PHYSIOLOGY AND AGRONOMIC USE OF *AZOLLA* SPECIES IN RICE CULTURE

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ABSTRACT

Although the value of *Azolla* species as a green manure for lowland agriculture is well established, environmental and management constraints have limited its use to a few countries. Cultivation of *Azolla* species may be extended to other areas if species and cultivars adapted to specific agricultural environments can be identified. Five accessions from three species of *Azolla* were identified as being relatively tolerant of high light and temperature. The nitrogen accumulation potential and physiological characteristics of these accessions were evaluated in a series of experiments.

The nitrogen and biomass accumulation by *Azolla caroliniana* (Brazil), *A. caroliniana* (Ohio, U.S.A.), *A. microphylla* (Galapagos), *A. pinnata* (Indonesia) and *A. pinnata* (Taiwan) were evaluated during summer in Taiwan. Although average air temperatures were above 30 C, *A. caroliniana* from Ohio, U.S.A. and *A. pinnata* from Indonesia accumulated more than 40 kg ha^{-1} of nitrogen in 20 days when intercropped with rice. Rice in plots fertilized with *Azolla* species had greater plant height, shoot weight, leaf area index, tiller number, and grain yield than the control plots. Rice growth and yield were not increased by treatments which included 25 kg ha^{-1} of fertilizer nitrogen with *Azolla*. The leaf area index and grain yield of rice fertilized with *Azolla* nitrogen were less than those obtained with a similar quantity of fertilizer nitrogen. The lower response to *Azolla* may be due to the delay in release of nitrogen by mineralization.

The relative growth rate of A. caroliniana (Ohio), A. microphylla (Galapagos) and A. pinnata (Indonesia) in greenhouse studies was negatively related to initial biomass when the biomass exceeded 600 g m^{-2}. An initial biomass of 600 g m^{-2} of A. caroliniana, A. microphylla or A. pinnata produced more fresh weight and nitrogen after 21 days of growth than did initial biomasses of 200 or 400 g m^{-2}. Fresh weight and nitrogen accumulation of all three species declined progressively with decreasing growth light level. With initial biomasses of 250 or 500 g m^{-2}, the two species accumulated almost the same biomass and nitrogen after 20 days when both were grown at 35% light. At higher light levels, A. microphylla accumulated significantly more fresh weight than A. caroliniana and A. pinnata, but had a significantly lower dry matter and nitrogen content.

Growth of A. caroliniana and A. microphylla in controlled-temperature chambers was greater at 20 C than at 33 C when harvested 10 days after planting (DAP); only the growth of A. microphylla was greater at 20 C than at 33 C at 20 DAP. Growth declined with decreasing light and the effect was generally much greater at 33 C than at 20 C.

The carbon dioxide exchange rate per unit weight (CER_W) of A. caroliniana was greater than rates measured for A. microphylla and A. pinnata. The greater CER_W was attributed to a greater specific leaf area and specific chlorophyll content. CER_W increased curvilinearly with increasing photosynthetic photon flux density up to about 1500 μmoles m^{-2} s^{-1}. The CER_W decreased as the biomass increased, probably due to increased mutual shading.

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CHAPTER I

INTRODUCTION

Significant advances in food production have been realized during this century mainly due to the success of scientific agriculture. A key to this success is the use of fertilizers and agro-chemicals. About one-third to one-half of the world cereal yield increase in the past 30 years can be attributed to the use of fertilizer nitrogen (Hardy, 1975). However in recent years, small farmers in developing countries have not been able to apply fertilizers at recommended rates because of high cost and limited availability. As a result, presently there is a greater emphasis on agricultural practices which would lessen a farmer's dependence on chemical fertilizers. Among these practices is the utilization of biologically fixed nitrogen which is an attractive alternative to fertilizer nitrogen because of its low cost. The amount of nitrogen biologically fixed in the biosphere every year is between 120 to 175 million metric tons. The quantity fixed in land under cultivated crops is estimated to be 45 million metric tons (Burns and Hardy, 1975; Burns, 1980).

Nitrogen-fixing plants, mostly legumes, have been used as green manures for centuries and played a major role in plant nutrition and soil fertility before the advent of chemical fertilizers. At present, there is renewed interest in green manuring and in cropping practices such as crop rotation and intercropping in which nitrogen fixed by one crop is made available to a second crop after mineralization in the

soil. Major research programs are underway to maximize the input from biological nitrogen fixation in crop fields. The greatest benefit from this technology would be expected in fields with low soil fertility that are planted to crops requiring higher amounts of nitrogen. This is often the case in rice-producing small farms in developing countries.

Rice constitutes nearly 20% of world food-grain production and is the major food crop in South and South-East Asia (Stangel, 1979). There is an urgent need to step up rice production to feed the ever-increasing population. Since the land area available to expand agriculture is limited in most regions of Asia, the grain yield of rice per unit area of land must be increased. A major achievement in this regard is the breeding of high-yielding rice varieties, particularly at the International Rice Research Institute (IRRI). An important characteristic of these varieties is their response to applied fertilizer nitrogen. IRRI and several national research institutes continue to make improved rice varieties available to farmers.

The yield potential of many of these improved varieties range from 12 to 15 tons ha^{-1}; however, national average yields in many countries are only 2.0 to 3.0 tons ha^{-1} (IRRI,1979). Gap analysis studies conducted to determine the reasons for this discrepancy have identified fertilizer application and insect control as the two most important factors limiting yield potential of these new rice varieties. Since new varieties have been selected for response to higher levels of nitrogen, significant yield gains are likely to be achieved only by increasing the supply of this essential nutrient. Fertilizer nitrogen rates of over 225 kg ha^{-1} are applied by some Japanese farmers to produce yields of

over 9.0 tons ha^{-1}. In the majority of developing countries, however, average nitrogen use is below 50 kg ha^{-1} and consequently rice yields are below 3.0 tons ha^{-1} (Matsushima, 1976; Stangel, 1979). Large increases in yields may be possible by the application of higher rates of nitrogen, provided the other conditions for growth are satisfactory. Nevertheless, high prices and non-availability of nitrogen fertilizer often prevent farmers from fertilizing at recommended rates. According to Burgess (1981), the situation may get worse if the production cost of nitrogen fertilizer continues to rise.

An alternative source of nitrogen in rice culture is the aquatic fern of the genus Azolla which has been receiving a great deal of attention lately because of its symbiotic association with a nitrogen fixing blue-green algae. It has been used in China (Chu, 1979) and Vietnam (Tuan and Thuyet, 1979) as a green-manure for centuries. In recent years, researchers in many other countries are exploring the potential of Azolla sp. primarily as a source of nitrogen for rice (Lumpkin et al., 1982; Rains and Talley, 1979; Singh, 1977; Watanabe et al., 1977). Seven Azolla species have been recognized (Lumpkin and Plucknett, 1981) and several accessions have been identified within some of these species (Watanabe and Berja, 1983). These species and accessions respond differently to environmental variations (Watanabe et al., 1977; Lumpkin, 1983).

The successful use of Azolla as a green manure for rice will depend on its adaptation to the environmental conditions in which rice is grown. Azolla can be grown either as a monocrop prior to planting rice or as an intercrop that is incorporated within the first month after

planting the rice crop. In either of these cropping systems, Azolla may be exposed to sub or supra-optimal temperature and light regimes.

A significant constraint to the wider use of Azolla in tropical rice-growing areas is the low productivity during periods of warm weather. Species used during the summer must be productive in environments having temperatures of 30 C or greater and high solar radiation. Information is lacking on species response to high temperature and solar radiation and to management practices that would result in the maximum accumulation of nitrogen for rice during the summer season. Supplemental fertilizer nitrogen is likely to be needed to assure high yields because the time available for the growth of a green manure for a summer rice crop is relatively short. Appropriate management practices such as timing of fertilizer nitrogen application and the relationship between initial Azolla biomass and growth and nitrogen accumulation need to be investigated.

To test the hypothesis that Azolla species and management practices exist which would make Azolla a viable greenmanure crop for the warm summer rice season, a series of experiments were conducted with the following objectives:

1. to evaluate in the field the effectiveness of five Azolla accessions representing three species as green manure for a summer rice crop in Taiwan;

2. to examine the influence of light, temperature and initial plant biomass on dry matter and nitrogen accumulation of Azolla in greenhouse experiments;

3. to study the influence of light on carbon assimilation by Azolla.

CHAPTER II

REVIEW OF LITERATURE

2.1 Azolla-Anabaena symbiotic system

Azolla (the genus name will be used in the generic sense for convenience) is a free-floating fern that is native to Asia, Africa and the Americas. **Azolla** species have been dispersed by man and natural means to various parts of the world (Lumpkin and Plucknett, 1982). Physiological processes such as photosynthesis and nitrogen fixation occur only during the vegetative phase of azolla which is represented by the sporophyte. The azolla sporophyte has fronds and roots. The fronds are usually 1.0 to 3.0 cm in diameter. It is classified as a fern based on the type of spores it produces (Table 1). Further classification into individual species takes into account morphological characteristics of fronds and spores. The cavity within the dorsal lobe of the fern leaf contains the nitrogen fixing blue-green algae Anabaena azollae, a cyanobacterium. In their symbiotic association, azolla provides shelter and products of photosynthesis to the algae in exchange for reduced nitrogen.

2.2 Influence of environment on the life cycle of azolla

While the azolla sporophyte multiplies by vegetative reproduction, the onset of the gametophytic cycle can be induced by a number of environmental factors. Extreme stress induces sporulation to ensure survival during temporarily unfavorable conditions (Ashton, 1977). Becking (1979) obtained sporocarp formation in A. filiculoides and

TABLE 1. Classification of Azolla.

DIVISION	Pteridophyta	
CLASS	Filicopsida	
ORDER	Salviniales	
FAMILY	Azollaceae	
GENUS	*Azolla*	
SECTIONS	*Azolla*	*Rhizosperma*
SPECIES	*A. caroliniana*	*A. nilotica*
	A. filiculoides	*A. pinnata*
	A. mixicana	
	A. microphylla	
	A. rubra	
VARIETIES		*A. pinnata* var. *imbricata*
		A. pinnata var. *pinnata*

Source: Lumpkin and Plucknett (1982).

A. caroliniana with high light intensities and relatively low night temperatures. Karamyshev (1957; quoted by Becking, 1979) observed sexual reproduction in A. pinnata during the hot summer months whereas for the same species Moore (1969) reported sporocarp formation in cold weather in China. Talley and Rains (1980) reported that high temperature accelerated sporulation of A. filiculoides. A. pinnata and A. nilotica produced sporocarps during periods of low night temperature and short days in Hawaií (personal observation). The optimum conditions that resulted in the highest percentage of plants bearing sporocarps in A. filiculoides were 14 hour photoperiod, 9.5 pH, 27 C and a nitrate nitrogen concentration of 1 mg l^{-1} (Ashton, 1977). The spores survive submerged in water and complete their life cycle to form the sporophyte when environmental conditions return to normal. For this reason, the first appearance of the sporophyte and subsequent abundance of azolla in its natural habitats were strongly correlated with seasonal changes (Gopal, 1967; Holst and Yopp, 1979a).

2.3 Influence of environment on vegetative growth of azolla

The agronomic value of azolla depends on dry matter and nitrogen accumulation. Rate and duration of dry matter and nitrogen accumulation are dependent on photosynthesis and nitrogen fixation. These physiological processes are influenced to a great extent by light and temperature. T. A. Lumpkin (personal communication) attributed the higher nitrogen accumulation of A. filiculoides compared to four other azolla accessions to its ability to maintain the immature phase of its life cycle throughout the growing season. The amount of nitrogen

accumulated was positively and exponentially related to time to sporocarp formation and negatively and exponentially related to average maximum temperature prevailing from 'planting' to sporocarp formation (Talley and Rains, 1980).

2.3.1 Light

Growth and nitrogen accumulation generally increase up to an optimum light level and then decline. The growth rate in A. filiculoides was positively correlated with sunlight up to 50% and then declined with further increase in light (Ashton, 1974). Light saturation of photosynthesis in A. caroliniana was observed at 400 $\mu E\ m^{-2}\ s^{-1}$ by Ray et al. (1979), but the CO_2 fixation rate did not decline appreciably even at 2000 $\mu E\ m^{-2}\ s^{-1}$. The optimum range of light levels for growth of azolla in China was 20 to 50 klux (FAO, 1979) while Vietnamese researchers have reported a light intensity for maximum growth of 15 to 18 klux (Tuan and Thuyet, 1979). The wide discrepancies observed by various workers may be due to species and accession differences and differences in biomass per unit area at the time of sampling. Nutrition can be another reason for these differences. For example, Tung and Shen (1981) observed the highest growth rate at 50% light when the culture medium contained less than four ppm phosphorus. Addition of 20 ppm phosphorus resulted in maximum growth at full sunlight.

The optimum temperature for growth of A. filiculoides increased from 22 C at a light intensity of 5 klux to 26 C when the intensity was increased to 20 klux (Ashton, 1974). Similar results were reported by

Talley and Rains (1980) who obtained the highest growth rate for day-night temperatures of 10/1.0 C at 100 $\mu E\ m^{-2}\ s^{-1}$; but at 35/25 C, the highest growth rate was at 1000 $\mu E\ m^{-2}\ s^{-1}$.

Solution pH interacts with light to influence azolla growth. The growth rate of A. filiculoides cultured at solution pH values ranging from 3 to 6 was negatively correlated with increasing light from 15 klux to 75 klux. However the growth rate was positively correlated with light up to 60 klux when pH ranged from 7 to 11. These results were obtained with a nutrient solution containing 10 ppm nitrogen as nitrate; Ashton (1974) concluded that uptake of combined nitrogen was greatest at acidic pHs and very low at basic pHs, while nitrogen fixation was highest in the basic range. The light-pH interaction has not been explained. Growth at 75 klux light intensity was lower than 60 klux at all pH values. Somewhat contradictory results have been reported from Vietnam where high light intensity enhanced azolla growth at pH 5, but inhibited growth at pH 6 and 7 (Tuan and Thuyet, 1979). Peters et al. (1980) found no interaction between light and pH. The available data suggest that generally azolla grows better under partial shade than in full sunlight. Deep shade may reduce growth.

In general, high light intensities appear to be detrimental to nitrogen fixation. Unlike legumes where root nodules are located away from leaves, there is no spatial separation of photosynthetic and nitrogen fixation systems in azolla. In addition, the endophyte also contains chlorophyll and phycobilin pigments which enable it to absorb light. Okon and Hardy (1983) consider this the ideal nitrogen-fixing system as both organisms of the symbiosis are phototrophic and possess a

large light harvesting surface. Although this increases the efficiency of utilization of light energy by the azolla-anabaena system, it may expose the endophyte to strong solar radiation and reduce nitrogen fixation rate. No change in nitrogen fixation, as estimated by acetylene (C_2H_2) reduction, was observed by Becking (1976) when A. pinnata was exposed to light intensities of 14 to 27 klux. However, the rate of C_2H_2 reduction was considerably reduced at noon when light intensities of 80 to 90 klux were reached (Becking, 1979). Acetylene reduction by A. caroliniana was saturated at about 5.0 klux whereas CO_2 fixation saturated at 8.0 klux (Peters, 1976). Subsequently Peters et al. (1980) reported an increase in growth rate of five accessions of azolla with increasing light intensity up to 400 μmoles m^{-2} s^{-1}, while C_2H_2 reduction activity saturated at 200 μmoles m^{-2} s^{-1}. There was an increase in nitrogen fixation of about 30% in A. mexicana when light intensity was increased from 12 klux to 25 klux (Holst and Yopp, 1979b). Nitrogen fixation in A. filiculoides reached a peak at 250 μE m^{-2} s^{-1} at day-night temperatures of 30/20 C (Talley and Rains, 1980). Becking (1979) suggested that the lower light intensities under a rice canopy may favor nitrogenase activity in azolla. Exposure of A. pinnata for 5 hrs to light intensities of 16 to 57 % of full sunlight had very little effect on nitrogenase activity; however nitrogenase activity was reduced to 30% of control plants by 84% of full sunlight (Brotonegoro and Abdulkadir, 1976).

The light-harvesting pigments in azolla are chlorophyll a and b and carotenoids. Anabaena is composed of vegetative cells and heterocysts. Vegetative cells are the site of photosynthesis and contain chlorophyll

a, phycobilins and carotenoids (Peters et al., 1979). Heterocysts are specialized cells where nitrogen fixation occurs. Unlike the heterocysts of other symbiotic blue-green algae that are in association with green plants such as cycads and Gunnera, those of Anabaena azollae retain phycobilins and absorb light energy (Peters et al., 1980). Estimates indicate that 10 to 20% of chlorophyll a or 7.5 to 15% of total chlorophyll in the azolla-anabaena complex is contributed by the endophyte (Peters and Mayne, 1974a).

The azolla-anabaena symbiotic association's light absorption spectra for photosynthesis is very similar to that of other green plants with the maximum quantum yield occurring between 650 nm and 670 nm. In phycobilin pigments of the endophyte, the maximum quantum yield occurs between 580 nm and 640 nm which is the portion of the spectrum least efficiently absorbed by chlorophyll pigments (Ray et al., 1979; Tyagi et al., 1981).

2.3.2 Temperature

The successful use of azolla as a green manure will depend to a great extent on the growth and nitrogen fixation rates obtained under extremes of temperature. In fact, poor adaptability of azolla accessions grown in China and Vietnam to temperature extremes is one of the reasons for the non-adoption of this technology by other rice-growing countries in Asia. Azolla growth is usually retarded by high as well as low temperatures. The cardinal temperatures for azolla vary with species (Lumpkin, 1983). Data for A. filiculoides show that the cardinal temperatures are 5.0 C and 45 C (Ashton, 1974). In the studies

of Talley and Rains (1980), A. filiculoides had a biomass doubling time of 6.7 days when grown at day-night temperatures of 10/1.0 C or 15/5.0 C; the doubling time was only two to three days when grown in 30/20 C but growth was too low to assess quantitatively at 40/30 C.

Although azolla can grow at extremely low and high temperatures, the optimum temperature range for growth and nitrogen fixation is usually narrower. Peters et al.(1980) found growth of A. mexicana and A. pinnata was greatest at 30 C while for A. caroliniana and A. filiculoides, the highest growth rates were at 25 to 30 C and 25 C respectively. The optimum temperature for growth of A. pinnata in China was 20 to 25 C (FAO, 1979). Watanabe et al. (1977) reported that fresh weight accumulation of A. pinnata at 35/27 C was less than half of that at 32/24 C, although the average temperature of 31 C was not unusually high for A. pinnata. Peters (1980) suggested that exposure to 35 C for 12 hrs may have been responsible for the reduced growth.

The ideal temperature range for nitrogen fixation also appears to be in the 20 to 30 C range. Acetylene reduction activity of A. pinnata increased with increasing temperature, reaching a maximum at about 30 C but there was appreciable activity even at 40 C (Becking, 1979). Similar results were obtained with A. pinnata (Chapman et al., 1981) and A. mexicana (Holst and Yopp, 1979b), but in both studies, acetylene reduction activity almost ceased at 40 C. Acetylene reduction increased with increasing temperature up to 40 C in studies on A. pinnata; inhibition occurred only at 43 C (Brotonegoro and Abdulkadir, 1976). Nitrogenase activity was almost nil at 40/30 C in A. filiculoides; however, when azolla grown at 20/10 C or at 30/20 C was placed in an

environment where temperature was increased stepwise by 5 C every 2 hrs, nitrogenase activity increased with temperature up to 40 C and remained high at 45 C (Talley and Rains, 1980). In most of the studies discussed above, the C_2H_2 reduction rates were greatly reduced above 35 C. This may be due to the adverse effects of high temperature on synthesis of metabolites required for nitrogen fixation.

Temperature also influences the nitrogen and dry matter content of azolla plant material. Lumpkin and Plucknett (1980) in their review quoted a study in China where nitrogen concentration of dry azolla increased from 1.75 % to 4.5 % as temperature was increased from 5 C to 25 C; however at 40 C, the nitrogen concentration dropped to 2.5 %. Nitrogen concentration was inversely related to the dry matter content of the tissue. The dry matter concentrations at temperatures of 5, 25 and 40 C were 16, 6 and 10% respectively. Nitrogen content increased with temperature in A. filiculoides up to 30/20 C and then decreased at 35/25 C (Talley and Rains, 1980). In another study, nitrogen content of four accessions of azolla increased up to 30 C, while A. filiculoides had the highest nitrogen content at 25 C. Beyond the optimum temperature, there was a drop in nitrogen content in all species (Peters et al., 1980). A green manure containing a higher dry matter content and a higher nitrogen percentage is the objective in azolla culture. Since its tissue is very delicate, even azolla with a low nitrogen content would be expected to decompose rapidly but the total amount of nitrogen added per unit fresh weight would be low.

Low or high temperature stress causes azolla to change color from green to pink or red. This color change is believed to be brought

about by the accumulation of anthocyanin pigments. Pieterse et al. (1977) observed an acceleration in anthocyanin formation at 5.0 to 10 C in A. filiculoides and A. caroliniana. Peters et al. (1980) observed the following color responses to temperature among the species. Anthocyanin formation occurred at 15 C in A. mexicana and A. caroliniana; at 40 C, A. filiculoides was bleached, A. caroliniana was bright red, and A. mexicana was reddish brown. A. pinnata remained green at all temperatures.

Temperature is often correlated with pest and disease outbreaks. This is a serious problem in the cultivation of azolla during warm summer weather. Pests of azolla thrive in warmer temperatures and may devastate the entire crop within a short time. High temperature combined with high humidity and shading under the rice canopy provide an ideal environment for fungal disease development in dense azolla mats. Therefore azolla grown as green manure for summer crops should be incorporated when the mat biomass attains 1.5 to 2.0 kg m^{-2} (Lumpkin and Plucknett, 1982).

2.3.3 Species differences in azolla

If azolla is intercropped with rice, high light intensities may not be detrimental because the rice canopy provides some shade. Therefore, the potential of azolla as a green-manure crop for the summer months will depend largely on the average temperature prevailing during that time and on species adaptability. The variability in growth response of Azolla species to light and temperature may make it possible to identify species and accessions better suited to a given environment. Field

studies of Talley et al. (1977) showed that A. mexicana has greater tolerance to high light than A. filiculoides. However, Peters et al.(1980) found no difference in response to light among five accessions of azolla representing four species, including the two species mentioned above, in controlled environment studies. A. microphylla, A. caroliniana and A. pinnata var. imbricata were more heat-tolerant than A. filiculoides, A. mexicana, A. nilotica, A. pinnata var. pinnata and A. rubra (Lumpkin and Plucknett, 1982; Watanabe, 1984). Peters et al.(1980) found the heat tolerance of A. pinnata and A. mexicana to be highest, A. caroliniana was intermediate, and A. filiculoides the lowest.

Azolla species have also differed in tissue dry matter and nitrogen content (Peters et al., 1980; Peters and Calvert, 1982). Therefore a high growth or biomass accumulation rate for azolla does not necessarily mean a higher rate of nitrogen accumulation. Absolute values of accumulated nitrogen are the most reliable indicators of the potential of a species as a green-manure crop. Studies in China by Lumpkin et al. (1982) demonstrated that the quantity of nitrogen accumulated by Azolla species differs markedly; in monoculture as well as duel culture with rice, A. filiculoides accumulated more nitrogen than three other species investigated.

2.4 Photosynthesis and Nitrogen Fixation

The light energy absorbed by phycobilins is used for nitrogen fixation in heterocysts and for CO_2 fixation in vegetative cells of the endophyte (Tyagi et al., 1981). According to Ray et al. (1979), the

contribution of the endophyte to the association's CO_2 fixation capacity is relatively small because it contains less than 20% of the chlorophyll and approximately 50% of its cells may be heterocysts that lack CO_2 fixation capability. Recent estimates show that anabaena contributes 6.0 to 10% of the total photosynthetic capability of the association (Peters and Calvert, 1983).

The azolla-anabaena association exhibits characteristics of C_3 plants including the production of the typical Calvin cycle intermediates of CO_2 fixation. The CO_2 compensation concentration of azolla in air was 35 $\mu l\ l^{-1}$. Upon reducing the oxygen level to 2%, the CO_2 compensation concentration dropped to 3.0 $\mu l\ l^{-1}$. This suggests the presence of photorespiration. However, the isolated anabaena lacked photorespiration as is the case for other green and blue-green algae (Ray et al., 1979). Rates of CO_2 fixation of the association in air are about 40% less than those at 2% oxygen (Peters and Calvert, 1983).

In addition to CO_2 fixation, photosynthesis also provides ATP and reducing power for nitrogen fixation. Thus, nitrogen fixation in azolla is a light-dependent process as it relies on the products of current photosynthesis. Nitrogen fixation is a Photosystem 1-linked process and not directly dependent on Photosystem II (Peters et al., 1981). The majority of the ATP for nitrogenase activity is obtained from cyclic phosphorylation (Photosystem I) and not oxidative photophosphorylation. Photosystem II activity is required to generate photosynthate for reducing power, but it is not directly required for nitrogenase activity (Peters et al., 1979). In the absence of light, nitrogenase activity can continue until the ATP and reductants generated during the light

period are depleted. For this reason, the rates of dark nitrogen fixation are less than half of those obtained during light (Peters et al, 1981).

Ray et al.(1979) recorded maximum photosynthetic rates in air of 90 to 100 μmoles CO_2 mg^{-1} chlorophyll hr^{-1} for A. caroliniana while azolla freed of the endophyte had values of 75 to 80 μmoles CO_2 mg^{-1} hr^{-1}. Daylength did not have a significant effect on photosynthetic rate though slightly higher rates were recorded in azolla conditioned under 12/12 hr light-dark periods compared to 16/8 hr light-dark periods. The photosynthetic rates obtained in different experiments seem to vary widely. Peters (1975) reported a peak value of 40 μmoles CO_2 mg^{-1} hr^{-1} and later Peters et al. (1981) obtained values as high as 123 μmoles CO_2 mg^{-1} hr^{-1}. In measurements of photochemical activity, the range of values observed were 140 to 246 μmoles CO_2 mg^{-1} chlorophyll hr^{-1} and 32 to 150 μmoles mg^{-1} chlorophyll hr^{-1} for Photosystem I and II respectively. Similarly, extracts of Anabaena vegetative cells showed appreciable Photosystem I and lower Photosystem II activity (Peters and Mayne, 1974a).

2.5 Azolla in rice-based cropping systems

Improved irrigation facilities, mechanization and breeding of early-maturing crop varieties have resulted in more intensive use of land. Multiple cropping, which is the cultivation of more than one crop during a year, is becoming more popular among farmers in many countries. Most multiple cropping systems in Asia include at least one crop of

rice. Since the culture of paddy rice requires flooding, it is usually grown during the major rainy season in tropical Asian countries (R.I.C.E., 1967). This season is usually between June and December in the Northern Hemisphere (Mikkelsen and De Datta, 1982). In rice-based intensive cropping systems, azolla grown as a green-manure can be fitted into the annual cropping calendar as the crop preceding rice or as an inter-crop with rice. For it to be most effective, azolla should be monocropped just before rice is planted and incorporated within the first month of planting rice. This will allow sufficient time for mineralization and release of nitrogen for rice. However, the planting time that maximizes the effectiveness of azolla for rice may result in its culture at a time when the climate is not ideal for azolla growth. Therefore azolla may have to be grown under unfavorable conditions in multiple cropping systems.

Land is continuously used for food crop cultivation in areas where there is no pronounced winter. Farmers may not readily include a monocrop of azolla in their cropping cycle because the benefits from a green manure crop are indirect. Therefore, azolla culture should be scheduled in such a way that it does not compete with rice for land, water or other resources. In Vietnam, azolla is usually grown as a winter crop from November to January when most other crops cannot be grown. The lower mean daily temperatures prevailing during the winter months are favorable for the growth of azolla (Tuan and Thuyet, 1979). Azolla has been grown experimentally in the United States as a green manure in fallow flooded fields during fall or late winter to early

spring (Talley et al., 1981). In areas where the land is fallow for a long time, a crop of azolla can increase the soil nitrogen and organic matter status. Dry matter production of azolla in natural habitats reached a maximum in November and December in India (Gopal, 1967).

In very intensive cropping systems as they are practiced today in many developing countries, including a monocrop of azolla in the cropping cycle is almost impossible. In such instances, azolla can be grown as an inter-crop with rice until the rice canopy closes over (Lumpkin et al., 1982). It takes about four to six weeks for rice to develop a canopy that is dense enough to shade out azolla. In order to further prolong this period of intercropping, double narrow-row planting of rice is practiced in China. Compact canopies formed by erect leaves and short stature of improved rice varieties facilitate greater penetration of light (Chu, 1979). When grown as an intercrop with rice, the modified environment under the canopy of the main crop may not be suitable for azolla because sub-optimal light and temperature may limit its photosynthetic and nitrogen fixation rates. On the other hand, the partial shade under the canopy may favor growth and nitrogen accumulation of azolla during warm summer months. Shade may also reduce the air and water temperature which can also be beneficial to azolla.

Another possibility is to raise azolla in ponds or field plots set aside specifically for multiplication. The green manure can then be collected and incorporated into rice fields. The disadvantage of this method is that large quantities of fresh azolla material have to be collected, transported, and distributed to the field. The dry matter

content of azolla is around 5% and it contains 3.0 to 5.0% nitrogen on dry weight basis (Lumpkin and Plucknett, 1982). Thus, 500 kg fresh azolla has to be incorporated for every kg of nitrogen applied. Air drying of azolla before transportation could partly overcome this problem. It may also be possible to reduce the bulk by composting. Azolla can be grown in a multiplication plot and harvested periodically for air-drying or composting.

2.6 Nitrogen requirement of rice

Quantitative determination of the nitrogen requirement of any crop is difficult and often inaccurate. In the case of cereal crops, early top dressing or a high level of soil nitrogen stimulates tiller formation while late top dressing ensures survival of tillers and grain set. As a result, split applications of fertilizer nitrogen have proved to be the most efficient way to improve nitrogen use in cereals (Spiertz and De Vos, 1983). As fertilizer nitrogen remains in the soil only for a limited time, it should be applied at growth stages when it can be expected to profoundly influence yield components.

The components that establish the potential yield of rice can be expressed as follows (Ishizuka, 1971):

Yield Capacity = Panicles m^{-2} X Spikelets $Panicle^{-1}$ X Hull Size.

Number of panicles is determined by the number of productive tillers. Murayama (1979) identified two critical growth stages at which nitrogen influenced panicle number; at the early tillering stage when it promoted tiller production and at early panicle initiation stage when nitrogen

increased the number of tillers carrying productive ears. Consequently, the efficiency of fertilizer nitrogen utilization was higher when it was applied at or soon after transplanting and just before or at panicle initiation (De Datta et al., 1974). For this reason, usually nitrogen is provided in split applications.

The number of spikelets initiated and the percentage of ripened grains determine the number of grains per panicle. Studies by Matsushima (1976) indicate that excess nitrogen at panicle initiation promotes the development of a larger flag leaf. A larger flag leaf can reduce the number of grains per panicle by competing with the panicle for photosynthates at the time of spikelet differentiation and grain filling (De Datta et al., 1974). An excess of nitrogen during grain filling may also encourage vegetative growth and delay crop maturity. On the other hand, a short supply of nitrogen may reduce the leaf area duration and force the leaves to senesce early resulting in an increased number of unfilled grains (Spiertz and De Vos, 1983). Maintaining a nitrogen level that will bring about a balance between the vegetative and reproductive functions at this critical stage of growth may be difficult to achieve.

