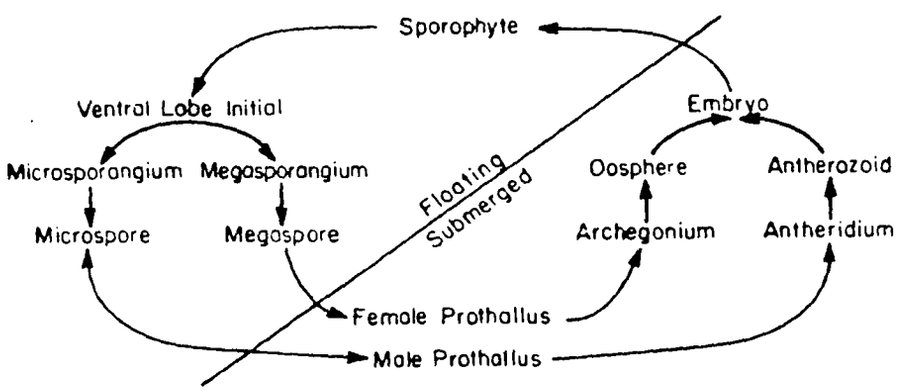

Fig. 3. Heterosporous life cycle of *Azolla*.

The genus is divided into 2 sections (subgenera) and 6 living species, primarily on the basis of reproductive organs, e.g., megaspore floats and glochidia (Svenson, 1944; West, 1953; Moore, 1969). The sections are Euazolla (3 floats) and Rhizosperma (9 floats). The glochidia of the species belonging to Euazolla (*A. filiculoides* Lamarck, *A. caroliniana* Willd., *A. microphylla* Kaulfuss and *A. mexicana* Presl.) are septate, while those of the Rhizosperma are simple in *A. pinnata* R. Brown (Hills and Gopal, 1967) or absent in *A. nilotica* DeCaisne (Demalsy, 1953). Use of septa in glochidia as a distinguishing characteristic was questioned by Godfrey et al. (1961) because of extensive morphological variation within a given species, which has given rise to contradictory observations by several authors (Clausen, 1940; Svenson, 1944; Hills and Gopal, 1967; Seto and Nasu, 1975).

The presence or location of glochidia on the massulae can also be a distinguishing characteristic (Konar and Kapoor, 1974). The species belonging to the Euazolla, *A. filiculoides*, *A. caroliniana*, *A. microphylla*, and *A. mexicana* have glochidia positioned on the total surface, while those of the Rhizosperma are located on the inner surface of *A. pinnata* and are absent in *A. nilotica*, but because sporocarps are usually absent, identification of *Azolla* species is often difficult.

The 6 living species are grouped in the sections Euazolla Sadeb. (3 floats) and *Rhizosperma* Sadeb. (9 floats). Euazolla replaced the earlier section Azolla introduced by Meyen (1836). Four new sections—Antiqua, Filipera, Florschuetzia, and Simplicispora (Martin, 1976; Follieri, 1977)—include only fossil species, of which 48 have been recorded (Fowler, 1975). Although the fossil record of the genus extends back to the late Cretaceous period (Martin, 1976), the stratigraphic range of individual species is relatively short (Fowler, 1975). According to Hills and Gopal (1967) the oldest living species are *A. filiculoides* of Euazolla and *A. pinnata* of Rhizosperma which date back to the Pleistocene era. On the basis of fossil evidence, most authors assume that *Azolla* species evolved in a sequential fashion, but Martin (1976) believes "that each arose separately out of the plastic cytological situation common to the whole genus."

There has long been confusion about the classification of Azolla species. For example, some *A. filiculoides* varieties were named as the species *A. rubra* and *A. japonica* (Moore, 1969) and some *A. pinnata* varieties as *A. africana* and *A. guineensis* (Sweet and Hills, 1971). In several cases the specific epithet was changed to a varietal name; e.g., *A. filiculoides* var. *rubra* and *A. pinnata* var. *imbricata* (Sweet and Hills, 1971). Christensen (1906) and Reed (1954) prepared lists of synonyms for the species which they recognized, but their lists are not in total agreement with current beliefs. References for current and unaccepted names of species may be found in papers by Mettenius (1847), Stapf (1929) and Reed (1954).

Distribution

The geographic distribution of *Azolla* has been reviewed by many authors. Papers have been published on the distribution of the 6 living species (Moore, 1969), the 4 species native to the New World (Svenson, 1944), the 3 species native to the United States, (Ott and Petrik-Ott, 1973), and *A. caroliniana, A. filiculoides, A. nilotica* and *A. pinnata* (Rao, 1936). Sculthorpe (1967) covered all species except *A. microphylla* and also included notes on the introduction of *A. caroliniana, A. filiculoides,* and *A. pinnata* into Europe. Sweet and Hills (1971) prepared a map showing the distribution of *A. pinnata* varieties.

Azolla occurs in ponds, ditches and paddy fields of warm-temperate and tropical regions throughout the world (Fig. 4). Prior to their dispersal by man, the species were endemic to the following areas: *A. caroliniana*, eastern North America and the Caribbean; *A. filiculoides*, southern South America through western North America including Alaska; *A. microphylla*, tropical and subtropical America; *A. mexicana*, northern South America through western North America; *A. nilotica*, upper reaches of the Nile to Sudan; and *A. pinnata*, most of Asia and the coast of tropical Africa (Sculthorpe, 1967; Svenson, 1944).

According to Sculthorpe (1967), *A. filiculoides* was formerly native to Europe, but probably died out during the last Ice Ages. In the 19th century, it was reintroduced into western Europe, along with *A. caroliniana* and *A. pinnata*, as an ornamental (Saccardo, 1892; Marsh, 1914; Chevalier, 1926; Sculthorpe, 1967), and it spread unchecked until it became a nuisance (Bolos and Masclans, 1955; Sculthorpe, 1967). This sequence of events was repeated in South Africa, New Zealand and elsewhere (Matthews, 1963; Fosberg, 1942; Oosthuizen and Walters, 1961; Ashton and Walmsley, 1976).

The world distribution of the species according to the literature is recorded as follows:

1. Section Euazolla.

a. *A. caroliniana*. ASIA: Canton (Saver, 1947), Hong Kong (Herklots, 1940). EUROPE: Belgium (Lawalree, 1964), Bulgaria, Czechoslovakia (Lawalree, 1964), Denmark (Olsen, 1972), France (Sculthorpe, 1967), Germany (Sculthorpe, 1967), Holland (Lawalree, 1964), Hungary (Lawalree, 1964), Italy (Avena et al., 1974), Portugal (Reed, 1962), Romania (Lawalree, 1964); Spain (Garcia Nova, 1969). LATIN AMERICA: Antilles (Herter, 1928), Argentina (Sota, 1976), Brazil (Sadebeck, 1902), Cuba (Svenson, 1944), Guyana (Anonymous, 1960), Jamaica (Svenson, 1944), Mexico, Uruguay (Sota, 1976), Venezuela (Herter, 1928). NORTH AMERICA: Delaware (Cohn and Renlund, 1953), Florida (Godfrey et al., 1961), Georgia (Duncan, 1960), Kentucky (McCoy, 1950), Maryland (Reed, 1951–53), Massachusetts (Ott and Petrik-Ott, 1973), Nebraska (Peterson, 1935), New Jersey (Cohn and Renlund,

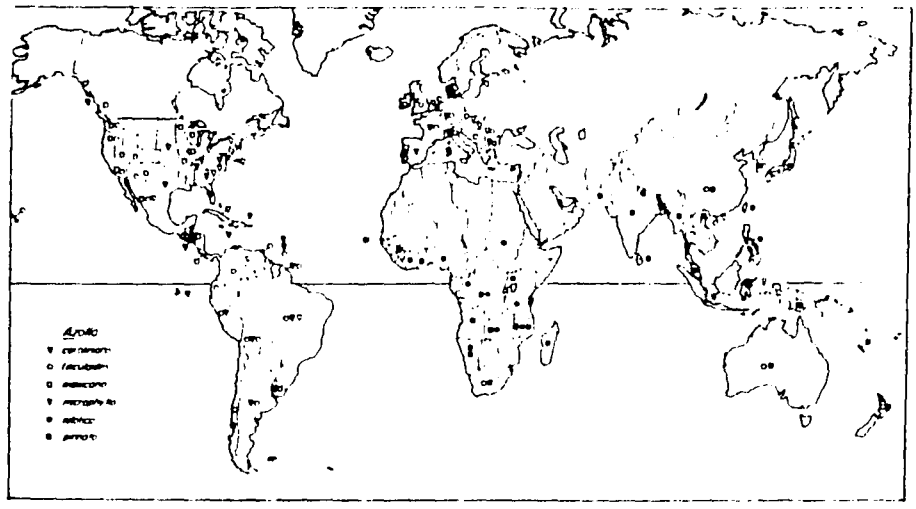

Fig. 4. Distribution of *Azolla* species.

1953), New York (Ott and Petrik-Ott, 1973), North Carolina (Ott and Petrik-Ott, 1973), Ohio (Ott and Petrik-Ott, 1973), South Carolina (Svenson, 1944), Tennessee (Shaver, 1954), Texas (Correll, 1956), Virginia (Ott and Petrik-Ott, 1973).

b. *A. filiculoides* (*japonica*, *rubra*). AFRICA: South Africa (Ashton and Walmsley, 1976). ASIA: Australia (Kleinschmidt, 1969), China (Reed, 1954), Japan (Seto and Nasu, 1975), New Zealand (Matthews, 1963). EUROPE: Belgium (Lawalree, 1964), Britain (Williams and Dollman, 1940), Bulgaria, Czechoslovakia (Sourek, 1958), France (Chevalier, 1926), Germany (Birkenbeil, 1974), Holland (Sculthorpe, 1967), Italy (Sculthorpe, 1967), Ireland (Sculthorpe, 1967), Portugal (Reed, 1962), Romania (Lawalree, 1964), Sardinia (Lawalree, 1964), Yugoslavia (Jalas and Suominen, 1972). LATIN AMERICA: Argentina (Tur, 1971), Bolivia (Svenson, 1944), Brazil (Reed, 1965), Chile (Reed, 1965), Colombia (Svenson, 1944), Ecuador (Herter, 1928), Guatemala (Svenson, 1944), Guyana (Reed, 1965), Honduras (Correll and Knobloch, 1962), Mexico (Svenson, 1944), Peru (Reed, 1965), Trinidad (Reed, 1965), Uruguay (LeGrand and Lombardo, 1958). NORTH AMERICA: Alaska (Svenson, 1944), Arizona (Svenson, 1944), California (Svenson, 1944), Hawaii (Neal, 1965), Oregon (Ott and Petrik-Ott, 1973), Washington (Ott and Petrik-Ott, 1973).

c. *A. mexicana*. LATIN AMERICA: Bolivia (Svenson, 1944), Costa Rica (Svenson, 1944), French Guiana (Correll and Knobloch, 1962), Honduras (Svenson, 1944), Mexico (Svenson, 1944). NORTH AMERICA: British Columbia (Ott and Petrik-Ott, 1973), California (Ott and Petrik-Ott, 1973), Illinois (Gunning and Lewis, 1957), Minnesota (Ott and Petrik-Ott, 1973), Missouri (Svenson, 1944), Nevada (Svenson, 1944), New Mexico (Ott and Petrik-Ott, 1973), Oregon (Svenson, 1944), Utah (Svenson, 1944), Washington (Svenson, 1944), Wisconsin (Ott and Petrik-Ott, 1973).

d. *A. microphylla*. LATIN AMERICA: Bolivia (Svenson, 1944), Brazil (Svenson, 1944), Dominican Republic (Svenson, 1944), El Salvador (Svenson, 1944), French Guiana (Svenson, 1944), Galapagos Islands (Morton and Wiggins, 1971), Guyana (Svenson, 1944), Peru (Svenson, 1944).

II. Section Rhizosperma

a. *A. nilotica*. AFRICA: Congo (Wild, 1961), Malawi (Reed, 1965), Mozambique (Reed, 1965), Namibia (Wild, 1961), Sudan (Wild, 1961), Tanzania (Demalsy, 1953), Uganda (Reed, 1965), Zaire (Demalsy, 1953, Zambia (Kornas, 1974).

b. *A. pinnata* (*africana*, *imbricata*). AFRICA: Angola (Sadebeck, 1902), Gambia (Reed, 1965), Ghana (Sweet and Hills, 1971), Guinea (Sadebeck, 1902), Ivory Coast (Sweet and Hills, 1971), Madagascar (Reed, 1954), Mozambique (Reed, 1965), Namibia (Reed, 1965), Nigeria (Sweet and Hills, 1971), South Africa (Oosthuizen and Walters, 1961), Zaire (Reed, 1965), Zambia (Reed, 1965). ASIA:

Australia (Sweet and Hills, 1971), Bangladesh (Sweet and Hills, 1971), Burma (Sweet and Hills, 1971), China (de Vol, 1945), India (Sweet and Hills, 1971), Indonesia (Soubert, 1949), Japan (Seto and Nasu, 1975), Korea (Mori, 1922), Malaysia (Chevalier, 1926), Nepal (Ohashi, 1975), New Caledonia (Chevalier, 1926), New Guinea (Sweet and Hills, 1971), New Zealand (Eady, 1974), Pakistan (Sweet and Hills, 1971), Philippines (Copeland, 1960), Sri Lanka (Svenson, 1944), Taiwan (Shen, 1960), Thailand (Sweet and Hills, 1971), Vietnam (Anonymous, 1918).

Anatomical studies

The anatomy of *Azolla* has been studied by Strasburger (1873), Queva (1910), Sud (1934), Rao (1936), Demalsy (1953, 1958), Bonnet (1957), Sweet and Hills (1971) and Konar and Kapoor (1972). The electron microscope has been used to observe leaves (Kawamatu, 1965b), megaspores (Martin, 1976), root caps (Kawamatu, 1962), chloroplasts in root hairs (Kawamatu, 1961, 1963), cortical microtubules (Gunning et al., 1976, 1977), the role of transfer cells in the symbiosis (Peters, 1976; Duckett et al., 1975a, 1975b) and *Anabaena azollae* heterocysts (Lang, 1965; Lang and Whitton, 1973; Grilli, 1964; Kawamatsu, 1965a).

1. Morphology and cytology.—*Azolla* plants are triangular or polygonal in shape, and float on the water surface individually or in mats. They give the appearance of a dark green to reddish carpet, except *A. nilotica* which does not produce the red anthocyanin pigment. Plant diameter ranges from 1–2.5 cm for small species, such as *A. pinnata*, to 15 or more cm for *A. nilotica* (Ridley, 1930). The latter species has leafy fronds placed on a wide-trailing leafless stem (Baker, 1887; Sadebeck, 1902).

The main rhizome bears several alternating branches with attached lateral branches. At the point of attachment each branch has an abscission layer which is important in vegetative reproduction (Westermaier and Ambronn, 1881; Rao, 1936; Konar and Kapoor, 1972). Cuticulate leaves are alternately arranged and each consists of a thick aerial dorsal lobe and a thin floating ventral lobe of slightly larger size (Fig. 5, 6). The papillose dorsal lobes are chlorophyllous except in the colorless margin and contain the symbiont within an ovoid cavity (Fig. 2) connected to the atmosphere by a pore (Fig. 7). The translucent ventral lobes resting on the water surface support the frond and are nearly achlorophyllous.

Stomata are present in vertical rows on both surfaces of the dorsal lobe and on the superior surface of the ventral lobe (Demalsy, 1953; Inamdar et al., 1971). Initially, each stomata has two guard cells, but these fuse to form a single annular guard cell with a central pore (Fig. 8) (Sud, 1934). Seto and Nasu (1975) reported that *A. filiculoides* (*japonica*) has two guard cells, and *A. pinnata* (*imbricata*) has only one. Inamdar et al. (1971) counted 112 stomata/mm^2 on a leaf of *A. pinnata*.

Adventitious roots hang in the water or, when in shallow water, occasionally penetrate into mud. Root length ranges from 1.5 cm (*A. pinnata*) to 11 cm (*A. nilotica*) depending upon the species. The roots develop in an acropetal fashion from branch points on the lower surface of the stem and have an abscission layer at the point of attachment (Rao, 1936). The young root is covered by a cap which is shed during growth of the basal root hairs (Leavitt, 1902; Chauveaud, 1901, 1911). Sircar (1935) and Kawamatu (1960) found granular mitochondria in the root

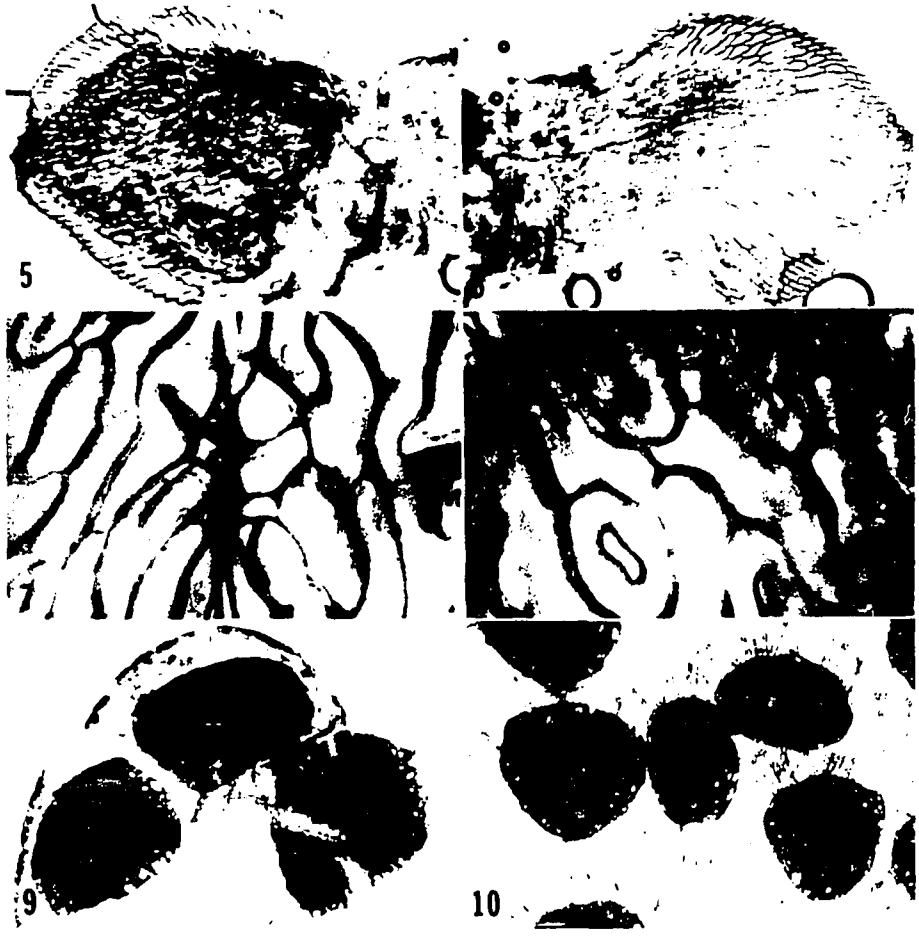

Fig. 5–10. Fig. 5. Vascular tissue and the colorless margin are visible on the dorsal lobe of an *Azolla filiculoides* leaf. Fig. 6. The achlorophyllous ventral lobe of an *Azolla filiculoides* leaf may play a role in flotation. Fig. 7. The large pore results when enclosing epidermal cells cap a leaf depression to form the algal cavity. Fig. 8. *Azolla* is unique in having a single annular guard cell surrounding each stoma. Fig. 9. Three massulae are visible within the crushed periplasmodium of an *Azolla pinnata* microsporangium. In this species, glochidia (protruding appendages) are somewhat pointed and cover only part of the massula surface. Fig. 10. Barbed glochidia, covering the total surface of *Azolla filiculoides* massulae, are representative of the section Euazolla.

hairs of *A. pinnata*. Numerous authors (Schimper, 1883; Rao, 1936; Atkinson, 1938; Kawamatu, 1961, 1965a, 1965b; Konar and Kapoor, 1972) have mentioned the existence of chloroplasts in the unicellular root hairs and in the cortical layers of the root. Transfer cells have also been detected in the roots (Duckett et al., 1975a, 1975b).

Using the acetocarmine squash technique, Loyal (1958) observed that the chromosomes of *A. pinnata* number 2n = 44 and are the smallest chromosomes in the ferns. The largest of these chromosome pairs measured only 2.08 microns

and the smallest was 1.04 microns (Loyal, 1972). Litardiere (1921) reported 2n = 48 in *A. caroliniana*, but his observation was disputed by Loyal (1958) because it was "based upon sectioned material which experience has shown may not be trustworthy." Duncan (1940) observed 18 and 20 chromosome pairs during meiosis in *A. filiculoides*.