The yield component that is least dependent on nitrogen nutrition is grain weight. The upper limit of grain growth in rice is determined by the size of the hull (Matsushima, 1980). Hull size is a varietal characteristic and is not significantly affected by nitrogen application. However, the grains may not attain their potential maximum size if the source capacity is inadequate. De Datta et al. (1974)

observed a reduction in 100 grain weight when nitrogen was applied after panicle initiation. This reduction was due to excessive vegetative growth. Thus, the relationship between nitrogen and size of grain appears to be related to nitrogen uptake by the plant up to the early reproductive phase. If the plant has accumulated sufficient nitrogen during early growth, further application may promote vegetative growth and reduce grain size. On the other hand, if the plant has formed a large number of grains with insufficient uptake of nitrogen, a late application of nitrogen may increase grain size (Murayama, 1979). Nitrogen supplied from a slow-release source during grain filling can prolong photosynthetic activity of leaves and produce a higher yield (Houng, 1976).

The nitrogen requirement of rice depends on several factors, the most important being variety and light intensity. High yielding varieties generally have a higher nitrogen requirement. Varieties such as IR-8 require 150 to 175 kg nitrogen ha^{-1} during the dry season and 100 kg ha^{-1} during the wet season; the dry and wet season yields were 6.0 and 4.0 tons ha^{-1} respectively (IRRI, 1975; 1976). Plant growth and yield during the wet season is lower because of reduced light availability and lower temperatures. Consequently, the crop requirement for nitrogen is also lowered. Data from many parts of the world show that the optimum nitrogen rate for modern varieties in farmers' fields is around 120 kg ha^{-1} for the dry season and 70 kg ha^{-1} for the wet season (Russell et al., 1970). Japonica rice varieties have always responded to well nitrogen application and farmers in Japan have used as

much as 230 kg nitrogen ha^{-1} to produce over 9.0 tons of brown rice ha^{-1} (Stangel, 1979).

2.7 Azolla in nitrogen nutrition of rice

The nitrogen requirement of rice can be satisfied by fertilizer or biologically fixed nitrogen. The in-situ sources of biological nitrogen fixation in paddy fields are anabaena in symbiosis with azolla, free-living blue-green algae and heterotrophic bacteria (Watanabe, 1984). Nitrogen fixing microorganisms are present in most paddy fields, though the extent of their contribution towards the overall nitrogen economy of the soil may not be significant. The annual input of nitrogen by microbial fixation in flooded paddies has been estimated to be 40 kg ha^{-1} in Japan (Yamaguchi, 1979). In subsistence agricultural systems where fertilizer nitrogen application is low, yields of lowland rice are higher than upland crops. A reason for this is better nitrogen conservation and nitrogen fixation by blue-green algae and heterotrophic bacteria (Buresh et al., 1980).

It is also possible to supply nitrogen by the incorporation of green manures, compost and crop residues (Patnaik and Rao, 1979). Azolla has been used as a green manure and the maximum nitrogen input by A. pinnata was estimated to be 335 to 670 kg ha^{-1} per year (Becking, 1972). A subsequent and more conservative approach by Becking (1976) estimated fixation at 103 to 162 kg ha^{-1} $year^{-1}$ under field conditions. Owing to the limited time available for its cultivation in an intensive cropping system and the unfavorable environmental conditions under which

azolla may have to grow, its maximum nitrogen accumulation potential may not be realised. In China, intercropping azolla with rice for 15 to 20 days before planting rice produced 37 to 45 kg nitrogen ha^{-1} (Chu, 1979) while 25 kg ha^{-1} was accumulated in 2 months in Vietnam (Tuan and Thuyet, 1979). Studies conducted by Lumpkin et al. (1982) in China showed that 150 kg nitrogen ha^{-1} could be fixed by monocropping A. filiculoides for 23 days and 100 kg ha^{-1} by intercropping for 20 days. Monocropping for 23 days followed by intercropping for 20 days fixed 200 kg nitrogen ha^{-1}. The large variations reported in different studies may be due to differences in Azolla species, season, nutrition and other management considerations.

Where the culture period for azolla is short, it must be considered a supplementary source of nitrogen and additional fertilizer nitrogen would be necessary to produce a high yield of rice. Moreover, unlike fertilizer nitrogen, azolla-nitrogen is not immediately available to rice because of the need for mineralization. Therefore, it might be necessary to allow sufficient time for azolla mineralization before planting rice or to apply fertilizer nitrogen for the early growth of rice until azolla-nitrogen becomes available. As discussed earlier, the slow-release of nitrogen from a source like azolla can be beneficial to rice during grain filling.

2.8 Management practices to maximize azolla growth and nitrogen fixation

When the surface cover of azolla is too sparse, biomass accumulation will be slower because of insufficient foliage area to

intercept available solar radiation. Once a satisfactory cover is formed, biomass accumulates at an exponential rate until interplant competition begins. Holst and Yopp (1979a) observed the exponential growth phase in A. mexicana to be from 5 to 12 days after 'planting' when the initial plant density was 1 plant per 10 cm^{-2}. Thereafter, crowding-caused mutual shading results in a growth rate decline. The relative growth rate of A. filiculoides remained above 0.23 g g^{-1} day^{-1} up to a plant density of 35 plants dm^{-2} when 2 g of azolla was introduced into 10 cm-diameter containers (Ashton, 1974). The growth rate declined sharply above this density as the water surface was fully covered. Therefore, in order to maximize production, the azolla surface cover should be dense enough to intercept light efficiently, but not too dense to reduce RGR. The desirable management of azolla would be to start with sufficient inoculum to provide a satisfactory cover and incorporate the green-manure crop into the soil at regular intervals so that competition for light and space are minimized.

Azolla multiplies by fragmentation and fronds tend to break up easily when handled. In a free-floating aquatic plant such as azolla, the biomass cover is better expressed as weight per unit area rather than number of plants per unit area. Depending upon whether the growth habit of the azolla species is erect (e.g.: A. microphylla) or prostrate (e.g.: A. pinnata), the optimum plant biomass may vary. Erect-growing species make use of more space above the water surface and may also utilize light more efficiently. An increase in plant biomass beyond the optimum could reduce the growth rate and the amount of fresh plant

material produced would be less. The growth habit of species should also be taken into account in multiplying azolla to be used as starting plant material. If a thick mat of A. microphylla forms, the shoot:root ratio is increased because of its erect growth habit. Inoculum from such dense mats exhibits slow early growth and requires a higher initial biomass where time is a constraint. Additional studies have to be conducted to determine the relationship between initial biomass and quantity parameters such as dry matter and nitrogen accumulation and quality parameters such as lignin content and carbon:nitrogen ratio of tissues.

The amount of nitrogen accumulated by azolla will depend on management practices such as initial biomass (inoculum rate) and interval between incorporations. A higher plant biomass may be preferable where the time available for growth of the green-manure crop is short. The accumulation of a given mass of azolla (W) can be described by the equation $W = W_o e^{Rt}$ where W is a function of the initial quantity of inoculum (W_o), the relative growth rate (R) and time (t). A higher inoculum rate can intercept more available light and fix more nitrogen within a shorter period of time. However, producing and handling large quantities of inoculum is labor intensive. Likewise, incorporation at shorter intervals may be more productive in terms of nitrogen, but prohibitive in terms of labor costs. The optimum plant biomass and incorporation interval may differ with species as their growth patterns and doubling times vary (Lumpkin and Plucknett, 1980; Peters et al., 1980). Studies on these management practices are necessary to economize on the use of inoculum and labor.

Initial azolla biomass of 750 g m^{-2} and 600 g m^{-2} are used in China for early-season and late-season planting, respectively, though the reason for the different rates was not given (Chu, 1979). Different amounts of biomass are recommended in Vietnam depending on the purpose for which azolla is grown. For green-manure production, 250 to 500 g per m^{-2} is used while the biomass of azolla for compost production is 500 to 750 g m^{-2} (Tuan and Thuyet, 1979). Depending upon the species, environment, and time available for culture, inoculum rates in other studies have varied from 50 to 400 g m^{-2} (Singh, 1979; Rains and Talley, 1979). Where more than 30 days are available for growth, even an inoculum biomass as low as 25 g m^{-2} is considered satisfactory (Lumpkin and Plucknett, 1982).

The response of azolla to temperature was also dependent upon plant biomass. Once azolla fully covered the surface and growth rate levelled off, it became more sensitive to high temperature damage. Therefore, Watanabe and Berja (1983) proposed that azolla should be contiunously harvested when grown at higher temperatures. In addition to the rapid senescence and dacay of plant tissue, pest and disease incidence can also be a serious problem under warm weather conditions.

Another consideration in azolla management is incorporation interval. Reducing the mat biomass by incorporating at shorter intervals will stimulate fresh growth and the amount of biomass and nitrogen accumulated will be greater. Therefore, use of a higher initial biomass of azolla will require incorporation at shorter intervals. Moreover, nitrogen from intercropped azolla will become

available to the main crop sooner, if incorporation is started early. Probably due to the above benefits, incorporation twice at 10 and 25 days after rice was transplanted increased the rice yield by 1.27 Mg ha^{-1} over azolla incorporated only once at 25 days after transplanting (Chu, 1979). The incorporation interval has generally varied from 10 to 20 days (Lumpkin et al., 1982; Singh, 1979; Chu, 1979). Incorporation is a costly labor-intensive task. If it becomes mechanized, shorter incorporation intervals may be possible.

2.9 Nitrogen mineralization from azolla green manure

The rate of mineralization is affected by the quality of the green manure. An indicator of quality is the ratio between carbon and nitrogen of plant tissue. A low C:N ratio is desired in a green manure crop as it decomposes easily. The tissue lignin content should be low because it resists microbial decomposition (Russell, 1973). Shi et al. (1981) have shown that the rate of nitrogen release was negatively correlated with the lignin content of plant tissue. Azolla would be expected to decompose readily as its tissue appears less fibrous than most plants. Studies of Watanabe et al.(1977) and Singh (1979) showed that over 50% of nitrogen in azolla was released as NH_4^+ three weeks after incorporation and as much as 75% was released six weeks after incorporation. Studies of Bellows (1981) showed that 42% of nitrogen from fresh azolla and 22% from air dried azolla was released in eight weeks. Although dried azolla tended to be mineralized slowly during the first four weeks, by eight weeks there was no significant difference in total nitrogen release between fresh and dried azolla.

2.10 Nitrogen transformations in paddy soil

The submerged nature of paddy soils creates unusual chemical and biological conditions which in turn influence nitrogen transformations. The flooded soil has an oxidized surface layer of less than three cm thickness and a reduced lower layer where most of the soil processes take place. The reduced layer is anaerobic and has a low redox potential, an indicator of the oxidation-reduction status of soil. Fertilizer nitrogen is usually added as ammonium sulfate or urea, both of which release nitrogen as NH_4^+. If nitrogen as NO_3^- is supplied to a submerged soil, it can be rapidly denitrified and the nitrogen lost to the atmosphere. If a field is allowed to dry, NH_4^+ can be oxidized to NO_3^- and on subsequent flooding nitrogen is lost through denitrification (Patrick and Reddy, 1976; Craswell and Vlek, 1979). Therefore poor water management resulting from repeated wetting and drying cycles can lead to severe loss of applied as well as mineralized nitrogen.

Fertilizer nitrogen can also become unavailable to plants as a result of immobilization. Yoshida and Padre (1975) reported microbial immobilization of 20% of the paddy nitrogen even without the addition of any organic matter. The amount fixed by microorganisms increased when organic matter was applied and the authors attributed this to the higher C:N ratio of added material. The nitrogen is not lost, but is unavailable to the plant for immediate use. Applied nitrogen can also become unavailable due to chemical immobilization. The major cause for this is the fixation of NH_4^+ ions between the interlayer space of mica (Kai and Wada, 1979). Chemical immobilization has been found to be

higher in kaolinite clay than in montmorillonite. Dry soils tend to fix more nitrogen than wet soils (Black and Waring, 1972). Hence, the fixation rates have been found to be lower under lowland conditions compared to upland soils. Volatilization is also a major source of loss of NH_4^+. Mikkelsen et al.(1978) suggested that as much as 20% of NH_4^+ is lost through volatilization in paddy soils. Craswell and Vlek (1979) have cited other studies where the reported loss was up to 60%.

Runoff losses can be high in paddy fields under certain conditions. The continuous flow of water through rice fields can carry away nitrogen and other nutrients. Patrick and Reddy (1976) reported that loss of fertilizer nitrogen can be decreased by deep placement and split application. Nitrogen, especially NO_3^-, can be lost through leaching. Similarly, deep placed urea may also be leached before it hydrolyses (Craswell and Vlek, 1979).

The slow release of NH_4^+ ions by mineralization is an important source of nitrogen for rice. Studies have shown that nitrogen supplied from soil by mineralization can play a more important role in the growth of rice than does fertilizer nitrogen (Houng, 1976). In fact, even at a rate of 120 kg nitrogen ha^{-1} of chemical fertilizer application, addition of compost increased rice yield (Oh, 1979). Other studies indicate that fertilizer nitrogen is exhausted by heading stage and what is absorbed thereafter is mineralized nitrogen (Yoshida and Padre, 1975). Hence supply of organic matter should be a routine practice for soils that are not fallowed to restore natural fertility.

2.11 Organic matter in rice soils

Oh (1979) reported that high rice yields in Korea were obtained when soil organic matter content ranged from 2.7 to 4.4%. The decomposition rate of organic matter under lowland conditions is lower than that in dryland soils. Paddy soils that are not allowed to dry and those in which minimum tillage is practiced would be expected to accumulate organic matter. Heavy application of organic matter to such soils may be undesirable as some products of decomposition are unfavorable to plant growth (Tanaka, 1978). Anaerobic microbes that decompose organic matter in flooded soils successively reduce soil compounds such as nitrates, sulfates and organic acids (Yoshida, 1978). Some end-products of decomposition in flooded soils are NH_4^+, CH_4, H_2S, Fe^{++}, CO_2 and organic acids. Some of these compounds can accumulate to toxic levels and cause nutritional disorders in rice (Tanaka, 1978).

The redox potential of the soil declines during organic matter decomposition. Fresh organic matter added to paddy fields causes a sharp drop in redox potential of the soil (Houng, 1976). Addition of azolla to a flooded paddy soil lowered the redox potential from 172 to 132 mV over a period of 28 days (Saha et al.,1981). Anaerobic bacteria break down organic matter slowly so that nitrogen immobilization in paddy soils is reduced relative to soils where aerobic organisms dominate. Hence, mineralization of organic matter of high C:N ratio can also proceed without a substantial drop in available nitrogen in flooded soils (Sanchez, 1976).

Nonetheless, addition of organic matter can be of immense benefit to most tropical paddy soils. The present land use pattern in rice-based cropping systems of small farmers is such that it favors rapid depletion of soil organic matter. An upland crop is often included in the cropping cycle, immediately after the harvest of the rice crop, which requires drying and tilling the land. It has been shown that soil organic matter decomposition is greatly enhanced by wetting and drying cycles (Arsjad and Giddens, 1966). According to Dei and Yamasaki (1979), alternate use of land for lowland and upland crops increases mineralization of organic matter and may increase crop yields in the short run; but eventually yields may decline as the organic matter in the soil is gradually depleted. Therefore paddy fields in which the cropping system includes planting of upland crops should receive sufficient organic matter or fertilizer to maintain their fertility.

CHAPTER III

EFFECTIVENESS OF FIVE AZOLLA ACCESSIONS AS GREEN MANURE FOR A SUMMER RICE CROP

INTRODUCTION

Azolla has been used as a green manure for rice in China (Chu, 1979) and Vietnam (Tuan and Thuyet, 1979) for many centuries. Although cultivation practices and environmental requirements for successful utilization of azolla in rice production are well documented (Lumpkin and Plucknett, 1982), the use of azolla has not spread to other rice-producing countries. One reason for this lack of interest in azolla is that the traditionally cultivated species in China and Vietnam, A. pinnata var. imbricata, does not perform well in warm tropical climates. Presently there are research programs underway to identify more promising species of azolla and accessions within species that are adapted to a wider range of environmental conditions (Lumpkin et al., 1981; Watanabe and Berja, 1983).

The main objective of the experiment reported herein was to identify five relatively heat-tolerant accessions of azolla and to test them as a source of nitrogen for a summer rice crop. The experiment was conducted in Taichung, Taiwan. As it was thought that azolla may not meet the total nitrogen requirement of the rice crop, a second objective was to study the effect of supplemental preplant fertilizer nitrogen on growth and yield of rice. It was believed that the preplant fertilizer nitrogen would enhance early growth of rice until nitrogen from incorporated azolla became available after mineralization.

MATERIALS AND METHODS

Ten accessions of azolla from the collection at the University of Hawaii were taken to the Taichung District Agricultural Improvement Station in Taiwan and multiplied in 40 x 25 cm plastic trays. Subsequently, eight of these accessions together with four others from the collection maintained at Taichung station were evaluated to identify those best adapted to warm weather. The average temperature during the evaluation period (July and August, 1982) was above 30 C (Table 2). The dimensions of the screening plots were 5 m x 3 m. About 1000 g of azolla was introduced into one end of the plot and restricted to a 3 m^2 area of the plot with a bamboo pole. As the azolla biomass increased, the pole was moved gradually towards the opposite end to permit azolla to spread over the water surface and thus minimize competition. The first five accessions to form a total biomass cover on the surface of the plot during this evaluation were chosen for the field experiment. They were A. caroliniana (Brazil), A. caroliniana (Ohio), A. microphylla (Galapagos Island), A. pinnata (Indonesia) and A. pinnata (Taiwan). The accessions not chosen were A. pinnata (Bangkok), A. pinnata-1 (China), A. pinnata-2 (China), A. pinnata (Ivory Coast), A. filiculoides (Hawaii), A. filiculoides (California), and A. mexicana (California).

The five accessions chosen as heat-tolerant were intercropped with rice under two different nitrogen application schedules, **azolla combined** and **azolla only**. In **azolla combined** treatments, the plots received 25 kg ha^{-1} fertilizer nitrogen before rice seedlings were transplanted and then they were inoculated with 0.25 kg ha^{-1} of azolla. The source of

TABLE 2. Mean temperature (TEMP), relative humidity (RH), rainfall (RF) and day length (DL) in Taichung, Taiwan during July, 1982 to December, 1982.

Period		TEMP C		RH	RF	DL
		Mean	Max	%	mm	hr
July	11 - 20	30.2	35.8	85.2	13	7.9
	21 - 31	28.6	34.0	78.4	312.7	6.5
Aug.	1 - 10	28.7	34.3	85.4	73.9	7.4
	11 - 20	27.2	33.5	85.5	12.9	7.7
	21 - 31	27.8	34.2	75.6	-	7.1
Sept.	1 - 10	27.6	35.0	73.7	2.8	7.0
	11 - 20	27.0	33.9	73.8	-	6.6
	21 - 30	25.3	33.2	71.0	-	8.7
Oct.	1 - 10	24.6	33.1	70.7	-	8.1
	11 - 20	24.0	31.5	71.1	-	8.5
	21 - 31	23.2	31.3	74.9	-	6.2
Nov.	1 - 10	22.0	29.9	78.6	1	5.1
	11 - 20	19.1	26.9	78.5	18.5	4.6
	21 - 30	19.3	27.3	77.8	10	5.6
Dec.	1 - 10	16.0	25.4	75.5	-	6.6

nitrogen thereafter was the intercropped azolla that was incorporated twice at 11 and 21 days after transplanting (DAT) the rice. No nitrogen fertilizer was applied to the plots receiving **azolla only** treatments where all nitrogen was supplied by the intercropped azolla inoculated and incorporated as described above. Three treatments receiving zero (control), 50 and 100 kg fertilizer nitrogen ha^{-1} respectively were included; these treatments were not intercropped with azolla. The 100 kg ha^{-1} treatment represents the quantity of nitrogen generally recommended for rice in Taiwan. The final treatment was 50 kg fertilizer nitrogen ha^{-1} and intercropped azolla. The 14 treatments (Table 3) were replicated three times and were arranged in a randomized complete block design.

The soil is classified as sand and shale alluvial with a pH of 5.5 and an organic matter content of 2.1%. The field was prepared by ploughing and then puddling. Excess water was drained off before individual plots were made. The size of each plot was 2.5 x 4 m. The plots were irrigated by lateral channels and each plot had a separate water inlet and outlet. Preplant applications of nitrogen, phosphorus and potassium (Table 4) were broadcasted after the final puddling and mixed with the soil during levelling. All plots received phosphorus and potassium prior to planting and also 11 DAT. Although the local recommendation calls for all the phosphorus to be added prior to planting, phosphorus and potassium were applied in two installments to promote growth of azolla (Table 4). Fertilizer application at 11 and 21 DAT was done immediately after the incorporation of azolla.

TABLE 3. Source and amount of nitrogen for rice in the 14 treatments.

Treatment number	Source of nitrogen	
	Azolla	Fertilizer N
		- - kg ha^{-1} - -
1	A. caroliniana (Brazil)[a]	25[b]
2	A. caroliniana (Ohio)	25
3	A. pinnata (Taiwan)	25
4	A. pinnata (Indonesia)	25
5	A. microphylla (Galapagos)	25
6	A. caroliniana (Brazil)	
7	A. caroliniana (Ohio)	
8	A. pinnata (Taiwan)	
9	A. pinnata (Indonesia)	
10	A. microphylla (Galapagos)	
11		0
12		50
13		100
14	A. pinnata (Indonesia)	50

[a]Amounts of nitrogen applied through Azolla species are given in Table 6.

[b]Fertilizer nitrogen was applied as ammonium sulfate (see Table 4).

TABLE 4. Timing and rate of application of nitrogen, phosphorus and potassium.

Fertilizer	Treatment number	Preplant	Postplant		
			11 DAT	21 DAT	45 DAT
		kg ha^{-1}			
[a]Nitrogen	1 to 5	25	-	-	-
	12 & 14	12.5	10	15	12.5
	13	25	20	30	25
	6 to 11	-	-	-	-
[b]Phosphorus	1 to 14	15	15	-	-
[c]Potassium	1 to 14	24	36	-	-

[a]Applied as ammonium sulfate containing 21% N.

[b]Applied as super phosphate containing 18% P_2O_5.

[c]Applied as potassium chloride containing 60% K_2O.

Fifteen-day old seedlings of rice variety Tainan-67, belonging to 3.5 to 4.0 month maturity group, were transplanted at a spacing of 21 x 21 cm on August 13, 1982. Each plot had approximately 200 hills and each hill had 4 to 7 rice seedlings.

The day after transplanting rice, azolla inoculum was collected from plots where it was being multiplied and drained in the shade for about one hour. Drained azolla was inoculated at 0.25 kg m^{-2} and uniformally distributed over the entire plot. Azolla was incorporated manually 11 and 21 DAT. At the time of first incorporation, it was found that the azolla growth rate was slow. Therefore, an additional 0.3 kg m^{-2} of azolla from the nursery was added to each plot. After incorporating part of it, approximately 0.5 kg ha^{-1} of azolla was retained as inoculum for the next cycle. The entire mat of azolla was incorporated at 21 DAT.

Measurements

The total quantity of nitrogen added by azolla was estimated by determining the fresh weight per area, and dry matter and nitrogen contents at each incorporation. Azolla collected from a 0.25 m^2 area was weighed and oven-dried at 70 C for 48 hrs. The nitrogen content of the dried azolla was determined by the method of Mitchell (1972) (Appendix A). The total fresh weight and total nitrogen from azolla incorporated into the soil was estimated as follows:

$$W_t = W_1 + W_2 + 0.3 - 0.5$$

$$W_n = W_t \times d \times p$$

where W_t is the total amount of fresh azolla incorporated in kg m^{-2}, W_1 and W_2 are the estimated fresh weights at the time of first and second incorporations respectively, 0.3 is the additional azolla introduced at first incorporation in kg m^{-2} and 0.5 is the amount in kg m^{-2} estimated to have been retained as inoculum for second growth cycle, W_n is the nitrogen added in kg ha^{-1}, d is the dry matter content per g fresh weight and p is the nitrogen content per g dry weight. The mean relative growth rates (RGR) of azolla for the periods 1 to 11 DAT and 11 to 21 DAT were estimated by the equation:

$$RGR = \ln W_2 - \ln W_1 / t$$

where W_1 and W_2 are the fresh weights at the beginning and end of each growth cycle lasting t days.

Plant height and tiller number of rice were taken from 12 hills per plot at 28, 48 and 75 DAT. These times represent the growth stages active tillering, panicle initiation and heading, respectively. The 12 hills were obtained by selecting three hills at random in each plot and making measurements on that hill and the three adjacent hills that made a square with the original hill. LAI and shoot dry weight were measured by harvesting plants at one side of the plot. Four vertically growing tillers, selected from four hills, were used for LAI determination. Leaf area was estimated by multiplying the product of leaf length x maximum leaf width by 0.75, the coefficient recommended for rice by Yoshida (1981). Two hills were cut at ground level to determine shoot dry weight. At each sampling, total shoot nitrogen content was also determined on a subsample obtained by compositing the samples from all three replicates.

Grain and straw yields were determined by harvesting the rice plants in a 6.0 m^2 area. Subsamples of 100 g of grain and straw were oven dried for 72 hours to determine dry weights. Based on the moisture percentage, grain and straw yields were adjusted to 14% moisture. Plant height and number of panicles per hill were measured from 12 hills per plot selected as explained above for tiller number. The percentage of filled grains was estimated by immersing grains from 15 panicles in salt water of specific gravity 1.06. Those grains that floated were separated as unfilled grains. Filled and unfilled grains were dried and counted with a seed counter. The filled grains were weighed to determine the 100 seed weight. In addition, shoot and grain nitrogen content, soil pH, and organic matter were also determined.

RESULTS AND DISCUSSION

Biomass and nitrogen accumulation by azolla accessions

The study reported here evaluated the biomass and nitrogen accumulation of azolla accessions in the field when intercropped with rice during summer. The initial rates of biomass accumulation for all accessions were low because only 0.25 kg m^{-2} of azolla was inoculated to start off the intercrop (Table 5). The range of RGRs measured for the five azolla accessions were 56 to 120 mg g^{-1} day^{-1} and 45 to 140 mg g^{-1} day^{-1} for the first and second cycles respectively (Table 5). In field culture of azolla in China, RGR values for most of the known species, including some of the accessions used in this study, were around 185 mg g^{-1} day^{-1}; even in intercropped azolla, Lumpkin (1983) obtained RGRs as high as 175 mg g^{-1} day^{-1}. The rice seedlings in that experiment were 36 days old at transplanting rather than 15 days, as was used in this study, and the environment beneath the rice canopy may have been more suitable for azolla growth as suggested by Becking (1979). The fifteen-day old seedlings used in this study evidently did not provide adequate shade for azolla. This was further aggravated by temperatures above 30 C (Table 1) during the growing season. The age of rice seedlings at transplanting and their subsequent vigor in canopy development determine light availability and growth of azolla.

The means for the two nitrogen application schedules were computed as follows:

azolla combined - mean of treatments one through five where nitrogen was supplied by a combination of preplant fertilizer and intercropped

TABLE 5. Fresh weight of azolla at 11 and 21 days after transplanting (DAT) and relative growth rate (RGR) of azolla accessions intercropped with rice as influenced by fertilizer nitrogen application.

Treatment	Fresh weight		RGR	
	11 DAT	21 DAT	1-11 DAT	11-21 DAT
	- - kg m^{-2} - -		- - mg g^{-1} day^{-1} - -	
a. **Azolla combined**				
A. caroliniana (Brazil)	0.70	0.80 b*	102	45 c
A. caroliniana (Ohio)	0.86	1.80 a	120	128 ab
A. pinnata (Taiwan)	0.67	1.16 b	91	84 b
A. pinnata (Indonesia)	0.77	2.03 a	112	140 a
A. microphylla (Galapagos)	0.44	1.21 b	56	82 b
b. **Azolla only**				
A. caroliniana (Brazil)	0.70	1.00 b	101	63 c
A. caroliniana (Ohio)	0.81	1.78 a	115	124 a
A. pinnata (Taiwan)	0.72	1.25 b	102	90 ab
A. pinnata (Indonesia)	0.66	1.77 a	94	126 a
A. microphylla (Galapagos)	0.64	1.21 b	87	87 ab
**SE (18)	0.13	0.15	18.93	12.68

*Means in a column and category (a or b) followed by the same letter are not significantly different at 0.05 level of probability as determined by Duncan's multiple range test.

**Standard error of mean with error degrees of freedom in parentheses.

azolla; **azolla only** - mean of treatments six through ten where the only source of nitrogen was intercropped azolla. Fresh weight and nitrogen accumulation in **azolla combined** treatments did not differ significantly from **azolla only** treatments. Applied fertilizer nitrogen is known to get into flood water in lowland paddies. Obcema et al., (1984) measured 78 to 98 ppm nitrogen when 100 kg ha^{-1} of fertilizer nitrogen was applied. Growth of A. pinnata was reduced to 43% of the nitrogen-free control in a culture solution containing 40 ppm nitrogen (Yatazawa et al., 1980). The lack of a difference between **azolla combined** and **azolla only** treatments suggests that applied nitrogen did not adversely affect growth. On the other hand, nitrogen fixation of azolla would be reduced if combined nitrogen is available for growth (Lumpkin and Plucknett, 1982). If that happens, the economic benefit of azolla would be lower as nitrogen is recycled and not newly fixed.

A. caroliniana (Ohio) and A. pinnata (Indonesia) had the highest RGRs during both growth cycles. These rates are similar to the results obtained with A. pinnata by Watanabe et al. (1977). The RGR of A. microphylla was lower than the other species during the first cycle, particularly in **azolla combined** treatments (1 to 11 DAT, Table 5). A. microphylla, the only erect-growing species included in this experiment, tended to have slow early growth partly due to dieback at the mature end of the fronds. The RGR of A. caroliniana (Brazil) declined during the second growth cycle (11 to 21 DAT) and it had the lowest fresh weight accumulation at the second incorporation. Older lower portions of the frond mat of A. caroliniana (Brazil) tended to die quickly and a multi-

layer mat was not formed. Moreover, fresh growth on a decaying older mat appeared to be slower, perhaps due to production of toxic substances. The other accessions maintained their growth rates during second cycle.

The amount of nitrogen from azolla incorporated into the soil was approximately proportional to the total mat fresh weight (Table 6) because nitrogen concentration of azolla was approximately the same. A. caroliniana (Ohio) and A. pinnata (Indonesia) produced significantly greater fresh weights than others at both incorporations and consequently the amount of nitrogen added was also higher. The nitrogen input by these two species was in the 40 to 50 kg ha^{-1} range. Considering the warm weather and the low inoculum level, this is a significant contribution.

Effect of azolla and fertilizer nitrogen on growth of rice.

The treatment effects on plant height, leaf area index (LAI) and shoot dry weight are presented in Tables 7, 8 and 9, respectively. Since no significant interaction was obtained between azolla accession and preplant nitrogen application (Appendix B), only the main effects are presented. Sections a and b in each table represent the treatment means when the data for the five azolla accessions and two nitrogen application schedules (**azolla combined** and **azolla only**) were analyzed as a 5 x 2 factorial experiment. The data for all 14 treatments were also analyzed as a randomized complete block design and the means for the

TABLE 6. Total fresh weight and nitrogen added by intercropping azolla accessions with rice for 20 days.

Treatment	Total fresh weight	Total nitrogen added
	-- Mg ha^{-2} --	--- kg ha^{-1} ---
a. Azolla combined		
A. caroliniana (Brazil)	1.31 c*	25.48
A. caroliniana (Ohio)	2.46 a	50.40
A. pinnata (Taiwan)	1.63 c	22.07
A. pinnata (Indonesia)	2.60 a	44.23
A. microphylla (Galapagos)	1.46 c	21.89
b. Azolla only		
A. caroliniana (Brazil)	1.49 c	29.11
A. caroliniana (Ohio)	2.40 a	49.16
A. pinnata (Taiwan)	1.77 bc	23.88
A. pinnata (Indonesia)	2.23 ab	37.91
A. microphylla (Galapagos)	1.66 c	24.89
**SE (18)	0.16	2.78

*Means in a column and category (a or b) followed by the same letter are not significantly different at 0.05 level of probability as determined by Duncan's multiple range test.

**Standard error of mean with error degrees of freedom in parentheses.

supplemental treatments (Treatments 11 to 14) are presented in Section c.

Rice plants in **azolla combined** treatments were significantly taller than those in **azolla only** treatments at all growth stages except at harvest although the differences were small (115 DAT) (Table 7 a). The shorter plants in **azolla only** treatments at all stages of growth probably was due to the fact that there was less nitrogen available for growth in these treatments.

There was no significant difference in height of rice plants due to azolla treatment at the active tillering stage (28 DAT) and at panicle initiation (48 DAT) (Table 7 b). The differences in plant height among the treatments became more marked at heading (75 DAT) though differences were relatively small. At this sampling, the rice plants in the A. caroliniana (Brazil) and A. microphylla (Galapagos) treatments were significantly shorter than those in the other azolla treatments. This difference was still evident at the final harvest (115 DAT). These two accessions accumulated less biomass and nitrogen than others (Table 6). Therefore the quantity of biomass and nitrogen added to the soil through azolla incorporation seemed to have had a positive effect on plant height of rice.

Plants in control plots were shorter than in all other treatments. This shows that rice plants benefited from nitrogen, either from the incorporation of azolla or from fertilizer. Throughout the experimental period, plants fertilized with 100 kg nitrogen ha^{-1} were taller than

TABLE 7. Effect of azolla and fertilizer nitrogen application on plant height of rice at active tillering (28 DAT), panicle initiation (48 DAT), heading (75 DAT) and at maturity (115 DAT).

Treatment	Plant height			
	28 DAT	48 DAT	75 DAT	115 DAT
	- - - - - - - - - - cm - - - - - - - - - -			
a. Nitrogen application schedules				
Azolla combined	57.36 a*	79.20 a	85.14 a	87.18 a
Azolla only	54.06 b	76.78 b	82.60 b	86.32 a
b. Azolla selections				
A. caroliniana (Brazil)	54.93	75.71	82.92 b	83.38 b
A. caroliniana (Ohio)	56.04	79.71	85.56 a	88.85 a
A. pinnata (Taiwan)	57.00	78.50	84.29 ab	87.99 a
A. pinnata (Indonesia)	55.97	78.67	84.14 ab	87.94 a
A. microphylla (Galapagos)	54.61	77.38	82.43 b	85.60 ab
**SE (18)	1.46	1.37	1.11	1.43
c. Supplemental treatments				
Zero	51.92 de	72.33 d	78.64 e	81.78 d
50 kg ha^{-1}	59.72 ab	79.00 ab	85.78 ab	89.75 a
100 kg ha^{-1}	61.14 a	80.17 a	88.00 a	90.00 a
50 kg+A. pinnata (Indonesia)	56.81 abcd	77.67 abc	84.97 ab	90.05 a
SE (26)	1.48	1.38	1.02	1.40

*Means in a column and category (a, b or c) followed by the same letter are not significantly different at 0.05 level of probability as determined by Duncan's multiple range test.

**Standard error of mean with error degrees of freedom in parentheses.

others. The differences among the treatments became less pronounced at 115 DAT, although the general trend remained the same.

The effect of azolla treatments on LAI was not significant at all three sampling times (Table 8). There was a trend towards increasing LAI with increasing nitrogen from azolla but differences were small. The control treatment attained maximum LAI at 48 DAT while LAI increased with age up to 75 DAT in other treatments. Due to the time taken for mineralization and the slow-release of nitrogen, leaf area increase may have been delayed in azolla treatments.

Plots that received 50 kg ha^{-1} fertilizer nitrogen and intercropped A. pinnata (Indonesia) received about 75 kg ha^{-1} of nitrogen by 28 DAT. The 100 kg of nitrogen ha^{-1} treatment had also received about the same amount of nitrogen up to 28 DAT. However, the LAI of the 100 kg ha^{-1} treatment was about 23% greater than the azolla treatment. This is further evidence that the response of rice to azolla nitrogen was slower than fertilizer nitrogen. The treatment fertilized with 50 kg nitrogen ha^{-1} had a larger LAI at 48 DAT than the treatment fertilized with 50 kg nitrogen plus azolla. It is also possible that some of the applied fertilizer nitrogen and other nutrients were taken up by the fast-growing azolla, reducing the availability of these nutrients to rice during early growth. Eventually the nutrients would have been released on mineralization to promote development of leaf area during the period between 48 DAT and 75 DAT. A reduction in nitrogen availability to rice caused by intercropped azolla has been reported by Lumpkin et al. (1980) and Bellows (1981).

TABLE 8. Effect of azolla and fertilizer nitrogen application on leaf area index of rice at active tillering (28 DAT), panicle initiation (48 DAT) and heading (75 DAT).

Treatment	Leaf area index		
	28 DAT	48 DAT	75 DAT
a. Nitrogen application schedules			
Azolla combined	2.07	3.89	4.30
Azolla only	1.94	3.67	3.98
b. Azolla selections			
A. caroliniana (Brazil)	1.88	3.74	3.94
A. caroliniana (Ohio)	2.00	3.88	4.45
A. pinnata (Taiwan)	2.08	3.78	3.96
A. pinnata (Indonesia)	2.08	3.89	4.44
A. microphylla (Galapagos)	1.99	3.63	3.92
**SE (18)	0.21	0.20	0.26
c. Supplemental treatments			
Zero	1.54 c*	2.99 d	2.81 e
50 kg ha^{-1}	2.34 ab	4.71 a	4.73 abc
100 kg ha^{-1}	2.70 a	4.65 ab	5.49 a
50 kg + A. pinnata (Indonesia)	2.11 abc	4.01 abc	4.82 ab
SE (26)	0.22	0.23	0.26

*Means in a column and category (a, b or c) followed by the same letter are not significantly different at 0.05 level of probability as determined by Duncan's multiple range test.

**Standard error of mean with error degrees of freedom in parentheses.

At 48 and 75 DAT, LAI showed a typical response to an increasing supply of nitrogen and the curves were best fit by a quadratic function (Figure 1 and 2). The fertilizer nitrogen curve was above that of azolla treatments. As discussed previously, the lower LAI values for the azolla treatments may be attributed to the lag in nitrogen availability from azolla. As more azolla nitrogen mineralized from 48 DAT to 75 DAT, the gap between the curves narrowed (contrast Figures 1 and 2).

The LAI did not exceed 6.0 even at 100 kg ha^{-1} of fertilizer nitrogen though an LAI of 6.0 is considered necessary for maximum yield (IRRI, 1972). The low LAI can be attributed to the fact that the crop was planted late. The summer crop in Taichung is usually planted by early July and harvested by mid-November. Rice in the present experiment was planted on July, 30 and harvested on December 06. During the period of maximum vegetative growth in September and October, the average temperature became progressively cooler and dropped to 23 C by late October (Table 2) which may have affected leaf area development. **Azolla combined** treatments had significantly higher shoot dry weight than **azolla only** treatments at 48 and 75 DAT (Table 9 a). Differences among azolla treatments were evident only 75 DAT (Table 9 b). Incorporation of A. caroliniana (Ohio), A. pinnata (Taiwan) and A. pinnata (Indonesia) resulted in greater shoot weights than the other two accessions. Among the supplemental treatments, shoot dry weight increased with increasing amount of nitrogen application and significant

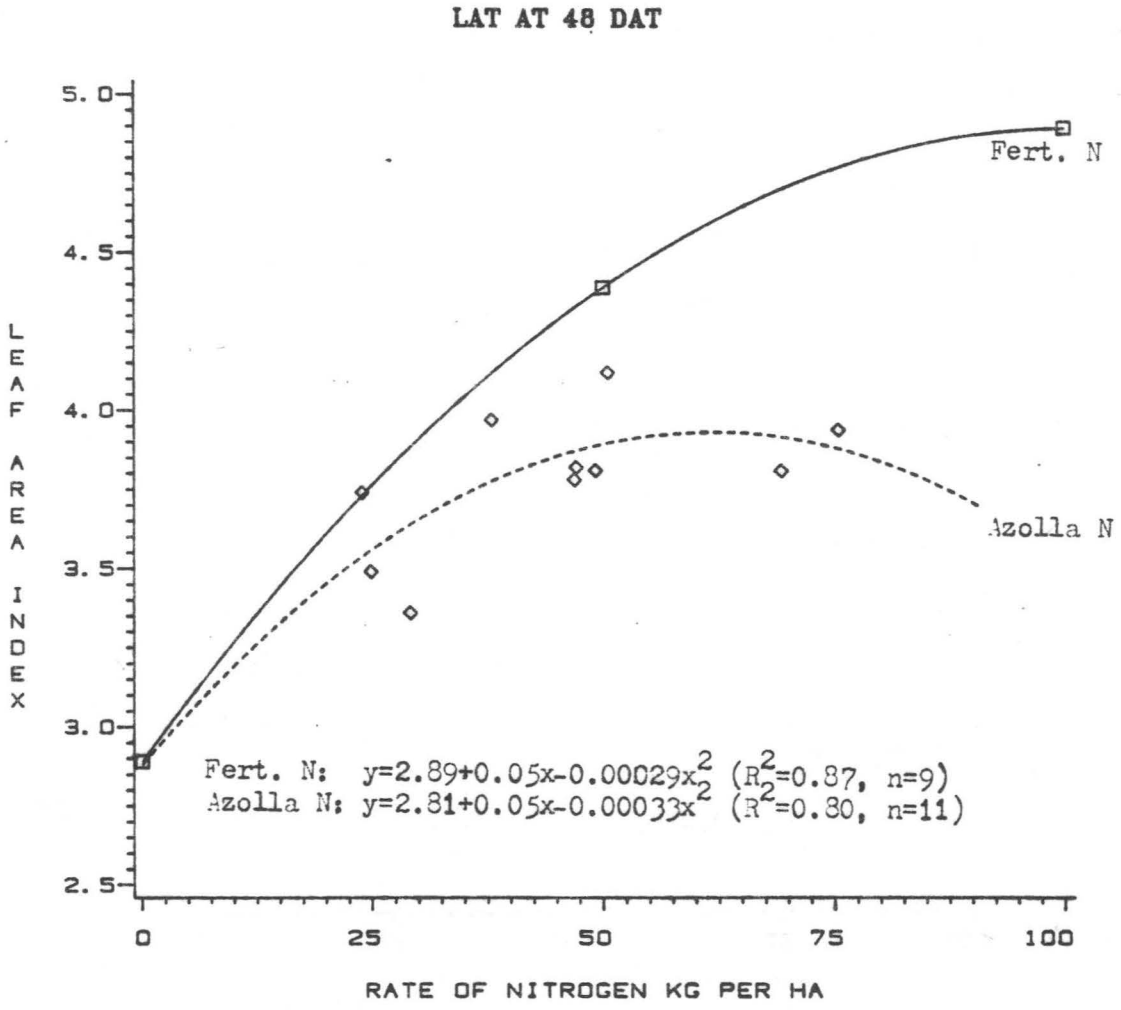


FIGURE 1. Effect of azolla and fertilizer on the leaf area index of rice at 48 days after transplanting.

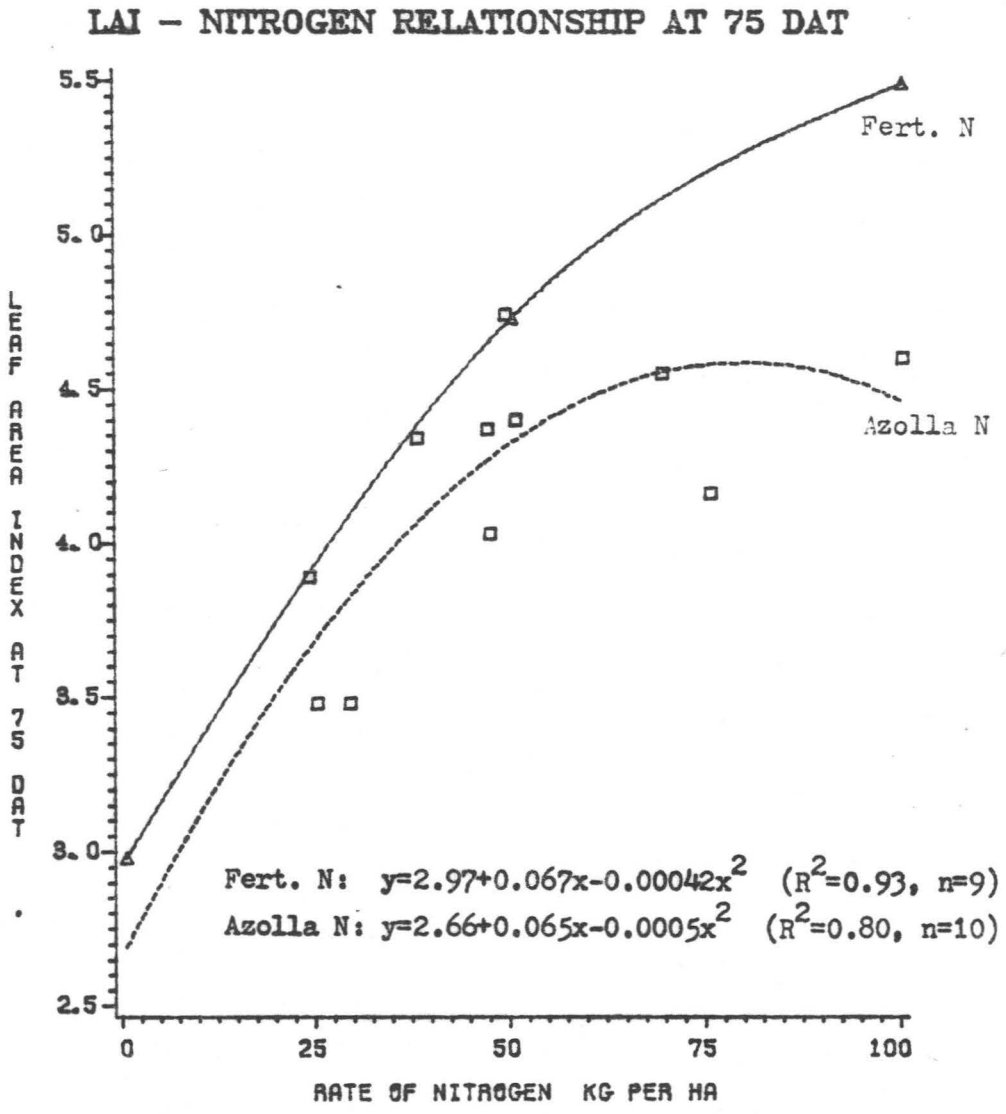


FIGURE 2. Effect of azolla and fertilizer on the leaf area index of rice at 75 days after transplanting.

TABLE 9. Effect of azolla and fertilizer nitrogen application on shoot dry weight of rice at active tillering (28 DAT), panicle initiation (48 DAT) and heading (75 DAT).

Treatment	Shoot dry weight		
	28 DAT	48 DAT	75 DAT
		g m^{-2}	
a. Nitrogen application schedules			
Azolla combined	282	804 a*	886 a
Azolla only	262	733 b	839 b
b. Azolla selections			
A. caroliniana (Brazil)	263	739	827 b
A. caroliniana (Ohio)	276	806	898 a
A. pinnata (Taiwan)	287	790	876 ab
A. pinnata (Indonesia)	261	772	875 ab
A. microphylla (Galapagos)	273	734	836 ab
**SE (18)	21.4	32.1	28.2
c. Supplemental Treatments			
Zero	250 b	618 e	685 d
50 kg ha^{-1}	299 ab	876 a	834 abc
100 kg ha^{-1}	370 a	904 a	877 a
50 kg + A. pinnata (Indonesia)	278 b	830 abc	850 ab
SE (26)	23.6	58.0	33.6

*Means in a column and category (a, b or c) followed by the same letter are not significantly different at 0.05 level of probability as determined by Duncan's multiple range test.

**Standard error of mean with error degrees of freedom in parentheses.

differences between the control and 100 kg nitrogen were observed at all sampling times (Table 9 c).

Tiller number in azolla combined treatments did not differ significantly from azolla only treatments (Table 10 a). There was no significant effect of azolla treatment on tiller number at 48 DAT (Table 10 a). Tiller number dropped slightly in all treatments from 48 to 75 DAT due to tiller mortality. Since A. caroliniana (Ohio) and A. pinnata (Indonesia) added more nitrogen than other accessions, they had the highest tiller number and lowest tiller mortality among azolla treatments at 75 DAT. The control treatment had significantly fewer tillers than all other treatments and the largest number of tillers were obtained with 100 kg of fertilizer nitrogen. Tiller mortality decreased with increasing rate of fertilizer nitrogen application (Table 10 c). Tiller production of rice has been shown to be influenced greatly by nitrogen nutrition (Ishizuka, 1971).

According to Murayama (1979), about 50 to 60% of total nitrogen in high-yielding rice varieties is absorbed by the panicle initiation stage. In the present study, even though differences in growth parameters were observed at panicle initiation (48 DAT) in response to nitrogen supply, some of the treatment effects became pronounced only at heading (75 DAT) (Tables 7 to 10). This suggests that nitrogen from azolla probably continued to become available to the rice crop after panicle initiation. Watanabe et al.(1977) and Bellows (1981) have reported mineralization rates of 59% and 42% respectively for fresh azolla nitrogen during the first four weeks after incorporation.

TABLE 10. Effect of azolla and fertilizer nitrogen application on tiller production of rice at panicle initiation (48 DAT) and heading (75 DAT) and tiller mortality from 48 to 75 DAT.

Treatment	Tiller number		Tiller
	48 DAT	75 DAT	mortality
			- - % - -
a. Nitrogen application schedule			
Azolla combined	17.08	15.78	7.15
Azolla only	16.23	15.46	4.50
b. Azolla selections			
A. caroliniana (Brazil)	17.02	15.28 ab*	9.80
A. caroliniana (Ohio)	16.53	16.43 a	0.33
A. pinnata (Taiwan)	16.48	14.89 b	9.43
A. pinnata (Indonesia)	16.78	16.11 ab	3.78
A. microphylla (Galapagos)	16.48	15.39 ab	5.80
**SE (18)	0.69	0.55	4.17
c. Supplemental treatments			
Zero	15.47 c	13.22 f	14.54 a
50 kg ha^{-1}	18.47 ab	16.83 ab	8.70 ab
100 kg ha^{-1}	18.78 a	17.58 a	5.19 ab
50 kg+A. pinnata (Indonesia)	17.31 abc	16.64 abc	3.69 ab
SE (26)	0.80	0.50	4.40

*Means in a column and category (a, b or c) followed by the same letter are not significantly different at 0.05 level of probability as determined by Duncan's multiple range test.

**Standard error of mean with error degrees of freedom in parentheses.

Assuming a comparable rate of mineralization in the study reported here, about 50% of the nitrogen would have become available only after panicle initiation.

Effect of azolla and fertilizer nitrogen on yield components of rice

The number of panicles per hill is dependent on the number of fertile tillers. Tillers per hill declined between 48 and 75 DAT with the result that the correlation between number of panicles and number of tillers increased from 0.64 at 48 DAT to 0.85 at 75 DAT. Supplementing azolla with 25 kg fertilizer nitrogen (**azolla combined** vs **azolla only** treatments) had no effect on tiller number or on the number of panicles produced (Table 11 a).

Although there was a small but significant effect of azolla on tiller number at 75 DAT (Table 10 b), there was no significant effect of azolla accessions or nitrogen application schedules on number of panicles per hill at harvest (115 DAT) (Table 11 b).

The control treatment had significantly fewer panicles than all other treatments while the highest number of panicles per hill was obtained by fertilizing at 100 kg nitrogen ha^{-1}. This is consistent with the results of Fagi and De Datta (1981) showed that the number of panicles per hill increased with nitrogen from zero to 90 kg ha^{-1}.

Grains per panicle is determined during the time of spikelet differentiation which occurs between panicle initiation and heading. This period is critical in relation to the nitrogen balance within the plant. Excessive nitrogen can promote vegetative growth at the expense

TABLE 11. Effect of azolla and fertilizer nitrogen application on yield components of rice.

Treatment	Panicles per hill	Grains per panicle	Filled grains	#)Grains per hill
			- - % - -	- - g - -
a. Nitrogen application schedule				
Azolla combined	15.83	85.23	75.80	1346
Azolla only	15.46	84.02	74.49	1300
b. Azolla selections				
A. caroliniana (Brazil)	15.12	80.29 b*	74.89 ab	1213 c
A. caroliniana (Ohio)	15.83	91.36 a	70.57 b	1445 a
A. pinnata (Taiwan)	15.32	82.17 ab	76.18 ab	1257 bc
A. pinnata (Indonesia)	16.24	86.58 ab	75.88 ab	1403 ab
A. microphylla (Galapagos)	15.72	82.72 ab	78.23 a	1299 abc
**SE (18)	0.48	4.50	3.16	20.63
c. Supplemental treatments				
Zero	12.97 e	84.17 abc	78.18 abc	1092 d
50 kg ha^{-1}	15.86 abc	86.60 abc	75.41 abcd	1376 c
100 kg ha^{-1}	17.42 a	87.77 abc	71.21 bcd	1496 ab
50 kg + A. pinnata (Indonesia)	17.17 ab	96.52 a	68.90 d	1632 a
SE (26)	0.42	4.18	2.36	22.14

*Means in a column and category (a, b or c) followed by the same letter are not significantly different at 0.05 level of probability as determined by Duncan's multiple range test.

**Standard error of mean with error degrees of freedom in parentheses.

#Product of panicles per hill and grains per panicle.

of spikelet formation while very low nitrogen can reduce spikelet formation. A satisfactory nitrogen level ensures formation of sufficient spikelets to achieve a high yield (Murayama, 1979). Application of preplant fertilizer nitrogen to supplement azolla did not have an effect on grains per panicle (Table 11 a). There were significant differences in number of grains per panicle among azolla accessions (Table 11 b). Azolla treatments where the quantity of nitrogen added was low had fewer grains per panicle than all other treatments (Table 11). The control treatment produced more grains per panicle than some of the azolla treatments, but the differences were not statistically significant. Nutrient availability to each panicle during grain formation may have been greater in the control treatment than in the azolla treatments because it produced significantly fewer panicles.

Since mutual compensation of yield components occurs in cereals like rice, the total number of grains per hill, which is the product of panicles per hill and grains per panicle, is a better index of potential yield than either panicles per hill or grains per panicle. Number of grains per hill for all azolla and nitrogen treaments was greater than the control (Table 11). Approximately 78% of the variation in grains per hill was accounted for by the amount of nitrogen applied (Table 12). The number of grains per hill was proportional to the amount of nitrogen applied, regardless of source (Figure 3). This suggests that the yield potential established at spikelet differentiation in azolla treatments followed the same trend as fertilizer nitrogen treatments.

TABLE 12. Regression models and coefficients of determination (R^2) for the relationship between growth and yield parameters of rice and total nitrogen application for all 14 treatments. The models express the component (y) as a function of total nitrogen applied (x).

Component	Equation	R^2
Grains per panicle	$y = 77.69 + .15x$	$.48^*$
Filled grain %	$y = 79.08 - .08x - .0001x^2$	$.47^*$
Dry matter yield	$y = 9.69 + .045x - .0002x^2$	$.81^{**}$
Straw yield	$y = 4.93 + .028x - .00015x^2$	$.74^{**}$
Grain yield	$y = 4.76 + .017x - .000072x^2$	$.41^*$

*Significant at P=.05

**Significant at P=.001

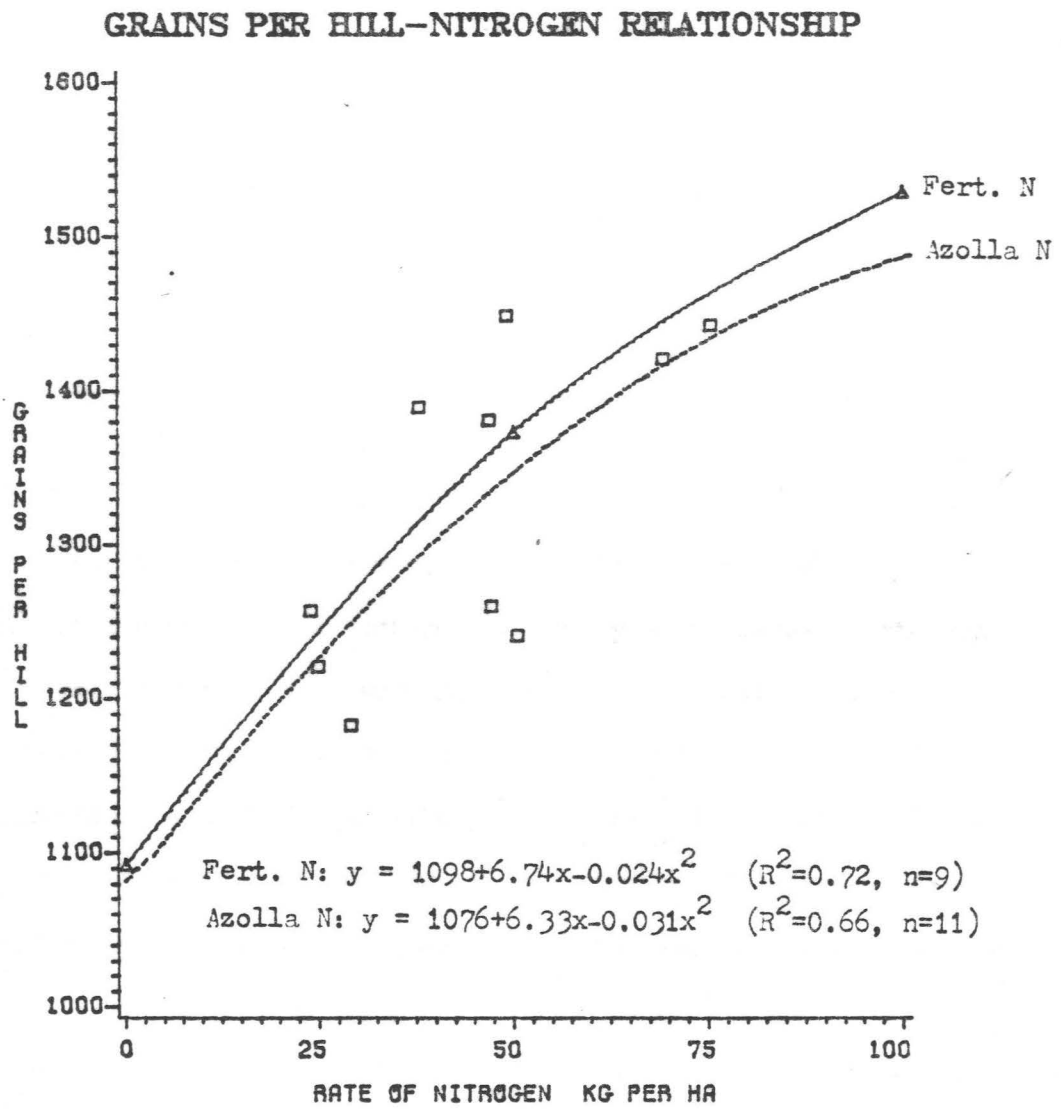


FIGURE 3. Effect of azolla and fertilizer on grains per hill of rice.

In high yielding rice varieties, 70 to 80% of total nitrogen absorbed is taken up by the time the heading stage is reached (Murayama, 1979). Significant correlations have also been reported by Matsushima (1980) between nitrogen uptake up to late spikelet differentiation and number of grains m^{-2}. Nitrogen uptake up to 75 DAT (x), the product of plant nitrogen concentration and shoot dry weight per unit area, was linearly related to grain yield and grains per hill with 34% of the variation in grain yield and 63% of the variation in grains per hill being accounted for by nitrogen uptake up to 75 DAT.

Grain yield = 4.60 + .013x r^2=.34 (P=.05)

Grains per hill = 852 + 7.97x r^2=.63 (P=.01)

Vlek et al.(1979) found nitrogen uptake to be curvilinearly related to grain yield and number of grains m^{-2}; the increase was linear up to 80 kg nitrogen ha^{-1}. The results reported here are in agreement with their observations.

The filled grain percentage reflects the ability of the leaves and other photosynthetic structures to supply assimilates to the grains for storage. Murata and Matsushima (1975) stated that a filled grain percentage of less than 80 could mean a source limitation while a percentage of over 80 was likely due to sink limitation. Filled grain percentages were less than 80 for all treatments in the present study (Table 11) and were negatively correlated with number of grains per hill (Figure 4). Treatments receiving larger amounts of nitrogen had lower filled grain percentages than those receiving smaller amounts of nitrogen. This may be due to a greater sink capacity because number of

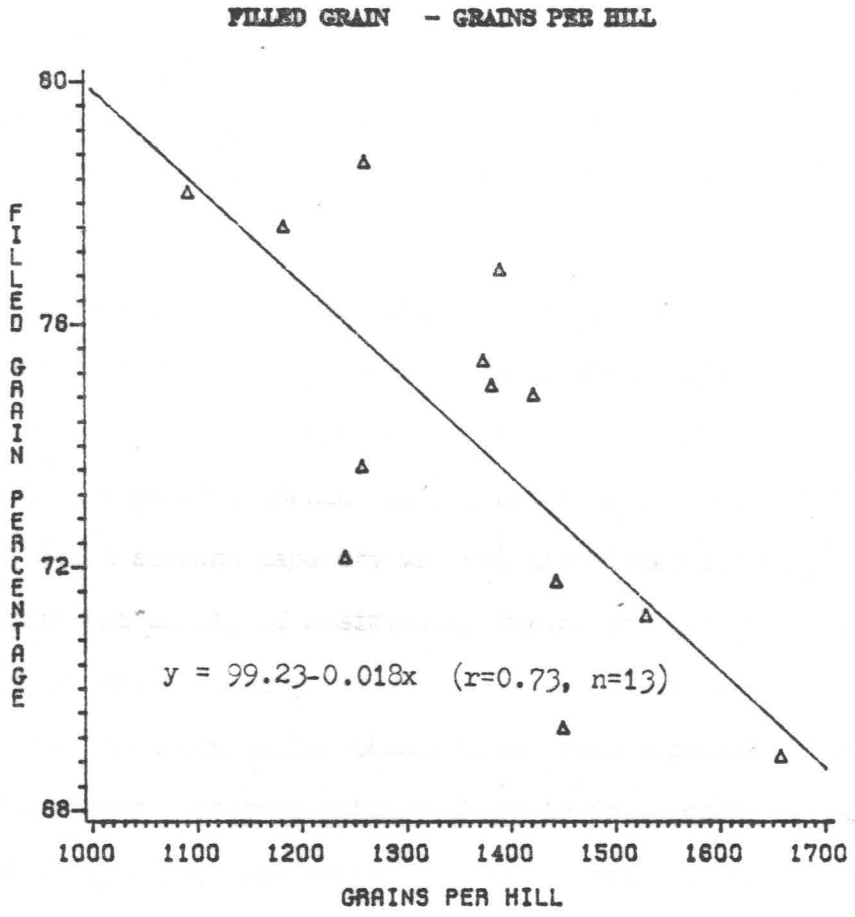


FIGURE 4. Relationship between grains per hill and filled grain percentage.

panicles per hill and number of grains per panicle were increased by nitrogen application in the current study (Table 11 c). In the study of 15 genotypes, Jones et al.(1979) concluded that grain filling rate was negatively correlated with panicles per unit area. This was due to the distribution of photosynthetic products among a larger number of sinks. As a result, the filled grain percentage tended to be lower in treatments where the number of grains per hill was higher.