2. Reproductive biology.—Commencing with Meyen (1836) and Griffith (1845), numerous authors have described the sporophytic cycle of *Azolla* (Fig. 3). Although no authors have mentioned methods of inducing sporocarp development, in *A. filiculoides* it is known to be associated with mat formation (Talley et al., 1977; T. Lumpkin, pers. obs.) and summer months in temperate regions (West, 1953; Correll, 1956; Ashton, 1974). *Azolla pinnata* sporocarp development is associated with winter months in both India (Konar and Kapoor, 1974) and Taiwan (Shen, 1960). Singh (1977d) reported that sporocarp development seems to retard growth of the fronds.

Sporocarp development on individual species was described by the following authors: Berggren (1882), Pfeiffer (1907) and Bergad (1972) described sporocarp development in *A. caroliniana*. Strasburger (1873), Rose (1883, 1888), Campbell (1893), Sadebeck (1902), Hannig (1911), Duncan (1940), Smith (1955), Bonnet (1957), Demalsy (1958), and McLean and Ivimey-Cook (1960) described the sporocarps of *A. filiculoides*. Meyen (1836), Baillon (1876) and Morton and Wiggins (1971) wrote brief reports about the sporocarps of *A. microphylla*. Rao (1936), Mulay (1938), Demalsy (1958), Shen (1960) and Konar and Kapoor (1974) described the sporocarps of *A. pinnata*. Demalsy (1953) wrote a commendable monograph on *A. nilotica* including information on its sporocarps. The article by Konar and Kapoor (1974) is one of the best on the subject. Sweet and Hills (1971), Seto and Nasu (1975) and Follieri (1977) prepared short glossaries of new terminology specifically for the sporocarps of *Azolla*.

Sporocarps are borne by short stalks on the first ventral lobe initial of a lateral branch and occur in pairs, except in *A. nilotica* in which they occur in tetrads (Demalsy, 1953). A sporangial pair may be of the same or opposite "sex." Microsporangia (male) are large and globular relative to the small and ovoid megasporangia (female).

As first observed by Strasburger (1873), the primordia of both microsporangia and megasporangia develop from megasporocarps. Sporangial development begins in a leptosporangiate fashion (Pfeiffer, 1907) when a ventral lobe initial divides (Bonnet, 1957) and eventually gives rise to 32 megaspore nuclei. If a megaspore develops, all but one of the apical nuclei abort. If all of the megaspore nuclei abort, microsporangial initials arise from basal outgrowths on the stalk of the megasporangium (Konar and Kapoor, 1974).

A megasporocarp takes about one week to mature (Campbell, 1893) and produces only one megaspore. A megaspore initial is embedded in the nutritious periplasmodium and is eventually covered by a thick perispore. Vacuoles form within the periplasmodium and give rise to the characteristic float corpuscles. The 3 floats in Euazolla or the 9 floats (3-float tier above a 6-float tier) in Rhizosperma are borne on a filamentous columella. Together, the floats and columella constitute the so-called "swimming apparatus" described by Strasburger (1873). Both megasporocarps and microsporocarps dehisce at maturity and sink to the

TABLE 1. CELL DIMENSIONS OF *ANABAENA AZOLLAE* IN VARIOUS *AZOLLA* SPECIES (μm).

Azolla species	Vegetative cells		Heterocysts		Source
	Diameter	Length	Diameter	Length	
A. caroliniana	5	8		10	Tilden, 1910
A. caroliniana	5	8	10		Toni, 1907
A. filiculoides	4–6	6–12.5			Hill, 1977
A. filiculoides	5	9	7	11	Pers. obs., T. Lumpkin
A. pinnata	4–5	5–7	6–7.5	7.5–8.5	Shen, 1960
A. pinnata	6–8	10–12			Singh, 1977a
Unknown	4–5.5	5–9.5	9.5	11.5	Geitler, 1925
Unknown	4–5	6–9.5	6–9.5	9–11.5	Prescott, 1951

bottom (Sud, 1934; Konar and Kapoor, 1974). After a dormant period, the submerged megaspores germinate and produce female prothalli (gametophytes), each of which produces one or more archegonia.

When microsporangial initials start to develop, additional initials appear. At maturity, as few as 8 (Svenson, 1944) or as many as 130 (Duncan, 1940) stalked microsporangia may occupy a microsporocarp. Within the periplasmodium of a microsporangia, 32 or 64 microspores develop and are aggregated into 3 (Godfrey et al., 1961; Demalsy, 1953) or 4–10 (Svenson, 1944) alveolar massulae which are homologous to float corpuscles in the megaspore. Massulae are created from vacuoles lined by a hardened network of cytoplasmic threads (Konar and Kapoor, 1974). Massulae are either bald (*A. nilotica*), partially covered (*A. pinnata*, Fig. 9) or totally covered (Euzolla, Fig. 10) with barbed protruding appendages (glochidia). After a microsporangium disintegrates and massulae are released, glochidia anchor massulae to megaspore entanglements. Next, microspores germinate and release antherozoids which escape through the gelatinized massula to fertilize the egg (oospore). Fertilization takes place underwater (Bierhorst, 1971).

The embryo produces a root and foot from hypobasal cells and a shoot and cotyledon from epibasal cells. As the cotyledon and first or second leaf emerge from the archegonium neck, the seedling floats to the surface (Campbell, 1893). The cotyledon lacks a cavity for the symbiont, but the succeeding dorsal lobes and shoot apex entrap *Anabaena* hormogonia (short filaments) surviving under the indusium cap. *Anabaena* hormogonia differentiate rapidly and begin growing in harmony with the fern.

Anabaena and morphology of the symbiosis

1. Taxonomy.—*Anabaena azollae* Strasburger is the only species mentioned in symbiotic association with *Azolla* (Fig. 9). However, Fjerdingstad (1976) claimed that the alga is actually an ecoform of *Anabaena variabilis* and should, therefore, be called *A. variabilis* status *azollae*. His proposal was based on second-hand information and a specimen of *Azolla* supposedly containing heterocyst-free algae. Wide variation within *A. azollae* is probably found within the 6 *Azolla* species, but information on this subject has not been reported.

Taxonomists place *Anabaena azollae* within the phylum Cyanophyta, order Nostocales, family Nostocaceae. The species has sinuous trichomes (threads) composed of bead-like or barrel-shaped cells without a sheath (Tilden, 1910;

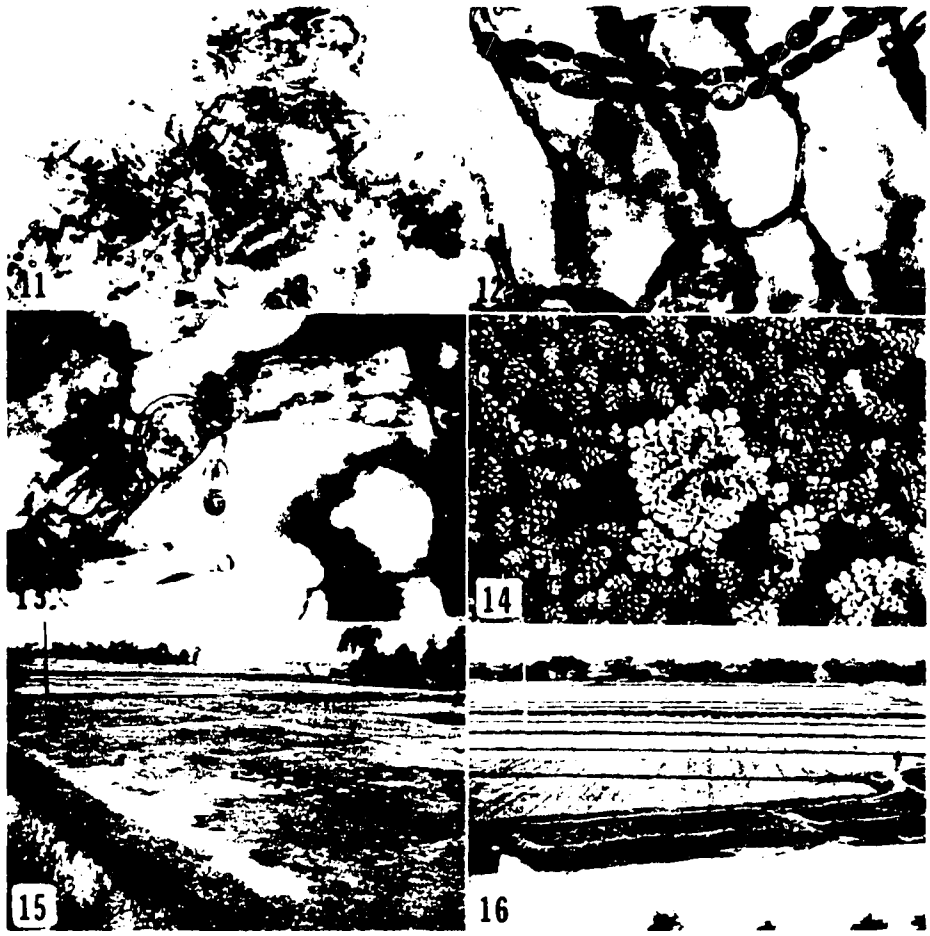

Fig. 11–16. Fig. 11. These *Anabaena azollae* and a few transfer hairs are the contents of one *Azolla pinnata* leaf cavity. Fig. 12. Two *Anabaena azollae* filaments within an *Azolla pinnata* leaf cavity. A transfer hair attachment point is visible in the center of the photograph. Fig. 13. An *Azolla pinnata* transfer hair with an *Anabaena* garland is shown partially torn from its anchorage (lower left). These transfer hairs are covered with rod-shaped bacteria. Fig. 14. A healthy *Azolla pinnata* plant was placed in the center of phosphorus deficient heat-stressed plants. The healthy plant is green and spreading compared to the crimson and compact phosphorus-deficient heat-stressed plants. Fig. 15. An *Azolla pinnata* nursery in Kiangsu province, People's Republic of China. *Azolla* is multiplied here before being introduced into newly-planted rice fields. Fig. 16. *Azolla* being cultivated in rice fields prior to the transplanting of rice seedlings. Note the rice-row barriers to prevent *Azolla* from drifting.

Geitler, 1925; Shen, 1960). There are three types of cells—vegetative cells (primary site of photosynthesis), heterocysts (site of nitrogen fixation, Fogg et al., 1973) and akinetes (thick-walled resting spores formed from vegetative cells). Several authors have not observed spores (Tilden, 1910; Prescott, 1951; Hill, 1977). The dimensions of *A. azollae* vegetative cells and heterocysts were partially reviewed by Fjerdingstad (1976). Table 1 presents cell dimensions of *Anabaena azollae* in relation to their associated *Azolla* species.

2. Morphology of the symbiosis.—*Sexual phase.* In illustrations by Strasburger (1873), Campbell (1893), Smith (1955), Bonnet (1957), Shen (1960), and Konar and Kapoor (1974), *A. azollae* akinetes are contained under the developing indusium (cap) of both the microsporocarps and megasporocarps. *Anabaena azollae* persists in the mature megasporocarps, but its fate in the microsporocarps is unknown. When an akinete germinates, its contents divide and form a short filament (hormogonium). The spore membrane becomes mucilaginous, swells, and then ruptures releasing its contents (Fritsch, 1904; Shen, 1960). After various attempts, Shen (1960) found she could induce formation of akinetes by running tap water over *Azolla* fronds. As is obvious from the literature, the life cycle of *A. azollae* is poorly understood.

Vegetative phase. During differentiation of the dorsal lobe primordia of the leaf, the cavity occupied by the symbiont is created by an epidermal cell growth covering a depression on the basal half of the lobe's ventral surface (Konar and Kapoor, 1972). Over the center of the depression, epidermal cells meet and form a large pore (Strasburger, 1873; Sadebeck, 1902; Rao, 1936; Shen, 1960; Konar and Kapoor, 1972) which may allow gaseous exchange between the cavity and the atmosphere (Fig. 7). Several *A. azollae* cells sheltered in the shoot apex are entrapped by the enclosing epidermal cells and begin colonizing the cavity particularly on its dorsal surface (Fig. 12). These algal cells are considered generative in function since they are composed largely of dividing cells which do not contain heterocysts nor fix nitrogen (Hill, 1977). The alga and fern develop in synchrony; as the leaf primordia develop, algal vegetative cells enlarge and a few differentiate into heterocysts (Fig. 12) which begin fixing nitrogen.

3. Heterocyst frequency and nitrogen fixation.—Lang (1965) and Grilli (1964) observed the sequential development of heterocysts from vegetative cells in *A. azollae* under the electron microscope. Lang (1965) noted similarities to heterocyst development in *A. cylindrica*. In *Azolla* fronds, heterocyst frequency increases from near zero at the shoot apex to a plateau of 29–33% in the 15th leaf, but after the 20th leaf, heterocysts begin to senesce (Hill, 1975). Hill (1977) measured nitrogen fixation at each leaf position on an *Azolla* frond, starting from the apex to the 32nd leaf and found that the rate of nitrogen fixation reaches a maximum at about the 12th leaf and declines with leaf senescence at about leaf 20 to 24. The average frequency of heterocysts observed by Peters (1975) was 23.1%, with the remainder composed of 60.9% vegetative cells and 16% akinetes. Other reported heterocyst frequencies are: 15–20% (Becking, 1976a) and 22–30% (Singh, 1977a). In comparison to *A. azollae*, the heterocyst frequency of free-living *A. cylindrica* was reported to be about 6% (Hill, 1975).

4. Transfer hairs.—The interior surface of a mature leaf cavity is lined with an envelope (Peters, 1976) and covered by a mucilaginous layer of unknown composition in which *A. azollae* filaments, multicellular transfer hairs and a few bacteria are found (Bottomley, 1920; Gregor, 1938; Grilli, 1964; Wieringa, 1968; Peters, 1976). Moore (1969) presumed that the mucilage was secreted by the transfer hair, but Duckett et al. (1975a) found that cavities freed of the symbiont did not contain mucilage. They speculated that mucilage normally found in the cavities was probably derived from the symbiont. Schaede (1947) and Grilli (1964)

claimed that liquid fills the whole cavity, but observations by the senior author indicate that the cavity is lined with mucilage and largely filled with gas.

Transfer hairs (Fig. 13) within the cavity appear to be organs of metabolic exchange between the fern and *A. azollae*. They demonstrate the transfer cell morphology of a dense cytoplasm containing abundant reticulum and numerous mitochondria (Duckett et al., 1975a), as defined by Gunning and Pate (1969). Konar and Kapoor (1972) observed that transfer hairs in the cavities are composed of several branched or unbranched cells and suggested that they are secretory in function. Duckett et al. (1975a) believed that the main function of the hairs is absorption of the nitrogenous products of *A. azollae*.

The senior author has counted 8–21 evenly spaced epidermal hairs per leaf in a small sample of *A. filiculoides*. Paradoxically, development of transfer hairs (and the cavity) is not dependent upon the presence of *A. azollae* (Peters and Mayne, 1974a; Peters, 1976; Ashton and Walmsley, 1976).

5. Bacteria.—*Anabaena azollae* shares the leaf cavity with small populations of bacteria. Isolated cultures of *A. azollae* were reportedly freed of bacteria by either ultraviolet radiation (Venkataraman, 1962) or heat treatment at 47°C for 100 minutes (Wieringa, 1968).

Bottomley (1920) mentioned isolating *Pseudomonas* and *Azotobacter* from the cavity. Peters and Mayne (1974b) conducted acetylene reduction assays on subcultures of the bacteria and concluded that they were non-nitrogen fixing. They also noted that *Azolla* fronds freed of *A. azollae* but supposedly containing bacteria do not fix nitrogen. Furthermore, bacteria are not involved in nitrogen fixation because nitrogen fixation in *Azolla* requires light and is inhibited by chloramphenicol (Peters, 1976; Peters et al., 1976; Peters and Mayne, 1974b).

Isolation and growth

1. Alga-free *Azolla*.—Naturally occurring alga-free *Azolla* have occasionally been reported (Marsh, 1914; Fremy, 1930; Hill, 1977) but are extremely rare. Moore (1969) reviewed the early methods claiming to produce alga-free *Azolla* fronds. These methods involved growing *Azolla* under conditions of environmental stress, such as cold, low light, and nutrient deficiency (Limburger, 1925; Huneke, 1933). Nickell (1958) pioneered the development of a dependable method for producing alga-free *Azolla* through the use of antibiotics. He treated *Azolla* sequentially in potassium penicillin, Terramycin, and streptomycin sulfate for one week each until *Azolla* was freed of *A. azollae* and contaminating microorganisms. His method was successfully employed by Johnson et al. (1966), Peters and Mayne (1974a) and Ashton and Walmsley (1976). Hill (1975, 1977) produced alga-free *Azolla* by first growing *Azolla* under low light intensity (1250 lux) and then under high light intensity (10,000 lux), a method similar to that reported by Schaede (1947). Alga-free *Azolla* is characteristically more compact, tends to have more roots and requires nitrogen fertilizer (Peters, 1976; Ashton and Walmsley, 1976; Hill, 1975).

Two techniques have been developed for isolating experimental quantities of *A. azollae* from *Azolla* fronds. One technique requires squashing the fronds with a teflon roller, followed by centrifugation and coarse filtering (Peters and Mayne,

1974a). The other technique utilizes repeated enzymatic digestion of *Azolla* fronds followed by several cycles of vortexing and screening until only algal packets surrounded by a filmy limiting envelope remain (Peters, 1976).

2. *Anabaena azollae*.—The difficulty of culturing *A. azollae* in isolation has been a major obstacle in elucidating the symbiosis. Numerous authors (Vouk and Wellisch, 1931; Huneke, 1933; Tuzimura et al., 1957; Shen, 1960; Venkataraman, 1962; Wieringa, 1968; Ashton and Walmsley, 1976; Becking, 1976a, 1976b) claimed to have grown *A. azollae* in isolation, but these reports were disputed by many who were unable to culture the alga in isolation (Singh, 1977a; Peters, 1976; Hill, 1975; Lang, 1965; Bortels, 1940; and Oes, 1913). Huneke (1933) and Bortels (1940) attempted to recombine isolated *A. azollae* and alga-free *Azolla*, but their attempts were unsuccessful.

PHYSIOLOGY AND BIOCHEMISTRY

Environmental factors

1. Nutrient requirement.—*Azolla* can be readily cultured in inorganic nutrient solution, and most researchers have used a nitrogen-free formula. Many use some dilution of a modified Knop's, Hoagland's, or Crone's formula. Watanabe and Espinas (1976) used a culture solution similar to that used for rice except that the solution included a double concentration of phosphorus (20 ppm), a triple concentration of molybdenum (0.1 ppm) and was nitrogen-free. Nickell (1958) added sucrose, thiamine, pyridoxin, nicotinamide, and potassium nitrate to his culture solution for aseptic *Azolla*. In a later study with aseptic *Azolla*, Nickell (1961) demonstrated that sucrose had a beneficial effect. Olsen (1972) added sodium to his nutrient solution because it was reported to be required by free-living *Anabaena cylindrica* and also he demonstrated that manganese was essential. Finally, nitrogen fixation by *Azolla-Anabaena* has been shown to require cobalt (Johnson et al., 1966; Olsen, 1972) and molybdenum (Bortels, 1940; Olsen, 1972).

Phosphorus is probably the most common factor limiting the growth of *Azolla* (Fig. 14). Fronds placed in a phosphorus deficient solution decreased or ceased growth, became red in color and developed curled roots (Cohn and Renlund, 1953; Watanabe and Espinas, 1976). Although the minimum phosphorus concentration for optimum growth is not known, Olsen (1972) found that *Azolla* thrives in Danish lakes with 1.1 mg P/l.

Another common limiting element is iron. In an acidic solution, Watanabe and Espinas (1976) have shown that 1 ppm Fe was sufficient for rapid growth, but in an iron deficient solution *Azolla* fronds became yellow. Deficiency problems arise in neutral to alkaline solution because ferric ions precipitate. In his definitive study on iron deficiency in *Azolla*, Olsen (1972) found that several elements interact with iron to affect its availability. His results showed competition between ferrous and manganous ions in neutral solution and reduction in absorption of both iron and manganese in a solution with high calcium concentration. At pH 4, ferric ions were so readily available that a high concentration of calcium was required to balance the increased absorption of iron; otherwise the fronds suffered from iron toxicity.

2. Light.—Ashton (1974) reported that relative growth rate and nitrogenase activity of *A. filiculoides* were maximum at 50% of full sunlight (40–57.5 klux). Also, nitrogenase activity declined more rapidly when light intensity increased in comparison to when light intensity decreased. Ahmad (1941a) found maximum growth in the range of 500–2000 lux. Others (Peters, 1975, 1976; Peters et al. 1976) reported that CO_2 fixation saturated at 8000 lux and nitrogen fixation saturated at 5000 lux in *A. caroliniana*. Reports from China (Lu et al., 1963; Anonymous, 1975b, 1975c) indicate that 25,000 lux resulted in the highest nitrogen content (4.8%), while 47,000 lux resulted in the highest rates of growth and nitrogen fixation for *A. pinnata*. They also reported that *A. pinnata* survived in a range from 3,500–120,000 lux but 20,000–40,000 was preferable since the nitrogen content was higher. Lu et al. (1963) showed that *Azolla* Leaf Area Index declined to zero as the LAI of rice increased to a maximum when heavy shading occurred.