Another reason for the lower filled grain percentages in the present study was the unfavorable weather during grain ripening. The crop matured at the onset of winter and therefore during the grain ripening period the crop experienced very cold weather (Table 2). The average temperature and sunshine hours for the last 30 days was 19 C and 5.5 hrs respectively; at times the day temperatures were below 15 C. Consequently, crop maturity was delayed by about 10 days, that is, 115 days from planting to harvest as opposed to 105 for a summer crop planted by early-July. Hence the potential of those treatments that had a higher grain storage capacity was not translated into grain yield due to insufficient supply of assimilates during the grain filling period. As a result, the highest grain yield obtained in this study was about 2 Mg ha^{-1} less than the yields obtained in other experiments at Taichung where the summer rice crop matured three to four weeks earlier.

The seed weight is a varietal characteristic and is not usually influenced by environmental factors including nitrogen nutrition. None of the treatments significantly influenced seed weight in the present study.

Effect of azolla and fertilizer nitrogen on grain and straw yield

Despite significant differences among treatments in some growth parameters and yield components, the differences in grain yield were small and for the most part, nonsignificant (Table 13). Incorporation of azolla or application of preplant fertilizer nitrogen increased grain yield by about 0.8 Mg ha^{-1} over the control. The control treatment yielded 4.63 Mg ha^{-1} of rice which was quite high. Improved rice cultivars, such as the one used here, can produce high yields with residual nitrogen. Since the field used in this study had been planted to rice previously, the rice crop of this experiment appears to have benefited from the residual fertility. On the other hand, grain yields in the 50 and 100 kg nitrogen ha^{-1} treatments were reduced by the lower filled grain percentage. Therefore the fertilizer nitrogen-yield relationship was almost linear, though the quadratic model slightly improved the fit. The grain yield-nitrogen application relationships for fertilizer nitrogen and azolla treatments were close to each other at the lower levels of applied nitrogen (Figure 5). However, with higher amounts of nitrogen, the distance between the curves widened. This may be due to a) over estimation of nitrogen input in azolla combined treatments or b) lower availability of nitrogen to rice plants from azolla due to slower mineralization and immobilization.

Only 41% of the variation in grain yield was accounted for by the amount of nitrogen applied (Table 12). As discussed already, the cold spell and shorter days during grain ripening could be responsible for the relatively low coefficient of determination for the relationship

TABLE 13. Effect of azolla and fertilizer nitrogen application on grain yield, straw yield and grain:straw ratio.

Treatment	Grain yield	Straw yield	Grain:straw ratio
	- - - - Mg ha^{-1} - - - -		
a. Nitrogen application schedule			
Azolla combined	5.48	5.98 a*	0.92
Azolla only	5.28	5.64 b	0.94
b. Azolla selections			
A. caroliniana (Brazil)	5.07	5.70 ab	0.89 b
A. caroliniana (Ohio)	5.46	6.15 a	0.89 b
A. pinnata (Taiwan)	5.46	5.89 ab	0.93 ab
A. pinnata (Indonesia)	5.47	5.87 ab	0.93 ab
A. microphylla (Galapagos)	5.44	5.45 b	1.07 a
**SE (18)	0.17	0.23	0.040
c. Supplemental treatments			
Zero	4.63 d	5.00 c	0.93 ab
50 kg ha^{-1}	5.59 ab	6.03 a	0.93 ab
100 kg ha^{-1}	5.90 a	6.34 a	0.93 ab
50 kg + A. pinnata (Indonesia)	5.72 ab	5.92 ab	0.97 ab
SE (26)	0.16	0.21	0.036

*Means in a column and category (a, b or c) followed by the same letter are not significantly different at 0.05 level of probability as determined by Duncan's multiple range test.

**Standard error of mean with error degrees of freedom in parentheses.

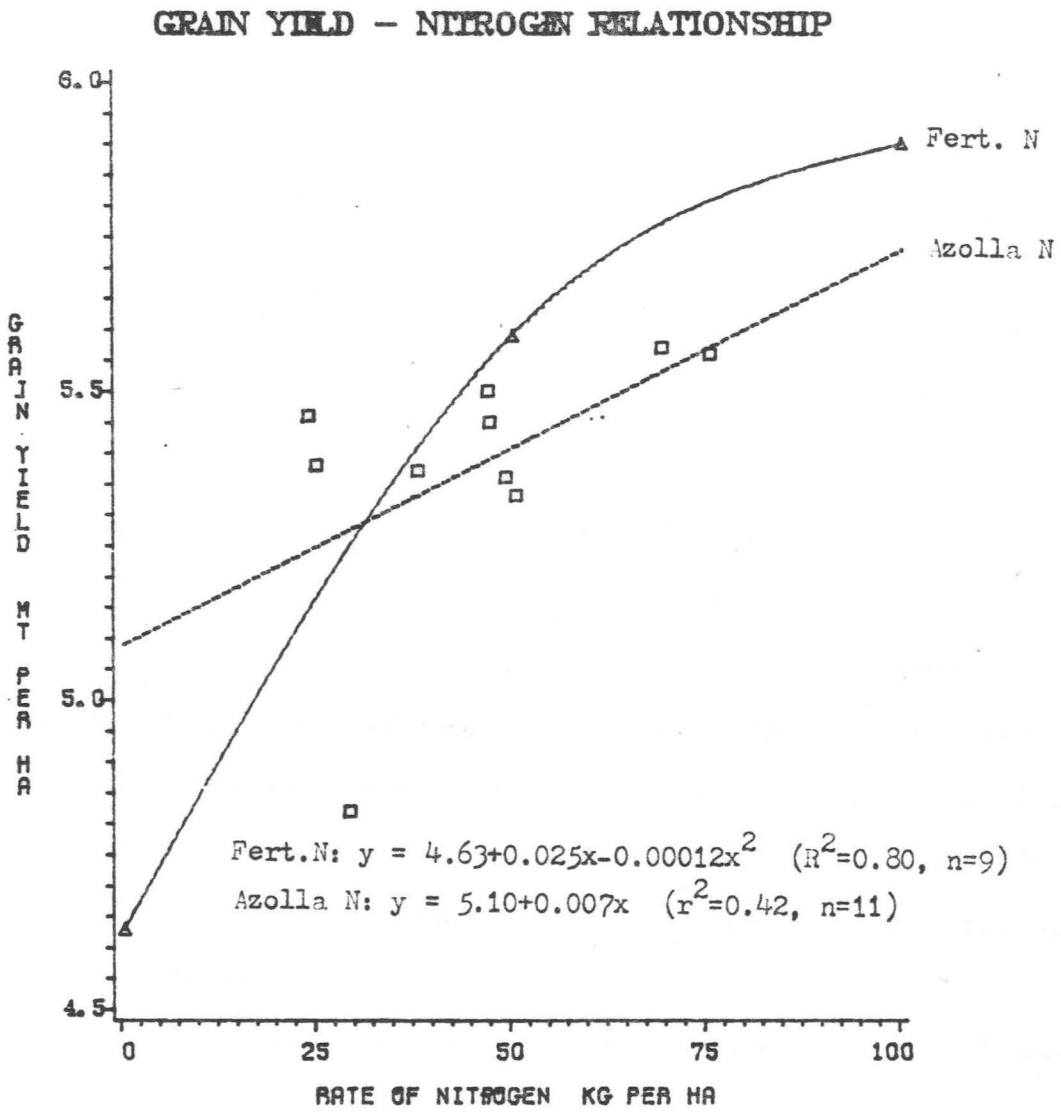


FIGURE 5. Effect of azolla and fertilizer on grain yield of rice.

between grain yield and added nitrogen (Table 12). Fage and De Datta (1981) observed that under low solar radiation increasing nitrogen levels above 60 kg ha^{-1} did not give a proportional yield increase. They also attributed lower yields of wet season plantings to low temperature and high humidity. These same reasons can be ascribed to the relatively small increase in grain yield with increasing nitrogen in the current study.

Ishizuka (1971) stated that growth during any given stage is mainly influenced by the ability of the leaves formed at one stage to contribute to the growth of the next. Usually LAI in cereals increases as the amount of nitrogen increases (Wells and Faw, 1978). Therefore, an important management objective is to attain the optimum LAI required to produce the maximum yield. During the ripening phase, the leaves supply assimilates to the developing grains which are the sink. Thorne et al. (1979) observed a positive correlation between grain yield and LAI at anthesis in wheat. A similar correlation existed between grain yield and LAI at 75 DAT (Figure 6). Studies at IRRI (1972) showed that grain yield does not change substantially when LAIs range from 6.0 to 10 and yields may be lower if LAI is below 6.0 due to source limitation. The LAI in the present experiment never exceeded 6.0 and this may have limited grain yield.

Total dry matter production was more highly correlated with added nitrogen than all other parameters (Table 12). Application of nitrogen, either in the form of azolla or as fertilizer, promoted vegetative growth and dry matter production of rice. As a result, the vegetative

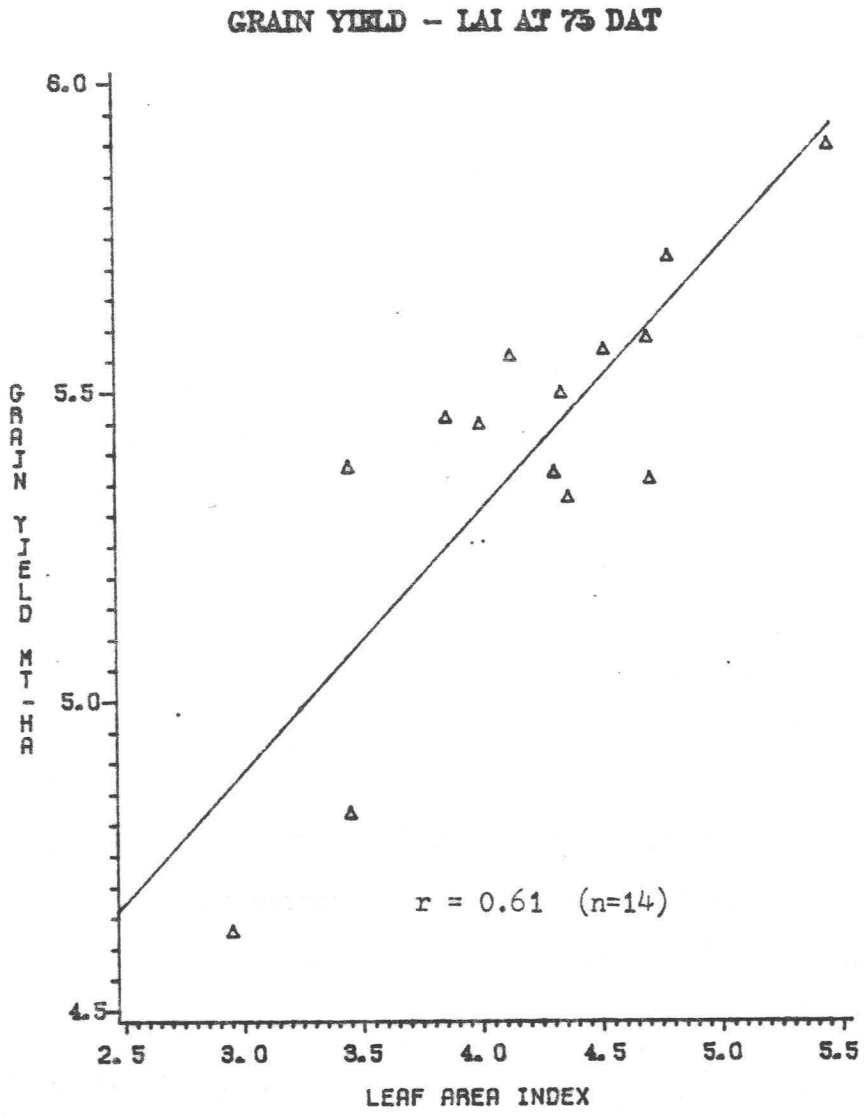


FIGURE 6. Relationship between leaf area index (LAI) at 75 days after transplanting (DAT) and grain yield of rice.

organs required to produce a high yield were formed in the nitrogen treatments during early growth. However, environmental limitations apparently were in part responsible for the failure to realize the yield potential established at earlier stages of growth. The grain:straw ratio was typical of improved rice varieties which partition more dry matter into grain than do traditional cultivars. The mean grain:straw ratio of highly nitrogen responsive varieties was 1.13 and many dwarf varieties had ratios above 1.20 (Chandler, 1969). The ratios obtained in this study were mostly less than one. Therefore dry matter distribution was less efficient than in some improved cultivars. This cannot be due to excessive vegetative growth, because the maximum amount of nitrogen applied was only 100 kg ha^{-1} and the maximum LAI reached was 5.9. It is concluded that adverse weather conditions during the grain filling phase reduced carbohydrate synthesis and the partitioning of dry matter into grain.

Effect of treatment on plant nitrogen content

The nitrogen concentration of plants is usually higher in young seedlings and then declines with the development of mechanical tissue. Nitrogen concentration is an index of nitrogen availability to plants. Treatments where higher amounts of nitrogen were applied generally had higher plant nitrogen concentrations at all stages of growth (Table 14). Nitrogen determinations were done on composite samples and were not replicated; thus the results were somewhat inconsistent.

TABLE 14. Effect of total nitrogen application on plant nitrogen content at active tillering (28 DAT), panicle initiation (48 DAT) and heading (75 DAT).

Treatment	Plant nitrogen content*		
	28 DAP	48 DAP	75 DAP
	- - - - - - - - - - % - - - - - - - - - -		
a. Nitrogen application schedule			
With nitrogen	2.40	0.90	0.57
Without nitrogen	2.49	0.75	0.50
b. Azolla selections			
A. caroliniana (Brazil)	2.60	0.65	0.60
A. caroliniana (Ohio)	2.60	0.95	0.53
A. pinnata (Taiwan)	2.70	0.70	0.60
A. pinnata (Indonesia)	2.55	0.95	0.55
A. microphylla (Galapagos)	1.80	0.90	0.40
c. Control			
Zero	2.30	0.80	0.35
50 kg ha^{-1}	2.60	0.75	0.60
100 kg ha^{-1}	2.70	0.85	0.70
50 kg + A. pinnata (Indonesia)	2.80	0.90	0.50

*Not statistically analyzed.

The total amount of fresh biomass added to soil by azolla during the 20 days of intercropping was between 14 to 24 Mg ha^{-1} (Table 6). The addition of such large quantities of organic matter might be expected to increase soil organic matter content. In most of the plots in this study, soil organic matter increased during the cropping period by about 0.3%.

CONCLUSIONS

The effects of azolla and fertilizer nitrogen supply on rice growth and yield may be summarized in the following manner. Azolla caroliniana (Ohio) and A. pinnata (Indonesia) produced more biomass and accumulated more nitrogen than the other azolla accessions during the warm summer months at Taichung. Azolla combined treatments where 25 kg fertilizer nitrogen per ha was applied prior to planting resulted in better early growth of rice but did not significantly increase rice yield. The potential yield that was established at heading was highest in plots fertilized with 50 and 100 kg nitrogen ha^{-1} followed by azolla accessions which produced greater amounts of biomass and fixed more nitrogen. There was an inverse relationship between number of grains per hill and filled grain percentage at harvest and consequently the rice yield response to applied nitrogen was small. Nevertheless, nitrogen supplied either as fertilizer or as azolla produced relatively higher yields than control. Azolla nitrogen can replace requirement for fertilizer nitrogen, especially at later stages of growth.

CHAPTER IV

GROWTH RESPONSE OF AZOLLA SPECIES TO FROND BIOMASS, LIGHT AND TEMPERATURE

INTRODUCTION

Dry matter and nitrogen accumulation by azolla are influenced by environment and management factors. The important environmental factors influencing the physiology of azolla are light and temperature. The response of azolla to light has been studied by several researchers (Ashton, 1974; Becking, 1976; Peters et al., 1980). Studies on the influence of temperature on azolla have been reported by Lumpkin (1983) and Watanabe and Berja (1983).

A completely formed azolla canopy is a dense biomass mat composed of fronds. The mat can reach about 5.0 cm in thickness and cover the water surface fully. The microenvironment of this mat with regard to light and temperature is dependent on the azolla biomass present at any given time. Initial biomass cover can be regulated by management to maximize fresh weight and nitrogen accumulation by azolla. Holst and Yopp (1979) found that growth of azolla is reduced with time by the progressive increase in biomass. The initial biomass used to inoculate fields in China and Vietnam range from 250 to 750 g m^{-2} (Lumpkin and Plucknett, 1982). Usually at least 250 g m^{-2} of azolla is preferred to provide a satisfactory surface cover. In the field experiment reported in Chapter III, 250 g m^{-2} of azolla was applied at the first inoculation and approximately 500 g m^{-2} was

used for the second cycle. In that experiment, in spite of the warm summer weather, A. caroliniana (Ohio) and A. pinnata (Indonesia) performed well enough to accumulate over 40 kg nitrogen ha^{-1} within a period of three weeks. A. microphylla (Galapagos) also was very productive during the second cycle. This species also differed from others as its fronds grew upright once the mat became dense. To more fully characterize the responses of the above three species to light, temperature and initial biomass, a series of greenhouse experiments were conducted.

MATERIALS AND METHODS

Four experiments were conducted at the greenhouse facilities of the Department of Agronomy and Soil Science, University of Hawaii. The treatments and some other particulars specific to an experiment are included under the experimental details at the beginning of each experiment. Information pertaining to plant material, culture solution, experimental site and measurements are given below.

Plant material

Azolla inoculum was obtained by multiplying azolla in plastic buckets containing a nitrogen-free culture solution (Table 15). The biomass was reduced periodically during multiplication to prevent the mat from getting too dense; this was essential in A. microphylla as erect-growing fronds tended to show slow early growth.

TABLE 15. Chemicals used and nutrient concentration of culture solution.

Chemical formula	Formula weight	Stock solution	[a]Nutrient concentration	
		- g l^{-1} -	- - ppm - -	
KH_2PO_4	136.09	43.85	5	P
			6.25	K
$MgSO_4.7H_2O$	246.50	135.75	7.5	Mg
			8.5	S
NaCl	58.44	12.72	2.5	Na
			4	Cl
$CaCl_2.2H_2O$	147.02	55.20	7.5	Ca
			13	Cl
Iron sequestrene	930.83	41.67	1.25	Fe
$CoCl.6H_2O$	237.95	0.04	0.005	Co
$CuSO_4.5H_2O$	249.69	0.4	0.05	Cu
H_3BO_3	61.84	0.56	0.05	B
$MnCl_2.4H_2O$	197.91	3.6	0.5	Mn
$Na_2MoO_4.2H_2O$	241.96	0.25	0.05	Mo
$ZnSO_4.7H_2O$	287.56	2.2	0.5	Zn

[a]Nutrient concentration in culture solution when one ml of stock solution was diluted with 2000 ml of water.

Culture solution

The chemical composition and nutrient concentration of the culture solution are given in Table 15. One ml of stock solution was diluted to 2000 ml with tap water to prepare the culture solution containing the nutrients at the required concentrations. The culture solution had a pH of 6.0 and an electrical conductivity of 650 µmhos. The solution was replaced every six days at the time of sampling in Experiment 1. There was no culture solution replacement in Experiments 2 to 4. It was assumed that the nutrients added at the beginning of the experiment were sufficient to sustain growth until the final harvest. This assumption was based on observations that normal growth was obtained in culture solutions that were reused after azolla was harvested.

Experimental site

Experiments 1 to 3 were conducted in a greenhouse while Experiment 4 was carried out in temperature-controlled acrylic plastic chambers sited close to the greenhouse. The mean temperature ranged from 28 to 34 C during the period of experimentation (Appendix C). The diurnal temperature fluctuation was 20 to 38 C during the period of experimentation while the temperature extremes within the nutrient solution were 25 to 35 C. The temperature of the atmosphere was similar to that of culture solution in the plastic chambers. The total solar radiation received inside the green house and plastic chamber was measured with a LI-COR model PY4690 pyranometer. Solar

radiation inside the greenhouse was 65% of that received outside. The corresponding figure for the plastic chambers was 80%.

The experimental units used for Experiment 1 were one-liter containers with a surface area of 85 cm^2. For Experiments 2 to 4, azolla was grown in five-gallon plastic buckets. The buckets were lined with black polythene bags to prevent entry of light through the sides to minimize growth of green algae. The surface of each bucket was divided in half with vertical fiberglass sheets. Each half was taken as an experimental unit and it had a surface area of 230 cm^2.

Measurements

The entire azolla mat within the experimental unit was removed from the culture solution, the excess moisture blotted away and fresh weight was taken. In Experiment 1, the fronds were returned to the culture solution and arranged to reconstruct the mat. The rest of the experiments required destructive sampling; 1.0 g of fresh weight was saved for chlorophyll determination and the remainder oven dried for dry weight and nitrogen estimation. Chlorophyll content was determined by the method of MacKinney (1941) as modified by Arnon (1949). The procedure of Mitchell (1972) was used to determine total nitrogen. These two methods are detailed in Appendix B. The temperature and relative humidity were recorded with a hygro-thermograph for the experiments conducted in the greenhouse (Appendix C).

EXPERIMENT 1: BIOMASS ACCUMULATION OF THREE SPECIES OF AZOLLA

EXPERIMENTAL DETAILS

The objective of this experiment was to determine the growth curves of A. caroliniana (Ohio), A. microphylla (Galapagos) and A. pinnata (Indonesia) in 65 and 100% light starting from a single frond. The azolla was grown until there was no further increase in biomass. The 65% light level was obtained by using neutral-density shade-cloth. The treatments consisted of a three species by two light levels factorial arranged in a randomized complete block design with three replicates. Fresh weight was measured every three days until 27 days after 'planting' (DAP) when biomass accumulation levelled off.

RESULTS AND DISCUSSION

Typical sigmoid growth curves were obtained for all treatments (Figure 7). A. microphylla accumulated more fresh weight than the other two species at all measurement days. A single frond of A. microphylla weighed 0.06 g while those of A. caroliniana and A. pinnata weighed 0.05 g. This initial advantage and the upright-growth habit of A. microphylla may have enabled it to produce greater fresh weight than others. The largest difference in weights was at 24 DAP when A. microphylla fresh weight was 38% greater than A. caroliniana. In general, the time taken to completely cover the surface for control and shaded treatments was 12 and 15 DAP respectively. Fresh weights in the shade treatment was generally less than half that of control. Growth was slow during the first 12 days. The 12 to 21-DAP interval

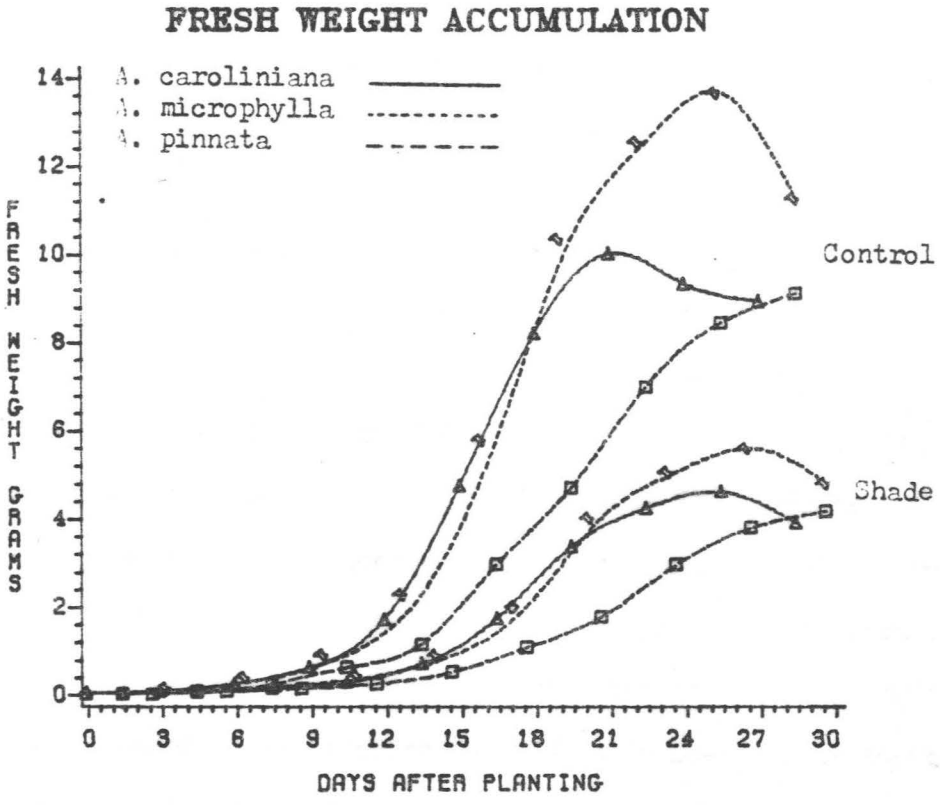


FIGURE 7. Growth curve of three Azolla species grown under 65% light (shade) and 100% light (control).

was the grand period of growth for the control treatment and 15 to 21 DAP for shade treatment in A. caroliniana and A. microphylla; in A. pinnata, the onset of the grand period of growth was delayed by about two days. Thus biomass accumulation under shade was limited by rate of growth and duration of grand period of growth.

The azolla biomass at the onset of rapid growth was 200 g m^{-2} (Figure 7). Thus, the lag phase of growth can be by-passed by starting with a biomass of 200 g m^{-2} or more. Azolla often encounters competition from green-algae in field conditions. A complete surface cover of azolla would minimize light availability for green-algae and suppress its growth. The container was completely covered by a single layer of fronds at about 400 g m^{-2}. A biomass of over 200 g m^{-2} would be required to bring about rapid growth in azolla. Since the environment under field conditions may not be the optimum for growth of azolla, a slightly higher frond biomass may be necessary.

The RGR of azolla increased slightly and then decreased and the decrease with time was curvilinear (Figure 8). This is the relationship commonly observed in other plants as well. Upon developing a complete canopy over the surface, competition for environmental resources causes a reduction in RGR. In addition, the proportion of non-photosynthetic tissue in most plants increases as they grow older and consequently RGRs decline. An increase in non-photosynthetic tissue with age does not seem to occur in azolla because fronds from dense mats resume vigorous growth upon reduction

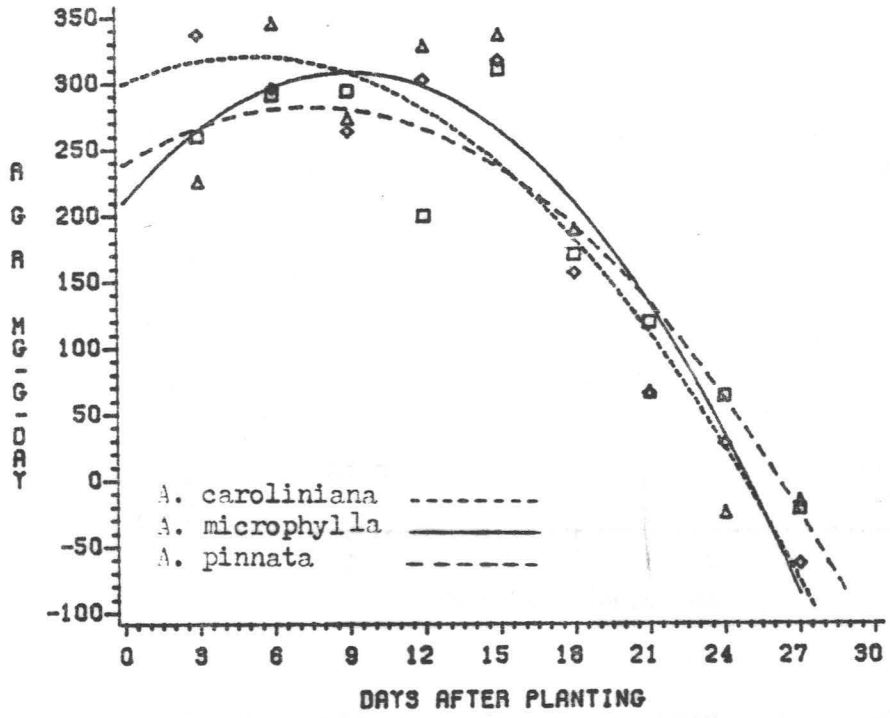


FIGURE 8. Relative growth rate of Azolla species grown under 100% light.

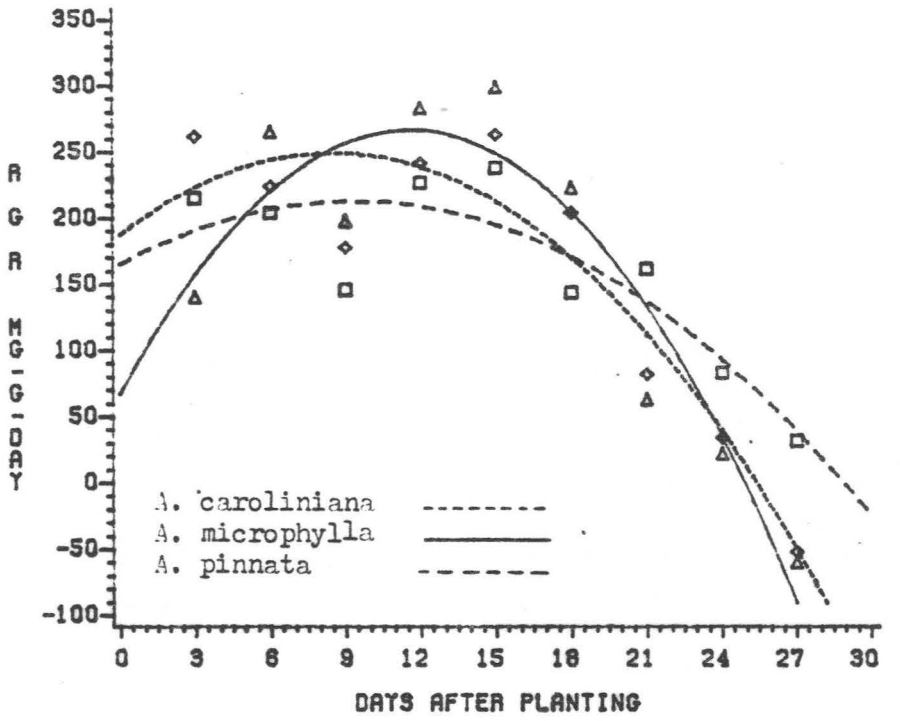


FIGURE 9. Relative growth rate of Azolla species grown under 65% light.

in mat biomass. Thus, the decline in RGR appears to be caused mainly by competition for light and space.

The RGRs during the first two weeks of growth were about 300 mg g^{-1} day^{-1} and these values were similar to those reported by Brotonegoro and Abdulkadir (1976) for A. pinnata, but lower than the 413 mg g^{-1} day^{-1} for A. caroliniana quoted by Becking (1979). The lower RGRs in this study may be due to warm temperatures in the greenhouse (Appendix C). Generally the RGR started to decline at 9 DAP when the mat biomass exceeded 600 g m^{-2} (Figures 8 and 9). As discussed above, at least 400 g m^{-2} was required to efficiently intercept light. Thus the optimum biomass required to maintain the maximum growth rate was 200 to 600 g m^{-2}. In order to maintain the biomass within this range, excess azolla would need to be removed every 2 to 3 days which is impractical in agricultural conditions. As the biomass range for maximum growth is very narrow, it will be difficult to maintain an azolla crop at its maximum biomass accumulation potential.

EXPERIMENT 2: EFFECT OF INITIAL FROND BIOMASS ON GROWTH OF AZOLLA SPECIES

EXPERIMENTAL DETAILS

It was found in Experiment 1 that highest growth rate of azolla occurred at frond biomasses between 200 to 600 g m^{-2}. The initial biomass used in many cropping situations have been within this range (Chu, 1979; Tuan and Thuyet, 1979). Factors such as planting material

availability, duration of cropping and handling cost would determine the actual initial biomass for any field situation. The objective of the experiment reported in this section was to study the effect of three initial frond biomasses on the growth of three Azolla species. The species chosen for the study were A. caroliniana (Ohio), A. microphylla (Galapagos) and A. pinnata (Indonesia) and the initial biomasses were 200, 400 and 600 g m^{-2}. A 3 x 3 factorial arrangement of treatments was used with three replicates in a randomized complete block design. Three harvests were taken at weekly intervals. The experiment had to be terminated at 21 DAP because mats in several treatments developed necrotic spots when the culture period was extended further. This was suspected to be a rotting disease, but the causal organism could not be identified. The warm greenhouse temperatures (Appendix C) may have been conducive to disease outbreak in dense mats.

RESULTS AND DISCUSSION

Biomass accumulation

The fresh weight increase with time was generally curvilinear in A. caroliniana and A. microphylla (Figures 10, 11). A. microphylla was superior to the other two species in biomass production. Growth of A. pinnata levelled off early (Figure 12) as some fronds died after 14 days. Frond death occurred with the other two species also when the culture period was extended beyond 21 days. The biomass accumulation in the 600 g m^{-2} treatment continued to be higher than

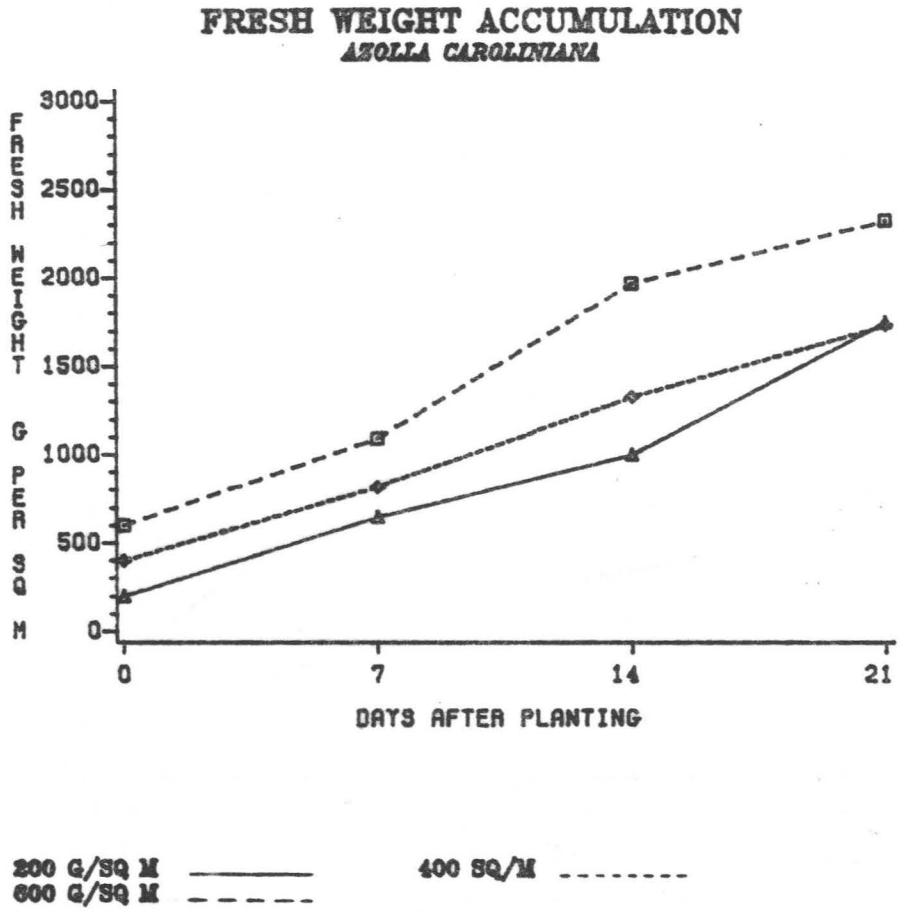


FIGURE 10. Effect of initial biomass on fresh weight accumulation of Azolla caroliniana.

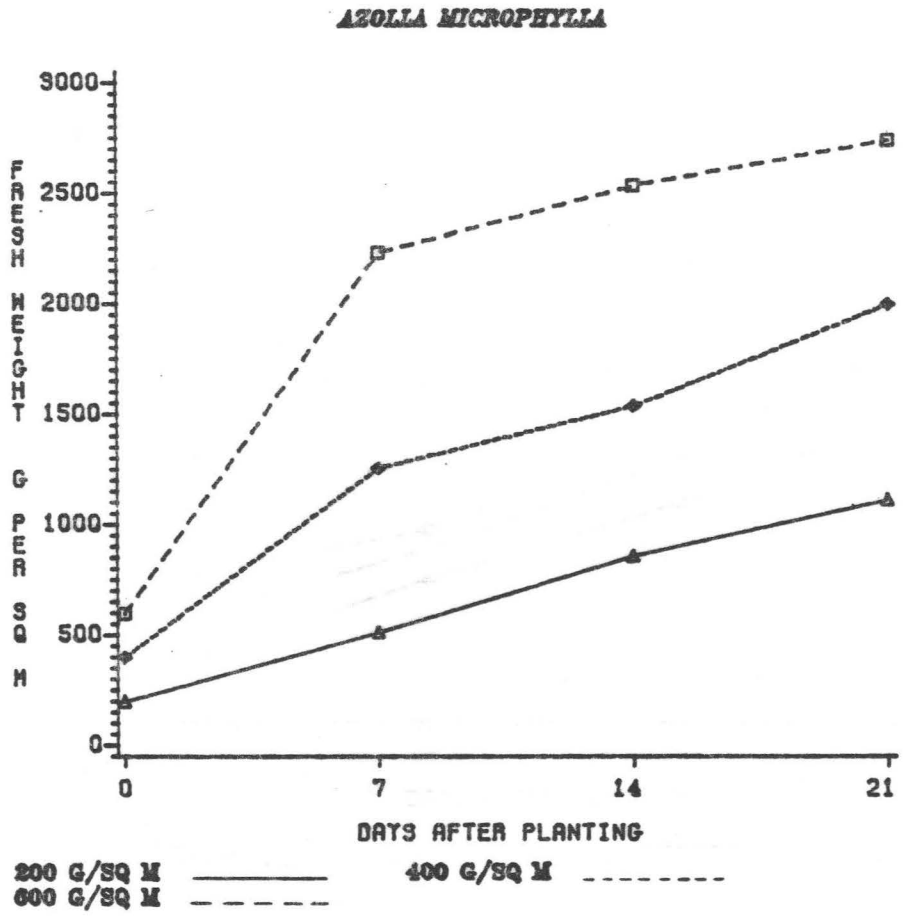


FIGURE 11. Effect of initial biomass on fresh weight accumulation of Azolla microphylla.

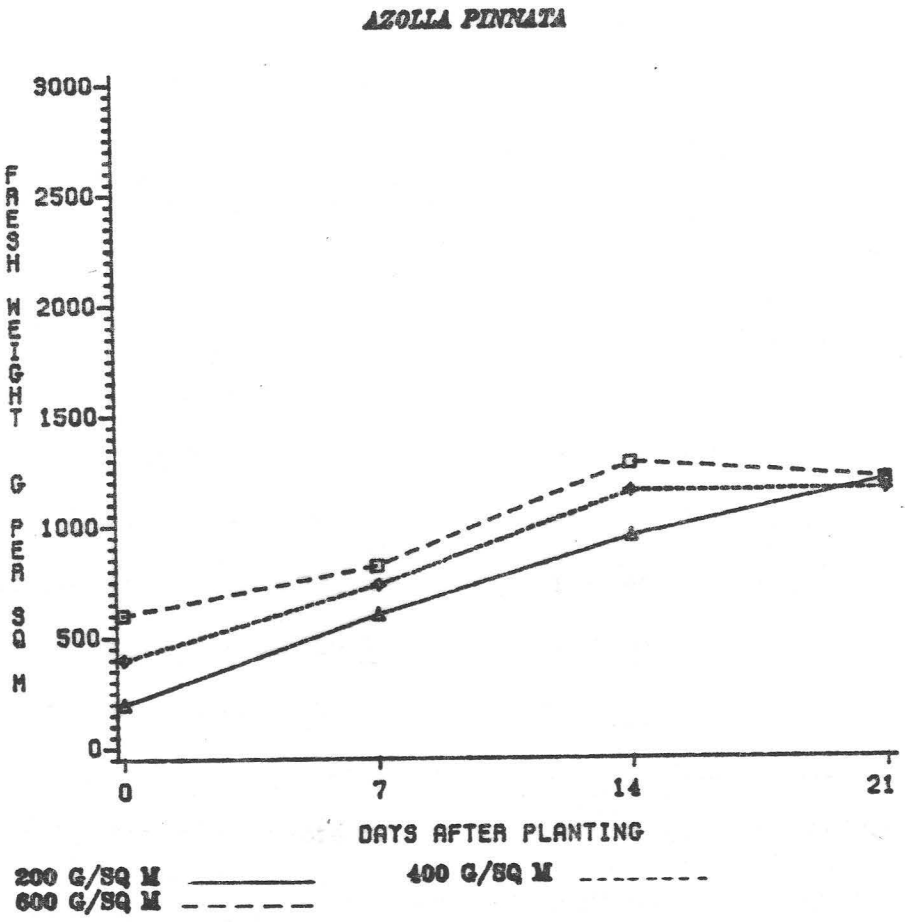


FIGURE 12. Effect of initial biomass on fresh weight accumulation of Azolla pinnata.

the other two initial biomasses even at 21 DAP in A. caroliniana and A. microphylla (Figures 10, 11), but in A. pinnata there was no such difference (Figure 12). It was assumed that the mat weights at the lower two biomasses would eventually reach values comparable to the 600 g m^{-2} treatement but would take more than 21 days. In contrast with the results obtained in this study, Brotonegoro et al. (1982) who studied the same three initial biomasses in the field, found that A. pinnata inoculated at 400 g m^{-2} produced the highest fresh weight yield when harvested at 12 DAP. Since azolla multiplies by fragmentation, minor disturbances are beneficial because they cause the break up of fronds. Wind action brings about fragmentation in nature. Since this does not happen under greenhouse conditions, growth may have been retarded due to lack of new growing points.

RGR decreased with time and with increasing initial biomass (Table 16). Increasing initial biomass reduced RGR, especially during the 0 to 7 day period. There was a significant species x initial biomass interaction on RGR; the decline in RGR with increasing initial biomass was more gradual in A. micorphylla than in A. caroliniana and A. pinnata (Figure 13). RGR of A. microphylla also decreased more gradually than the other two species with time (Table 16). As discussed already in Chapter 3, this species tended to dieback when newly introduced at lower densities and grew vigorously after recovery. The difference in growth habit can be attributed to the ability of this species to maintain greater RGRs over a longer period of time. In general, RGR at any time was inversely related to the

TABLE 16. Effect of initial biomass and time on relative growth rate of three Azolla species.

Treatment	Relative growth rate	
	7-14 DAP	14-21 DAP
	----μg g^{-1} day^{-1}----	
Azolla species		
A. caroliniana	99	31 ab*
A. microphylla	91	49 a
A. pinnata	88	18 b
Initial biomass g m^{-2}		
200	104 a	53 a
400	85 b	33 ab
600	89 b	12 b
SE (16)**	8.16	11.4

*Means in a column within a category followed by the same letter are not significantly different at the 0.05 level of probability as determined by Duncan's multiple range test.

**Standard error of mean with error degrees of freedom in parentheses.

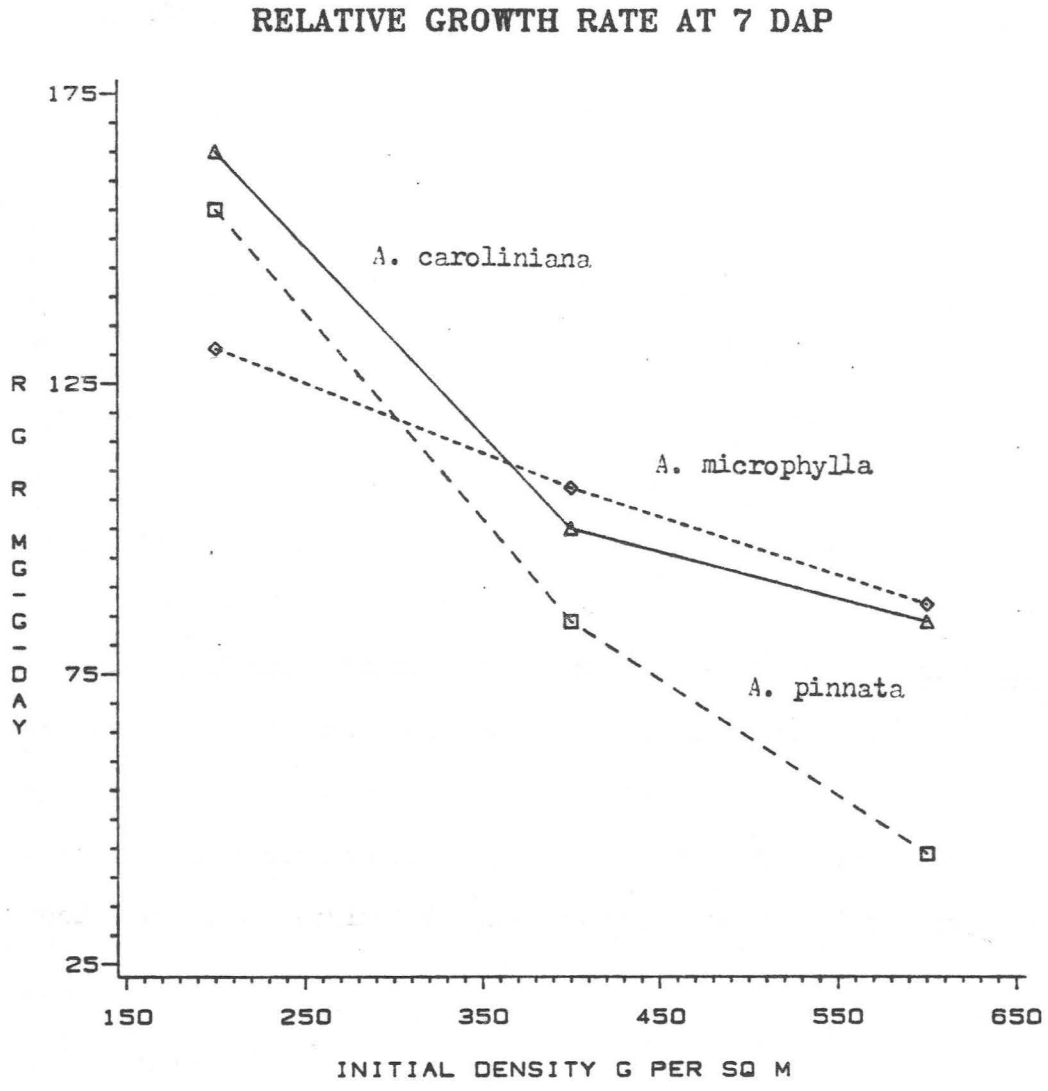


FIGURE 13. Effect of initial biomass on relative growth rate of three Azolla species at 7 days after planting (DAP).

plant biomass present at that time. The following RGR-biomass relationships were obtained by pooling the data for all three samplings. RGR of azolla expressed as a function of initial biomass (x) can be described by the following equations:

A. caroliniana: RGR = 170 - .062 x r^2=.60 (P=.05)

A. microphylla: RGR = 146 - .038 x r^2=.83 (P=.001)

A. pinnata : RGR = 190 - .122 x r^2=.38 (Not significant)

The greater fresh weight accumulation of A. microphylla did not result in higher dry matter production than the other two species because it had a lower dry matter content (Table 17). This appears to be an inherent characteristic of the species. The dry matter content of A. microphylla declined with age unlike that of the other two species. Kaplan and Peters (1981) reported a decrease in dry matter content with increasing leaf age in single fronds of A. caroliniana. However, generally an older mat would be expected to contain more dry matter than a young one because it can contain dead tissue with lower moisture content. The fronds in the uppermost layer of A. microphylla were growing vertically at the time of final harvest and these fronds appeared more succulent which may be a reason for the decrease in dry matter content with increasing biomass. A slower growth rate may also result in less succulent fronds. This may have been the case with A. pinnata in the present study whereas luxurious growth in A. microphylla may have resulted in succulent fronds with a lower dry matter content.

TABLE 17. Effect of initial biomass on dry matter accumulation and dry matter content of three Azolla species.

Treatment	Dry weight at 21 DAP	Dry matter content 7 DAP	Dry matter content 21 DAP
	- g m^{-2} -	- - - - % - - - -	
Azolla species			
A. caroliniana	107.01 a*	5.12 b	5.61 b
A. microphylla	109.50 a	5.28 b	4.40 c
A. pinnata	86.87 b	5.86 a	7.43 a
Initial biomass g m^2			
200	93.06 b	5.36	5.76
400	100.94 ab	5.50	6.06
600	109.37 a	5.40	5.62
SE (16)**	6.71	0.14	0.26

*Means in a column within a category followed by the same letter are not significantly different at the 0.05 level of probability as determined by Duncan's multiple range test.

**Standard error of mean with error degrees of freedom in parentheses.

Chlorophyll content

Chlorophyll content appeared to be species-specific and was not influenced by either initial biomass or age (Table 18). Chlorophyll content increased slightly with increasing biomass at the first sampling perhaps because the shading associated with increased frond biomass enhances the development of chlorophyll. A characteristic feature of A. microphylla, besides its larger fronds, is the light green color. The light color was due to a lower chlorophyll content (Table 18). The chlorophyll contents obtained in this study were similar to those reported for A. caroliniana by Peters et al.(1976) and Ray et al.(1978).

The chlorophyll a:b ratio of 1.70 obtained in this study is much lower than the ratios of 3.9 to 4.3 reported by Peters et al. (1980). Ratios calculated from chlorophyll a and b values reported by Sahai and Khosla (1980) for A. pinnata were less than 1.5 as they were in this study. The discrepencies in the ratios among different studies may be due to the different methods used for chlorophyll determination. Peters et al. (1980) used the Wintermans and DeMots (1965) method for chlorophyll determination. The chlorophyll a:b ratio in A. microphylla was found to be significantly higher than in the other two species. Chlorophyll a is found in both azolla as well as anabaena cells of the symbiotic association while only azolla contains chlorophyll b (Peters et al., 1979). Therefore, the higher chlorophyll a:b ratio in A. microphylla compared to the other two species could be because the endophyte makes up a larger proportion of

TABLE 18. Effect of initial biomass on chlorophyll content and chlorophyll a/b ratio of three Azolla species.

Treatment	Chlorophyll content		Chlorophyll a/b ratio	
	7 DAP	21 DAP	7 DAP	21 DAP
	- ug g^{-1} fr. wt.-			
Azolla species				
A. caroliniana	350 a*	311	1.36 b	1.44**
A. microphylla	259 b	241	1.70 a	1.62
A. pinnata	355 a	402	1.40 b	1.34
Initial biomass g m^2				
200	302	322	1.55	1.49
400	324	316	1.45	1.47
600	339	318	1.47	1.43
SE (16)***	.026	-	.094	-

*Means in a column within a category followed by the same letter are not significantly different at the 0.05 level of probability as determined by Duncan's multiple range test.

P=0.05

**Chlorophyll content determined only for one replicate.

***Standard error of mean with error degrees of freedom in parentheses.

the biomass. If the chlorophyll content was low, a greater endophyte density would help meet the energy requirements for nitrogen fixation.

Nitrogen accumulation

The ultimate objective in azolla management is to produce as much nitrogen as possible per unit area per unit time. The total nitrogen accumulated is a function of dry matter accumulation and tissue nitrogen content. Significant differences among the species were found in tissue nitrogen content (Table 19). Nitrogen content of the three species in descending order were A. caroliniana > A. pinnata > A. microphylla. Nitrogen content increased with age in all three species. This observation does not agree with that of Talley and Rains (1980) who reported a gradual drop in nitrogen content with age up to about 40 DAP. The nitrogen content of higher plants generally decreases with age because of the increase in non-photosynthetic tissue. As non-photosynthetic tissue does not appear to increase with age in azolla, nitrogen content would not be expected to decrease in mature tissue.

Nitrogen accumulation doubled during the period from 7 to 14 DAP in all three species (Table 20). In spite of the very high fresh weight accumulation (Figure 11), A. microphylla accumulated significantly less nitrogen than A. caroliniana because tissue dry matter (Table 17) and nitrogen content (Table 19) were lower. The fresh weight yield of A. microphylla at the final harvest was 23% higher than A. caroliniana, but the nitrogen yield was 33% lower.

TABLE 19. Effect of initial biomass and time on tissue nitrogen content of three Azolla species.

Treatment	Days After Planting		
	7	14	21
	- - - - - - - - % - - - - - - - -		
Azolla species			
A. caroliniana	4.42 a*	5.04 a	5.08 a
A. microphylla	3.55 b	3.55 c	3.75 c
A. pinnata	3.64 b	4.37 b	4.34 b
Initial biomass g m^2			
200	3.89 ab	4.53 a	4.57 a
400	3.52 b	4.20 a	4.21 b
600	4.19 a	4.22 a	4.39 ab
SE (16)**	0.30	0.20	0.14

*Means in a column within a category followed by the same letter are not significantly different at the 0.05 level of probability as determined by Duncan's multiple range test.

**Standard error of mean with error degrees of freedom in parentheses.

TABLE 20. Effect of initial biomass on nitrogen accumulation of three Azolla species.

Treatment	Nitrogen accumulation		
	7	14	21
	- - - - - - - - $g\ m^{-2}$ - - - - - - - -		
Azolla species			
A. caroliniana	1.94 a*	4.36 a	5.47 a
A. microphylla	1.53 a	2.81 c	4.12 b
A. pinnata	1.55 b	3.37 b	3.77 b
Initial biomass $g\ m^{-2}$			
200	1.22 c	2.91 c	4.27 a
400	1.53 b	3.40 b	4.24 a
600	2.26 a	4.24 a	4.85 a
SE (16)**	0.17	0.26	0.38

*Means in a column within a category followed by the same letter are not significantly different at the 0.05 level of probability as determined by Duncan's multiple range test.

**Standard error of mean with error degrees of freedom in parentheses.

Therefore, both dry matter and nitrogen contents should be considered as important quality parameters in evaluating the productivity of a green-manure crop such as azolla. With an initial biomass of 600 g m^{-2} azolla accumulated significantly more nitrogen than the lower densities at the first two samplings (Table 20). At the final harvest, however, no significant difference among the treatments having different initial biomass was detected in total nitrogen accumulation though the quantity of nitrogen in the 600 g m^{-2} treatment was higher. Thus, starting the growth cycle with a higher frond biomass was beneficial when azolla green manure was harvested at intervals of about 7 days. This advantage was minimized if the crop was allowed to grow for a longer time.

EXPERIMENT 3: EFFECT OF THREE LIGHT LEVELS AND TWO INITIAL BIOMASS ON GROWTH OF AZOLLA SPECIES

EXPERIMENTAL DETAILS

The experimental results discussed previously in this chapter indicated that the RGR of azolla was reduced when frond biomass exceeded 600 g m^{-2}. Mutual shading would be expected to increase with increasing biomass and consequently light availability could become a growth-limiting factor. Light availability is also likely to be reduced when azolla is intercropped with rice or taro. Hence, the objective of Experiment 3 was to examine the growth of azolla at two initial biomasses under three levels of light. The same three species investigated in Experiments 1 and 2 were grown at initial biomasses of

250 and 500 g m^{-2}. The number of initial biomasses was reduced to two to limit the total number of treatments. The light levels were control (no shade), 65% light and 35% light. Different light levels were obtained by covering the buckets with neutral-density shade cloth. A split-plot design was used where the species and shade treatments were main-plots and initial biomass was sub-plots. The treatments were replicated three times. Azolla was harvested 10 DAP and the experiment was repeated for the harvest interval of 20 DAP.

RESULTS AND DISCUSSION

Shading reduced biomass accumulation in all three species. In addition, light level interacted significantly with species and biomass in influencing dry matter, nitrogen and chlorophyll contents of azolla. Growth in regard to species and plant biomass followed the same trends evident in previous experiments. Therefore, the discussion of this study focuses more on the interactions between light and species and light and initial biomass. There was no significant interaction between species and biomass (Appendix D).

Biomass accumulation

The fresh weight increase at 10 DAP was similar for all three species; however, A. microphylla grown at 100% and 65% light accumulated significantly greater fresh weights than the other two species at 20 DAP (Figure 14). The differences among the species were very small at 35% light. The suppression of growth by shade was more

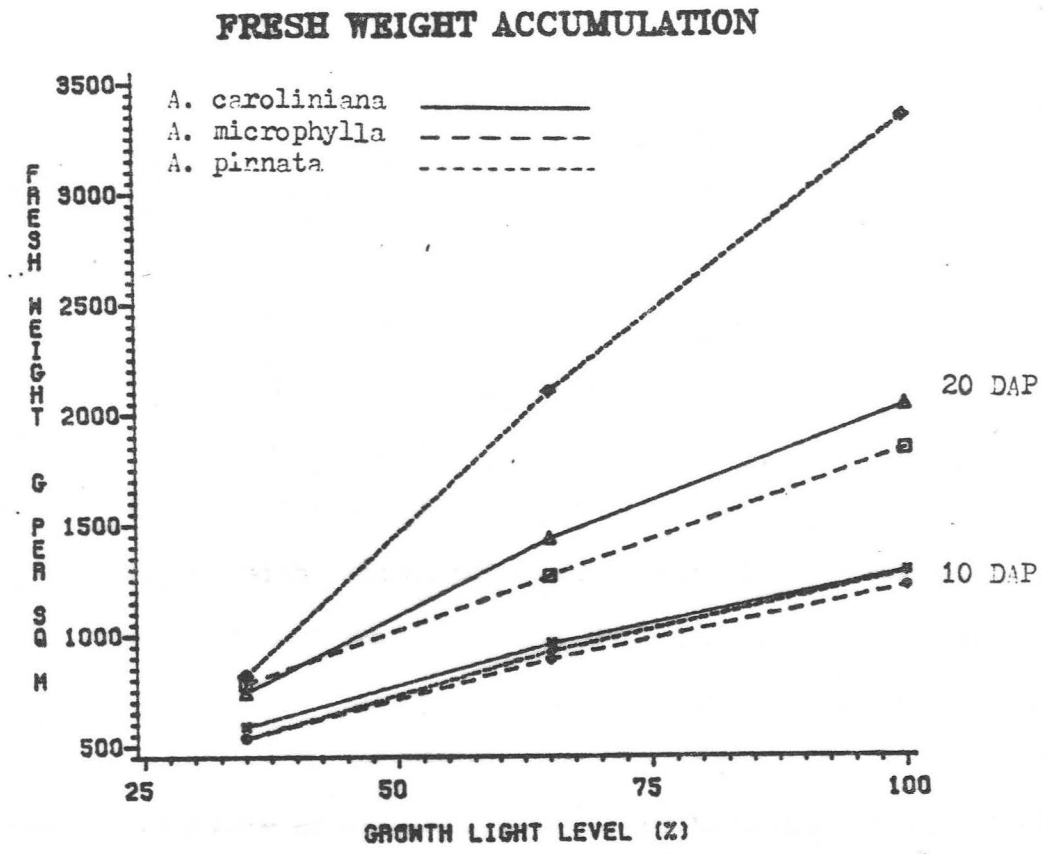


FIGURE 14. Influence of light on fresh weight accumulation of three Azolla species at 10 and 20 days after planting (DAP).

pronounced at 20 DAP than at 10 DAP, especially at 35% light. Growth of A. filiculoides (Ashton, 1974) and A. mexicana (Holst and Yopp, 1979a) has been greater in shade than in full sunlight. In the present experiment, all three species of azolla grown without shade consistently produced higher fresh and dry matter than shaded treatments. The disagreement may be due to the differences in experimental conditions. This experiment was conducted in a greenhouse where light availability was 65% of that received outside. When 35% shade was applied inside the greenhouse, the resulting light level was only about 42% of full sunlight. Therefore the light level in the control treatments may have been sufficiently low for maximum biomass accumulation. Another influencing factor may be nutrient supply. Tung and Shen (1981) obtained maximum growth at full sunlight when 20 ppm of phosphorus was supplied; growth was maximum at 50% light in the absence of phosphorus. The phosphorus level in the present study was 5.0 ppm (Table 15).

Data on dry weight accumulation showed that light interacted with initial biomass at both sampling times (Figure 15). The increase in dry weight at 65 and 100% light was more pronounced at 20 DAP than at 10 DAP, especially with an initial biomass of 500 g m^{-2}. The higher biomass yielded more at all light levels when harvested 10 DAP. This difference in yield at the two biomasses diminished in shaded treatments at 20 DAP. A similar trend was observed in the species x biomass x light interaction at 20 DAP (Figure 16). Differences in dry weight among species and biomasses were apparent only at 100% light.

TABLE 21. Effect of growth light level on relative growth rate of three Azolla species.

Treatment	Days After Planting	
	7	14
	- - - ug g^{-1} day^{-1} - - -	
Azolla species		
A. caroliniana	92	52 b*
A. microphylla	87	68 a
A. pinnata	84	58 b
SE (18)**	4.7	5.1
Growth light level		
35%	43 c	49 c
65%	94 b	59 b
100%	126 a	70 a
SE (16)**	3.4	7.4

*Means in a column within a category followed by the same letter are not significantly different at the 0.05 level of probability as determined by Duncan's multiple range test.