3. pH.—*Azolla* can survive within a pH range of 3.5–10, but optimum growth is observed in the range of 4.5–7 (Nickell, 1961; Le Van and Sobochkin, 1963; Lu et al., 1963; Ashton, 1974; Anonymous, 1975a, 1975b, 1975c). Ashton (1974) found that relative growth rate is influenced by a direct relationship between light intensity and pH; high light intensity (60,000 lux) with high pH (9–10) and low light intensity (15,000 lux) with low pH (5–6) allowed maximum relative growth rates.

An inverse relationship between pH and temperature influences nitrate reduction and nitrogen fixation. Nitrate reduction was optimal at pH 4.5 and 30°C while nitrogen fixation was optimal at pH 6.0 and 20°C (Ashton, 1974; Holst and Yopp, 1976). Both Ashton (1974) and Watanabe et al. (1977) reported that nitrogen fixation decreased at neutral pH.

4. Temperature.—The most favorable temperature for growth and nitrogen fixation by *A. pinnata* is between 20–30°C. Outside of this range, growth decreases until the plant begins to die at temperatures below 5°C and above 45°C (Lu et al., 1963; Tran and Dao, 1973; Anonymous, 1971c, 1975a, 1975b, 1975c). Temperature affects both nitrogen and water content. *Azolla pinnata* grown at 5°C contained 1.75% N (dry wt.) and 84% H_2O (fr. wt.); at 25°C, it contained 4.5% N and 94% H_2O; and at 40°C it contained 2.5% N and 90% H_2O (Anonymous, 1975b). Talley et al. (1977) reported that *A. filiculoides* could withstand temperatures as low as −5°C without apparent harm but was less tolerant than *A. mexicana* to high temperature. Cold tolerance increased with pH and was highest in the pH range of 8–10 (Ashton, 1974).

5. Salinity.—The growth rate of *Azolla* gradually declines as salinity increases. At about 1.3% salt (33% of sea water) the growth of *Azolla* ceases and higher concentrations result in death (Haller et al., 1974). Tran and Dao (1973) suggested that the optimal concentration of mineral nutrients should be within the range of 90–150 mg/l. Le Van and Sobochkin (1963) reported that *Azolla* did well in lake water with a salt concentration of 160–380 mg/l but wilted in full Knop's solution (1500 mg/l) and in some rice fields where the salt concentration reached 1480–1872 mg/l during the summer season. Salinity is a factor which should be considered wherever the introduction of *Azolla* is being considered.

Life processes

1. Photosynthesis.—*Azolla* chloroplasts are comparable to spinach chloroplasts. They have a chlorophyll a/b ratio of 2.78 and demonstrate photosystem II and photosystem I activity as high as 45 (diphenyl carbazide → DCIP) and 246 (ascorbate + $DCIPH_2$ → $NADP^+$) μmol/mg chl·hr, respectively (Peters and Mayne, 1974a). As stated earlier, CO_2 fixation by both the symbiont and the association saturates at 8,000 lux (Peters, 1975).

Anabaena azollae contains the photosynthetic pigments, phycocyanin and chlorophyll a (Becking, 1976a; Peters and Mayne, 1974a). Chlorophyll a is the only chlorophyll pigment found in the Cyanophyta. The symbiont contains 10–20% of the association's chlorophyll a or 7.5–13% of the total chlorophyll (Peters and Mayne, 1974a). Phycocyanin, a water-soluble phycobilin pigment present in blue-green algae, traps light of low intensity inside the lobe cavity and passes the trapped energy on to chlorophyll.

Photosynthesis in the alga produces the reductant utilized for nitrogen fixation. This is illustrated by three findings: phycocyanin is concentrated in algal vegetative cells adjacent to the heterocysts (Becking, 1976a); CO_2 fixation occurs only in vegetative cells (Peters, 1975); and vegetative cells have a low chl/P700 ratio (Peters and Mayne, 1974a).

Even though the symbiont has a significant proportion of the total chlorophyll, Peters (1977) believes that the symbiont cannot photosynthetically support its level of nitrogen fixation and must supplement its energy requirements by either mixotropic activity (combining holophytic with saprophytic nutrition) or by cross-feeding of a carboxylated compound from the fern.

2. Light compensation point.—The light compensation point, defined as the light intensity where photosynthesis and respiration are in equilibrium, was determined for *A. filiculoides* by Ahmad (1941a, 1943) using light intensity and temperature as variables. He found that the light compensation point at all light intensities (400–2,000 lux) was reached when the temperature was reduced to 5°C or increased to 35°C. None of the light intensities used was low enough to determine the light compensation points between 5° and 35°C.

3. CO_2 compensation concentration.—Peters (1977) reported that an increase in the partial pressure of oxygen from 2% to 20% caused an increase in the CO_2 compensation concentration of the *Azolla* association from 19 ppm CO_2 to 51 ppm CO_2; the same increase had no effect on isolated *Anabaena azollae*. He interpreted these results to indicate that photorespiration does not occur in the symbiont.

4. Anthocyanin.—During periods of stress, anthocyanin is thought to protect the photosynthetic apparatus from damaging high light intensities by absorbing some of the light and converting it to heat. *Azolla* often exhibits a red color under field conditions, especially where phosphorus is deficient (Cohn and Renlund, 1953; Talley et al., 1977). Other authors (Olsen, 1972; Moore, 1969) have noted that fronds turn brick red in strong sunlight while fronds under shade retain their green color.

TABLE 2. EFFECT OF CHEMICALS ON THE GROWTH OF *AZOLLA*.

Growth regulators	Sources*	Remarks and recommendations
Gibberellin (GA)	(1)	reduces root number, roots appear tentacle-like
	(2)	inhibits natural fragmentation of frond, lethal at 100 mg/l in 7 days.
Indole acetic acid (IAA)	(1)	stimulatory at 0.1 ppm, inhibitory at 1 ppm and above.
	(2)	little growth effect, 1 mg/l, and less reduces fragmentation, 10 mg/l increases fragmentation, lethal at 100 mg/l in 24 h.
IAA + GA	(2)	fragmentation effect of 50 mg/l IAA is suppressed by 1–10 mg/l GA.
Maleic hydrazide	(1)	strong inhibition of growth
	(2)	no growth effect, except lethal at 10 mg/l in 48 h.
AMO 1618	(1)	ineffective at all levels
2,4-D	(1)	ineffective at all levels, except lethal at 100 ppm.
Diquat/paraquat	(3)	at 0.05–1.0 ppm becomes chlorotic and dies; starch granules and osmiophilic globules accumulate.
Herbicide studies		
2,4-D	(4)	not recommended, 1,000–10,000 ppm results in proportional necrosis.
	(5)	ineffective, 2,2-DPA-Amitrole-2,4-D.
	(6)	ineffective.
	(7)	effective, good control with granular butoxy ethanol ester of 2,4-D at 270 kg/ha of 2% w/w product.
Diquat	(8)	effective, 0.25–1.0 ppm active ingredient, foliar spray.
Diquat/paraquat	(9)	effective, 100% chlorotic at 1.0 ppm.
Paraquat	(5)	effective, surface application at 0.56–2.24 kg/ha.
Diesel 0:1	(6)	effective, foliar application, 1:1 with water.
	(5)	adequate, with PCP.

*Sources

(1) Nickell, 1961
(2) Dusek and Bondes, 1965
(3) Lang and Seaman, 1964
(4) Cohn and Renlund, 1953
(5) Matthews, 1963
(6) Oosthuizen and Walters, 1961
(7) Kleinschmidt, 1969
(8) State of Florida, 1973
(9) Blackburn and Weldon, 1965

As stated previously, greater than 50% of full sunlight reduces photosynthesis (Ashton, 1974). Personal observations (T. Lumpkin) suggest that the formation of anthocyanin is also caused by stress factors which limit photosynthesis, such as insect damage, high pH, or the low temperature associated with onset of winter, any of which limits the ability of the fern to utilize strong sunlight. *Azolla* anthocyanin pigment resembles luteolinidin-5-glucoside (Shimura and Terada, 1967; Holst, 1977).

5. Growth-regulating compounds.—The findings reported in published papers on the effect of growth regulating compounds on *Azolla* (Nickell, 1961; Dusek and Bondes, 1965; Lang and Seaman, 1964) are summarized in Table 2. Bottomley

(1920) and Ahmad (1941b) reported that auximone, derived from bacterized peat or from yeast, promoted the growth of *A. filiculoides*. Nickell (1961) used *Anabaena*-free *Azolla* in his study of growth regulators, while other researchers apparently used the intact association.

Nitrogen fixation

Although *Azolla* can extract nitrogen from its aquatic environment, the algal symbiont is capable of meeting the entire nitrogen requirement of the association. Nitrogen fixation by the *Azolla-Anabaena* symbiosis has been demonstrated indirectly by the use of nitrogen-free nutrient solution, acetylene reduction-gas chromatography, and assay of H_2 production and directly by the use of $^{15}N_2$. Nitrogen can be supplied to the association by N_2 fixation, by absorption from the aqueous medium, or by any combination of the two without the loss of nitrogenase activity (Peters et al., 1976). Nitrogenase activity of the endophytic *Anabaena azollae* is protected by the fern from combined nitrogen in the medium. Even after 6–7 mo of growth in a medium containing nitrogen, *Azolla* fronds still exhibited appreciable nitrogenase activity (Peters and Mayne, 1974b).

1. Acetylene reduction.—The acetylene reduction technique has been used to estimate nitrogenase activity of the association, the algal symbiont, and alga-free fronds. Although results shown in Table 3 verify that the symbiont is the agent of N_2 fixation, there is considerable variation among the figures.

Acetylene reduction by the association in the dark was reduced to 25–30% of the activity in the light (Becking, 1976a). Reduction by the isolated symbiont is negligible in dark anaerobic conditions, but reduction under dark aerobic conditions is 40% of light aerobic production until the endogenous substrates are depleted (Peters, 1975).

Nitrogenase requires 2 electrons to reduce C_2H_2 to C_2H_4 and 6 electrons to reduce N_2 to $2NH_3$. Theoretically, a conversion ratio of $3C_2H_2$ reduced per N_2 fixed should exist (Becking, 1976a; Brotonegoro and Abdulkadir, 1976). However, an atmosphere with C_2H_2 as a substrate suppresses H_2 production, while an atmosphere with N_2 as a substrate continues to use electrons to produce hydrogen. Therefore, determination of H_2 production in conjunction with C_2H_2 reduction and N_2 fixation is desirable. Peters et al. (1977) compared the partial pressure of 0.1 atmosphere C_2H_2 (95% inhibition of H_2 production) and various partial pressures of a mixture of $^{14}N_2$ and $^{15}N_2$, with H_2 production. They concluded that the conversion factor for C_2H_2/N_2 is actually between 1.6 and 2.0 for the association and 2.5 and 3.0 for the symbiont. Watanabe et al. (1977) found C_2H_2/N_2 conversion ratios for *A. pinnata* of 3.4, 1.6 and 2.4 after 14, 19 and 22 days of growth, respectively.

Considering that the alga's portion of the total plant nitrogen is about 10–17%, Becking (1976a) estimated that the nitrogenase activity (C_2H_2) of the alga is 6–10 times higher than the activity of the association and 12–20 times higher than the activity of free-living blue-green algae.

2. Effect of nitrogen fertilizer.—Nitrogen fertilizers usually have an adverse effect on the growth of *Azolla* (Oes, 1913; Le Van and Sobochkin, 1963; Anonymous,

TABLE 3. NITROGEN FIXATION RATES OF *AZOLLA*, ALGA-FREE *AZOLLA*, AND *ANABAENA AZOLLAE* UNDER VARIOUS LIGHT AND TEMPERATURE CONDITIONS.

Azolla spp.	Ethylene production	The association		Temp°C	Source
		%C_2H_2[a]	Light		
caroliniana	1.7–2.6 nmol/mg dry wt. min[b]	11–20	27 klux	29	Becking, 1976a
caroliniana	20–60 nmol/mg total chl. min	10	5 klux	24/28	Peters, 1976
caroliniana	25–60 nmol/mg total chl. min	10	8 klux	23	Peters and Mayne, 1974b
caroliniana	41 nmol/mg total chl. min	10	5.4–7.5 klux	26–28	Peters et al., 1976
caroliniana	13.96 nmol/mg total chl. min	10	5.4 klux	28	Peters et al., 1977
filiculoides	0.96 nmol/mg dry wt. min	20	40–57.5 klux	25	Ashton, 1974
filiculoides	0.58 nmol/mg dry wt. min	20	80–115 klux	25	Ashton, 1974
filiculoides	2.7–3.8 nmol/mg dry wt. min	9.8[b]	14 klux	27	Becking, 1976a
filiculoides	0.773 nmol/mg dry wt. min	*	300 μE/m^2/sec	26	Talley et al., 1977
filiculoides	0.559 nmol/mg dry wt. min	*	midday	*	Talley et al., 1977
mexicana	0.800 nmol/mg dry wt. min	*	300 μE/m^2/sec	26	Talley et al., 1977
mexicana	0.848 nmol/mg dry wt. min	*	midday	*	Talley et al., 1977
pinnata	0.6–1.4 nmol/mg dry wt. min	8.5[b]	midday	28–30	Becking, 1976b
pinnata	1.4–2.1 nmol/mg dry wt. min	12–21[b]	14 klux	29	Becking, 1976a
pinnata	21.6 mg/g dry wt. day	*	50–70 klux	24–35	Brotonegoro and
pinnata	2.25 mg/g dry wt. hr	*	60 klux	36	Abdulkadir, 1976
pinnata	0.9–1.1 nmol/mg fr wt. hr	10	4–5 klux	31–32	Watanabe and Epinas, 1976
		Algal-free Azolla			
caroliniana	0 nmol/mg total chl. min	10	8 klux	23	Peters and Mayne, 1974b
*	0 nmol/g fr wt. hr	10	*	25	Newton, 1976
		Isolated Anabaena azollae			
	0.9–3.1 nmol/mg protein min	14	19 klux	29	Becking, 1976a, 1976b
	50–150 nmol/mg algal chl. min[c]	10	4.3 klux	28	Peters, 1975
	50–150 nmol/mg algal chl. min	10	5/8 klux	24/28	Peters, 1976
	45 nmol/mg algal chl. min	10	8 klux	23	Peters and Mayne, 1974b
	92.97 nmol/mg algal chl. min	10	5.4 klux	28	Peters et al., 1977
	179 nmol/mg algal chl. a min	10	4.3 klux	28	Peters et al., 1976

[a] Percent acetylene in incubation atmosphere.

[b] 58 mg dry weight contained 11.6 mg protein (Becking, 1976a, 1976b)

[c] *A. azollae* contains 7.5–15% of the association's total chlorophyll or 10–20% of its chlorophyll a (Peters and Mayne, 1974a).

* Unknown.

1975b; Singh, 1977a) although other authors (Bortels, 1940; Tuzimura et al., 1957) have reported that low concentrations of nitrate and especially ammonium promote growth in culture solution. When *Azolla* is subjected to cold stress in the range of 5–15°C, nitrogen fixation declines sharply and nitrogen fertilizer is required (Lu et al., 1963; Anonymous, 1975b). Personal experience of the senior author (T. Lumpkin) indicates that nitrogen fertilizer per se does not have an adverse effect on the growth of *Azolla* but increased competition from other organisms stimulated by nitrogen fertilizer does have an adverse effect.

Reports on the effect of nitrogen fertilizer on acetylene reduction activity are inconsistent. Peters and Mayne (1974b) found a 30% decrease in acetylene reduction after 35 days and a 90% decrease after 6–7 mo for the association grown on a culture solution containing nitrate or urea. They also reported that ammonium chloride was inhibitory. Newton (1976) reported an 87% decrease in acetylene reduction by *Azolla* after growth in medium containing nitrate for an unreported length of time. However, Becking (1976a) reported that in the light, nitrate-grown *A. filiculoides* reduces twice as much acetylene compared to *A. pinnata* and *A. caroliniana* grown on nitrogen-free Crone's solution.

Peters (1977) used his data and the data from Newton (1976) to calculate the relative efficiency of nitrogen fixation for the association grown on N_2 and on nitrate. His data resulted in nitrogen fixation efficiency values of 0.94–0.99 for N_2-grown *Azolla* and 0.60–0.84 for nitrate-grown *Azolla*, while Newton's data resulted in efficiency values of 1.0 and 0.89 for N_2-grown and nitrate-grown *Azolla*, respectively.

3. Hydrogen production.—The *Azolla* association is capable of significant light-dependent, nitrogenase-catalyzed H_2 evolution (Peters, 1975, 1976; Peters et al., 1976, 1977; Newton, 1976). In an argon atmosphere, hydrogen production by the association and by the isolated alga was measured to be 21.7 and 88.6 nmol H_2/mg chl a·min, respectively (Peters et al., 1976). Newton (1976) measured levels as high as 760 nmol H_2/g fr wt·hr. Both articles reported that alga-free *Azolla* does not produce H_2 nor reduce acetylene, and that CO inhibits all reductions catalyzed by nitrogenase except the production of H_2. Peters et al. (1976) suggested that H_2 production is the result of a nitrogenase catalyzed, ATP-dependent reaction by the algal symbiont, since H_2 production by the isolated symbiont is inhibited by N_2 and C_2H_2. Also, he reported that H_2 evolution and acetylene reduction were completely inhibited by 5 μmol *m*-chlorocarbonyl cyanide phenylhydrazone (an uncoupler of phosphorylation) and partially inhibited by 12 μmol DCMU (diuron). This inhibition was also reported by Holst and Yopp (1976) and Newton (1976). Most authors believe that H_2 is produced from H_2O by reductant supplied by photosystem II.

4. Ammonia excretion and assimilation.—The isolated symbiont not only fixes nitrogen, but it also excretes ammonia (Peters, 1976; Ashton and Walmsley, 1976) and continues to excrete ammonia in an environment with ammonium chloride concentrations as high as 5 mmol (Peters, 1975). Peters (1977) used $^{15}N_2$ to determine the distribution of nitrogen compounds produced by the algal symbiont. From preliminary data he reported that the $^{15}N_2$ labeled products were 49.9% extracellular ammonia, 6.4% intracellular ammonia, 5.6% extracellular organic

nitrogen, and 38.1% intracellular organic nitrogen. Newton and Cavins (1976) reported that 43% of the total intracellular nitrogen in the free nitrogen pools of *Azolla* was in the form of ammonia, and the protein constituent of the pools contained 24% glutamine and 8% glutamate, which are carriers of ammonia. Venkataraman and Saxena (1963) listed aspartic acid, glutamic acid and alanine as the extracellular products of their independently cultured *Anabaena azollae*.

Ammonia produced by nitrogen fixation in the symbiont is excreted into the leaf cavity and is adsorbed by ammonia-assimilating enzymes in the fern. Glutamine synthetase (GS) is thought to be the principal ammonia-assimilating enzyme (Peters, 1977). Rhodes and Stewart (1974) have developed a procedure for the in vivo determination of GS activity by freezing *Azolla* with liquid nitrogen to render the cells permeable. They found GS activity as high as 0.78 μmol/min·gram fresh weight. Peters (1977) reported high GS activity in the association and low activity in the symbiont. Because the symbiont excretes ammonia, GS activity is expected to be high in the host, especially in transfer hairs.

Some *Azolla* species are thought to release nitrogenous compounds into their aquatic environment. Shen et al. (1963) reported that a Chinese variety of *Azolla* (Whole River Red) released 14–21% of its fixed nitrogen into the water. Saubert (1949) reported that 2% of the nitrogen assimilated by *A. pinnata* was released. Brill (cited by Peters, 1977) mentioned that a researcher working in his laboratory found a specimen of *A. mexicana* which excreted about 20% of its fixed nitrogen as ammonia. Talley et al. (1977) also speculated that *A. mexicana* released fixed nitrogen. However, Watanabe et al. (1977) found only 1 ppm ammonia in an originally nitrogen-free solution taken from a container where *A. pinnata* had been grown, and Peters (1977) did not find any nitrogen compounds in a solution where *A. caroliniana* had been grown.

AZOLLA IN AGRICULTURE

History of cultivation

1. Vietnam.—*Azolla pinnata* has been used as a green manure crop in Vietnam for centuries, long before French colonial rule (Bui, 1971). There are numerous embellished legends about its domestication and many conflicting reports about its subsequent use. Supposedly, 'Beo Giong' *Azolla* was discovered and domesticated in La Van village, Thai Binh province, by a peasant woman called Ba Heng (Le Van and Sobochkin, 1963). This domesticated *Azolla* was so effective for spring rice that, after Ba Heng's death, the villagers erected a pagoda in her honor and offered prayers and sacrifices to her spirit.

'Beo Giong' *Azolla* multiplied rapidly only during the colder seasons, particularly before and after transplanting of the spring rice crop in January. When the average temperature rose to about 22°C in April, 'Beo Giong' *Azolla* withered away and after 5–7 days released its nutrients as the rice crop entered the stage of maximum tillering (Duong, 1971). Wild types of *Azolla* either did not die, or did not die early enough to fertilize the standing crop (Burkill, 1966) because they were resistant to higher temperatures (Chevalier, 1926).