**Standard error of mean with error degrees of freedom in parentheses.

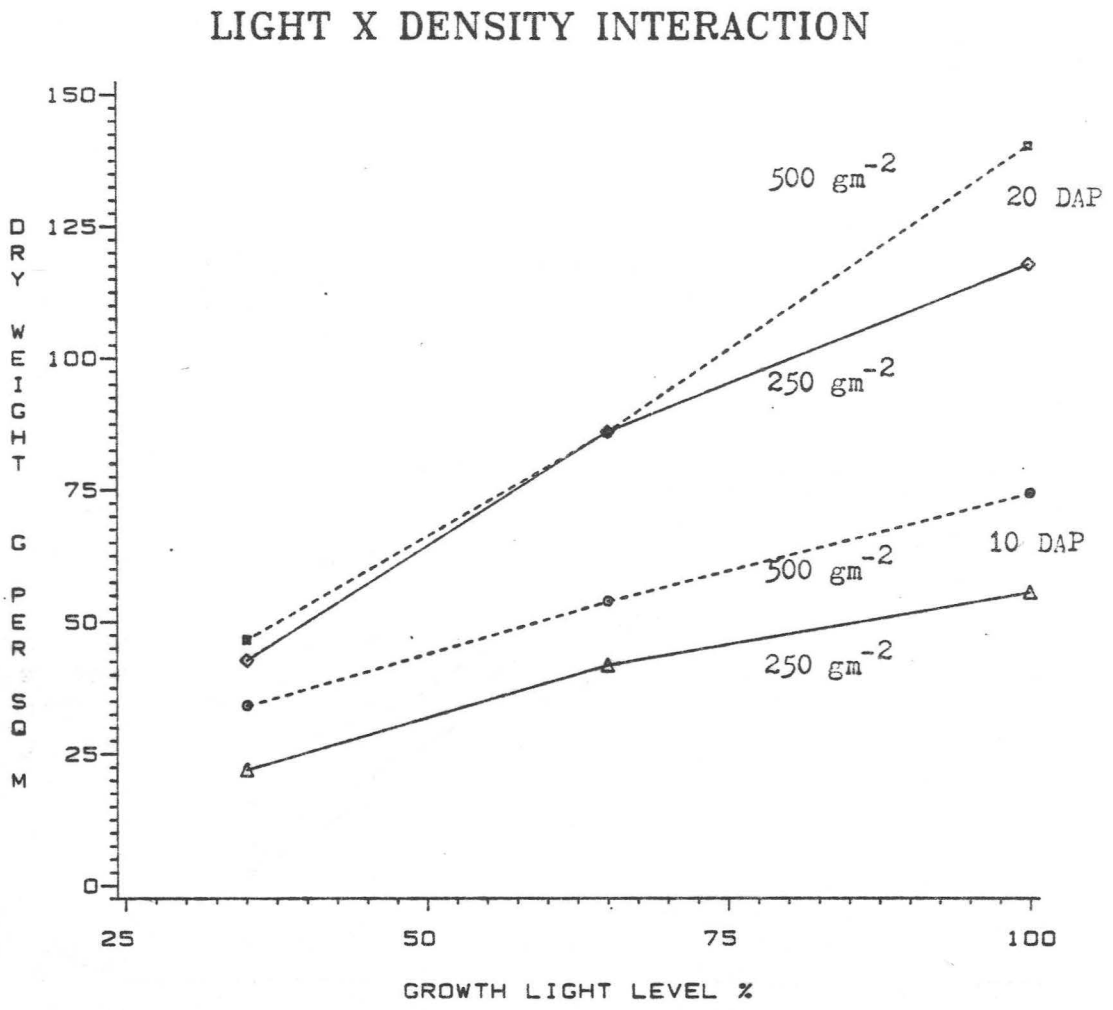


FIGURE 15. Influence of light and initial biomass on dry weight accumulation at 10 and 20 days after planting (DAP).

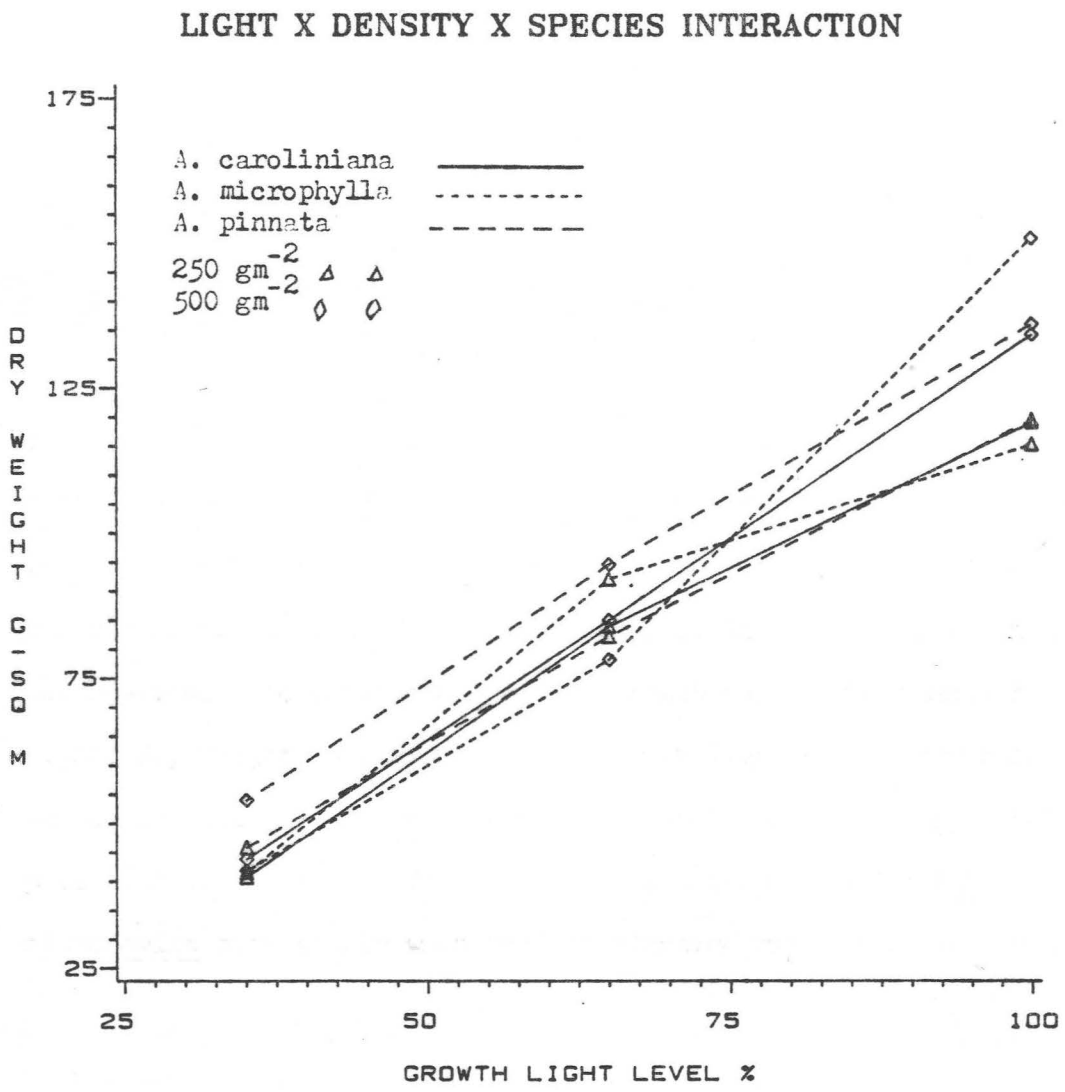


FIGURE 16. Species x initial biomass x light interaction on dry weight accumulation at 20 days after planting.

The maximum dry matter per unit area appears to be influenced more by light and less by species or initial biomass when light is limiting as it was here. Thus if azolla is grown under low or no shade, it is desirable to start with a higher biomass. On the other hand, if the shade is heavy, a higher initial biomass would not be advantageous, particularly where the harvest interval is long. The superior biomass accumulation potential of A. microphylla was realized only when it was grown without shade at a higher initial biomass and harvested 20 DAP.

The changes in tissue dry matter content of the three species with increasing light level is shown in Figure 17. The species were significantly different with A. pinnata having the highest dry matter content at all light levels. Whereas the dry matter contents of A. caroliniana and A. pinnata increased slightly with increasing light, that of A. microphylla declined considerably when light was increased from 35 to 65%. A. microphylla grown in 35% light did not have upright growth as it did in 65% and 100% light and resembled the other two species. The absence of upright growth may be the reason for higher dry weight in 35% light than in 65% light or the control. Dry matter content may also be important in determining the regeneration potential of azolla fronds as vertically growing fronds of A. microphylla grew slowly when used as planting material.

Chlorophyll content

Tissue chlorophyll content decreased with increasing light (Figure 18). Plants growing in shade usually contain more chlorophyll

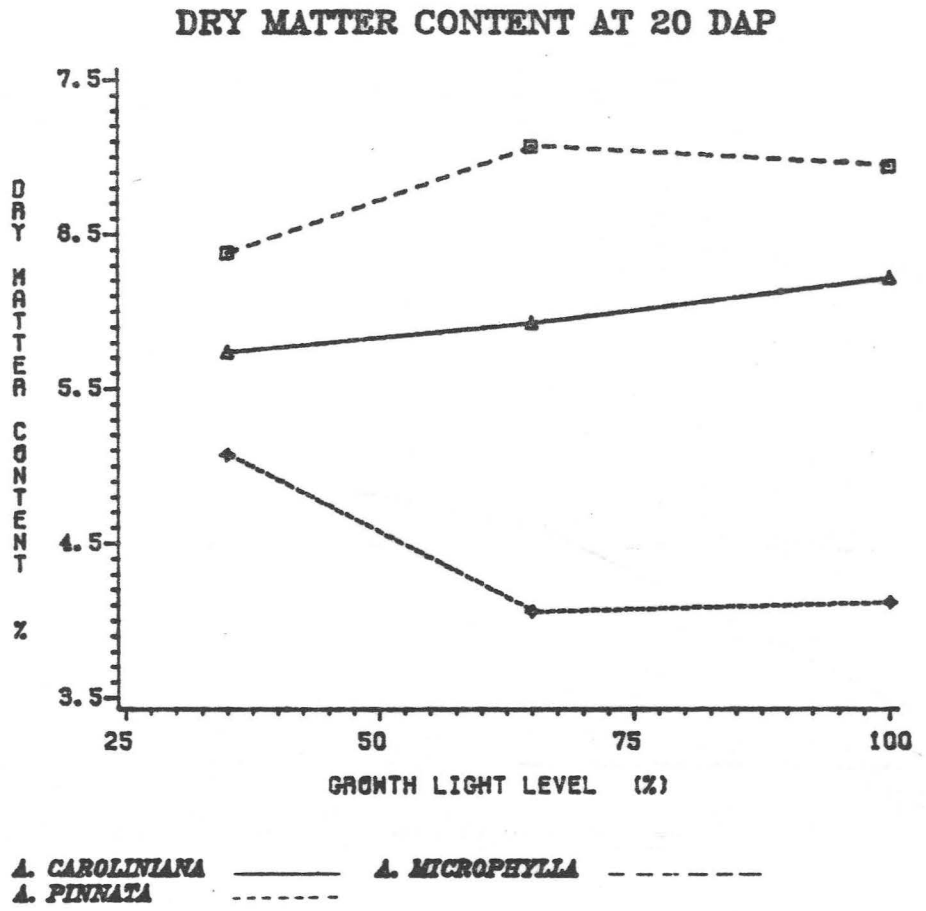


FIGURE 17. Influence of growth light level on dry matter content of Azolla species.

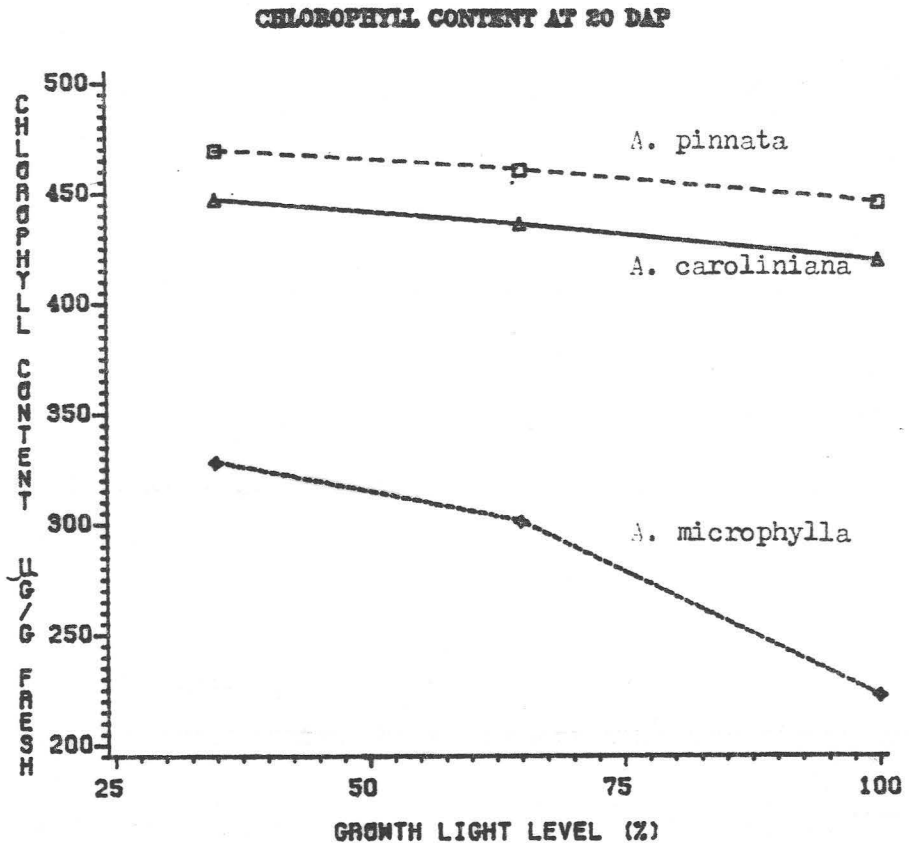


FIGURE 18. Effect of growth light level on plant chlorophyll content of Azolla species.

per unit weight which enables them to make maximum use of the available sunlight. For instance, there was a three-fold increase in chlorophyll content when light intensity was lowered from 660 to 20 μE m^{-2} s^{-1} in Tradescantia albifora (Adamson, 1978). The response of A. caroliniana and A. pinnata was somewhat similar though their chlorophyll contents were less influenced by light level than that of A. microphylla. The chlorophyll content of A. microphylla was much lower than that of other two species and it decreased about 50% when light was increased from 35% to 100%. Although both chlorophyll a and b increased with increasing shade, the relative increase in chlorophyll b was higher (Table 22), a common result in higher plants and green algae (Falkowski and Owens, 1980). It is believed that chlorophyll b synthesis from chlorophyll a is enhanced in the dark (Tanaka and Tsuji, 1981). The chlorophyll a:b ratio of A. microphylla increased with increasing light (Figure 19). This increase was more pronounced in A. microphylla because it had 38 and 65% more chlorophyll a and b respectively, in 35% light than in 100% light (Table 22).

Nitrogen accumulation

Tissue nitrogen content of A. pinnata increased significantly with increasing light, but the trend was reversed for A. microphylla (Figure 20). Much smaller but significant effects of light on the nitrogen content of A. caroliniana were also observed. A. caroliniana dry matter contained more nitrogen than the other two species and it

TABLE 22. Effect of light on chlorophyll a and b content of three *Azolla* species.

Light level	Chlorophyll content		Expressed as % of control	
	a	b	a	b
	- µg g^{-1} fr. wt. -		- - - - - - % - - - - - -	
A. caroliniana - 100	239	180	100	100
65	246	190	103	106
35	250	197	105	109
A. microphylla - 100	140	81	100	100
65	183	118	131	146
35	194	134	138	165
A.pinnata - 100	252	193	100	100
65	253	207	100	107
35	257	212	102	110

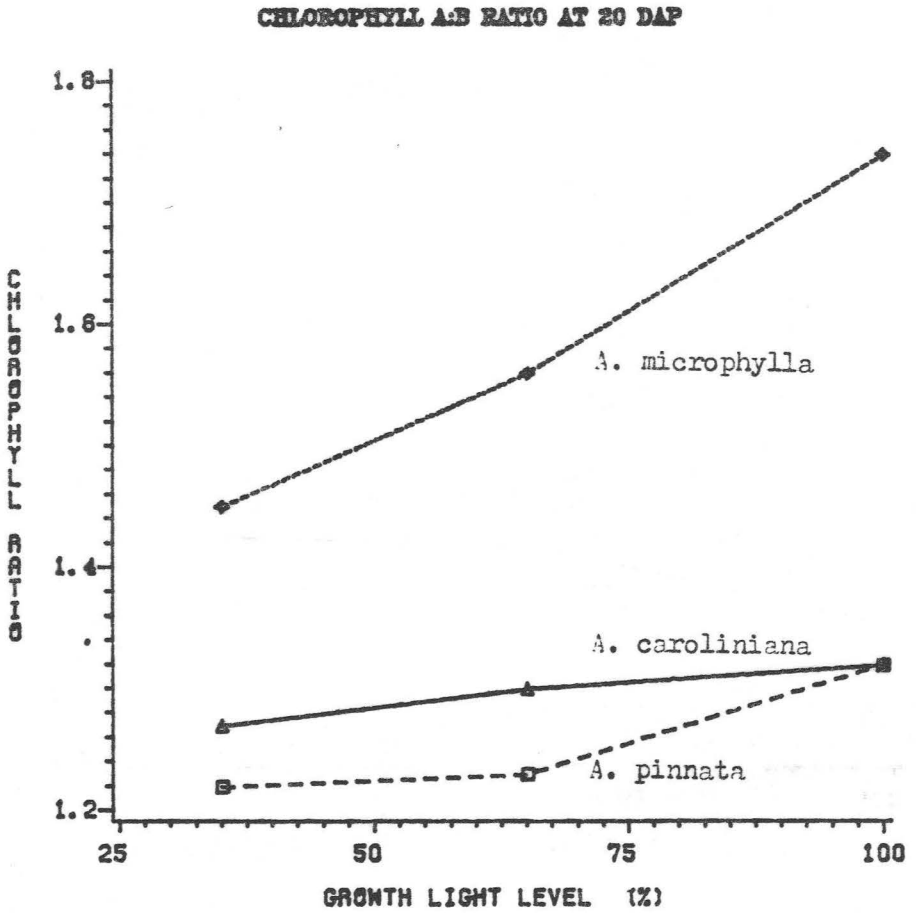


FIGURE 19. Effect of growth light level on chlorophyll a:b ratio of Azolla species.

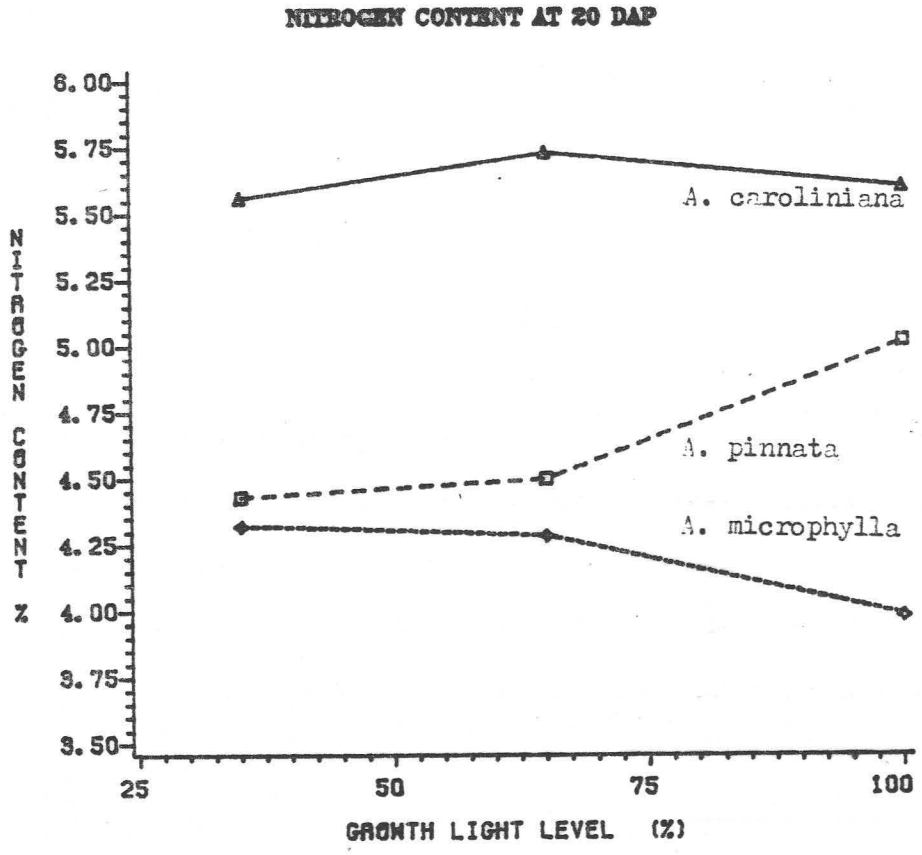


FIGURE 20. Effect of growth light level on tissue nitrogen content of Azolla species.

was less sensitive to light level. Shade should promote nitrogen fixation and improve tissue nitrogen content because light intensities above 50% of full sun reduced nitrogen fixation in A. filiculoides (Ashton, 1974). On the other hand, A. mexicana grown in sun had higher nitrogen fixation activity than those in shade (Holst and Yopp, 1979b). The energy required for nitrogen fixation is obtained through photosynthesis. Energy supply and consequently nitrogen fixation can be reduced when azolla is grown under heavy shade. If the dry matter accumulation rate proceeds faster than nitrogen accumulation rate, the fixed nitrogen would be translocated to a larger number of sinks resulting in lower tissue nitrogen content. Hence, the lower nitrogen content of A. microphylla in control treatments may be the result of a low nitrogen fixation rate and/or higher dry matter accumulation rate.

Nitrogen accumulation which is the product of dry matter accumulation and nitrogen content also increased with light (Figure 21). The differences among treatments were less pronounced in 35% light and became progressively larger with increased light. A higher initial biomass was not beneficial when azolla was grown under shade for 20 DAP. The rate of increase in nitrogen accumulation was greater for A. caroliniana than for the other two species, especially in the higher biomass treatment. Although azolla grown under shade had a darker green color and appeared healthier than control plants, fresh weight and nitrogen accumulation were not enhanced. Similar results have been reported by Brotonegoro et al. (1982) in field studies with A. pinnata.

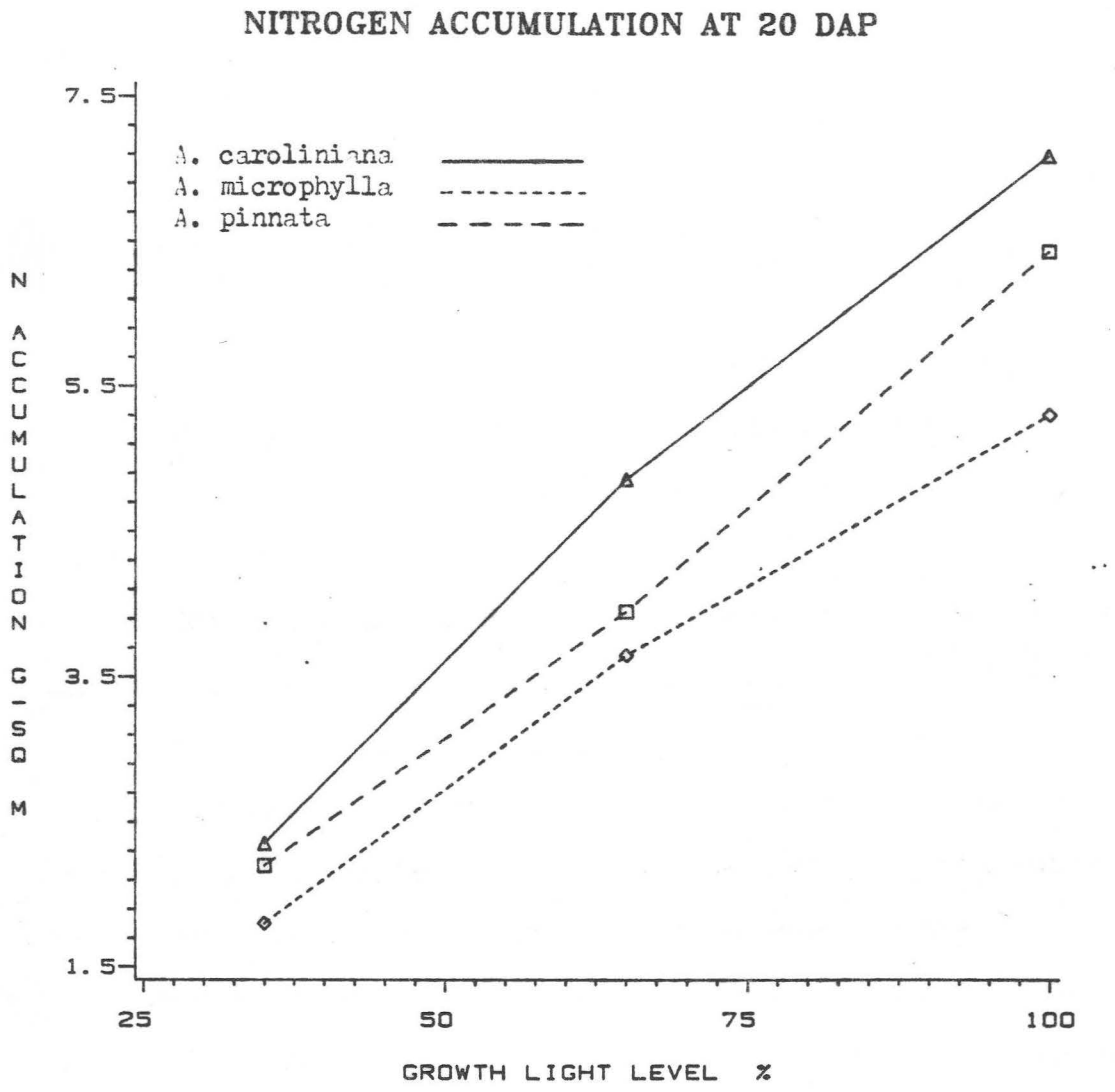


FIGURE 21. Effect of growth light level on nitrogen accumulation of Azolla species at 20 days after planting (DAP).

EXPERIMENT 4: INFLUENCE OF LIGHT AND TEMPERATURE ON GROWTH OF TWO AZOLLA SPECIES

EXPERIMENTAL DETAILS

The results of Experiment 3 clearly demonstrated that azolla growth was reduced by shading. Temperature also influences plant growth and Azolla species have exhibited differential responses to temperature (Lumpkin and Plucknett, 1982; Watanabe and Berja, 1983). The adaptability of azolla to non-optimal light and temperature regimes is an essential requirement for its use in tropical environments. Therefore, the objective of the experiment reported here was to examine the influence of temperature and its interaction with light on growth of azolla. Two species of azolla, A. caroliniana and A. microphylla, were grown at initial biomass of 500 g m^{-2} in 15-liter buckets as described in Experimental site on page 74. The temperature treatments were 20 and 33 C and the light levels were 35, 65 and 100% of available light in the greenhouse. The experiment was carried out in two temperature-controlled acrylic plastic chambers maintained at nearly constant temperatures of 20 and 33 C. In each chamber, treatment arrangement was such that light level was the main-plot and species was the sub-plot. The treatments were replicated three times within each chamber. The pooled data from the two chambers were statistically analyzed as nested-plot treatments to compare temperature effects. A sample ANOVA is presented in Appendix E. After the harvest at 10 days, the experiment was repeated for 20 DAP harvest.

RESULTS AND DISCUSSION

Biomass accumulation

The main effects of the three factors, temperature, light and species, on fresh weight accumulation at 10 and 20 DAP and dry matter content at 10 DAP were significant. The main effect of species on dry matter content at 20 DAP was also significant. In addition, several significant interactions among the three factors were also detected. Fresh weight was higher at 20 C than at 33 C for both *A. caroliniana* and *A. microphylla* at 10 DAP (Figure 22). Heavy shade depressed growth in both species and the growth reduction was more severe at 33 C than at 20 C. As a result of the light x temperature interaction, the reduction in fresh weight accumulation at 33 C was greater in 100% light than in 35 and 65% light. Fresh weight increased almost linearly with increasing light at 20 C, but the increase was curvilinear at 33 C.

The fresh weight of *A. microphylla* was lower than *A. caroliniana* at 10 DAP because of the slow early growth of the former. However at 20 DAP, *A. microphylla* produced significantly higher fresh weights than *A. caroliniana* at all temperature and light levels (Figure 23). Its growth was inhibited only by a combination of high temperature and heavy shade. In fact, *A. microphylla* fresh weights were more than double those of *A. caroliniana* in most treatments. Species x light and species x temperature interactions were significant at 20 DAP. The increase in fresh weight with increasing light was more gradual in *A. caroliniana* than in *A. microphylla*. Temperature did not have a

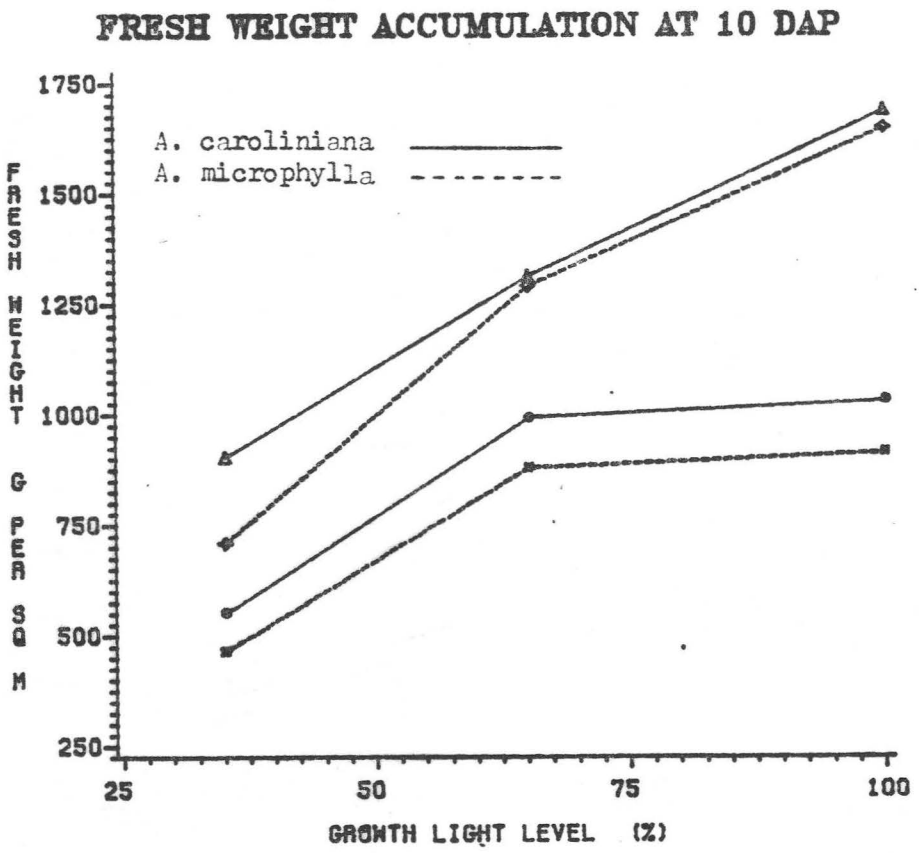


FIGURE 22. Effect of growth light level and temperature on fresh weight accumulation of Azolla species at 10 days after planting (DAP).

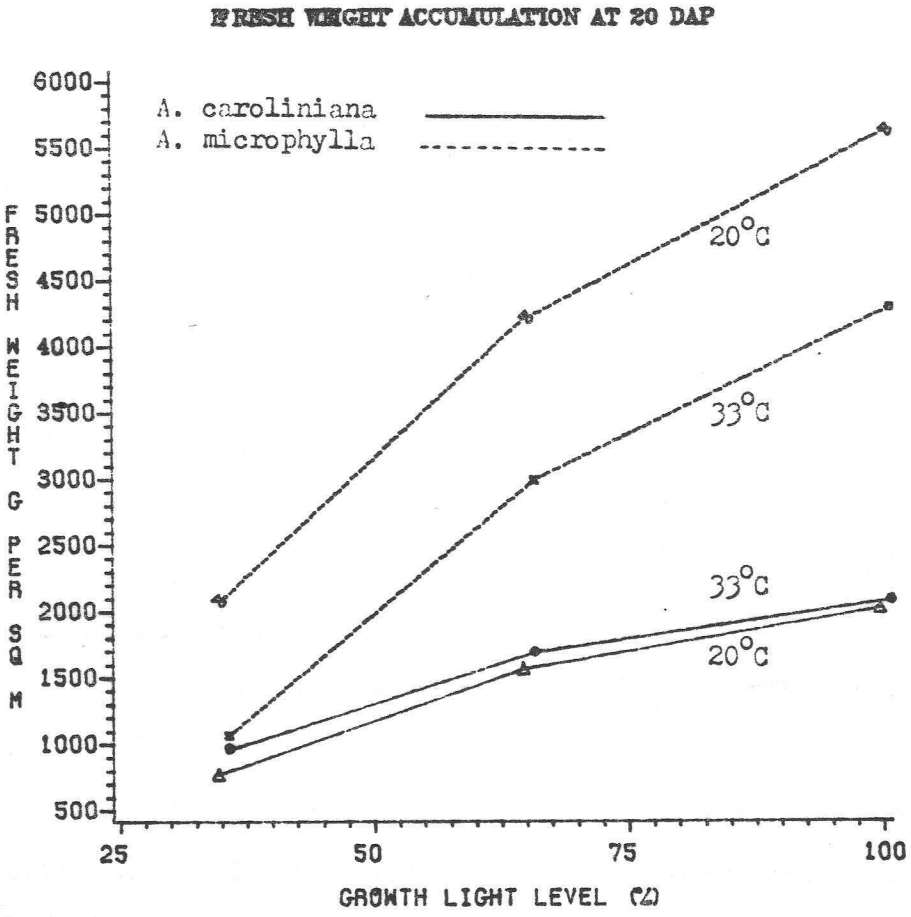


FIGURE 23. Effect of growth light level and temperature on fresh weight accumulation of Azolla species at 20 days after planting (DAP).

significnt effect on fresh weight accumulation of A. caroliniana at 20 DAP, but there was a significant reduction at 33 C in A. microphylla. Nevertheless the fresh weight produced by A. microphylla at 33 C was higher than that of A. caroliniana at both temperatures.

Dry matter content was higher in A. caroliniana than in A. microphylla regardless of temperature or light treatment (Table 23), a result consistent with those observed in Experiment 2 (Table 17) and Experiment 3 (Figure 14). In general, azolla grown at 33 C contained more dry matter than when grown at 20 C. The dry matter content of A. microphylla decreased with age and the rate of decline was sharper at 20 C, probably because of profuse growth. However, the dry matter content of A. caroliniana increased with age and the increase was much greater at 20 C than at 33 C. Peters et al. (1982) reported a drop in dry matter content with increasing temperature up to 30 C in A. caroliniana and an increase at temperatures above that level.

Chlorophyll content

The chlorophyll contents of both species were lower at 20 C than at 33 C (Figure 24). This may be due to fresh weight accumulating at a faster rate than the rate chlorophyll could be synthesized. The efficiency of light utilization at any time depends on the chlorophyll index, which is the chlorophyll content per unit land area. In spite of its significantly lower chlorophyll content, A. microphylla grown at 20 C had the highest chlorophyll index at 20 DAP (Figure 25). A well formed A. microphylla cover may be as efficient as that of

TABLE 23. Effect of temperature and light on dry matter content of two Azolla species at 10 and 20 days after planting (DAP).

Treatment		A. caroliniana		A. microphylla	
		10 DAP	20 DAP	10 DAP	20 DAP
		- - - - - - - - - % - - - - - - - - -			
Temperature	20 C	4.85	6.52	4.27	3.81
	33 C	5.45	5.73	4.54	4.10
Light	35%	4.72	5.92	4.00	4.23
	65%	5.17	6.18	4.41	3.76
	100%	5.55	6.25	4.80	3.88
*SE - Temperature (4)		0.36	0.24		
Light (8)		0.27	0.18		
Species (12)		0.29	0.23		

*Standard error of mean with error degrees of freedom in parentheses.

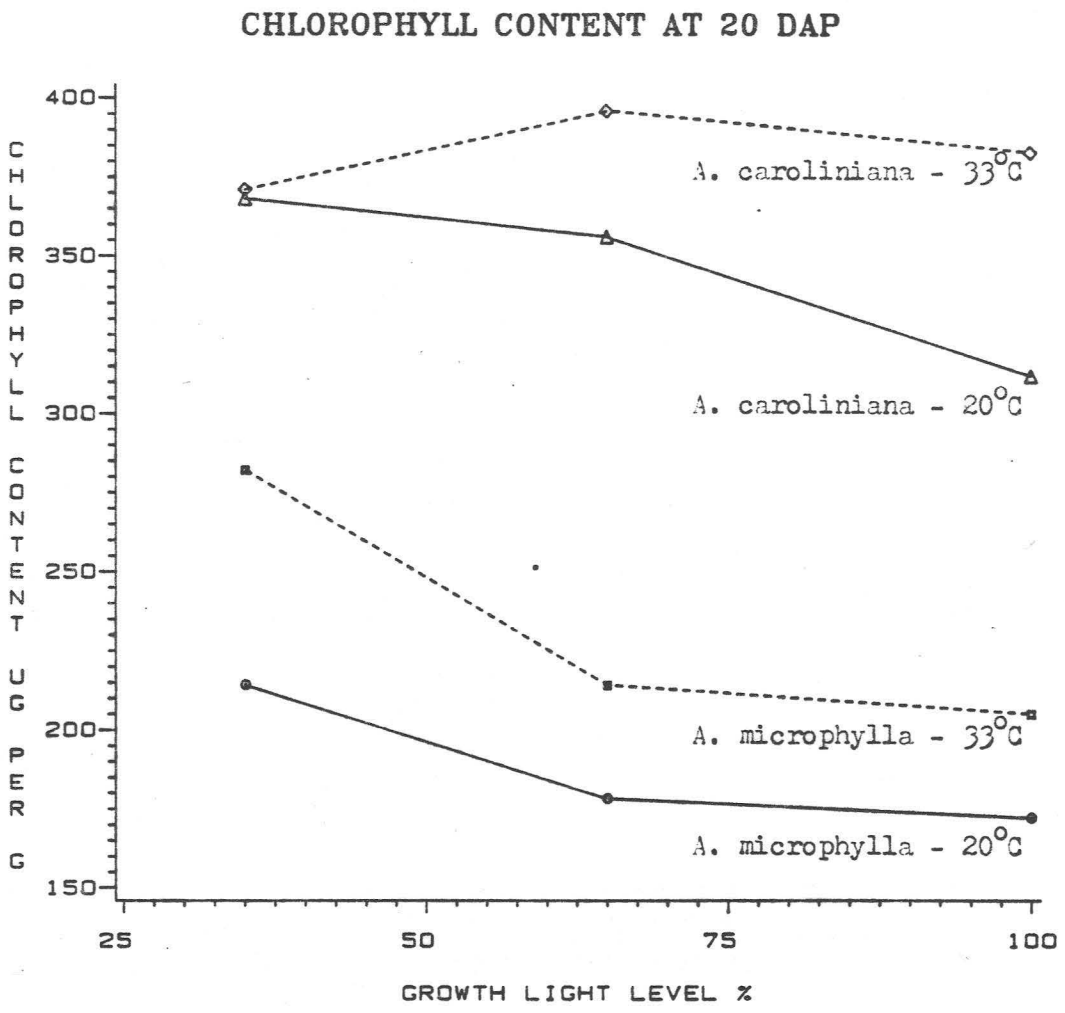


FIGURE 24. Effect of growth light level and temperature on chlorophyll content of Azolla species at 20 days after planting (DAP).

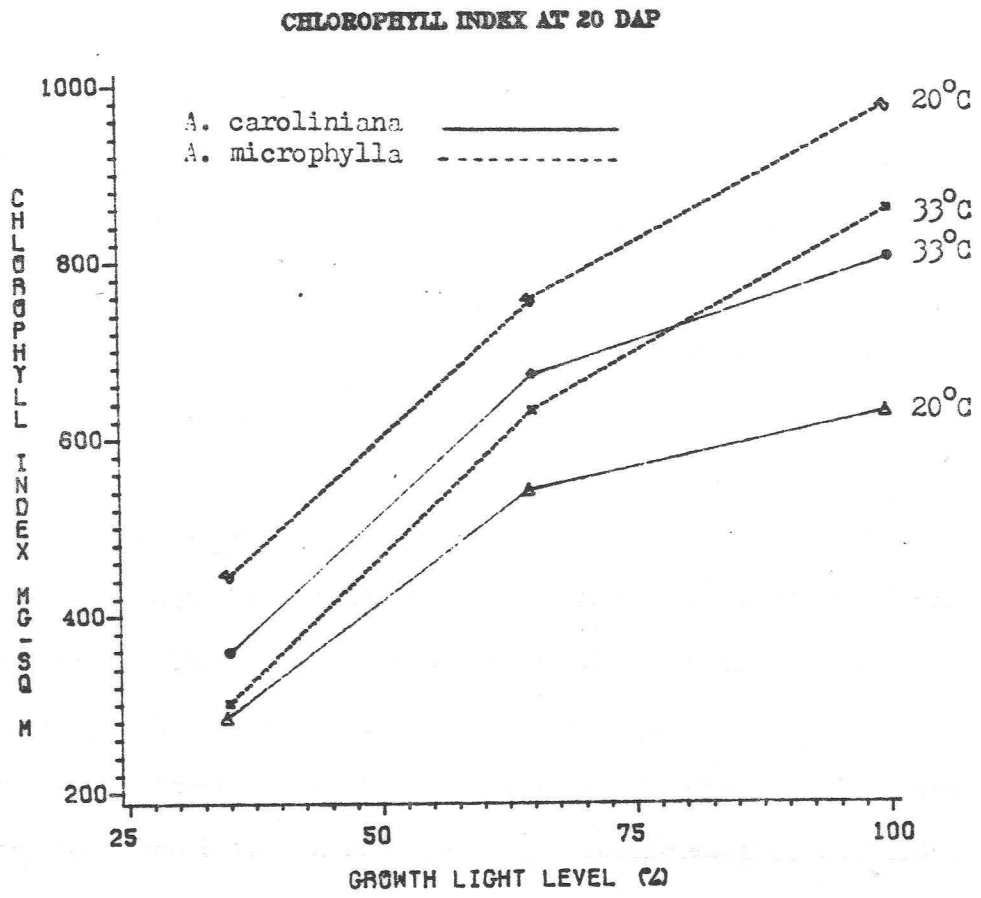


FIGURE 25. Effect of growth light level and temperature on chlorophyll index of Azolla species at 20 days after planting (DAP).

A. caroliniana in intercepting light because it compensates for low chlorophyll content by accumulating more biomass per unit area. Since the chlorophyll content of A. microphylla was low, a higher initial level of biomass would be required to have a chlorophyll index comparable to that of other species.

The chlorophyll a:b ratios were similar to those of Experiments 1 and 2. A. caroliniana had a lower chlorophyll a:b ratio than A. microphylla and the ratio changed very little with increasing light (Figure 26). The ratio in A. microphylla increased sharply with increasing light from 35% to 65%, particularly in azolla grown at 33 C. The temperature effect on the chlorophyll a:b ratio did not follow any particular pattern. Peters et al. (1982) found the chlorophyll a:b ratio of A. caroliniana to be insensitive to light and temperature in the 15 to 30 C range.

Nitrogen accumulation

The nitrogen content was higher at 20 C than at 33 C in both species at all light levels when harvested at 10 DAP (Table 24). When harvested at 20 DAP, nitrogen contents were higher at 33 C. As discussed previously under Experiment 3, rapid growth at 20 C may dilute the fixed nitrogen because of the requirement of new tissue for nitrogen. The data for A. microphylla at 20 DAP show this clearly. When A. microphylla had the highest fresh weights (Figure 23), it had the lowest nitrogen contents (Table 24) at all light levels. In other studies, nitrogen content increased with increasing temperature from 5

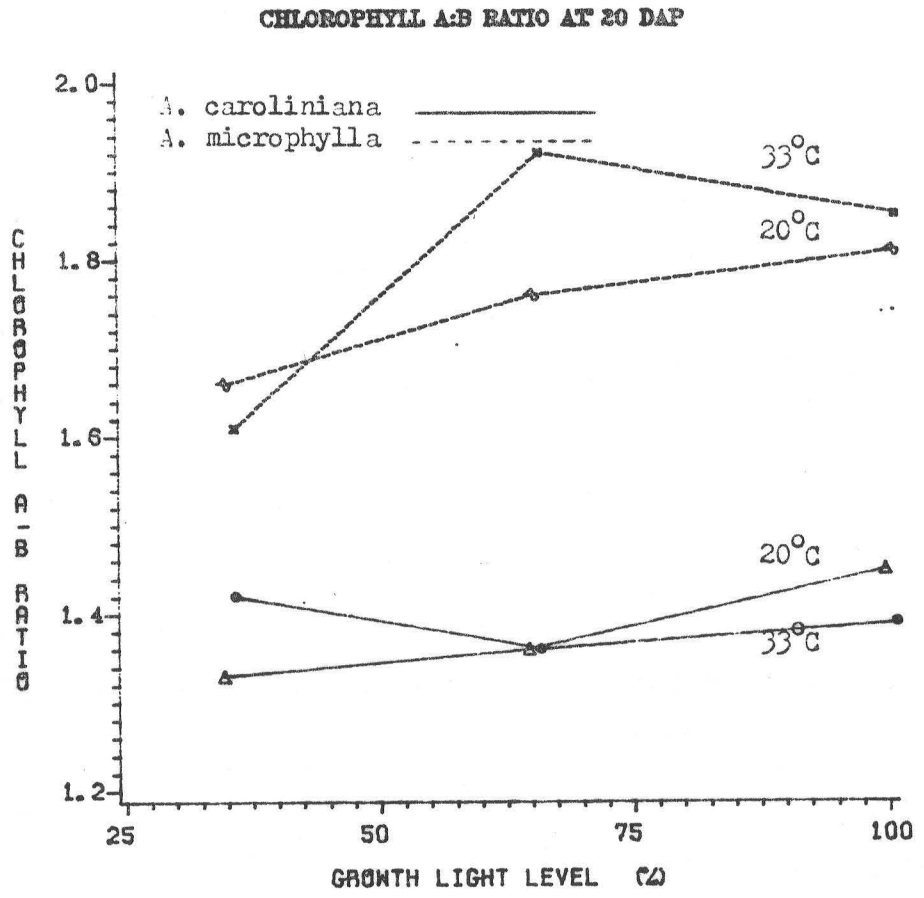


FIGURE 26. Effect of growth light level and temperature on chlorophyll a:b ratio of Azolla species at 20 days after planting (DAP).

TABLE 24. Effect of temperature and light on nitrogen content of two Azolla species at 10 and 20 days after planting (DAP).

Treatment		Light level		
		35	65	100
		- - - - - - - - - % - - - - - - - - -		
10 DAP				
A. caroliniana	20 C	5.15	5.31	4.80
	33 C	4.16	4.34	4.59
A. microphylla	20 C	4.58	4.11	3.75
	33 C	3.84	3.85	3.84
*SE - Temperature (4)		0.23		
Light (8)		0.22		
Species (12)		0.28		
20 DAP				
A. caroliniana	20 C	4.82	5.16	4.75
	33 C	5.47	5.82	5.69
A. microphylla	20 C	2.95	3.52	3.13
	33 C	4.54	4.50	3.95
SE - Temperature (4)		0.38		
Light (8)		0.14		
Species (12)		0.20		

*Standard error of mean with error degrees of freedom in parentheses.

to 30 C and then declined at higher temperatures (Peters et al. 1980; Talley and Rains, 1980).

A. caroliniana accumulated more nitrogen than A. microphylla at both temperatures when harvested at 10 DAP (Figure 27). However, rapid growth of A. microphylla during the 11-20 DAP period resulted in non-significant differences in accumulated nitrogen between the species at 20 DAP (Figure 28). Moreover at 20 DAP, nitrogen accumulation increased with increasing light level though the increase was not as great from 65 to 100% as it was from 35 to 65%.

When azolla is grown in the field, the harvest interval usually is kept short to prevent disease outbreaks and to maintain rapid growth. In such situations, knowledge of the differential response of species to light and temperature could be useful for species selection for a given environment.

CONCLUSIONS

The nitrogen production by a crop of azolla is a function of initial biomass, the RGR, period of cropping, tissue dry matter content and nitrogen content. The RGR at any given time was inversely related to azolla biomass present at that time. A biomass of 200 to 600 g m^{-2} covered the water surface and at the same time had RGRs above 300 mg g^{-1} fresh weight day^{-1}. Initial biomasses of 200 and 400 g m^{-2} had less fresh weight than an initial biomass of 600 g m^{-2} up to 21 DAP.

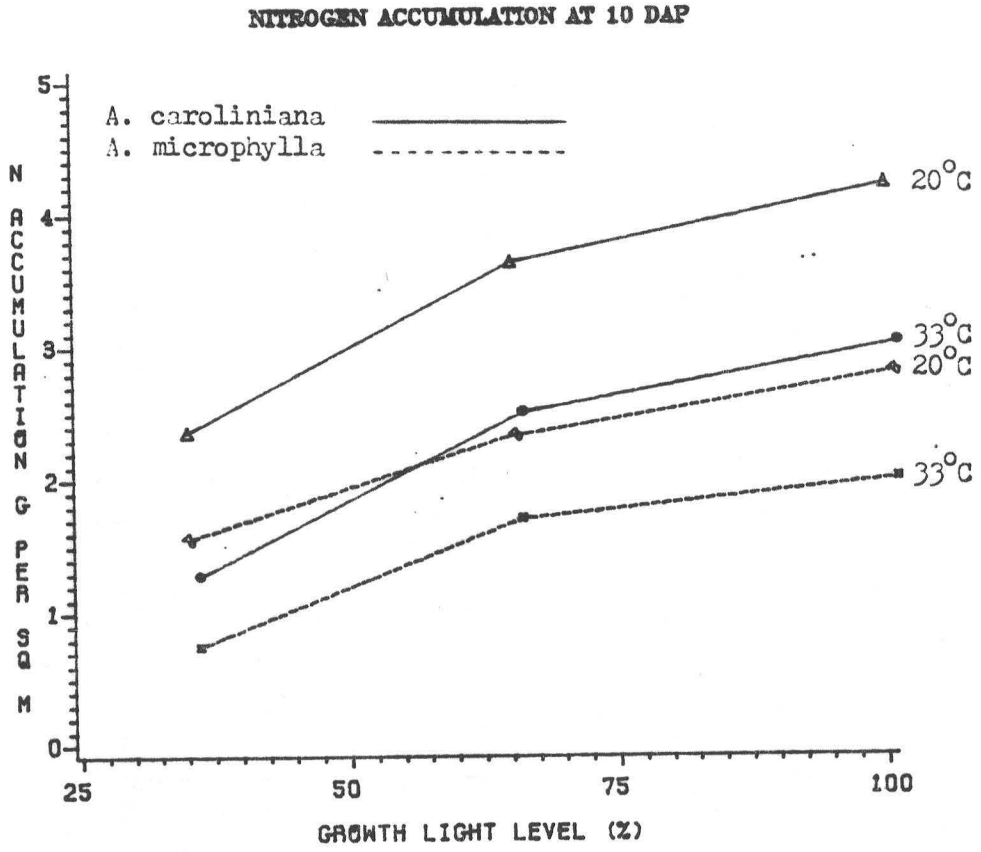


FIGURE 27. Effect of growth light level and temperature on nitrogen accumulation of Azolla species at 10 days after planting (DAP).

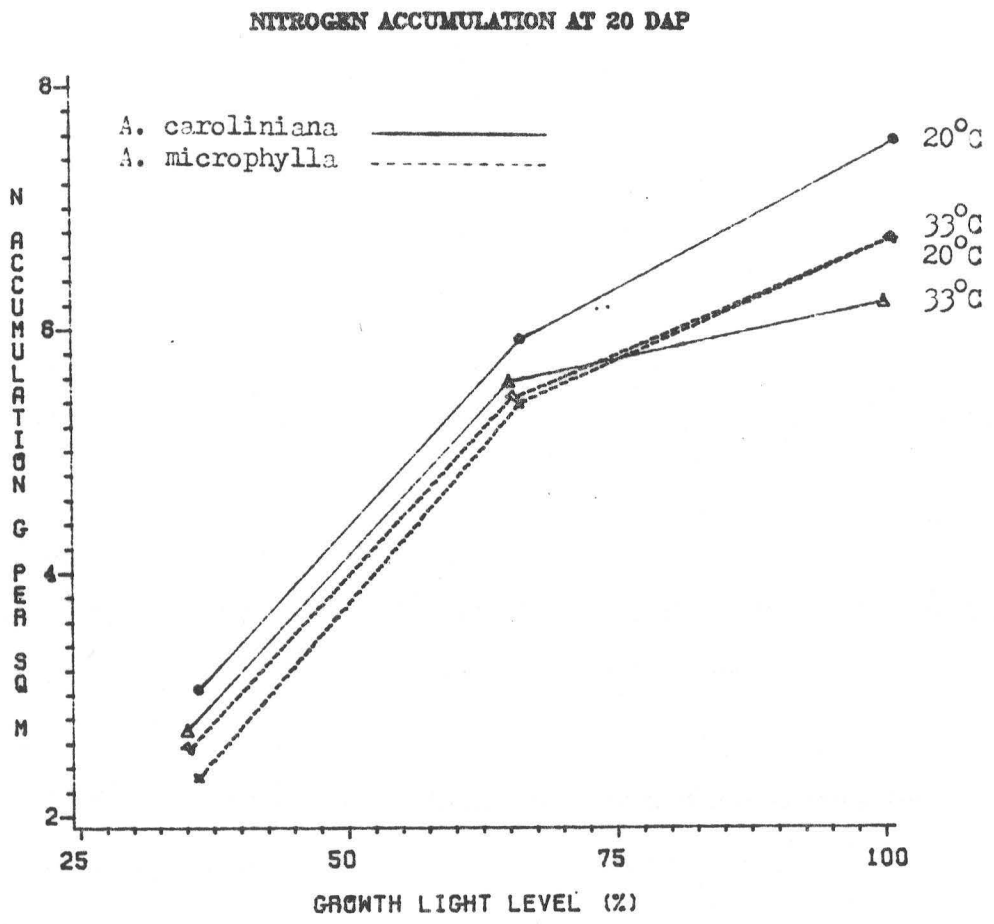


FIGURE 28. Effect of growth light level and temperature on nitrogen accumulation of Azolla species at 20 days after planting (DAP).

Among the three species that were investigated, A. microphylla accumulated more fresh weight than A. caroliniana and A. pinnata, particularly when the harvest interval was 20 DAP. A reason for this was that the fronds of A. microphylla grew upright following the formation of a dense surface mat. However, the fronds of this species had lower dry matter and nitrogen content than the other two species. Consequently, nitrogen accumulation in A. microphylla was generally lower than A. caroliniana despite its greater fresh weight accumulation.

Growth rate and fresh weight accumulation were reduced by shade in all species. As a result, species and initial biomass effects on fresh weight accumulation were evident only at 100% light. Contents of dry matter, chlorophyll and nitrogen were less influenced by light level in A. caroliniana and A. pinnata; but in A. microphylla, all three of these parameters declined with increasing light because of the upright growth at 100% light.

When the cropping period was 10 days, fresh weight accumulation was higher at 20 C in A. caroliniana and A. microphylla than at 33 C. This same trend continued at 20 DAP in A. microphylla, but A. caroliniana grew well at 33 C when the growth period was long. The higher temperature combined with heavy shade depressed biomass production in both species. Nitrogen accumulation was higher in all A. caroliniana treatments at 10 DAP, but no significant differences were observed between the species at 20 DAP.

CHAPTER V

INFLUENCE OF PHOTOSYNTHETIC PHOTON FLUX DENSITY, GROWTH LIGHT LEVEL AND FROND BIOMASS ON CARBON ASSIMILATION OF AZOLLA

INTRODUCTION

Most of the biomass accumulated in green plants is derived from carbon dioxide assimilated in photosynthesis. The carbon dioxide exchange rate (CER) provides a means of explaining the response of plants to environmental variation. CER is also related to transpiration rate as these two processes occur simultaneously in plants.

The rate of photosynthesis is usually expressed as the amount of CO_2 taken up per unit time per unit area of photosynthetic surface. The photosynthetic surface in azolla is difficult to measure accurately because the leaves are small and arranged compactly on the fronds. As biomass increases, the fronds become tightly arranged to form a mat. Therefore, the CER of azolla is more easily expressed on the basis of unit fresh weight, unit frond area, or unit chlorophyll content. Ray et al. (1979) presented CER of azolla on the basis of chlorophyll content. The frond area exposed to light can change if the mat is disturbed. For the rates to be representative of an undisturbed mat, CER should be measured without disturbance to the mat. Since evaporation from the azolla growth medium cannot be prevented during gas exchange measurements, the water vapor loss from

the canopy represents evapotranspiration (ET) rather than transpiration.

The effect of growth light level on chlorophyll content was discussed in the experiments presented in Chapter IV. Growth light level causes changes in chlorophyll content and frond characteristics such as thickness that may affect CER and overall dry matter accumulation. The objective of the studies reported in this chapter was to examine the influence of light and frond biomass on CER and ER of three azolla species and to relate the differences to foliar characteristics such as frond area and thickness.

MATERIALS AND METHODS

Experimental details

The plant material and culture solution used were similar to that described in Chapter IV. The plants were raised in the greenhouse and CER measurements were carried out in the laboratory in one-liter containers. In order to study the effect of growth light level on CER, azolla was multiplied under the respective light levels for one week. The azolla biomass was then reduced to 4 g per container on the day of 'planting'. Planting was staggered in such a way that six azolla containers were available for CER measurement on any given day. The treatments consisted of three species, three growth light levels and three photosynthetic photon flux densities (PPFD) replicated three times. The species were A. caroliniana, A. microphylla and A. pinnata and the growth light levels were 35%, 65% and 100% (control). The 35

and 65% light levels were established by covering the containers with neutral-density shade cloth. The containers were brought to the laboratory six days after planting and CER was measured under PPFD of 300, 800 and 1300 $\mu E\ m^{-2}\ s^{-1}$.

After CER was measured, fresh weight, chlorophyll content and frond area were determined. The method for chlorophyll determination is described in Appendix B; frond area of a 1.0 gram sub-sample was measured with a LI-COR LI-3100 area meter and total area was calculated by multiplying frond area per gram fresh weight by total fresh weight.

There appears to be some confusion regarding the terminology in azolla morphology. The structure containing the cyanobacterium is referred to as a leaf by Calvert and Peters (1981) and leaflet by Bozzini et al. (1982). The leaf of a fern is usually determinate and is called a frond. In azolla literature, however, a frond is a structure containing several indeterminate 'main stem axis' each bearing many leaves or leaflets. In the present study, the terminology used by Calvert and Peters (1981), as illustrated in Figure 29, is followed. The actual leaf area depends on leaf size and the number of leaves per unit frond length (leaf density). Leaf length and leaf density were measured with an optical micrometer.

The effect of azolla biomass on CER was measured for three species of azolla at three levels of biomass. Plant material for the study was raised in 15-liter buckets for 10 days. On the day of CER measurement, azolla was collected from the buckets and weighed

A. An azolla frond (Watanabe et al., 1977).

B. A 'main stem axis' with leaves; numerals refer to leaf age (Calvert and Peters, 1981).

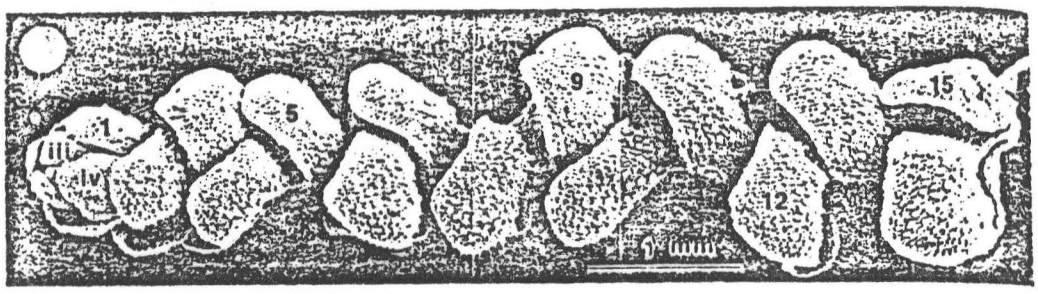


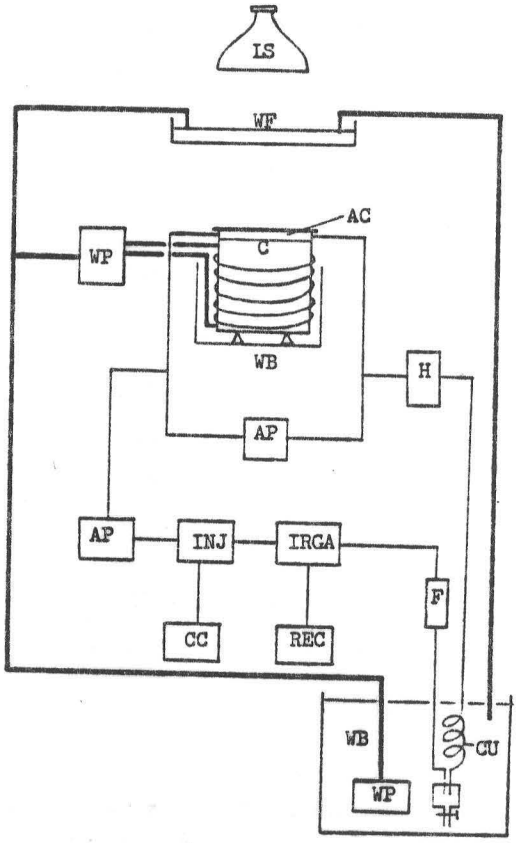
FIGURE 29. Illustration of azolla frond and leaves.

quantities were introduced into one-liter containers filled with culture solution. The quantities of azolla represented biomasses of 500, 1000, 1500 g m^{-2}. Azolla fronds were arranged on the culture solution surface to construct a frond mat. The CER of azolla in each container was measured under PPFDs of 500, 1000, 1500, 2000 and 2500 $\mu E\ m^{-2}\ s^{-1}$.