By the early part of this century, utilization of *Azolla* had spread only to the adjacent provinces of Nam-Dinh, Hai-Duong and Hung-Yen because each autumn

TABLE 4. ESTIMATES OF THE AREA UNDER *AZOLLA* CULTIVATION IN NORTHERN VIETNAM.

Year	Hectares	Source
1954	5,000	Bui and Tan, 1976
1955	40,000	Tran and Dao, 1973
1957	90,000	Karamyshev, 1957
1971	300,000	Bui, 1971
1973	500,000	Gigineishvili, 1973
1976	400,000	Bui, 1976

rice farmers had to go to La Van for starter colonies of 'Beo Giong' since their stock had perished in the spring. Only a few families in La Van knew the secret techniques of selecting and multiplying 'Beo Giong' *Azolla* during the hot summer. These secret techniques were retained by the families' males through a strict system of taboos and inheritance (Galston, 1975). There are two theories about what comprised these secret techniques.

One theory is based on sexual reproduction. 'Beo Giong' *Azolla* produces sporocarps prior to senescence in April (Nguyen, 1930). In July, the developing *Azolla* embryos float to the surface of ponds. Experts in La Van identified and collected 'Beo Giong' *Azolla* plants in these ponds. Supposedly, this *Azolla* had dark green stripes with thick leaves and transparent roots (Nguyen, 1930). These plants were propagated behind high fences in ponds especially designed for this purpose. The ponds were cleaned of all fish and aquatic animals and then the water level was brought to a depth of 1 m. The young *Azolla* plants were placed in floating bamboo frames to keep them from being blown about and were regularly fertilized with pig manure, ash, urine, and or castor oil cake (Nguyen, 1930).

Another theory involved preserving a stock of 'Beo Giong' *Azolla* by regulating the acidity of propagation ponds or vats to prevent *Azolla* senescence (Galston, 1975). Many paddy fields become increasingly alkaline as the growing season progresses, making iron and phosphorus unavailable to floating *Azolla*. A secret formula of acidification kept 'Beo Giong' *Azolla* alive until it was sold as a starter stock in the autumn.

In either case, the villagers of La Van began selling starter stocks of 'Beo Giong' *Azolla* in November to regional propagators at high prices which declined during the season (Chevalier, 1926). Peasants who bought early, later sold stocks to their neighbors. The fern was multiplied in old rice seedling beds or in some other highly nutritious environment. Then it was placed in corners of the rice fields for propagation and surrounded by a low dike made of earth, straw or bamboo to prevent the fronds from being scattered by the wind (Nguyen, 1930) which retarded their growth (Braemer, 1927a). As the *Azolla* multiplied, enclosures in each corner were enlarged daily as needed until most of the rice field was covered by a dense carpet (Nguyen, 1930). A 3 kg stock cultivated in November would yield 2.5 tons of fresh *Azolla* by February (Nguyen, 1930).

The present government of Vietnam became interested in the potential of both *Azolla pinnata* and *Sesbania cannabina* in 1954 when the secrets involved in the

cultivation of these green manures were made public (Pham, 1971). An extension network was organized to stimulate the utilization of *Azolla*, and over 1,000 depots for its multiplication were established (Moore, 1969), resulting in its spread throughout the northern part of the country (Bui, 1967) (Table 4).

In the mid-1960s the Vietnamese government made renewed efforts to extend the area under *Azolla* cultivation. Some cooperatives experienced repeated failures before cultivation of *Azolla* was established (Pham, 1971), especially in summer. Growth rates of *Azolla* are actually higher in the summer (Dao and Tran, 1966), but, because of the extreme parasite problem and high temperatures during summer, cultivation is rarely practiced then (Tran and Dao, 1973). Because of this problem Chevalier (1926) proposed the use of heat tolerant "wild" *Azolla* and Bui (1966) suggested planting the "wide-spreading" strain during summer. Nevertheless, *Sesbania cannabina* has replaced *Azolla* as a green manure crop for the summer rice crop in Thanh Oai district (Nguyen, 1971; Pham, 1971).

Dao and Tran (1966) described a 6-step approach for cooperatives to introduce *Azolla* cultivation into rotation with their rice. In essence the 6 points are as follows:

(1) Rearrange the crop rotation schedule to accommodate *Azolla* production in all seasons, especially during the gaps between rice crops. Five to 10 percent of the summer paddy fields should be used to produce the quantity of *Azolla* needed to fertilize the spring rice crop. Rice seedling fields are useful for this purpose.

(2) Take measures to provide a year-round water supply for *Azolla* production. The use of silty water is recommended to reduce dependence on fertilizers for *Azolla*.

(3) The cooperative should organize an *Azolla* team and train them for 3–6 mo on the difficult techniques and meticulous care required for multiplying *Azolla*. Training should emphasize sowing densities, fertilizer requirements, and protection from insects.

(4) To insure a high yield and a low price, labor should be organized with the principle of product consignment in mind. Each member of the *Azolla* team should be responsible for 0.5–1 ha of *Azolla*, while other cooperative members should take care of 0.3–0.5 ha of *Azolla* when it is introduced into the rice fields.

(5) A good technical and material foundation for the production of *Azolla* must be insured. The annual requirements for one hectare of *Azolla* are 360–500 kg P_2O_5 (158–220 kg P), 3600–5000 kg ash (on poor land) and 5–7 l of "Vofatoc" insecticide.

(6) The schedule for raising and harvesting *Azolla* must be closely coordinated with the sowing and transplanting of rice in order to attain 100% coverage of the rice area.

Two varieties of *A. pinnata* are presently recognized in northern Vietnam—"green *Azolla*" with green dorsal lobes and white ventral lobes, and "purple *Azolla*" with green dorsal lobes, which turn red-violet in case of insufficient nutrition, and pink ventral lobes (Tran and Dao, 1973).

2. China.—Short histories of *Azolla* cultivation in China are provided by Lu et al. (1963) and by the Chekiang Agricultural Academy (Anonymous, 1975b). The cultivation of *A. pinnata* (syn. *A. imbricata*) in Chinese rice fields was reported

to extend back two centuries, with long histories of utilization in Chekiang, Fukien, Szechuan and Kwangtung (Canton) provinces.

In Chekiang province, the use of *Azolla* expanded gradually after 1948 when about 16,200 ha were cultivated. By 1962, when major research efforts began, only 24,200 ha were under *Azolla*. But within a few years, research overcame the two major obstacles impeding the spread of *Azolla*. First, new techniques in overwintering the *Azolla* were developed (Anonymous, 1974a, 1975b) to supplement the ancient use of hot springs for this purpose. The second obstacle was conquered in 1961 when techniques were developed to prevent the "summer death" of *Azolla*.

In conjunction with these efforts, selections from wild *Azolla* were identified and cultivated, the life cycle and activities of pests infesting *Azolla* were observed, and experiments were conducted to determine proper methods of fertilizing and utilizing *Azolla*. The success of these efforts made possible additional use of *Azolla* during the summer and fall and allowed its cultivation to spread inland and northward into cooler regions.

In China, cultivars of *A. pinnata* are divided into four categories, and their characteristics and usage are described in scientific magazines and provincial and national pamphlets. These cultivars and their pertinent publications are: "Red *Azolla*" (Anonymous, 1975a, 1975c, 1976c), "Green *Azolla*" (Anonymous, 1971b, 1974c, 1975b, 1976c), Wild *Azolla*—"Whole River Red" (Shen et al., 1963; Anonymous, 1974b, 1975a, 1975b), and "Vietnam *Azolla*" (Anonymous, 1975a).

Green manure

Azolla is primarily grown as a green manure for rice, but it is also grown with water bamboo (*Zizania aquatica*), arrowhead (*Sagittaria sagittifolia*) and taro (*Colocasia esculenta*) (Anonymous, 1975b). The Kiangsu People's Publishers (Anonymous, 1976b) recently released a booklet about producing compost for use on any crop from *Azolla*, water hyacinth, and other aquatic weeds.

Ngo (1973) examined the positive effect of *Azolla* green manure on the number of shoots, length of longest leaf, fresh weight, and dry weight of rice plants. Shen et al. (1963) compared the nitrogen fixation ability of "Red *Azolla*" with alfalfa (*Medicago sativa*) and soybean (*Glycine max*). They discovered that 1.5 mo of *Azolla* cultivation increased the nitrogen content of the soil to a level equal to that produced by a crop of soybeans, but to only 40% of the level produced by alfalfa. Their calculations were based on alfalfa, soybean and "Red *Azolla*" nitrogen contents 2.87%, 2.90% and 3.5%, respectively (dry weight basis). Also, they pointed out that since cultivation of *Azolla* is combined with rice, it does not occupy extra land. Talley et al. (1977) reported that *A. filiculoides* could provide one half of the nitrogen requirement for California rice if it were grown as a green manure before rice seeding. Tran and Dao (1973) reported that two successive *Azolla* layers, incorporated into the soil before rice transplanting, can supply 50 percent of the nitrogen necessary to produce 5 tons of rice per hectare.

1. Yield.—The maximum density of an *Azolla* layer is subject to considerable variation. Gopal (1967) reported a maximum yield of 37.8 tons/ha fresh weight containing 2.78 tons dry weight of *A. pinnata* growing on a temporary pond in

India. Talley et al. (1977) reported maximum yields of 0.98 ton/ha dry weight (24–45 kg N/ha) for *A. mexicana* and 1.8–2.57 tons/ha dry weight (58–105 kg N/ha) for *A. filiculoides*. A yield of 3 tons/ha dry weight (90 kg N/ha) of *A. filiculoides* was obtained in Denmark (Olsen, 1972). Under Asian rice field conditions, *A. pinnata* could realistically produce yields of 8–10 tons/ha (25–50 kg N/ha) per crop fresh weight (Tran and Dao, 1973).

2. Annual N_2 fixation.—Estimates of the annual nitrogen fixation potential and rate vary widely. Becking (1972) estimated 335–670 kg N/ha·year for *A. pinnata* in Indonesia, but later revised his estimate (Becking, 1976a) to a more conservative 103–162 kg N/ha·year. Talley et al. (1977) reported that *A. filiculoides* and *A. mexicana* produced 52 kg N/ha and 41 kg N/ha, respectively, in 35 days under field conditions. Both species fixed nitrogen at a rate of 1.2 kg/ha·day between 10–35 days after field inoculation. Watanabe et al. (1977) estimated a daily rate of 1.1·kg N/ha or 120 kg N/ha in 106 days by *A. pinnata*. Moore (1969) estimated potential fixation values of 100–160 kg N/ha in 3–4 mo. The highest estimates of annual fixation potential come from the two countries with the longest experience of cultivation. The Vietnam Institute of Agriculture (Pham, 1971) suggested an approximate potential of 1000 kg N/ha, while the People's Republic of China published figures of 92.7–151.8 kg N/ha in 1.5 mo (Shen et al., 1963) and 59.2 kg N/ha in 30 days (Lu et al., 1963).

The Institute of Soils and Fertilizers in the Chekiang Agriculture Academy, China (Anonymous, 1975b), reported that *Azolla* used as a green manure decreased specific gravity, increased porosity (3.7–4.2%) and increased organic matter in soils. Also, in one of their experiments, growth of *Azolla* reduced evaporation by 11%, water salt content by 0.012–0.049%, and soil salt content by 0.014–0.048%.

3. Rice yields.—The effect of *Azolla* on rice yields has been the primary focus of most research. In his excellent review, Moore (1969) cited rice yield increases of 14, 17, 22 and 40 percent with the cultivation of *Azolla*. Talley et al. (1977) achieved 1) rice yield increases of 112% over the control by incorporating one 60 kg N/ha layer of *A. filiculoides* into the paddy soil and 2) a 216% increase (over 4 metric tons) by first incorporating one layer (as in 1 above) and then growing *Azolla* as a dual crop with rice. Singh (1977a) obtained a 6% rice yield increase when *A. pinnata* was grown with rice but was not incorporated and increases ranging from 9–38% when *Azolla* was incorporated into the soil. Watanabe (1977) reported a 13% increase in grain yield with incorporated *A. pinnata*, but recently he has observed considerably higher yields (personal communication), possibly due to a cumulative effect of slow nitrogen release from successive manuring with *Azolla*. Scientists in the People's Republic of China (Anonymous, 1975b) reported rice yield increases of 0.4–158%, with an average of 18.6%, from the results of 422 field experiments. An *Azolla*-rice experiment in Sri Lanka (Kulosorriya and de Silva, 1977) produced 32% more filled grains per panicle compared to control; unfortunately, grain yields were not reported.

4. Growth rate.—*Azolla* has an exponential growth potential which is subject to numerous environmental variables. *A. pinnata* can double its biomass in 3–5

TABLE 5. ELEMENTAL ANALYSES OF *AZOLLA* TISSUE ON DRY WEIGHT BASIS.

% Total dry wt.	Percent								PPM				Species	Remark	Reference
	N	P	K	Ca	Mg	S	Na	Cl	Mn	Fe	Cu	Zn			
6.4–7.2	3.6–4.75	0.12–0.95	0.88–6.52	0.60–2.49	0.25–0.41								Mixed	Lit. review	Moore, 1969
6–7	3–4	0.6–1.5	1.5–2.5										*A. pinnata*		Anonymous, 1975a,b,c
	4.47	0.49		0.97									*A. filiculoides*		Buckingham et al., 1977
	3.44	0.43	1.98	0.61	0.62	0.61	0.92	2.50	430.	5,870.	14.	79.	*A. filiculoides*	Taro field	Unpublished data, T. Lumpkin
	2.16	0.16	1.99	0.79	0.43	2.63 ~~6.30~~			1,456.	3,045.	12.	392.	*A. filiculoides*	Red	Unpublished data, T. Lumpkin
	2.77	0.21	2.57	0.65	0.54	0.37			1,911.	3,022.	39.	487.	*A. filiculoides*	Red center	Unpublished data, T. Lumpkin
	3.46	0.43	2.56	0.65	0.47[a]	0.47			2,331.	5,426.	29.	135.	*A. filiculoides*	Green	Unpublished data, T. Lumpkin

TABLE 6. PERCENTAGE OF SPECIFIC FREE AMINO ACIDS IN *AZOLLA*.

Species	Ala	Arg	Asn	Asp	Cys	Cyth	Gln	Glu	Gly	Leu Ile	Met	Phe	Pro	Ser	Thr	Try	Val	*	Chromatographic solution	Reference
A. filiculoides	13.5	1.1	11.6	6.4	0.8		37.5	12.9	1.9	1.1	0.1	—	0.1			0.2	4.7	7.7	Thin layer Knop's	Lahdesmaki, 1968
A. caroliniana	5.5		1.8	3.6		12.7	43.6	14.5				3.6		7.3	1.8			5.5	Column N-free Hoagland's	Newton & Cavins, 1976[b]

[a] Y-amino butyric acid

[b] Percentages calculated from data reported in reference

days, but in the field 5–10 days is more likely (Duong, 1971; Tran and Dao, 1973; Watanabe et al., 1977; Watanabe, 1977). The senior author has observed a doubling time of 2.8 days with *A. pinnata* in the phytotron at the International Rice Research Institute (IRRI). Talley et al. (1977) measured a similar doubling time (2.8 days) for *A. mexicana* but a longer doubling time of about 7 days for *A. filiculoides*. Mitchell (1974) reviewed the mathematical formulae used to analyze the exponential growth rate of *Azolla* and other aquatic weeds. He discussed the importance of a stable hydrological regime for good growth of aquatic weeds.

5. Composition.—On a dry weight basis (Tables 5, 6), *Azolla* has a substantial protein content, with reports as high as 13% (Tran and Dao, 1973), 16.1% (Anonymous, 1975a), 22.6% (Fujiwara et al., 1947) and 23.4% (Buckingham et al., 1977). Buckingham et al. (1977) conducted amino acid analyses and rat feeding experiments with *A. filiculoides*. Amino acid analysis indicated lysine, methionine and histamine were probably limiting. Feeding trials with *Azolla* verified the preceding point and indicated that indigestible fiber and minerals were excessive. Their experiments also indicated that *Azolla* did not contain growth inhibitors or toxins for rats.

On a dry weight basis, other reported constituents are: lignin 9.3% and cellulose 15.2% in *A. filiculoides* (Buckingham et al., 1977); ash 23.8%, fat 4.42%, fiber 9.5%, and starch 6.38% in *A. pinnata* (Fujiwara et al., 1947) and, also in *A. pinnata*, ash 9.7%, carbohydrate 61%, crude fat 6.3%, and protein 23% (Varghese et al., 1976).

A carbon to nitrogen ratio (C:N) of 15:1 for *A. pinnata* was reported by Chinese scientists (Anonymous, 1975b). Peters (1977) found that *A. caroliniana* grown on different sources of nitrogen resulted in different C:N ratios: 18.1 with N_2 only, 15.4 with urea and N_2, and 10.4–10.6 with nitrate and air or argon.

6. Decomposition.—Talley et al. (1977) and others have recognized an increase in nitrogen recovery when *Azolla* is incorporated into soil rather than allowed to decompose into water. *Azolla* nitrogen is released slowly and its availability to the first rice crop is only about 70% of that of ammonium sulfate (Watanabe et al., 1977). Singh (1977d) observed that *Azolla* decomposed after 8–10 days in Indian paddy soil, and the rice crop benefited noticeably after 20–30 days. Other authors have reported that two-thirds of *Azolla* nitrogen was released after 6 weeks (Watanabe et al., 1977) or 5–8 weeks (Tuzimura et al., 1957) in paddy soil.

Management practices

As a green manure for rice in tropical Asia, *Azolla* is cultivated in essentially two ways (Bui, 1966; Duong, 1971; Anonymous, 1975a, 1975b, 1975c, 1976c). In brief, one way is to set aside approximately 5–10% of the crop area for year-round *Azolla* production for each crop of rice to be grown. In consequence, two crops of rice would require 10–20% of the cultivated area in *Azolla*. *Azolla* is cultivated in special fields (Fig. 15) or ponds and later added as compost to the rice or other crops. In the second way, *Azolla* is cultivated in the rice fields (Fig. 16) and incorporated into the paddy soil during intervals before and/or after the rice crop and between crops. Preferably, *Azolla* is grown and incorporated sev-

eral times before transplanting the rice seedlings. As *Azolla* approaches maximum density and its growth rate begins to decline, ⅓–½ of the *Azolla* is retained as seed for the next crop, and the remainder is incorporated into the paddy soil. In both methods, *Azolla* is usually cultivated as a dual crop with rice.

1. Planting density.—The inoculum rate or planting density is an important factor in the efficient production of *Azolla*. Since *Azolla* is propagated vegetatively, the planting density should be high enough to intercept most sunlight, yet low enough to permit a several-fold multiplication before high density reduces its growth rate. The following recommended planting densities (as kilograms fresh weight) are subject to modification due to labor costs and environmental factors. The Vietnamese (Tran and Dao, 1973) recommended an *A. pinnata* planting density of 0.5 kg/m^2 which increases to a density of 1–1.6 kg/m^2 in winter and 1–1.4 kg/m^2 in summer. If algal blooms are expected to be a problem, they recommend a rate of 0.7–0.8 kg/m^2 to prevent sunlight from reaching the algae. Singh used planting densities of 0.1–0.3 kg/m^2 (1977b), 0.37 kg/m^2 and 0.4 kg/m^2 (1977a). The last-mentioned density resulted in 8–15 tons/ha of green manure in 8–20 days with a nitrogen content of 30–50 kg/ha. Talley et al. (1977) followed a Vietnamese recommendation (Bui and Tan, 1976) of 0.5 kg/m^2 for their experiments with *A. mexicana* and *A. filiculoides* in California.

2. Fertilizer requirement.—*Azolla* is notably responsive to phosphorus fertilizer and requires a continuous supply of the element for rapid propagation. Talley et al. (1977) applied phosphorus fertilizer at the rate of 7.2 kg P/ha as KH_2PO_4 in four equal doses 7 days apart. They concluded that each kg of phosphorus resulted in more than 5 kg of additional nitrogen in the *Azolla* biomass after 35 days of growth. Their conclusion followed an earlier report by Tran and Dao (1973) that 1 kg of P_2O_5 (440 g P) resulted in a quantity of *Azolla* equivalent to 2.2 kg of nitrogen. The Vietnamese recommended 5–10 kg of superphosphate (1–2 kg P_2O_5) per ha every 5 days. Singh (1977a) recommended 4–6 kg P_2O_5/ha/week.

Shen et al. (1963) conducted a phosphorus fertilizer experiment on *Azolla* with 1.5, 3 and 6 kg of superphosphate (0.3, 0.6 and 1.2 kg P_2O_5) in plots of 8.5 m^2. The resulting nitrogen increases were 35, 76, and 190 percent, respectively, after 8 days. Tuzimura et al. (1957) conducted phosphorus fertilizer experiments using 0.5 m^2 buried earthenware pipes as a growing container. An inoculum of 0.4 g *A. filiculoides* per pipe resulted in the following weight increases above the zero P rate after 40 days: 69% (20 kg P_2O_5/ha), 349% (40 kg P_2O_5/ha), 948% (80 kg P_2O_5/ha) and 879% (160 kg P_2O_5/ha). Ideally, phosphate fertilizer should probably be applied as a solution in frequent small doses into neutral to acidic paddy water.

On light soils with low organic matter and mineral nutrients, Tran and Dao (1973) recommended 5 kg of K_2O per ha (4.1 kg K/ha) every 5 days. Also, these authors suggested that microelements can be applied as ash at the rate of 100 kg per ha every 5 days. Singh (1977b) used 5–8 kg K_2O (4.1–6.56 kg K) per ha per week and 50 kg ash per ha to supply microelements. In another experiment (Singh, 1977a), 0.125 kg/ha of molybdenum was applied per month to enhance nitrogen fixation. Talley et al. (1977) obtained a significant *Azolla* growth response from a single application of 0.8 kg of iron per hectare as 0.01 M ferric ethylenediaminetetracetate (EDTA), but they did not believe that the iron deficiency in

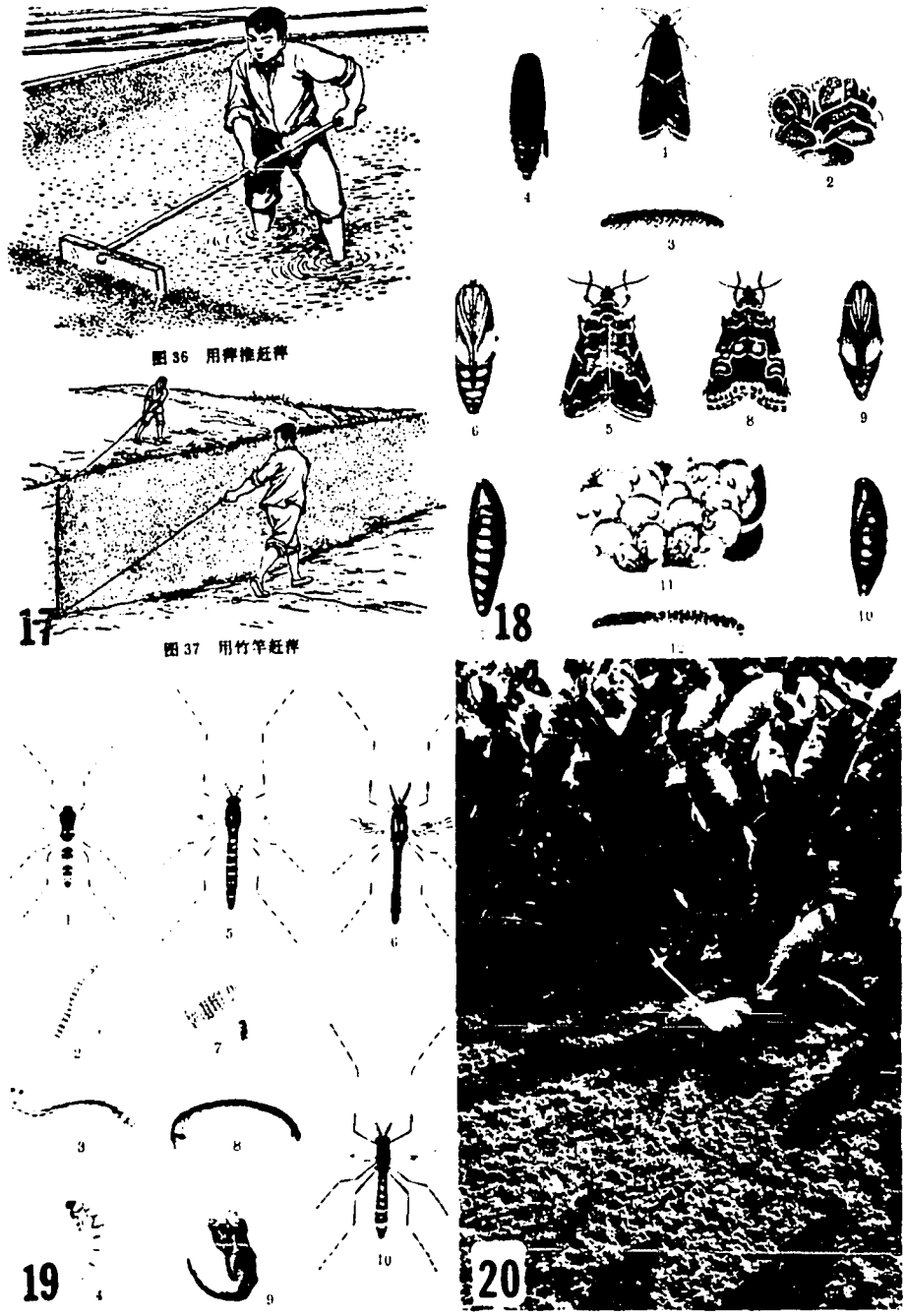

Fig. 17–20. Fig. 17. Chinese (PRC) illustration of an *Azolla* pusher and bamboo pole used for amassing and transporting *Azolla* (Anonymous, 1975b). Fig. 18. An illustration of *Azolla* moth life-cycles in a Chinese (PRC) publication. Larva of *Pyralis* sp. (above) and *Nymphula* spp. (below) of the Pyralidae family attack *Azolla* mats (Anonymous, 1975b). Fig. 19. Chinese (PRC) illustration on

their test paddies was a widespread nutrient problem. Finally, boron deficiency has been observed in paddy fields of southern China (Lumpkin, 1977).

3. Water control.—Water control is critical, especially for year-round cultivation of *Azolla*. A water level which allows *Azolla* roots to touch the soil surface will often cause mineral deficiencies to disappear. This phenomenon has been observed in Hawaiian taro fields and was observed in rice fields by Saubert (1949) and Talley et al. (1977). The latter authors recommended low water levels and rough plowing to protect *Azolla* from wind and wave action, which can eventually fragment and kill *Azolla* (Ashton, 1974). *Azolla filiculoides* was seen surviving on moist soil under Hawaiian taro plants, but dispersion was limited since standing water was not present. Nguyen and Nguyen (1934) suggested that *Azolla* could be kept alive during the hot season by growing it on mud-covered floating rafts under a sun screen.

4. Tools.—An *Azolla* publication by the Chekiang Academy of Agricultural Science (Anonymous, 1975b) included a section on hand tools useful for intensive small scale cultivation of *Azolla*. This is the only publication known to include any information on tools. An *Azolla* basket, pusher, scooper, scrape board and beater are described (Fig. 17).

5. Rotation.—Vietnam has shifted to intensive farming and multiple cropping in order to increase their annual rice yields from 5 or fewer tons/ha to 15 or more tons/ha. Inclusion of *Azolla* in their rotation patterns has been a key point in this shift. Duong (1971) listed three rotations where *Azolla* was included: (1) dry ploughing + *Azolla* + spring rice + *Sesbania cannabina* + "tenth-moon" rice, (2) *Azolla* + spring rice + *Azolla* + "tenth-moon" rice, and (3) *Azolla* + spring rice + *Azolla* + rice with a short duration of growth (with late transplantation in August so that *Azolla* may give a high yield). Duong (1973) listed rotations which included *Azolla* for clay soil: (1) spring rice + "tenth-moon" rice + winter farming + *Azolla* and (2) spring rice + "tenth-moon" rice + *Sesbania cannabina* + *Azolla*. During a visit to IRRI, Bui and Tan (1976) described the following rotation: spring rice (February–June) + summer rice (June–November) + *Azolla* inoculum grown in summer rice seedbeds (September–November) + *Azolla* grown in rice paddies and incorporated twice (November–February).

Chinese rotations are numerous and complex, but those mentioned in the available literature can be divided into *Azolla* rotations for the subtropical south (Anonymous, 1975a, 1975c) and those appropriate for the temperate Chekiang region (Anonymous, 1971c, 1974a, 1975b) where overwintering techniques have been developed.

←

the lifecycle of the *Azolla* midges, family Chironomidae. Red and white larvae of these midges attack the roots of *Azolla* (Anonymous, 1975b). Fig. 20. A thick mat of *Azolla filiculoides*, as seen in Hawaiian taro fields, is known to suppress the growth of some weeds because of its shading effect.

Insects and disease

A key element in successful *Azolla* cultivation is effective prevention and control of insects and disease. Fortunately, the insects which attack *Azolla* are different from those attacking rice, yet most *Azolla* pests are controlled by pesticides used for rice (Bui and Tan, 1976). Insect attacks are more prevalent during the summer, especially when the temperature rises above 28°C. If care is not taken, an *Azolla* crop can be destroyed by insects in 3–5 days (Anonymous, 1971c). Singh (1977b, 1977c, 1977d) recommended 2.5–3 kg active ingredient of Furadan per hectare to control *Azolla* insects. In contrast, Reed (1965), Singh (1977b), and Knoff and Habeck (1976) recommended utilizing insects as a biocontrol in locations where *Azolla* is a weed.

1. Vietnam.—The earliest report of insects attacking *Azolla* was by Nguyen (1930). He mentioned two different larvae of Microlepidoptera which attacked *Azolla* during its vegetative period. One larva species was white with a black head and the other was brown with a red head. The presence of these insects was noticed when infested plants were found to be stuck together. At that time, control measures amounted to drowning the insects by submerging overnight, baskets of *Azolla* planting material. Tran and Dao (1973) described the main insect pests of *Azolla* in Vietnam as larvae of *Chironomus*, *Pyralis*, and *Nymphula* species. These insects were controlled by spraying affected fields with organic phosphate or organic chloride pesticides such as DDT, "666" (BHC) or "Vofatoc" (Tran and Dao, 1973; Galston, 1975) and by adding pesticide to a slurry of superphosphate fertilizer and mixing it with *Azolla* just before seeding (Bui and Tan, 1976).

2. China.—Scientists in the People's Republic of China are by far the most advanced in the study of insects attacking *Azolla*. A substantial number of publications have been produced at the provincial level detailing *Azolla* insects, their life cycle and methods of control (Anonymous, 1971c, 1974a, 1975a, 1975b, 1975c, 1976a).

Since all of the above publications contain similar information, none will be referred to individually, except at the end of this section where deviations will be noted. Three members of the moth family, Pyralidae, are the most prevalent and destructive insects attacking *Azolla* (Fig. 18). The larval forms of the species *Nymphula turbata* Butler and *Nymphula enixalis* Swinhoe of the subfamily Nymphulinae and a species of the genus *Pyralis*, hatch from eggs laid on the ventral surface of *Azolla* leaves. The young larvae eat the terminal buds and new shoots of *Azolla*. The *Nymphula* species form oval cocoons by surrounding themselves with stem and leaf portions of *Azolla* fronds. A single larva, inside its cocoon can become as large as a peanut and can consume 9–14 *Azolla* plants each day.

Another very destructive group of *Azolla* pests is the aquatic red larval and white larval forms of undetermined species of *Chironomus*, *Microspector* or *Polypedium* of the midge family, Chironomidae (Fig. 19). The larvae reside in underwater tubes in the paddy soil and swim up to feed on *Azolla* roots. Some members of the Chironomidae are known to damage rice seedlings.

Two other pests of *Azolla*, noted by the Chinese, are the "*Azolla* Elephant

Beetle" and several species of snails. The beetle and its larvae consume the fronds, and the snails consume both the fronds and the roots.

A Kwangtung (Canton) province pamphlet (Anonymous, 1975a) and a Shanghai handbook (Anonymous, 1976a) both mentioned a decay or mold disease and appropriate control measures, but they did not identify the specific cause of the disease. This disease may be similar to a mold observed by the senior author at the IRRI in the Philippines and in Thailand. The Kwangtung pamphlet also discussed damage by algae and agricultural chemicals. The Shanghai handbook and a Chekiang Agriculture Academy handbook (Anonymous, 1975b) both report pest life cycles and the dates and timing of pesticide applications. All of the above-mentioned Chinese articles detail pesticide application for *Azolla*. Commonly used pesticides for *Azolla* in China are organic phosphates and organic chlorides such as "666" (BHC), and "223" (DDT).

3. Other countries.—The following pests or diseases have been found in association with *Azolla*: the neotropic water mites, *Arrenurus* (*Megaluracarus*) *triconicus* Marshall and *Arrenurus* (*Megaluracarus*) *epimerosus* Marshall, in Argentina (de Ferradas, 1973); the weevil *Stenopelmus rufinasus* Gyllenhal (Richerson and Grigarick, 1967); larvae of the moth *Nymphula responsolis* Walker in India (Vergis, 1976); larvae of the moth *Sumea multiplicalis* in Florida (Knopf and Habeck, 1976); adults of *Paulina acuminata* in Trinidad (Reed, 1965); aphids and larvae of the moth *Agrotis ipsilon* Hufnagel on *Azolla* mats in Hawaiian taro fields (pers. ob., T. Lumpkin), and mold on *Azolla* mats grown in the IRRI phytotron, tentatively identified as a *Penicillium* spp. (Pers. comm., I. Watanabe, IRRI).

Weed or weed suppressor?

1. World opinion.—In many regions of the world, an understanding of *Azolla*'s usefulness is lost in ambivalence. The maxim that "any plant is a weed when it grows where it isn't wanted" is seldom appropriate for *Azolla*. Usually *Azolla* is considered a weed where ignorance prevents man from exploiting its presence.

As a weed, *Azolla* has been reported to disrupt fishing and livestock watering (Florida Dept. of Nat. Resources, 1973), clog pumps (Chomchalow and Ponepangan, 1973), impede water flow in ditches, clog pipes and floodgates (Oosthuizen and Walters, 1961; Matthews, 1963; Blackburn and Weldon, 1965; Kleinschmidt, 1969; Eady, 1974; Edwards, 1975), and interfere with watercress cultivation in Maryland (Reed, 1951) and Hawaii (personal observation, T. Lumpkin).

Taro growers in Hawaii have divided opinions over the usefulness of *Azolla* (Fosberg, 1942). Regardless of the growers' opinions, *Azolla* is found in nearly all flooded Hawaiian taro fields as a result of heavy fertilization (Fig. 20).

Outside of Vietnam and China, rice growers also have divided opinions. Tuzimura et al. (1957) mentioned that *Azolla* was an unpopular weed in Japan because it covered rice seedlings immediately after transplanting if the water level rose; this phenomenon was also observed by Singh (1977c). Nishida (1974) mentioned two kinds of *Azolla*, "Ooakaukikusa" and "Akaukikusa," as being weeds in Japan. Yet Fujiwara et al. (1947) mentioned a Japanese rice farmer who purposely cultivated *Azolla* in his rice fields for use as a green manure. *Azolla* was mentioned as a rice weed in Guyana (Anonymous, 1960), but in references concerning

the presence of *Azolla* in rice fields in Italy (Saccardo, 1892), Spain (de Bolos and Masclans, 1955), Portugal (Wild, 1961), and Java (Burkill, 1966; Becking, 1976b), opinions concerning its effect were not expressed.

Although the Vietnamese and Chinese have developed management practices for the cultivation of *Azolla*, they still recognize certain *Azolla* strains as weeds. In colonial Vietnam, one strain of *Azolla* hindered rice cultivation because it grew throughout the year instead of dying in the spring like the domesticated variety (Chevalier, 1926; Braemer, 1927a, b). In China, a strain of *A. pinnata* called "Whole River Red" was recognized as a weed (Anonymous, 1974b, 1975a, 1977), but an earlier article (Shen et al., 1963) mentioned it as a valuable suppressor of other weeds. Recently, a handbook (Anonymous, 1975b) recommended collecting this strain for local use.

Azolla has often been found growing in association with other floating aquatic weeds. *Lemna* was almost always mentioned (Shaver, 1954; Torrey, 1934; Neal, 1965; Birkenbeil, 1974) as well as *Salvinia* (Chevalier, 1926; de Vol, 1945; Bonetto, 1970), *Eichhornia* (Chevalier, 1926; Bonetto, 1970), *Pistia* (Chevalier, 1926) and *Spirodela polyrhiza* (Cohn and Renlund, 1953).

2. Weed suppression.—The ability of a thick, light-proof *Azolla* mat to suppress weed development has long been observed (Braemer, 1927a, b; Nguyen, 1930; Fosberg, 1942; Shen et al., 1963; Olsen, 1972; Anonymous, 1975b), but little hard evidence has been collected. In some rice fields, the benefit from *Azolla* weed suppression may even surpass the benefit from nitrogen fixation. Of course weeds with strong stature and abundant food supply can push through an *Azolla* mat, and weeds or rice growing above the water surface before and after mat development and large floating weeds will not be affected. Nguyen (1930) reported that a thick *Azolla* mat caused the death of *Utricularia flexuosa*, *Echinochloa crusgalli* and *Sagittaria* species. Olsen (1972) reported that *A. caroliniana* successfully displaced *Lemna* on Danish lakes but could not survive the cold winter. Ngo (1973) provided a graph showing the suppressive effect of different *A. pinnata* mat densities on the quantity of *Echinochloa crusgalli* found in a paddy field. After a 6-week period, the 50% *Azolla* cover plot had 70% fewer *E. crusgalli* and the 100% *Azolla* cover plot had 93% fewer *E. crusgalli* than the control. Talley et al. (1977) found that early development of a thick *A. filiculoides* mat successfully suppressed *Cyperus difformis*. They also demonstrated the effect of different *Azolla* treatments on rice yield and distribution of *Cyperus difformis*, *Echinochloa crusgalli* and *Polygonum* sp.

3. Chemical control of *Azolla*.—The complete eradication of *Azolla* may be very difficult, especially in locations such as New Zealand where it is a serious pest. Eady (1974) stated, "It is necessary to completely eradicate all plants from a given area, otherwise a rapid reinfestation will result. The chances of eliminating all living plants from an area by either chemical or mechanical means are not good, furthermore if nearby waterways remain infested it is very likely that reintroduction will take place in a very short time." *Azolla* literature often mentions that infestation was commonly caused by waterfowl (Eady, 1974; Sculthorpe, 1967).

Matthews (1963) recommended controlling *Azolla* by surface application of

0.56–2.24 kg/ha of paraquat PCP preparations applied in diesel oil, but he discouraged the use of 2,2-DPA-amitrole/2,4-D mixtures since they have little or no effect. Patnaik (1976) also recommended paraquat which was effective at 0.2 kg/ha and was not toxic to fish. Cohn and Renlund (1953) and Oosthuizen and Walters (1961) also discouraged the use of liquid 2,4-D, but Kleinschmidt (1969) obtained good control by applying a granular formulation of the butoxy ethanol ester of 2,4-D at the rate of 1 pound of 20% w/w product per 200 ft² (270 kg/ha). The Florida Department of Natural Resources (1973) recommended foliar spray or injection into non-flowing water of 0.25–1 ppm diquat. Oosthuizen and Walters (1961) recommended spraying diesoline, either undiluted or mixed with water in a ratio of 1:1, to destroy *Azolla*. Table 2 summarizes chemical control measures for *Azolla*.

The physiological effects of diquat, paraquat (Lang and Seaman, 1964; Blackburn and Weldon, 1965), and diuron (Peters, 1976) were discussed in a previous section.

4. Biological control of *Azolla*.—Biological and physical means are preferred for continued control of *Azolla*. Richardson (1975) described a laminar flow system which skimmed *Azolla* and other undesirable plants from the water surface of a Louisiana bayou. As mentioned earlier, Knoff and Habeck (1976) reviewed efforts to use the moth *Samea multiplicales* to control *Salvinia* and reported that the moth is commonly found on *A. caroliniana*. *Paulinia acuminata* may also prove useful in controlling *Salvinia* and *Azolla* (Reed, 1965). On a local basis, initial efforts should first concentrate on the prevention of water pollution which may be providing nutrients, especially phosphorus, that are conducive to *Azolla*'s growth. The use of fish for biological control is discussed in a later section.

Azolla is and may become a weed in certain special locations and/or situations, but its fragile structure and high nutrient requirement prevent its invasion of most waterways. Nutrient-rich bodies of water created by man are susceptible to invasion. The presence of *Azolla* in canals, watercress fields, and fields where rice is seeded by broadcasting is especially undesirable. With proper planning and management *Azolla* should not become a threatening weed; on the contrary, it can be managed to suppress other weeds.

Other uses

1. Fishfood and weed control.—The grass carp, *Ctenopharyngodon idella*, has been studied as a biological control for aquatic weeds (Anonymous, 1971a; Edwards, 1974, 1975; Varghese et al., 1976). These herbivorous fish have a short, inefficient digestive system and, at suitable water temperatures, will consume daily more than their own weight of aquatic weeds. These fish normally will not spawn outside of their native Amur river unless pituitary hormones are administered; thus their numbers can be controlled. Grass carp show a marked preference for *Azolla*, *Lemna*, and other small floating weeds (Anonymous, 1971a; Edwards, 1974, 1975; Varghese et al., 1976). A hybrid cross of grass carp (*Ctenopharyngodon idella*) and Israeli carp (*Cyprinus carpio*) was found to prefer *A. caroliniana* and even the root system of *Pistia stratiotes* (Duthu and Kilgen, 1975). Another fish, *Tilapia mossambica*, has been shown to be an efficient de-

stroyer of aquatic vegetation. In feeding trials, it always consumed *Azolla* and *Lemna* first when these plants were provided in any combination of 12 other aquatic weeds (Lahser, 1967).

2. Mosquito control.—Around the turn of the century, a strong international interest developed in the use of *Azolla* (often called mosquito-fern) for mosquito control. *Azolla* mats were thought to prevent mosquitoes from laying eggs and prevent larvae from coming up for air (Benedict, 1923; King et al., 1942; Cohn and Renlund, 1953; Shaver, 1954; Neal, 1965; Burkill, 1966), but little hard evidence is available to support this claim.

A 1909 report by the U.S. Department of Commerce and Labor (cited by Howard, 1910) described research at a malaria station in Wilhelmshaven, Germany. *Azolla* was found to suffocate mosquito larvae and prevented the insect from depositing eggs; subsequently, it was used successfully by a mosquito-destroying commission on the Rhine. However, a German official involved with mosquito extermination in the African colonies of Germany was far from enthusiastic about its use. He believed *Azolla* could only be used in special places since the plant would not grow under the dense or even moderate shade of tropical forests, would not grow in brackish water or along seacoasts, and would die from drought and thus necessitate restocking.

3. A fodder crop.—*Azolla* has a tremendous potential as a fodder crop if the previously mentioned growth rate and protein content are considered. Tran and Dao (1973) reported that one hectare of *Azolla* can produce 540–720 kg of assimilable protein per month. It has traditionally been used as a fodder throughout Asia and parts of Africa (Chevalier, 1926) and was fed to pigs, ducks and chickens (Chevalier, 1926; Fujiwara et al., 1947; Dao and Tran, 1966; Burkill, 1966; Anonymous, 1975a, b); cattle (Le Van and Sobochkin, 1963; Dao and Tran, 1966, 1973; Sculthorpe, 1967) and fish (Le Van and Sobochkin, 1963; Sculthorpe, 1967). When pigs were fed *Azolla*, their manure contained 0.87% nitrogen, compared to 0.42% for pigs on regular diets (Dao and Tran, 1966).

4. Miscellany.—*Azolla* has been found to help purify water (Cohn and Renlund, 1953); to be an ingredient in soap production by some African tribes (Chevalier, 1926) and was chewed to cure sore throat in New Zealand (Usher, 1974). Bui (1966) implied that with suitable processing *Azolla* could become a good source of human food. Dr. P. K. Singh (pers. comm.) wrote from Cuttack, India, that he has eaten *Azolla* regularly in several fried preparations; he reports that these preparations are tasty and do not cause digestive difficulties. He was taking steps to popularize the cultivation of *Azolla* in small trays for human consumption.

5. Genetic material.—Selection and collection of *Azolla* genetic material has not received sufficient attention. Several institutions have begun to collect the 6 species and promising varieties. China (Anonymous, 1975b, 1975c; Lumpkin, 1977) and Vietnam (Tran and Dao, 1973) are selecting varieties of *A. pinnata* to meet their regional requirements. In California Talley et al. (1977) have used *A. filiculoides* and *A. mexicana* in agronomic studies, but most other researchers utilize only *A. pinnata*, which is indigenous to Asia.

CONCLUSIONS

Azolla is useful to man because of the following attributes: 1) the ability to fix atmospheric nitrogen in nitrogen-deficient water commonly found in unfertilized paddy fields, 2) the ability to grow in flooded paddy conditions where traditional nitrogen-fixing green manures cannot grow, 3) the ability to suppress development of submerged paddy weeds without affecting the growth of transplanted rice, 4) the inability of native *Azolla* species to pose a serious weed threat. (At least one species is present in most paddy regions but is considered innocuous or unobtrusive.), 5) the ability to provide secondary benefits, e.g., compost for upland crops, high protein fodder for carp and pigs, and, possibly, food for human consumption, and 6) the potential of the algal symbiont, if it can be cultured independently, to be significant as a photosynthetically-driven nitrogen, hydrogen, and/or protein factory.

Some reasons for *Azolla*'s limited popularity as a green manure are: 1) unfavorable environmental conditions which limit *Azolla*'s growth potential throughout the entire year, 2) the need for maintenance of an off-season starter stock, so that *Azolla* cultivation can begin before rice seedlings are ready to transplant, instead of waiting for naturally occurring species to germinate later in the growing season, 3) lack of a sufficient research base, both in terms of information about the plant as well as financial support (Effective mass selection, cross breeding, and germ plasm storage techniques have not been developed.), 4) ignorance of *Azolla*'s potential and of the methods required for its cultivation (Only China and Vietnam have full scale extension programs.), 5) farmer resistance to learning the management system, increasing his work load, and purchasing necessary inputs such as pesticides and phosphorus fertilizer, and 6) use of inorganic nitrogen fertilizer, when it is cheap relative to the labor cost required for *Azolla* cultivation.

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Appendix B.

TABLE 16. Mean relative growth rates ($mg \cdot g^{-1} \cdot day^{-1}$) at the mid-date of growth cycles (**DATES**) for eight azolla accessions grown in pot culture at Hangzhou, China in 1980. Table parameters include mean values for maximum air temperature (**MAXA**), minimum air temperature (**MINA**), maximum surface water temperature (**MAXW**), minimum surface water temperature (**MINW**), percent relative humidity (**RH**), light intensity in Klux (**KLUX**), minutes of daylight (**MOD**), estimated surface solar radiation as $cal \cdot cm^{-2} \cdot day^{-1}$ (**ECR**), solar radiation above the atmosphere as $cal \cdot cm^{-2} \cdot day^{-1}$ (**CSR**), centimeters of precipitation (**PRE**), and mean relative growth rates for the azolla accessions A. caroliniana (**CAR**), A. filiculoides (**FIL**), A. mexicana (**MEX**), A. microphylla (**MIC**), A. nilotica (**NIL**), A. pinnata var. pinnata (**PIN**), A. pinnata var. imbricata (**IMB**), and A. rubra (**RUB**).

DATES	MAXA	MINA	MAXW	MINW	RH	KLUX	MOD	ECR	CSR	RAIN	CAR	FIL	MEX	MIC	NIL	PIN	IMB	RUB
1/13	11.6	1.3	8.4	2.6	65	19.5	629	280	410	2.0	5	5	.[a]	.	.	.	2	10
2/13	5.5	0.2	7.2	2.2	60	25.2	668	328	498	1.6	0	3	.	.	.	.	0	0
4/01	18.3	8.2	20.4	8.8	60	50.1	750	421	644	3.4	63	65	.	.	.	.	33	59
5/07	23.0	14.3	26.1	14.5	61	22.3	809	445	721	4.8	132	79	17	109	44	43	103	76
5/20	26.6	16.3	30.4	17.5	56	40.1	829	519	729	4.8	138	124	73	116	77	31	87	82
6/03	30.1	20.2	30.4	20.8	55	53.0	840	598	732	2.0	166	121	107	139	130	180	184	92
6/12	29.6	22.9	30.5	23.1	64	33.0	842	462	739	10.3	179	144	101	150	85	98	167	101
6/20	29.4	23.0	30.2	23.3	68	16.8	845	418	737	3.6	203	163	155	191	142	159	204	109
6/27	35.7	23.7	34.6	26.1	62	58.4	844	513	733	7.3	239	193	210	238	141	219	268	67
7/04	29.1	23.1	29.3	24.5	80	23.6	840	352	731	11.2	191	103	131	228	94	148	202	62
7/11	30.7	23.9	33.4	25.6	79	31.1	836	463	728	7.9	166	180	189	247	121	156	210	.
7/20	36.7	25.5	36.5	25.9	69	48.8	828	601	715	2.7	178	114	162	235	138	94	218	.
7/26	36.2	27.6	36.6	28.1	68	58.0	820	699	710	0.0	.	.	.	.	.	.	.	.
8/03	29.0	22.3	29.5	24.3	80	27.4	811	348	704	14.3	129	103	102	145	.	.	141	.
8/11	28.7	22.2	31.3	24.2	79	24.4	799	355	693	9.2	153	141	144	172	99	.	167	.
8/18	26.9	21.9	27.3	22.8	83	26.1	789	317	668	14.3	158	139	151	198	99	.	157	.
8/26	34.2	24.0	32.6	25.0	73	30.0	779	436	656	7.2	117	126	125	174	123	.	178	.

TABLE 16. Continued.

DATES	MAXA	MINA	MAXW	MINW	RH	KLUX	MOD	ECR	CSR	RAIN	CAR	FIL	MEX	MIC	NIL	PIN	IMB	RUB
9/04	27.4	22.7	30.4	23.4	74	29.3	765	389	635	1.1	132	131	111	150	80	118	152	.
9/14	31.3	19.2	30.7	20.3	57	53.3	743	573	614	0.0	178	181	174	186	137	166	190	.
9/22	21.2	16.1	22.7	18.3	81	9.4	730	292	592	9.3	204	197	165	214	182	166	170	117
9/29	26.3	15.3	26.7	17.6	67	53.7	715	485	571	0.0	257	257	243	254	220	177	252	176
10/07	27.8	18.9	28.3	20.0	73	32.4	704	.	548	0.0	215	229	214	224	220	217	230	.
10/14	22.9	14.8	22.1	17.5	73	.	698	.	527	7.6	183	195	162	172	144	148	182	159
10/23	23.9	9.9	23.1	14.3	61	.	675	.	482	7.2	116	142	98	89	53	106	127	99
11/03	21.3	8.7	21.1	12.8	65	.	656	.	465	0.0	78	142	61	80	79	79	114	124
11/13	19.0	6.8	20.5	11.7	60	.	645	.	446	0.0	113	125	52	122	109	95	107	93
12/16	15.2	7.7	13.1	5.2	66	16.9	617	.	377	1.9	67	87	.	.	.	23	48	57

[a] Accession could not survive or climatic variable not measured.

Appendix C.

TABLE 17. Diurnal nitrogenase activity as indicated by acetylene reduction for seven Azolla species during 16 - 17 June 1980 at Hangzhou, China. Results are means of two samples.

SPECIES	STARTING TIMES OF SAMPLING												
	8 am	10am	noon	2 pm	4 pm	6 pm	8 pm	10pm	12pm	2 am	4 am	6 am	8 am
	nM $C_2H_4 \cdot mg^{-1}$ dry wt $\cdot minute^{-1}$												
A. caroliniana	0.77	3.50	2.50	2.19	0.97	0.24	0.12	0.15	0.28	0.22	0.18	0.84	0.58
A. filiculoides	0.83	2.16	2.21	1.91	0.73	0.30	0.13	0.21	0.14	0.17	0.10	0.58	0.64
A. mexicana	0.57	2.66	1.79	2.37	0.95	0.16	0.17	0.32	0.24	0.14	0.13	0.31	0.54
A. microphylla	0.50	0.90	0.55	0.67	0.21	0.09	0.03	0.10	0.11	0.05	0.05	0.13	0.16
A. nilotica	1.14	4.01	1.51	2.97	0.89	0.35	0.29	0.30	0.40	0.36	0.24	0.60	1.05
A. pinnata	0.83	2.08	2.21	1.40	0.56	0.15	0.14	0.16	0.18	0.17	0.09	0.48	0.33
A. rubra	1.41	1.15	0.89	1.11	0.70	0.06	0.08	0.11	0.14	0.12	0.11	0.32	0.52
INCUBATION CONDITIONS													
Time (minutes)	30	30	30	30	30	30	60	60	60	60	60	30	30
Water ^{o}C	20	29	32	30	22	16	13	12	10	9	8	9	16
Air ^{o}C	21	24	26	25	21	16	13	12	9	9	9	10	17
Light (Klux)	54	72	92	80	20	0	0	0	0	0	0	4	45

Appendix D.

TABLE 18. The influence of six azolla accessions and three azolla management treatments on grain (**GRHA**) and straw (**STHA**) yields in metric tons/ha, central tiller height (**TLHT**) in cm, and yield components of rice. Yield components are panicles/hill (**PAHI**), spikelets/panicle (**SPPA**), percent unfilled grains (**%UFG**), and weight of one hundred grains (**HUGR**). Data are shown for five replications (**REP**). Values for tiller height and components of yield represent a mean of twelve hills per replication.

TREATMENTS[a]						YIELD COMPONENTS			
AZOLLA	MANAGEMENT	REP	GRHA	STHA	TLHT	PAHI	SPPA	%UFG	HUGR
NONE	CONTROL	1	4.29	4.63	71.2	9.17	50.3	3.47	2.64
		2	4.36	4.35	73.6	8.92	51.1	6.83	2.65
		3	5.01	4.58	74.8	8.83	52.8	4.86	2.73
		4	5.12	5.40	76.1	10.6	49.5	5.12	2.64
		5	4.69	3.44	76.7	10.1	57.0	4.72	2.41
	60kgN	1	5.67	7.19	82.7	10.9	60.0	9.76	2.63
		2	5.57	6.15	79.9	11.2	57.2	6.50	2.66
		3	5.60	7.12	78.3	12.0	53.6	8.98	2.66
		4	5.85	7.56	82.4	10.2	69.0	7.10	2.55
		5	5.59	6.24	78.8	10.3	58.0	6.72	2.63
MIC	INTER	1	4.97	4.54	72.8	9.83	50.2	8.54	2.29
		2	5.07	5.48	75.6	11.1	51.1	10.6	2.64
		3	5.67	5.80	77.4	11.9	56.0	10.1	2.68
		4	5.62	6.35	76.8	11.0	53.6	7.20	2.68
		5	5.56	5.51	77.5	10.7	54.4	4.62	2.74
CAR	MONO	1	5.45	6.08	76.9	11.0	54.2	9.25	2.70
		2	4.98	5.91	77.9	11.3	59.2	5.20	2.60
		3	5.60	5.33	79.8	11.6	55.1	6.14	2.65
		4	5.55	5.83	77.5	11.0	53.6	6.89	2.71
		5	5.97	6.16	78.6	12.5	58.6	8.42	2.58
	INTER	1	4.85	5.09	74.1	11.1	44.3	4.92	2.71
		2	4.69	4.47	72.2	9.92	51.5	5.01	2.65
		3	5.23	5.48	74.7	9.67	51.7	5.54	2.82
		4	4.92	4.90	71.9	10.4	48.7	5.48	2.73
		5	5.04	5.48	77.0	10.8	56.3	4.50	2.69
	COMBI	1	5.85	5.87	76.0	12.3	43.1	5.70	2.70
		2	6.14	5.84	77.7	12.7	54.0	8.86	2.59
		3	5.99	5.90	76.6	12.8	49.1	10.1	2.59
		4	5.33	6.24	76.1	10.4	57.7	7.85	2.70
		5	6.15	5.21	78.7	11.1	56.2	6.03	2.68

TABLE 18. Continued.

TREATMENTS			YIELD COMPONENTS						
AZOLLA	MANAGEMENT	REP	GRHA	STHA	TLHT	PAHI	SPPA	%UFG	HUGR
FIL V1	MONO	1	5.79	5.61	78.4	10.8	58.6	6.52	2.62
		2	5.65	6.21	78.9	10.3	56.6	9.49	2.62
		3	6.06	6.16	77.8	11.9	52.6	2.98	2.64
		4	5.89	6.30	80.7	11.1	58.2	7.61	2.64
		5	5.97	6.12	78.0	11.3	54.2	6.30	2.61
	INTER	1	5.43	5.31	74.9	10.9	52.3	6.57	2.71
		2	5.16	4.95	75.5	11.2	48.8	7.96	2.70
		3	5.39	5.83	78.7	11.2	55.3	6.97	2.64
		4	5.43	6.66	73.2	11.7	47.5	7.67	2.62
		5	5.50	4.83	76.2	12.3	50.5	6.65	2.67
	COMBI	1	5.92	6.48	77.3	14.4	46.5	6.63	2.60
		2	5.98	7.04	77.9	14.0	47.3	6.80	2.61
		3	6.15	5.96	80.0	12.1	59.6	14.5	2.55
		4	5.71	6.11	79.1	11.0	59.3	17.0	2.66
		5	6.15	6.30	80.4	11.7	59.9	12.3	2.57
FIL V2	MONO	1	5.50	6.36	77.6	10.4	60.1	6.52	2.58
		2	5.20	5.25	77.0	10.6	55.3	6.48	2.64
		3	5.59	5.62	78.9	11.7	52.3	9.49	2.70
		4	5.60	5.26	78.8	11.3	58.0	6.20	2.66
		5	5.79	4.79	79.3	10.3	55.2	5.46	2.77
	INTER	1	4.93	4.20	75.2	10.3	47.9	5.83	2.69
		2	5.10	4.44	74.9	11.1	51.3	7.33	2.67
		3	5.10	5.51	75.8	10.0	56.5	5.66	2.65
		4	4.96	5.23	73.3	10.3	48.9	6.50	2.65
		5	5.32	5.43	76.0	10.8	55.3	6.22	2.70
	COMBI	1	5.70	5.23	77.3	13.5	52.4	7.28	2.62
		2	5.85	6.52	77.4	12.5	51.5	2.75	2.61
		3	5.14	4.11	76.8	13.8	48.0	11.5	2.58
		4	5.71	5.71	75.7	12.8	53.8	9.29	2.66
		5	5.75	6.56	77.3	14.0	50.3	12.8	2.60

TABLE 18. Continued.

TREATMENTS			YIELD COMPONENTS						
AZOLLA	MANAGEMENT	REP	GRHA	STHA	TLHT	PAHI	SPPA	%UFG	HUGR
IMB	MONO	1	5.28	5.56	75.7	11.0	53.0	5.36	2.63
		2	5.62	6.35	77.1	10.0	65.3	5.35	2.43
		3	5.61	6.62	76.4	9.67	58.0	7.78	2.68
		4	5.62	5.49	76.3	10.6	57.3	5.36	2.61
		5	5.96	5.30	77.7	10.7	57.1	5.24	2.71
	INTER	1	4.90	5.88	75.6	10.3	52.3	4.86	2.68
		2	5.41	5.16	75.9	11.6	52.9	6.05	2.70
		3	5.42	4.86	73.9	11.9	52.3	5.34	2.54
		4	4.93	5.45	75.0	12.5	48.6	6.16	2.65
		5	5.57	5.35	75.4	11.8	49.3	8.09	2.66
	COMBI	1	5.89	6.11	78.3	12.6	54.1	7.63	2.69
		2	5.85	5.55	78.9	12.3	52.2	6.55	2.58
		3	6.29	4.61	76.6	12.7	51.3	7.39	2.68
		4	6.18	5.45	78.5	12.4	51.9	8.60	2.59
		5	5.99	5.50	81.0	12.2	58.9	9.82	2.59
RUB	MONO	1	6.09	4.96	77.2	10.3	60.9	5.13	2.52
		2	5.75	5.25	78.6	10.5	58.5	5.34	2.64
		3	5.23	5.85	78.9	12.8	57.0	10.2	2.56
		4	5.78	5.80	80.0	11.0	56.4	8.66	2.66
		5	5.62	5.49	76.6	10.2	62.7	6.95	2.69
	INTER	1	4.96	3.94	74.1	11.3	51.7	7.05	2.71
		2	5.23	5.31	75.4	10.2	59.0	6.78	2.44
		3	5.32	5.22	75.1	11.7	47.1	7.62	2.88
		4	5.25	5.05	74.4	11.2	52.4	7.44	2.69
		5	5.30	5.80	78.0	10.1	54.6	5.37	2.62
	COMBI	1	5.75	5.28	77.2	11.2	54.7	10.0	2.68
		2	5.73	6.54	76.5	11.4	54.6	9.83	2.72
		3	5.90	5.42	78.2	13.8	52.8	10.8	2.63
		4	6.39	7.69	77.8	12.1	58.3	8.52	2.58
		5	6.01	5.46	76.9	12.0	51.8	9.08	2.64
MEAN OF MONO			5.64	2.80		11.0	57.1	6.73	2.63
MEAN OF INTER			5.15	2.49		11.0	51.5	6.30	2.68
MEAN OF COMBI			5.90	2.83		12.5	53.1	9.09	2.63

[a] Treatments include no fertilizer and no azolla (**NONE CONTROL**), a basal application of 60 kg N/ha as ammonium sulfate and no azolla (**NONE 60KGN**), the azolla accession A. microphylla (**MIC**) grown with the rice (**INTER**), and the azolla accessions A. caroliniana (**CAR**), A. filiculoides (**FIL V1** and **FIL V2**), A. pinnata var. imbricata (**IMB**), and A. rubra (**RUB**) grown prior to the rice (**MONO**), with the rice (**INTER**) or both (**COMBI**).

APPENDIX E.

TABLE 19. Growth in fresh weight (**FRWT**) and dry weight (**DRWT**) in t/ha, relative growth weight (**RGR**) in $mg \cdot g^{-1} \cdot day^{-1}$, and accumulated nitrogen (**KG N**) of five azolla accessions grown as a monocrop in field plots from 26 April to 19 May 1980 at Hangzhou, China. Symbols for azolla accessions are: A. caroliniana, **CAR**; A. filiculoides, **FIL V1** and **FIL V2**; A. pinnata var. imbricata, **IMB**; and A. rubra, **RUB**.

DATE EXPLANATION MEASUREMENT	AZOLLA CAR.	FIL.V1	FIL.V2	IMB.	RUB.	MEAN
26 April						
Inoculated field plots with azolla.						
FRWT	5.0	5.0	5.0	5.0	5.0	5.0
DRWT	0.33	0.25	0.27	0.26	0.23	0.27
KG N	14.0	13.1	12.3	10.4	10.7	12.1
2 MAY						
FRWT	13.4	15.4	14.4	11.7	14.1	13.8
RGR	164	187	176	142	172	168
6 MAY						
FRWT	22.5	25.7	26.7	19.8	24.7	23.9
FRWT[a]	16.7	19.9	21.0	14.1	19.0	18.1
RGR	129	128	155	132	140	137
9 MAY						
Incorporated part of the azolla mat into the soil.						
Before the incorporation.						
FRWT	24.3	29.9	31.9	19.9	28.3	26.8
DRWT	1.59	1.48	1.69	1.04	1.30	1.42
KG N	68.2	78.3	78.7	41.4	60.3	65.4
Azolla remaining after the incorporation.						
FRWT	3.2	2.2	4.8	1.6	3.0	3.0
FRWT[a]	9.0	8.0	10.5	7.4	8.8	8.7
Azolla incorporated into the soil.						
FRWT	21.1	27.7	27.1	18.2	25.2	23.9
DRWT	1.38	1.37	1.44	0.96	1.16	1.26
KG N	59.1	72.5	66.9	38.0	53.8	58.1
10 MAY						
FRWT	10.5	9.5	12.1	8.7	10.1	10.2
16 MAY						
FRWT	26.4	25.9	28.6	22.4	23.1	25.3
RGR	154	168	143	158	137	152
19 MAY						
Final incorporation of azolla into the soil.						
FRWT	41.9	42.8	44.0	36.1	34.9	39.9
DRWT	2.40	2.27	2.52	1.83	1.51	2.11
KG N	99.0	91.0	90.5	71.1	63.6	83.1

Total of 9 May and 19 May incorporations.						
FRWT	63.0	70.5	71.1	54.3	60.1	63.8
FRWT[b]	58.0	65.5	66.1	49.3	55.1	58.8
DRWT	3.77	3.64	3.96	2.78	2.68	3.37
DRWT[b]	3.44	3.40	3.69	2.52	2.45	3.10
KG N	158	163	157	109	117	141
KG N[b]	144	150	145	99	107	129

a On 6 May, 5.8 t/ha was removed from the plots for use to re-inoculate the plots after the first soil incorporation of azolla. However, heavy rains delayed the first incorporation from 6 May until 9 May, after which the 5.8 t/ha inoculum was placed back into the plots.

b Does not include FRWT, DRWT, or KG N of initial 5.0 t/ha of inoculum.

TABLE 20. Growth in fresh weight (**FRWT**) and dry weight (**DRWT**) in t/ha, relative growth weight (**RGR**) in $mg \cdot g^{-1} \cdot day^{-1}$, and accumulated nitrogen (**KG N**) of five plus one azolla accessions grown as an intercrop in field plots from 24 May to 14 June 1980 at Hangzhou, China. Symbols for azolla accessions are: A. caroliniana, **CAR**; A. filiculoides, **FIL V1** and **FIL V2**; A. pinnata var. imbricata, **IMB**; A. rubra, **RUB**; and A. microphylla, **MIC**.

DATE EXPLANATION / MEASUREMENT	AZOLLA						
	CAR.	FIL.V1	FIL.V2	IMB.	RUB.	MEAN	MIC.
24 MAY							
Inoculated azolla into field plots							
FRWT	5.0	5.0	5.0	5.0	5.0	5.0	5.0
KG N	7.5	7.7	10.3	8.2	7.7	8.3	7.6
30 MAY							
FRWT	15.7	11.9	13.4	15.3	11.9	13.8	15.3
RGR	190	144	187	186	144	173	186
4 JUNE							
FRWT	23.1	20.3	23.5	26.0	20.3	23.1	22.8
RGR	78	107	112	107	107	103	80
5 JUNE							
Incorporated part of the azolla mat into the soil.							
Before the incorporation.							
FRWT	25.0	22.6	26.3	28.9	22.6	25.6	24.7
Azolla remaining after the incorporation.							
FRWT	13.8	10.1	11.2	19.7	10.1	13.8	14.0
Azolla incorporated into the soil.							
FRWT	11.2	12.6	15.1	9.3	12.6	11.8	10.8
DRWT	0.42	0.47	0.81	0.34	0.47	0.52	0.40
KG N	16.8	19.2	30.9	15.2	19.2	21.0	16.4
7 JUNE							
FRWT	16.4	16.3	14.7	21.1	12.0	16.1	16.7
12 JUNE							
FRWT	25.3	22.4	28.9	25.2	18.7	24.1	26.2
RGR	87	64	135	36	89	82	90
14 JUNE							
Final incorporation of azolla into the soil.							
FRWT	30.1	25.4	37.9	27.1	22.3	10.3	31.4
DRWT	1.14	1.23	2.03	1.00	0.84	1.25	1.17
KG N	45.3	53.6	77.7	44.2	34.2	51.0	47.9
Total of 5 June and 14 June incorporations.							
FRWT	41.3	36.4	52.9	36.3	34.9	40.4	42.1
DRWT	1.57	1.77	2.84	1.34	1.31	1.76	1.57
DRWT[a]	1.38	1.52	2.57	1.16	1.12	1.55	1.38
KG N	62	77	109	59	53	72	64
KG N[a]	55	66	98	51	46	63	57

[a] Does not include **DRWT** or **KG N** of initial 5.0 t/ha of inoculum.

TABLE 21. Growth in fresh weight (**FRWT**) and dry weight (**DRWT**) in t/ha, relative growth weight (**RGR**) in $mg \cdot g^{-1} \cdot day^{-1}$, and accumulated nitrogen (**KG N**) of five azolla accessions grown as an intercrop in field plots from 24 May until 14 June 1980 at Hangzhou, China, after having been previously grown as a monocrop in the same field plots. Symbols for azolla accessions are: A. caroliniana, **CAR**; A. filiculoides, **FIL V1** and **FIL V2**; A. pinnata var. imbricata, **IMB**; and A. rubra, **RUB**.

DATE EXPLANATION MEASUREMENT	AZOLLA CAR.	FIL.V1	FIL.V2	IMB.	RUB.	MEAN
24 MAY						
Start of growth as an intercrop.						
FRWT[a]	12.6	13.1	9.0	16.4	9.8	12.2
30 MAY						
FRWT	16.7	18.5	15.3	18.9	14.5	16.8
4 JUNE						
FRWT	21.2	24.6	24.0	21.3	20.2	22.3
RGR	47	57	90	24	66	57
5 JUNE						
Incorporated part of the azolla mat into the soil.						
Before the incorporation.						
FRWT	22.2	26.1	26.3	21.8	21.6	23.6
Azolla remaining after the incorporation.						
FRWT	11.8	13.1	12.9	13.8	11.5	12.6
Azolla incorporated into the soil.						
FRWT	10.5	13.0	13.3	8.0	10.1	11.0
DRWT	0.40	0.63	0.71	0.30	0.38	0.48
KG N	15.6	27.3	27.3	13.1	15.5	19.8
7 JUNE						
FRWT	13.3	15.0	14.4	15.2	12.7	14.1
12 JUNE						
FRWT	18.1	21.1	18.8	19.3	16.4	18.7
RGR	62	68	53	48	51	56
14 JUNE						
Final incorporation of azolla into the soil.						
FRWT	20.5	24.2	20.9	21.2	18.2	21.0
DRWT	0.78	1.17	1.12	0.79	0.68	0.91
KG N	30.9	50.9	42.9	34.7	27.8	37.4
Total of 5 June and 14 June incorporations.						
FRWT	30.9	37.2	34.2	29.2	28.3	32.0
DRWT	1.17	1.80	1.83	1.08	1.06	1.39
DRWT[b]	0.99	1.56	1.57	0.90	0.87	1.18
KG N	46.6	78.2	70.2	47.8	43.3	57.2
KG N[b]	39.1	67.7	60.0	39.6	35.6	48.4

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[a] At the start of the intercrop portion of the combined monocrop and intercrop azolla management treatment, the plots were inoculated with 5.0 t/ha of nursery azolla. However, the plots had been reflooded soon after completion of the monocrop portion of the combined treatment, and some of the incorporated monocropped azolla floated to combine with the 5.0 t/ha intercrop inoculum.

[b] Does not include DRWT or KG N of 5.0 t/ha of inoculum.

TABLE 22. Totals of growth in fresh weight (**FRWT**) and dry weight (**DRWT**) in t/ha, relative growth weight (**RGR**) in $mg \cdot g^{-1} \cdot day^{-1}$, and accumulated nitrogen (**KG N**) of five azolla accessions grown as a combination of a monocrop and then an intercrop in field plots from 26 April to 14 June 1980 at Hangzhou, China. Symbols for azolla accessions are: A. caroliniana, **CAR**; A. filiculoides, **FIL V1** and **FIL V2**; A. pinnata var. imbricata, **IMB**; and A. rubra, **RUB**.

DATE EXPLANATION MEASUREMENT	AZOLLA CAR.	FIL.V1	FIL.V2	IMB.	RUB.	MEAN
19 MAY						
Total of 9 May and 19 May incorporations of monocropped azolla.						
FRWT	63.0	70.5	71.1	54.3	60.1	63.8
Amount of monocropped azolla that refloated to grow as an intercrop.						
FRWT	6.0	6.1	2.5	10.1	3.4	5.6
Total of incorporated azolla minus amount that refloated.						
FRWT	57.0	64.4	68.5	44.2	56.7	58.2
DRWT	3.43	3.32	3.81	2.27	2.53	3.07
KG N	144	151	152	89	111	129
Total of incorporated azolla minus refloated azolla and inoculum.						
FRWT	52.0	59.4	63.5	39.2	51.7	53.2
DRWT	3.10	3.07	3.55	2.01	2.30	2.81
KG N	130	137	140	79	101	117
Total of monocrop and intercrop incorporations.						
Total with 5.0 t/ha inoculums of monocrop and intercrop.						
FRWT	88	102	103	73	85	90
DRWT	4.60	5.12	5.64	3.35	3.59	4.46
KG N	190	229	222	137	155	187
Total without 5.0 t/ha inoculums of moncrop and intercrop.						
FRWT	78	92	93	64	75	80
DRWT	4.09	4.63	5.11	2.90	3.17	3.98
KG N	169	205	200	118	136	166

Appendix F.

TABLE 23. KCl extractable soil ammonia concentration (ppm) during development of a transplanted rice crop as influenced by azolla accessions and azolla management treatments. The plow layer was sampled from 30 May to 23 July 1980.

TREATMENT[a]		DATES (MONTH/DAY)									
AZOLLA	MAN.	5/30	6/04	6/09	6/14	6/19	6/24	6/30	7/05	7/11	7/23
NONE	CONT.	37.3	40.3	40.7	36.6	31.3	40.2	27.2	34.0	32.1	26.3
	60KGN	54.2	38.0	49.6	37.8	40.7	34.1	44.7	39.1	39.1	25.2
MIC	INTER	46.2	31.1	39.2	40.7	41.7	36.1	32.5	38.6	38.2	29.5
CAR	MONO	51.0	51.6	47.6	47.7	30.6	36.8	26.3	32.6	31.3	24.4
	INTER	28.7	32.3	41.4	25.0	39.0	28.9	27.9	30.3	25.6	32.6
	COMBI	53.9	51.8	45.2	38.3	36.7	29.0	22.2	37.3	28.7	25.7
IMB	MONO	48.8	28.6	50.1	38.9	38.6	27.5	38.2	37.1	33.1	25.5
	INTER	35.3	25.6	40.9	29.3	37.1	30.1	37.6	31.9	34.4	27.2
	COMBI	56.0	27.6	52.2	35.9	30.8	36.7	38.0	34.2	36.0	35.8
FIL V1	MONO	57.3	44.5	40.0	38.6	26.4	38.8	28.1	31.1	28.1	25.0
	INTER	39.7	27.1	37.8	35.0	36.5	33.5	22.7	29.6	25.4	24.5
	COMBI	36.8	41.3	45.9	38.7	30.8	50.2	29.9	33.8	31.8	33.9
FIL V2	MONO	56.9	54.6	58.7	45.1	31.9	36.8	41.2	33.2	32.7	36.0
	INTER	45.8	30.6	36.4	31.6	29.0	38.9	37.3	35.3	31.4	24.1
	COMBI	46.4	31.7	44.7	38.8	36.5	57.6	34.2	28.0	33.4	36.4
RUB	MONO	48.5	44.7	42.5	52.8	26.6	32.6	35.0	34.5	33.5	15.8
	INTER	41.0	31.0	42.7	28.5	32.3	33.4	29.8	38.3	37.2	29.3
	COMBI	43.5	41.1	47.7	35.2	31.8	33.3	32.3	37.8	37.8	26.2
MEAN OF	MONO	52.5	44.8	47.8	44.6	30.8	34.5	33.8	33.7	31.7	25.3
MEAN OF	INTER	38.1	29.3	39.8	29.9	34.8	33.0	31.1	33.1	30.8	27.5
MEAN OF	COMBI	47.3	38.7	47.1	37.4	33.3	41.4	31.3	34.2	33.5	31.6

[a] Treatments include no fertilizer and no azolla (**NONE CONT.**), a basal application of 60 kg N/ha as ammonium sulfate and no azolla (**NONE 60KGN**), the azolla accession *A. microphylla* (**MIC**) grown with the rice (**INTER**), and the azolla accessions *A. caroliniana* (**CAR**), *A. filiculoides* (*FIL V1* and *FIL V2*), *A. pinnata* var. *imbricata* (**IMB**), and *A. rubra* (**RUB**) grown prior to the rice (**MONO**), with the rice (**INTER**), or both (**COMBI**).

Appendix G.

TABLE 24. Elemental content of azolla grown in pots (**POT**) and field plots (**FIELD**), and whole rice plants (**PLANT**) and harvested straw (**STRAW**) and grain (**GRAIN**) grown in field plots at Hangzhou, China during 1979 and 1980. All pot material originated from nitrogen-free nutrient solution culture, while field material was obtained from rice paddy experiments. Elemental concentrations are calculated on an oven-weight basis.

SITE, DATE TISSUE TREATMENT	N	P	K	CA	MG	S	SI	NA	CL	MN	FE	CU	ZN[a]
	PERCENT									PPM			
POT, NOV 79													
NIL[b] NONE	4.01	1.46	4.03	0.80	0.34	0.43	0.60	0.27	.[c]	392	2763	264	989
PIN	2.62	1.07	4.34	0.76	0.65	0.59	0.32	0.23	.	389	3375	212	752
MIC	3.14	1.56	5.54	0.73	0.32	0.60	0.27	0.61	.	227	2116	190	761
CAR	3.63	1.29	5.34	0.59	0.31	0.46	0.32	0.55	.	267	2032	214	848
RUB	3.08	1.30	5.54	0.63	0.31	0.60	0.32	0.56	.	275	2563	210	887
MEX	4.08	1.40	3.83	0.94	0.33	0.38	0.39	0.42	.	258	1487	243	***[d]
FIL V1	4.60	1.57	5.12	0.52	0.36	0.51	0.26	0.61	.	153	2865	142	872
IMB	3.99	1.32	4.91	0.45	0.32	0.63	0.24	0.72	.	174	2006	168	687
FIL V2	4.54	1.55	5.97	0.47	0.40	0.50	0.17	0.34	.	118	1353	90	615
POT, FEB 80													
CAR NONE	2.68	0.83	1.03	0.87	0.42	0.37	0.34	0.66	.	611	2368	5	355
RUB	3.11	0.64	1.41	1.28	0.38	0.44	0.56	0.60	.	605	1972	11	622
FIL V1	3.85	0.95	1.30	0.96	0.24	0.36	0.37	0.81	.	459	2680	10	444
IMB	3.42	0.35	0.69	1.14	0.29	0.34	0.60	0.23	.	370	3542	0	255
FIL V3	2.88	0.45	0.71	0.85	0.30	0.32	0.35	0.34	.	365	2614	0	246
FIL V2	5.00	0.90	1.59	0.84	0.51	0.55	0.32	1.03	.	277	985	43	199

TABLE 24. Continued.

SITE, DATE TISSUE TREATMENT	N	P	K	CA	MG	S	SI	NA	CL	MN	FE	CU	ZN[a]
	PERCENT									PPM			
POT, 4 JUNE 80													
MIC NONE	4.41	0.45	2.56	1.59	0.34	0.89	1.40	1.00	2.53	0	1400	18	120
CAR	3.99	0.42	1.73	1.20	0.46	0.68	1.04	0.88	1.78	0	959	22	125
RUB	4.25	0.61	2.24	1.62	0.44	0.59	1.53	0.82	1.93	20	1435	11	178
FIL V1	4.35	0.48	1.62	1.59	0.50	0.54	1.32	0.66	1.72	306	1658	10	169
IMB	4.45	0.30	1.58	1.19	0.44	0.61	1.42	0.79	1.76	0	1382	18	114
FIL V2	3.54	0.30	1.64	1.30	0.60	0.49	1.41	0.52	1.86	896	1386	12	124
POT, 8 AUG 80													
MIC NONE	4.06	0.97	3.22	0.81	0.27	0.66	0.53	0.63	.	236	916	24	***
CAR	3.66	1.20	2.92	0.79	0.37	0.54	0.51	0.68	.	161	1152	22	***
IMB V2	4.20	0.71	2.11	0.88	0.38	0.54	1.59	0.45	.	300	1873	0	***
MEX	4.34	0.89	1.88	1.10	0.31	0.38	2.80	0.38	.	242	2082	0	***
FIL V1	2.40	0.98	1.85	1.27	0.40	0.33	1.68	0.38	.	2314	2087	0	***
IMB V1	4.01	0.87	2.29	0.89	0.36	0.50	1.10	0.45	.	178	1385	9	***
FIL V2	2.92	1.13	2.55	0.75	0.46	0.30	0.52	0.31	.	551	1270	20	***
POT, 10 SEPT 80													
NIL NONE	3.02	0.75	1.51	0.82	0.27	0.22	3.42	0.45	0.82	163	890	0	776
PIN	3.19	0.71	1.64	0.88	0.49	0.34	3.29	0.44	0.62	66	765	2	895
MIC	3.32	0.88	2.72	0.65	0.21	0.44	3.82	0.79	1.41	0	788	9	807
CAR	2.92	0.84	1.82	0.61	0.28	0.29	3.51	0.76	0.95	97	800	2	***
IMB V2	3.35	0.73	1.55	0.79	0.27	0.27	3.38	0.58	0.75	106	815	0	716
MEX	3.92	0.73	1.84	0.79	0.24	0.31	3.35	0.62	0.71	0	770	8	462
FIL V1	2.60	0.84	1.59	1.08	0.38	0.29	2.22	0.76	0.68	81	908	2	840
IMB V1	4.09	0.91	1.98	0.78	0.28	0.34	2.49	0.59	0.92	11	930	8	745
FIL V2	3.18	0.80	1.48	0.57	0.44	0.22	3.45	0.64	0.86	179	905	0	914

TABLE 24. Continued.

SITE, DATE TISSUE TREATMENT	N	P	K	CA	MG	S	SI	NA	CL	MN	FE	CU	ZN
	PERCENT									PPM			
FIELD, 26 APR 80													
CAR NONE	3.26	0.80	1.46	0.91	0.41	0.50	0.69	1.07	.	232	2390	14	181
FIL V1	5.16	0.76	1.72	1.18	0.38	0.63	0.83	1.31	.	344	2084	27	164
FIL V2	3.37	0.40	0.88	1.30	0.24	0.25	0.52	0.16	.	147	1161	0	325
MIC	4.19	0.64	3.17	1.06	0.22	0.68	0.26	0.97	.	796	658	32	200
IMB	3.33	0.75	1.80	1.17	0.34	0.38	0.78	0.72	.	577	1514	9	150
RUB	4.48	0.66	2.01	1.14	0.39	0.63	0.63	1.12	.	338	1360	31	439
FIELD, 6 MAY 80													
CAR NONE	4.29	0.50	2.87	0.71	0.28	0.73	0.17	0.84	.	66	574	34	200
RUB	4.63	0.46	3.58	0.78	0.25	0.69	0.16	0.76	.	91	399	38	224
FIL V1	5.30	0.55	2.72	0.76	0.28	0.66	0.18	0.81	.	78	476	38	216
IMB	3.97	0.79	3.16	0.67	0.34	0.55	0.19	0.74	.	106	468	33	204
FIL V2	4.66	0.44	3.06	0.74	0.38	0.57	0.17	0.54	.	126	399	42	203
FIELD, 19 MAY 80													
CAR NONE	4.14	0.28	1.37	1.03	0.37	0.60	1.11	0.88	.	772	2569	15	225
RUB	4.20	0.32	1.86	0.90	0.36	0.60	1.14	0.90	.	760	2551	18	244
FIL V1	4.00	0.25	1.36	0.85	0.44	0.46	1.95	0.71	.	856	3518	0	228
IMB	3.89	0.25	1.29	0.90	0.38	0.48	1.60	0.77	.	603	3143	0	218
FIL V2	3.59	0.23	1.07	0.74	0.53	0.35	2.52	0.47	.	737	3185	0	193
FIELD, 25 MAY 80													
MIC NONE	3.55	0.38	1.17	1.49	0.35	0.58	1.56	0.77	1.71	0	1678	16	227
CAR	3.82	0.79	0.80	1.10	0.63	0.58	0.67	0.92	1.29	0	628	26	481
RUB	4.31	0.65	0.98	1.62	0.54	0.60	1.15	0.88	1.52	0	945	21	226
FIL V1	4.57	0.93	0.84	1.70	0.55	0.55	0.84	0.96	1.14	0	950	23	359
IMB	4.42	0.53	0.92	1.30	0.33	0.56	0.52	1.04	1.16	0	650	22	400
FIL V2	3.84	0.72	1.15	1.18	0.66	0.43	1.56	0.61	1.31	410	1245	18	263

TABLE 24. Continued.

SITE, DATE / TISSUE	TREATMENT	N	P	K	CA	MG	S	SI	NA	CL	MN	FE	CU	ZN
		PERCENT									PPM			
FIELD, 25 MAY 80														
CAR	NORMAL FIELD ZN	3.95	0.38	2.34	0.68	0.28	0.63	0.39	0.75	.	149	1140	25	442
CAR	TOXIC FIELD ZN	1.96	0.51	2.38	0.71	0.27	0.54	0.64	0.76	.	195	1470	15	***
CAR	FERTILE	3.30	0.81	0.31	1.14	0.48	0.31	0.61	0.40	.	519	1468	0	117
FIELD, 2 JUNE 80														
MIC	NONE	4.10	0.43	2.09	1.44	0.33	0.81	1.63	1.08	.	1906	3621	11	234
CAR		3.97	0.43	1.54	1.10	0.43	0.63	1.04	0.96	.	954	2785	14	225
RUB		4.09	0.59	1.82	1.44	0.42	0.53	2.07	0.86	.	2260	3873	0	286
FIL V1		4.34	0.47	1.44	1.47	0.46	0.51	1.27	0.72	.	2944	4051	2	249
IMB		4.42	0.32	1.37	1.12	0.43	0.56	1.50	0.82	.	673	3491	3	227
FIL V2		3.83	0.29	1.45	1.20	0.58	0.43	1.81	0.52	.	1990	3275	0	211
FIELD, 8 AUG 80														
NIL	NONE	2.02	0.20	1.93	0.57	0.50	0.27	3.51	0.22	.	798	4205	0	67
MIC		3.10	0.19	1.90	0.85	0.36	0.43	3.28	0.33	.	1254	3967	0	112
CAR		3.17	0.31	1.41	0.76	0.45	0.34	3.34	0.31	.	1335	5608	0	98
IMB		3.21	0.18	1.21	0.47	0.46	0.31	3.30	0.26	.	617	5249	0	76
FIELD, 12 AUG 80														
NIL	NONE	2.60	0.29	2.13	1.08	0.46	0.35	3.48	0.36	1.20	741	1837	1	37
MIC		3.62	0.23	2.38	1.19	0.37	0.58	3.53	0.68	1.90	72	2095	4	26
CAR		3.81	0.37	2.19	1.27	0.41	0.47	2.65	0.56	1.19	98	1593	6	46
IMB		3.56	0.28	1.44	1.08	0.43	0.37	2.61	0.41	0.84	227	1580	5	45

TABLE 24. Continued.

SITE, DATE / TISSUE	TREATMENT		N	P	K	CA	MG	S	SI	NA	CL	MN	FE	CU	ZN
			PERCENT									PPM			
SPRING RICE FIELD, 5 JUNE 80															
PLANT	NONE	CONTROL	2.34	0.49	1.30	0.56	0.31	0.25	1.65	0.31	.	854	4112	0	136
	NONE	60KGN	3.03	0.52	1.46	0.49	0.29	0.30	1.91	0.35	.	767	4333	0	79
	MIC	INTER	2.20	0.48	1.15	0.50	0.30	0.26	1.72	0.31	.	562	4788	0	66
	CAR	MONO	2.70	0.54	1.20	0.57	0.35	0.24	1.92	0.37	.	972	5739	0	59
		COMBI	2.68	0.49	1.17	0.58	0.36	0.25	1.98	0.33	.	640	5799	0	62
		INTER	1.93	0.40	1.16	0.45	0.30	0.25	1.74	0.34	.	649	3020	0	81
	RUB	MONO	2.79	0.56	1.30	0.66	0.34	0.25	1.81	0.34	.	1052	5473	0	62
		COMBI	2.54	0.44	1.36	0.54	0.33	0.24	1.77	0.35	.	807	2837	0	74
		INTER	2.33	0.47	1.19	0.45	0.31	0.26	1.75	0.34	.	559	4214	0	70
	FIL V2	MONO	2.91	0.64	1.22	0.58	0.33	0.24	1.65	0.34	.	1025	6280	0	61
		COMBI	2.93	0.57	1.07	0.60	0.35	0.25	1.74	0.32	.	641	6158	0	68
		INTER	1.97	0.41	1.13	0.42	0.30	0.23	1.80	0.32	.	487	3062	0	68
	IMB	MONO	2.53	0.38	1.39	0.61	0.38	0.23	2.08	0.31	.	1260	2427	0	87
		COMBI	2.71	0.36	1.46	0.59	0.36	0.25	1.87	0.30	.	702	2097	0	69
		INTER	2.12	0.37	1.45	0.47	0.31	0.25	1.62	0.31	.	565	1513	0	89
	FIL V2	MONO	2.75	0.49	1.57	0.57	0.37	0.26	1.72	0.36	.	1031	2850	0	82
		COMBI	2.86	0.42	1.37	0.61	0.37	0.27	1.87	0.30	.	631	2266	0	86
		INTER	2.26	0.35	1.29	0.57	0.33	0.25	1.72	0.25	.	550	1652	0	90

TABLE 24. Continued.

SITE, DATE TISSUE	TREATMENT		N	P	K	CA	MG	S	SI	NA	CL	MN	FE	CU	ZN
			PERCENT									PPM			
SPRING RICE FIELD, 14 JUNE 80															
PLANT	NONE	CONTROL	2.75	0.49	1.76	0.34	0.24	0.23	2.16	0.23	0.97	281	1889	65	79
	NONE	60KGN	3.09	0.50	1.75	0.36	0.28	0.27	2.30	0.27	1.05	300	1624	73	78
	MIC	INTER	2.85	0.53	1.62	0.38	0.26	0.25	2.13	0.23	0.99	212	2049	61	72
	CAR	MONO	3.29	0.55	1.69	0.37	0.27	0.25	2.15	0.26	1.01	354	2932	57	68
		COMBI	3.30	0.59	1.83	0.37	0.25	0.25	2.15	0.24	1.04	204	2469	38	54
		INTER	2.74	0.52	1.74	0.36	0.25	0.24	2.02	0.23	0.98	230	2067	84	84
	RUB	MONO	2.91	0.53	1.71	0.36	0.26	0.23	2.19	0.27	0.98	409	2539	22	39
		COMBI	3.45	0.59	1.79	0.38	0.29	0.26	2.24	0.26	1.04	238	2665	50	63
		INTER	2.67	0.49	1.62	0.34	0.24	0.23	2.05	0.23	0.97	258	2063	96	94
	FIL V1	MONO	3.29	0.57	1.74	0.36	0.27	0.25	2.32	0.27	1.05	366	2445	55	62
		COMBI	3.52	0.58	1.80	0.36	0.27	0.25	2.22	0.25	1.05	211	2320	39	58
		INTER	3.00	0.57	1.66	0.39	0.27	0.24	2.22	0.24	1.01	220	2703	39	54
	IMB	MONO	3.09	0.53	1.79	0.36	0.27	0.24	2.31	0.25	1.01	367	2364	45	49
		COMBI	3.34	0.57	1.83	0.38	0.28	0.26	2.15	0.25	1.08	229	2339	41	55
		INTER	2.44	0.58	1.63	0.36	0.26	0.24	2.35	0.24	0.97	257	2735	82	88
	FIL V2	MONO	3.12	0.55	1.77	0.37	0.28	0.24	2.14	0.24	1.06	347	2361	106	103
		COMBI	3.18	0.57	1.75	0.36	0.27	0.25	2.10	0.23	1.02	222	2346	45	61
		INTER	2.74	0.53	1.55	0.38	0.26	0.24	2.18	0.25	0.97	254	2587	61	64

TABLE 24. Continued.

SITE, DATE TISSUE	TREATMENT		N	P	K	CA	MG	S	SI	NA	CL	MN	FE	CU	ZN
			PERCENT									PPM			
SPRING RICE FIELD, 9 JULY 80															
PLANT	NONE	CONTROL	1.18	0.25	1.26	0.19	0.19	0.14	2.81	0.16	0.70	303	536	21	39
	NONE	60KGN	1.29	0.27	1.24	0.21	0.22	0.14	2.99	0.15	0.70	330	478	63	57
	MIC	INTER	1.48	0.29	1.35	0.23	0.21	0.14	2.93	0.14	0.73	309	598	35	46
	CAR	MONO	1.35	0.27	1.32	0.21	0.21	0.14	1.76	0.14	0.74	351	444	90	89
		COMBI	1.56	0.28	1.37	0.21	0.22	0.16	2.73	0.15	0.79	342	551	36	55
		INTER	1.28	0.26	1.28	0.21	0.20	0.13	2.83	0.14	0.69	276	552	55	51
	RUB	MONO	1.42	0.30	1.43	0.22	0.23	0.16	3.12	0.14	0.84	437	684	215	182
		COMBI	1.57	0.30	1.45	0.22	0.23	0.16	2.88	0.15	0.85	357	471	82	81
		INTER	1.38	0.30	1.45	0.22	0.22	0.15	3.00	0.15	0.80	326	430	59	67
	FIL V1	MONO	1.37	0.29	1.31	0.21	0.23	0.15	3.10	0.15	0.78	387	496	50	59
		COMBI	1.81	0.35	1.51	0.24	0.27	0.18	3.24	0.15	0.92	436	630	81	78
		INTER	1.41	0.28	1.43	0.22	0.21	0.14	3.09	0.14	0.79	325	462	83	73
	IMB	MONO	1.30	0.27	1.31	0.21	0.23	0.15	3.11	0.14	0.75	374	479	63	79
		COMBI	1.39	0.31	1.34	0.22	0.24	0.15	2.98	0.16	0.79	342	578	46	54
		INTER	1.41	0.30	1.29	0.22	0.21	0.15	2.77	0.17	0.75	308	636	51	57
	FIL V2	MONO	1.33	0.28	1.33	0.22	0.22	0.15	3.03	0.15	0.77	359	576	67	74
		COMBI	1.46	0.30	1.44	0.20	0.22	0.15	3.14	0.12	0.77	373	460	90	87
		INTER	1.74	0.33	1.53	0.24	0.24	0.17	3.04	0.15	0.90	363	451	33	54

TABLE 24. Continued.

SITE, DATE TISSUE	TREATMENT		N	P	K	CA	MG	S	SI	NA	CL	MN	FE	CU	ZN
			PERCENT									PPM			
SPRING RICE FIELD, 26 JULY 80															
PLANT	NONE	CONTROL	0.78	0.00	1.41	0.33	0.23	0.14	3.62	0.15	0.91	500	943	6	30
	NONE	60KGN	0.76	0.01	1.52	0.32	0.24	0.16	3.68	0.16	1.00	570	740	7	30
	MIC	INTER	0.94	0.03	1.57	0.32	0.24	0.15	3.68	0.16	1.01	499	795	6	28
	CAR	MONO	0.82	0.00	1.73	0.32	0.26	0.16	3.75	0.15	1.10	547	570	6	29
		COMBI	1.08	0.03	1.66	0.35	0.28	0.18	3.76	0.15	1.11	608	821	10	32
		INTER	0.84	0.01	1.61	0.32	0.22	0.15	3.67	0.15	0.99	516	782	6	27
	RUB	MONO	0.90	0.03	1.54	0.32	0.26	0.17	3.72	0.14	1.06	605	636	7	34
		COMBI	0.92	0.06	1.55	0.36	0.28	0.18	3.74	0.16	1.08	603	916	7	37
		INTER	0.92	0.03	1.58	0.30	0.23	0.15	3.65	0.15	0.96	485	767	5	29
	FIL V1	MONO	0.95	0.00	1.49	0.33	0.27	0.17	3.70	0.14	1.03	581	757	6	33
		COMBI	1.10	0.05	1.53	0.35	0.30	0.18	3.79	0.16	1.16	705	939	6	35
		INTER	1.07	0.06	1.57	0.32	0.25	0.16	3.70	0.15	1.03	577	846	6	32
	IMB	MONO	0.80	0.00	1.59	0.31	0.25	0.16	3.69	0.15	1.02	543	820	6	29
		COMBI	1.01	0.04	1.66	0.33	0.29	0.16	3.75	0.14	1.09	684	692	6	31
		INTER	0.90	0.03	1.54	0.33	0.23	0.15	3.67	0.16	0.99	543	823	7	25
	FIL V2	MONO	1.01	0.03	1.57	0.33	0.27	0.17	3.73	0.15	1.08	595	671	7	34
		COMBI	1.27	0.05	1.65	0.35	0.30	0.18	3.79	0.13	1.16	730	620	8	40
		INTER	0.95	0.00	1.68	0.32	0.24	0.15	3.73	0.13	1.07	577	668	5	25

TABLE 24. Continued.

SITE, DATE TISSUE	TREATMENT		N	P	K	CA	MG	S	SI	NA	CL	MN	FE	CU	ZN
			PERCENT									PPM			
SPRING RICE FIELD, 8 AUG 80															
GRAIN	NONE	CONTROL	1.16	0.26	0.41	0.01	0.12	.	.	0.14	.	60	370	13	68
	NONE	60KGN	1.26	0.28	0.43	0.02	0.13	.	.	0.01	.	68	353	14	74
	MIC	INTER	1.36	0.27	0.38	0.01	0.11	.	.	0.01	.	48	260	12	47
	CAR	MONO	1.40	0.29	0.38	0.01	0.12	.	.	0.02	.	58	325	14	53
		COMBI	1.42	0.31	0.40	0.01	0.12	.	.	0.01	.	53	309	16	55
		INTER	1.30	0.28	0.39	0.01	0.11	.	.	0.02	.	52	301	12	57
	RUB	MONO	1.40	0.27	0.42	0.01	0.11	.	.	0.01	.	57	283	15	46
		COMBI	1.49	0.25	0.42	0.01	0.09	.	.	0.02	.	54	288	11	69
		INTER	1.28	0.28	0.36	0.01	0.11	.	.	0.03	.	56	303	14	54
	FIL V1	MONO	1.41	0.27	0.38	0.01	0.11	.	.	0.01	.	57	236	12	48
		COMBI	1.48	0.28	0.41	0.01	0.12	.	.	0.01	.	71	290	14	62
		INTER	1.40	0.30	0.41	0.01	0.11	.	.	0.01	.	59	246	18	44
	IMB	MONO	1.23	0.29	0.39	0.01	0.11	.	.	0.01	.	52	238	14	45
		COMBI	1.40	0.30	0.38	0.01	0.11	.	.	0.01	.	54	291	14	44
		INTER	1.31	0.30	0.39	0.01	0.12	.	.	0.01	.	50	263	13	44
	FIL V2	MONO	1.41	0.30	0.37	0.01	0.11	.	.	0.01	.	51	218	13	38
		COMBI	1.51	0.30	0.40	0.01	0.11	.	.	0.01	.	54	196	12	43
		INTER	1.28	0.28	0.39	0.01	0.12	.	.	0.00	.	49	281	14	37

TABLE 24. Continued.

SITE, DATE / TISSUE	TREATMENT		N	P	K	CA	MG	S	SI	NA	CL	MN	FE	CU	ZN
			PERCENT									PPM			
RICE SEEDLING NURSERY, AUG 80															
PLANT	NONE		1.39	0.28	1.31	0.30	0.30	0.19	3.16	0.17	0.77	518	1265	8	56
AUTUMN RICE FIELD, 2 SEPT 80															
PLANT	NONE	CONTROL	1.49	0.34	1.44	0.26	0.26	0.16	2.99	0.21	0.69	320	2294	3	27
	NIL	INTER	1.69	0.36	1.72	0.25	0.27	0.19	2.57	0.19	0.73	306	1922	5	34
	MIC		1.91	0.37	1.76	0.24	0.27	0.17	2.61	0.22	0.76	322	2197	6	31
	CAR		1.63	0.34	1.60	0.23	0.28	0.16	3.29	0.21	0.72	338	2266	3	24
	IMB		1.91	0.37	1.88	0.25	0.25	0.18	2.56	0.21	0.82	338	2023	7	37
	NONE	30KGN	2.04	0.37	1.78	0.24	0.28	0.20	2.89	0.22	0.83	361	1766	7	38
	NONE	60KGN	2.41	0.40	2.00	0.24	0.28	0.23	2.72	0.20	0.95	407	1507	10	40
AUTUMN RICE FIELD, 27 SEPT 80															
PLANT	NONE	CONTROL	1.15	0.28	1.22	0.21	0.20	0.13	2.21	0.12	0.66	568	743	9	31
	NIL	INTER	1.06	0.28	1.31	0.21	0.19	0.13	2.36	0.12	0.66	492	842	7	30
	MIC		1.26	0.30	1.46	0.23	0.22	0.15	2.22	0.13	0.73	624	566	12	38
	CAR		1.15	0.29	1.40	0.23	0.20	0.15	2.39	0.12	0.70	581	742	8	33
	IMB		1.22	0.30	1.52	0.23	0.20	0.15	2.35	0.13	0.74	597	706	10	42
	NONE	30KGN	1.44	0.30	1.43	0.24	0.22	0.17	2.24	0.14	0.76	624	651	10	35
	NONE	60KGN	1.37	0.30	1.54	0.21	0.23	0.18	2.17	0.11	0.78	676	596	12	38
AUTUMN RICE FIELD, 12 NOV 80															
STRAW	NONE	CONTROL	0.69	0.15	1.13	0.24	0.18	0.14	2.66	0.13	0.72	667	949	6	25
	NIL	INTER	0.63	0.17	1.18	0.24	0.16	0.14	2.51	0.12	0.66	578	750	6	25
	MIC		0.65	0.16	1.14	0.24	0.24	0.13	3.30	0.14	0.64	737	1401	2	18
	CAR		0.75	0.18	1.32	0.25	0.17	0.16	2.44	0.13	0.75	734	736	9	34
	IMB		0.64	0.17	1.43	0.24	0.16	0.15	2.48	0.13	0.78	682	598	9	33
	NONE	30KGN	0.83	0.17	1.33	0.26	0.19	0.16	2.45	0.12	0.79	729	803	7	36
	NONE	60KGN	0.89	0.19	1.19	0.24	0.23	0.17	2.81	0.14	0.74	788	1034	5	24

TABLE 24. Continued.

SITE, DATE / TISSUE	TREATMENT		N	P	K	CA	MG	S	SI	NA	CL	MN	FE	CU	ZN
			PERCENT									PPM			
AUTUMN RICE FIELD, 12 NOV 80															
GRAIN	NONE	CONTROL	1.16	0.35	0.31	0.08	0.17	0.09	0.77	0.07	0.14	85	119	3	26
	NIL	INTER	1.10	0.34	0.32	0.08	0.16	0.09	0.75	0.07	0.14	83	108	4	28
	MIC		1.14	0.36	0.33	0.08	0.17	0.09	0.80	0.07	0.13	93	108	3	28
	IMB		1.10	0.34	0.32	0.08	0.16	0.09	0.72	0.07	0.13	81	108	3	27
	NONE	30KGN	1.23	0.35	0.33	0.08	0.16	0.09	0.76	0.07	0.14	100	119	5	29
	NONE	60KGN	1.30	0.35	0.32	0.08	0.16	0.10	0.63	0.06	0.13	97	111	4	29

[a] Elemental analyses by micro-Kjeldahl and X-ray quantometer

[b] Treatments include no fertilizer and no azolla (**NONE CONTROL**), a basal application of 60 or 30 kg N/ha as ammonium sulfate (**60KGN** or **30KGN**), the azolla accession A. microphylla (**MIC**) grown with rice (**INTER**), and the azolla accessions A. caroliniana (**CAR**), A. filiculoides (**FIL V1** and **FIL V2**), A. mexicana (**MEX**), A. nilotica (**NIL**), A. pinnata var. pinnata (**PIN**), A. pinnata var. imbricata (**IMB**), and A. rubra (**RUB**) grown prior to the rice (**MONO**), with the rice (**INTER**) or both (**COMBI**).

[c] No analysis (.).

[d] Concentration beyond calibrated limits (***).

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