Gas exchange measurement

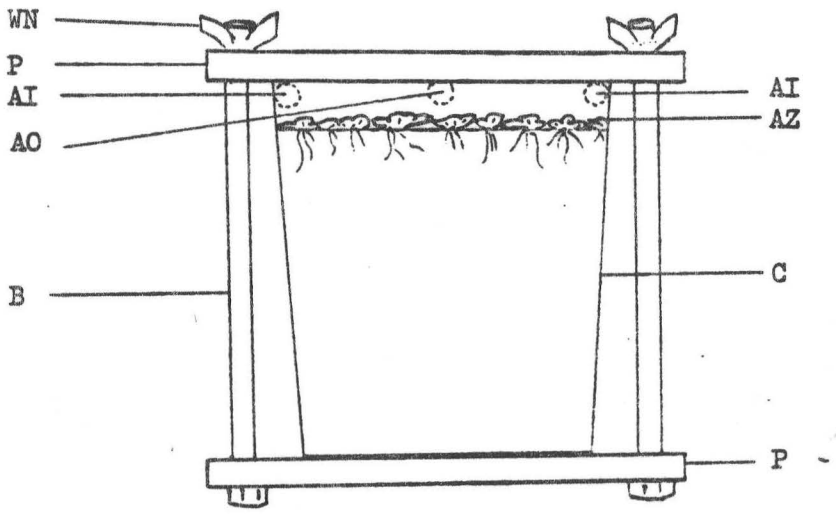
CER and ET were measured in a semi-closed gas exchange system. The components of the system were a Beckman IR 215A infrared gas analyzer (IRGA), a CO_2 injection apparatus, a General Eastern condensation dew point hygrometer, a copper coil condensor, a flow meter and the azolla assimilation chamber (Figure 30). The assimilation chamber (AC) was made by attaching a plexiglass cover to a cylindrical sleeve which was inserted into the one-liter azolla container and secured by bolts and nuts (Figure 31). The volume of AC was approximately 150 cm^3. An air pump, connected in series with the above components, circulated air through the system at 1.0 l min^{-1}. A second pump, connected only to the AC, moved air through the chamber at 8.6 l min^{-1} to minimize boundary layer effects.

The dew point temperature of air entering the assimilation chamber (D_i) was controlled by immersing the copper coil condensor in a temperature-controlled water bath (WB-1). The mean D_i was 14.9 C. The dew point temperature of air leaving the assimilation chamber (D_o) was measured with the condensation dew point hygrometer. The air



Air circulation system = ———— ;
Water circulation system = ———— ;
AC = assimilation chamber; AP = air pump; C = azolla container;
CC = CO_2 container; CU = condensation unit; F = flow meter;
H = dew-point hygrometer; INJ = CO_2 injector; IRGA = infra-red
gas analyzer; LS = light source; REC = recorder; WB = water bath;
WF = water filter.

FIGURE 30. Diagram of apparatus used for gas exchange measurements.



AI = air inlet; AO = air outlet; AZ = azolla mat;
B = bolt; C = azolla container; P = plexiglass;
WN = winged nut.

FIGURE 31. Diagram of assimilation chamber.

temperature (t) of the AC was maintained at 25 C by immersing the azolla container in a waterbath (WB-2).

Light from a 1000 watt Lucalox lamp housed in a parabolic aluminized reflector was filtered through a 3-cm water layer placed between the lamp and AC to reduce thermal radiation. A pump placed in WB-1 circulated cold water through the water filter to dissipate the heat. The light intensity on the surface of the azolla mat was varied either by changing the distance between AC and the lamp or by placing neutral-density shade cloth inside the water filter. PPFD was measured with a LI-COR Instruments LI-190SB quantum sensor and LI-1776 solar monitor.

The IRGA was calibrated daily with 99.9% N_2 and a standard gas containing 301 vppm of CO_2. The output of the IRGA was used to control the injection of CO_2 into the system to maintain the concentration between 275 to 350 vppm. The volume of CO_2 injected each time was 84 ul.

Azolla was equilibrated under the treatment light intensity for 10 to 15 minutes prior to making measurements. A steady-state CER of azolla was assumed to have been reached when D_o stabilized. The number of injections per unit time were obtained from a strip chart recording of the IRGA output.

Calculations

The following equations were used to calculate CER per unit fresh weight (CER_W) and per unit frond area (CER_A):

$$CER_W = (T/(T+t))(22.4\ V/t_h)(1/W)$$

$$CER_A = (T/(T+t))(22.4\ V/t_s)(1/A)$$

where CER_W is umol CO_2 g^{-1} fresh weight hr^{-1}, CER_A is µmol CO_2 m^{-2} frond area s^{-1}, T is absolute temperature (273.16 Kelvin), t is the air temperature in the AC = 25 C, V is the volume of pure CO_2 in ul injected into the system in t_h hours or t_s seconds, 22.4 is the uls per µmole, W is the total fresh weight in grams and A is the total frond area in m^2.

The difference in the dew point temperature of air entering (D_i) and leaving (D_o) the AC was used to calculate ET. The equations used in the calcualtion were:

$$P_i = 6.1078 \exp [17.269\ D_i/(D_i+237.3)]$$

$$P_o = 6.1078 \exp [17.269\ D_o/(D_o+237.3)]$$

$$W_i = P_i/[4618(t_a+273.16)]$$

$$W_o = P_o/[4618(t_a+273.16)]$$

$$ET = [F\ (W_o-W_i)]/A$$

where P is the water vapor pressure of air in mb, W is water vapor pressure in g cm^{-3}, F is flow rate through AC in cm^{-3} s^{-1}, A is frond area in m^2 and ET is evapo-transpiration rate in g m^{-2} s^{-1}. Subscripts i and o denote air entering and leaving the AC respectively.

The following parameters were derived based on the frond area and chlorophyll content:

1. Frond area index (FAI) = Frond area / Container surface area
2. Specific frond area (SFA) = Frond area / Fresh weight

3. Specific chlorophyll content (SCC) =

$$\frac{\text{Chlorophyll content}}{\text{Frond area}}$$

where SFA and SCC are expressed in $cm^2\ g^{-1}$ fresh weight and ug chlorophyll cm^{-2} frond area respectively.

RESULTS AND DISCUSSION

Influence of growth light level and PPFD on CER

The CER_W expressed on fresh weight basis represents canopy photosynthesis by azolla. When the three azolla species were grown at 100% light, A. caroliniana had a significantly higher CER_W than A. microphylla and A. pinnata at all light PPFDs (Table 25). Several factors may account for the higher CER_W of this species. A. caroliniana had a greater specific frond area (SFA) resulting in a significantly higher FAI than the other two species (Table 26). The compactly arranged leaves within a frond, as indicated by leaf density, further increased the photosynthetic area. The specific chlorophyll content (SCC) of A. caroliniana was comparable to that of A. pinnata and significantly higher than that of A. microphylla (Table 26). Thus, the greater CER_W of A. caroliniana is explained by the larger photosynthetic area and greater chlorophyll content of A. caroliniana. The lower CER_W of A. microphylla was associated with a significantly lower SFA and SCC. The CER_W of A. pinnata was lower than that of A. caroliniana because of lower SFA and leaf density despite the higher SCC.

TABLE 25. Effect of growth light level and photosynthetic photon flux density (PPFD) on carbon dioxide exchange rate of azolla expressed on fresh weight basis (CER_W).

Treatment	PPFD (uE m^{-2} s^{-1})		
	300	800	1300
	- - μmoles CO_2 g^{-1} fr. wt hr^{-1} - -		
a. Azolla species (averaged over growth light level)			
A. caroliniana	36.72 a*	56.32 a	66.55 a
A. microphylla	29.91 c	42.10 b	46.34 b
A. pinnata	33.58 b	48.57 b	50.67 b
b. Growth light level (averaged over species)			
35%	35.67 a	47.75	49.68 b
65%	35.68 a	51.34	61.13 a
100%	28.86 b	47.90	52.76 b
SE (16)**	1.61	4.28	4.55

*Means in a column within a category followed by the same letter are not significantly different at the 0.05 level of probability as determined by Duncan's multiple range test.

**Standard error of mean with error degrees of freedom in parentheses.

TABLE 26. Effect of Azolla species and growth light level on fresh weight, frond area index (FAI), specific frond area (SFA), specific chlorophyll content (SCC), leaf length and number of leaves per frond area (Leaf density).

Treatment	Fresh wt.	FAI	SFA	SCC	Leaf	
					Length	Density
	- g -		$cm^2\ g^{-1}$	$\mu g\ cm^{-2}$	mm	$no.mm^{-1}$
a. Azolla species (averaged over growth light level)						
A. caroliniana	6.55 b*	2.93 a	38.44 a	8.98 a	1.1**	3.28**
A. microphylla	6.88 ab	2.14 c	26.96 c	7.55 b	1.3	2.27
A. pinnata	7.20 a	2.46 b	29.33 b	9.07 a	1.6	2.22
b. Growth light level (averaged over species)						
35%	5.09 c	2.03 c	34.11 a	8.53	1.3	2.68
65%	6.98 b	2.55 b	31.25 b	8.68	1.4	2.63
100%	8.57 a	2.95 a	29.38 c	8.38	1.4	2.59
SE (16)***	.26	.12	.80	.34	-	-

*Means in a column within a category followed by the same letter are not significantly different at the 0.05 level of probability as determined by Duncan's multiple range test.

**Mean of 15 to 20 observations.

***Standard error of mean with error degrees of freedom in parentheses.

CER_W at all PPFDs increased with growth light level from 35% to 65% and declined at 100% (Table 25). Since CER measurement was done 6 DAP, the FAI at 100% light may have exceeded the optimum causing a decrease in CER_W. The azolla biomass in 100% light was above 1000 g m^{-2} for all species. Growth rates declined at biomasses greater than 600 g m^{-2} in greenhouse studies discussed in Chapter 4 (Figure 11). Therefore, the CER_W-biomass relationship may be similar to that between relative growth rate and biomass.

The SFA decreased with increasing growth light level (Table 26). An increase in leaf thickness with increasing light level is commonly observed in plants. A four-fold increase in light caused the leaf thickness to double in tomato (Charles-Edwards and Ludwig, 1975). Bannister and Wildish (1982) reported significantly lower SLAs in ferns from sunny habitats compared to the same species growing in shade. As shown in Table 26, the FAI was higher at 100% light despite the lower SFA due to the greater fresh weight at the highest light level. Growth light level did not affect SCC.

CER_W at a given growth light level increased with increasing PPFD (Table 25), but the increase from 800 to 1300 $\mu E\ m^{-2}\ s^{-1}$ was small. Light saturation may have occurred at 1300 $\mu E\ m^{-2}\ s^{-1}$ but no data were collected at higher PPFDs. Ray et al.(1979) observed light saturation at 400 $\mu E\ m^{-2}$ in A. caroliniana, but the azolla biomass and growth light level of their study is not given. Among other ferns, Botrychium virginianum and Pellaea atropurpurea light saturated at 800 and 2500 ft candles respectively (Ludlow and Wolf, 1975) while in

Pteris cretica saturation was at 20 to 25 W m^{-2} (Hariri and Prioul, 1978). Light saturation usually occurs at lower intensities when the measurement is made on a single leaf than when made on a plant canopy; because a plant canopy is multilayered, more light is required to saturate it (Gaastra, 1962).

There was no difference among the species at 800 or 1300 μE m^{-2} s^{-1} when CER was expressed on a unit frond area basis (Table 27). CER_A of A. caroliniana was significantly lower than the other two species at 300 μE m^{-2} s^{-1}. This may have been due to greater mutual shading in A. caroliniana which had a significantly greater FAI (Table 26). The maximal photosynthetic rates of ferns are generally low; the average is near 2 μmol m^{-2} s^{-1} (Nobel et al., 1984). The rates in the present study ranged from 2.7 to 5.5 μmol m^{-2} s^{-1}. The actual photosynthetic area of an azolla frond can be many times greater than the frond area because many leaves are borne on a frond. This may be a reason for the higher CER_A values in the present study than those reported for other ferns.

The evapo-transpiration rate (ET), which is expressed on a unit frond area basis, generally followed the same trend as CER_A and increased with increasing PPFD (Table 28). A. caroliniana had lower ET than the other two species. Whereas the CER_As of the three species were not different at the highest PPFD (Table 27), the ET rates differed significantly (Table 28). The ET of A. microphylla and A. pinnata were higher than A. caroliniana at all three PPFD. Since the CER_As of the three species were similar at 800 and 1300 μE m^{-2} s^{-1},

TABLE 27. Effect of growth light level and photosynthetic photon flux density (PPFD) on carbon dioxide exchange rate of azolla expressed on frond area basis (CER_A).

Treatment	PPFD (uE m^{-2} s^{-1})		
	300	800	1300
	- - μmoles CO_2 m^{-2} s^{-1} - -		
a. Azolla species (averaged over growth light level)			
A. caroliniana	2.66 b*	4.07	4.85
A. microphylla	3.08 a	4.41	4.82
A. pinnata	3.18 a	4.62	4.82
b. Growth light level (averaged over species)			
35%	2.93 b	3.90	4.05 b
65%	3.22 a	4.58	5.46 a
100%	2.77 b	4.62	5.00 a
SE (16)**	0.15	0.43	0.42

*Means in a column within a category followed by the same letter are not significantly different at the 0.05 level of probability as determined by Duncan's multiple range test.

**Standard error of mean with error degrees of freedom in parentheses.

TABLE 28. Effect of growth light level and photosynthetic photon flux density (PPFD) on evapo-transpiration rate of azolla.

Treatment	PPFD (uE m^{-2} s^{-1})		
	300	800	1300
	- - - - - - g m^{-2} s^{-1} - - - - - -		
a. Azolla species (averaged over growth light level)			
A. caroliniana	59.42 b*	67.14 c	69.26 c
A. microphylla	85.25 a	95.59 a	95.86 a
A. pinnata	78.83 a	79.86 b	83.55 b
b. Growth light level (averaged over species)			
35%	69.25 b	76.20 b	72.96 c
65%	79.04 a	73.26 b	81.96 b
100%	75.21 ab	93.12 a	93.75 a
SE (16)**	4.19	3.85	3.76

*Means in a column within a category followed by the same letter are not significantly different at the 0.05 level of probability as determined by Duncan's multiple range test.

**Standard error of mean with error degrees of freedom in parentheses.

the water use efficiency of A. caroliniana would be greater than the other two species. Although the water surface was completely covered by the azolla mats, evaporation would not have been totally prevented. The higher ET of the less compact canopy of A. microphylla suggests that evaporation was a significant component of ET.

Influence of frond biomass and PPFD on CER of azolla species

Significant differences were observed in CER_W among species, frond biomass and PPFD. As was shown previously (Table 25), the rates were higher for A. caroliniana than for A. microphylla and A. pinnata (Figure 32). CER_W increased with increasing PPFD and light saturation was attained around 1500 $\mu E\ m^{-2}\ s^{-1}$ in A. caroliniana and A. pinnata; A. microphylla saturated at about 1000 $\mu E\ m^{-2}\ s^{-1}$. CER_W was higher at the 500 g m^{-2} frond biomass than at 1000 and 1500 g m^{-2} biomasses (Figure 33). Since mutual shading would be greater in denser mats, the CER_W may have been reduced by competition for light. Light saturation at the lowest frond biomass occurred at around 1500 $\mu E\ m^{-2}\ s^{-1}$ while saturation was not attained in higher amounts of biomass. This is usually observed in canopies of higher plants because some leaves in the lower layers of the canopy are shaded and may never be light saturated.

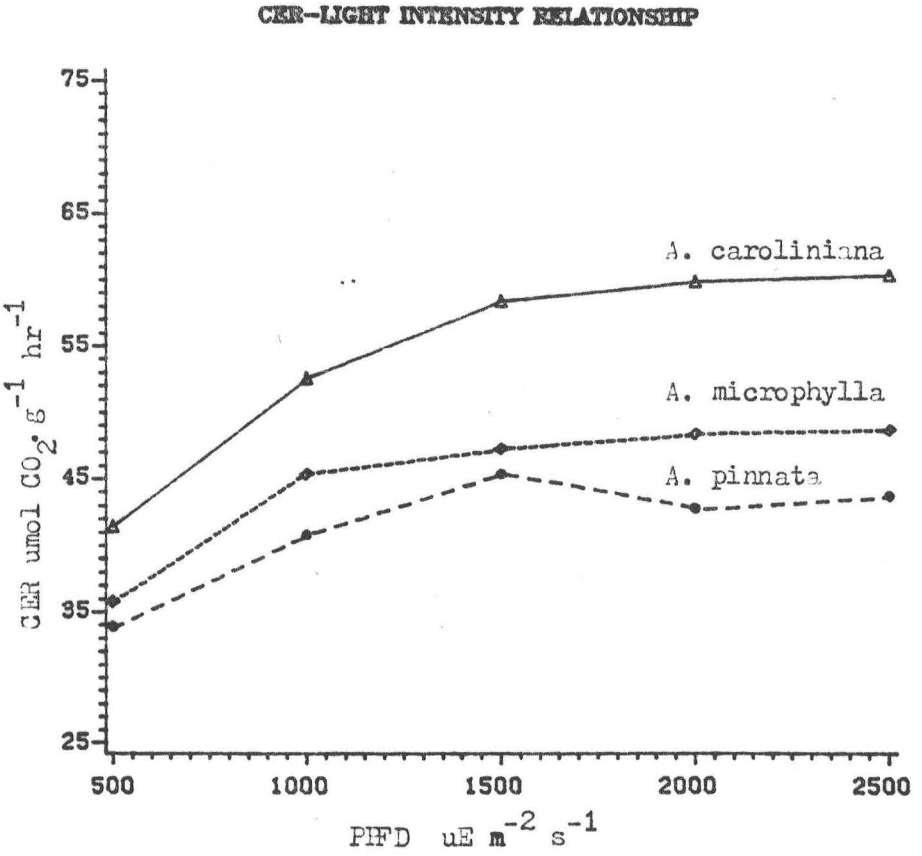


FIGURE 32. Effect of photosynthetic photon flux density (PPFD) on carbon dioxide exchange rate (CER) of Azolla species on fresh weight basis.

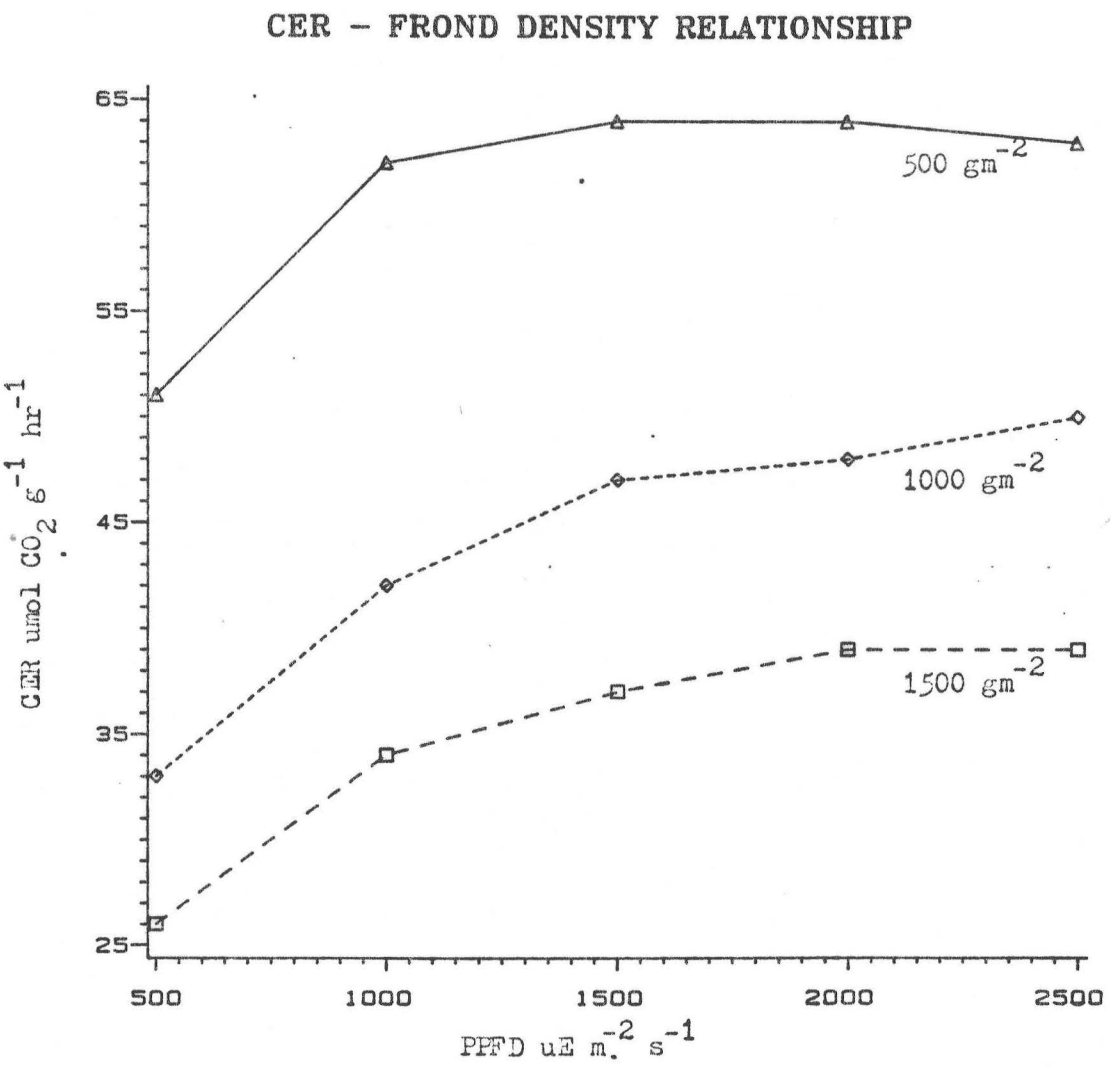


FIGURE 33. Effect of photosynthetic photon flux density (PPFD) and frond biomass on carbon dioxide exchange rate (CER) of azolla.

CONCLUSIONS

The differences in carbon dioxide exchange rate among azolla species were related to foliar characteristics. Specific leaf area, specific chlorophyll content and leaf density appear to be important determinants of CER as they are in other plant species. A. caroliniana had a higher SLA, SCC and leaf density which resulted in greater CER_W than A. microphylla and A. pinnata. All three of the above parameters were lower for A. microphylla which probably limited its CER_W. The limiting factors in A. pinnata were lower SFA and leaf density. The CER_W increased with growth light level from 35 to 65% and then declined, probably due to supra-optimal FAI in the control treatment. The ET of A. caroliniana was also lower than the other two species and therefore it may use water more efficiently in dry matter production. CER_W increased with increasing PPFD and decreased with increasing frond biomass. PPFD for saturation increased with increasing frond biomass.

CHAPTER VI

GENERAL DISCUSSION

Adaptation to warm climates is an essential requirement for the expansion of azolla cultivation in the tropics. The results of the field experiment with azolla showed that the azolla accessions A. caroliniana (Ohio) and A. pinnata (Indonesia) accumulated over 40 kg nitrogen ha^{-1} in 20 days when grown as an intercrop in the summer season (Table 6). In controlled-temperature experiments (Chapter IV), azolla growth was reduced by a combination of high temperature (33 C) and heavy shade (65%). Since azolla culture would be mostly limited to the first month after rice transplanting, heavy shade would not be a severe constraint when azolla is intercropped with rice. Fresh weight and nitrogen accumulation for the period from day 10 to day 20 was greater than for the period from day 0 to day 10 for A. microphylla at both 20 and 33 C and in A. caroliniana at 33 C when the growth light level was 65 or 100% (Figures 27 and 28). Therefore, a longer incorporation interval may be desirable, provided disease outbreaks are not encountered.

Nitrogen supplied to rice from intercropped azolla likely would be insufficient to meet the total nitrogen requirement of modern rice cultivars. Supplemental fertilizer nitrogen would be required to produce maximum yields. Timing of the application of fertilizer nitrogen is important to ensure that nitrogen from both sources are

fully utilized. If fertilizer nitrogen is added during intercropping, azolla may compete with the rice for nitrogen and the net gain in nitrogen would decrease. If fertilizer nitrogen application is delayed until after azolla incorporation, early growth of rice would be reduced due to nitrogen deficiency. An alternative would be to start the rice crop with preplant fertilizer nitrogen and introduce azolla 7 to 10 days after transplanting followed by incorporation at 10 day intervals over about three weeks. This may minimize absorption of fertilizer nitrogen by azolla. Moreover, the rice canopy would be sufficiently formed to provide some shade for azolla. However, this is possible only if rice seedlings are about 15 days old at transplanting; a heavier shade may develop sooner if older seedlings are transplanted. Another possibility would be to deep place fertilizer nitrogen so that its presence in flood water is minimized, but this may also reduce nitrogen availability to rice seedlings.

Initial azolla biomass and incorporation interval are the primary management considerations in maximizing nitrogen accumulation. In intensive cropping systems, management of the azolla biomass should be such that it is always maintained in the grand period of growth. This is achieved by using sufficient biomass to form a single-layer cover and reducing the mat thickness by incorporation when growth levels off. Based on the results of Experiment 3 in Chapter IV, the initial biomass for field conditions should be above 250 g m^{-2} and the incorporation interval about 7 to 10 days.

The time available for azolla culture has been an important factor in determining the initial biomass in China and Vietnam. A biomass cover sufficient enough to shade out green-algae is another consideration, especially during warm weather. The studies discussed in Chapters IV and V indicate that differences in frond characteristics should also be taken into account in determining the initial biomass. A. caroliniana had greater specific frond area than A. microphylla and A. pinnata (Table 26). Therefore, a comparatively lower amount of initial biomass of A. caroliniana would be required to obtain the same degree of surface cover formed by a higher amount of A. microphylla or A. pinnata.

Other frond characteristics observed in greenhouse experiments further confirmed that a higher initial biomass would be required for A. microphylla in order to efficiently intercept light. It had lower contents of dry matter (Figure 14), chlorophyll (Figure 15) and nitrogen (Figure 17) than A. caroliniana and A. pinnata. If the same initial biomass is used for all species, A. microphylla would have less chlorophyll per unit area than those species that have higher chlorophyll content per unit fresh weight; consequently, light interception and biomass accumulation would be lower.

The multiplication of azolla for field inoculation should also take into account the growth characteristics of the different species. Among the azolla species, A. filiculoides, A. microphylla and A. nilotica have an upright growth habit and form thicker frond mats than prostrate-growing species such as A. caroliniana, A. mexicana and A.

A. pinnata. Fronds from thicker mats of A. microphylla had lower dry matter and chlorophyll contents and their growth rate was slower when used as initial biomass. Therefore, precaution should be taken during multiplication of species such as A. microphylla not to permit the development of thick mats having high biomass.

Although fresh weight accumulation of A. microphylla was greater than A. caroliniana (figure 11), significant differences in dry matter accumulation was not observed (Figure 13) because of lower dry matter content in A. microphylla (Figure 14). Dry matter production is dependent on carbon assimilation rate. The carbon dioxide exchange rate per unit fresh weight (CER_W) was greater for A. caroliniana than A. microphylla (Table 25). Therefore, dry matter production of A. caroliniana would be expected to be higher than A. microphylla. This may be a reason for the lower growth rates of A. microphylla during the first few days after inoculation. However, when grown for 20 days, A. microphylla compensated for lower CER_W by having higher fresh weight per unit area. Both relative growth rate (RGR) (Figure 13) and CER_W (Figure 33) declined with increasing initial biomass probably due to mutual shading. Hence, the effect of an environmental factor on carbon assimilation rate is reflected in the growth rate.

Azolla is a source of low-cost nitrogen for lowland agriculture and also adds large amounts of organic matter which can be beneficial to continuously cropped soils. If an adaptable azolla accession is managed properly in a given environment, its contribution towards the nitrogen requirement of rice can be significant.

APPENDIX A. Analytical methods.

A. Determination of chlorophyll

Plant chlorophyll content was determined by the procedure of McKinney (1941) and Arnon (1949). Two grams of azolla was blended in a homogenizer with 40 ml of 80% acetone and 20 mg of $MgCO_3$ for two minutes. The homogenate was filtered through Whatman #1 filter paper and the extract was made to 50 ml with 80% acetone. The absorbence of the extract was determined with Spectronic-20 spectrophotometer at wavelengths of 645 and 663 nm. The total chlorophyll (C_t), chlorophyll a (C_a) and chlorophyll b (C_b) in mg l^{-1} were calculated according to the following equations:

$$C_t = 20.2\ A_{645} + 80.02\ A_{663}$$

$$C_a = 12.7\ A_{663} - 20.69\ A_{645}$$

$$C_b = 22.9\ A_{645} - 40.68\ A_{663}$$

where A_{645} and A_{663} were absorbance at 645 and 663 nm respectively. The values were converted to $\mu g\ g^{-1}$ fresh weight.

B. Determination of total nitrogen

Total nitrogen of plant tissue was determined by the method described by Mitchell (1972). The reagents required for this method are:

1. Salt mixture - 20:0.5:2.0 mixture of K_2SO_4, $FeSO_4$ and $CuSO_4$;
2. Solution A - 6.24 g NaOH in 1000 ml of water;
3. Solution B - 5 g phenol and 25 mg sodium nitroprusside in 500 ml of water;

4. Solution C - 2.5 g NaOH, 1.87 g Na_2HPO_4, 15.9 g $Na_3PO_40.12H_2O$ and 5 ml 5.25% sodium hypochlorite;

5. 1% EDTA solution adjusted to pH 10 with NaOH.

A sample of 0.05 g of oven-dried ground plant material was placed in a 75 ml digestion tube with one g of salt mixture and 5 ml concentrated H_2SO_4. The sample was digested at 375 C for three hours, allowed to cool and diluted to 75 ml with deionized water. An aliquot of 2 ml of the digest was transferred into a 100 ml volumetric flask followed by 1 ml EDTA, 5 ml of solution A, 10 ml of solution B and 10 ml solution C. The contents were diluted to 100 ml with deionized water and allowed to stand overnight for color development. The absorbence was then measured at 625 nm. The percentage nitrogen on dry weight basis was obtained from a standard curve. The standard curve was prepared by plotting the absorbance values for known nitrogen concentrations in the 10 to 50 ppm range.

APPENDIX A. (cont.)

Calender of events.

07.30.82 Planting pre-soaked rice seeds in nursery trays.

08.13.82 Transplanting of rice seedlings.

08.14.82 Introduction of intercrop azolla into rice plots.

08.24.82 First postplant fertilizer application.

First incorporation of azolla.

09.04.82 Second postplant fertilizer application.

Final incorporation of azolla.

09.10.82 Sampling at active tillering.

09.30.82 Sampling at panicle initiation.

10.27.82 Sampling at heading.

12.06.82 Harvesting of rice.

APPENDIX B. Significance probability values (PR>F) for F values.

Parameter	Azolla accession (A)	N application schedule (B)	A x B
a. Growth parameters			
Plant height - 48 DAT	0.082	0.012	0.477
75 DAT	0.081	0.002	0.302
115 DAT	0.008	0.357	0.110
Shoot dry wt - 48 DAT	0.732	0.155	0.311
75 DAT	0.146	0.003	0.200
LAI - 48 DAT	0.710	0.104	0.271
- 75 DAT	0.108	0.073	0.158
Tiller number- 48 DAT	0.918	0.068	0.152
Tiller number- 75 DAT	0.072	0.380	0.150
b. Yield and yield components			
Panicles hill^{-1}	0.202	0.235	0.170
Grains panicle^{-1}	0.152	0.675	0.715
Filled grain %	0.088	0.434	0.167
100 seed weight	0.403	0.962	0.759
Grain yield	0.145	0.082	0.668

APPENDIX C. Average temperature (TEMP) and relative humidity (RH) inside the greenhouse.

Experiment	TEMP	RH
	- C -	- % -
Experiment 1		
1 - 7 days	30.6	50
8 - 14 days	33.1	51
15 - 21 days	34.2	48
22 - 28 days	31.0	54
Experiment 2		
1 - 7 days	33.4	58
8 - 14 days	32.4	51
15 - 21 days	34.0	47
Experiment 3		
1 - 10 days	28.5	50
11 - 20 days	28.3	48

APPENDIX D. Significance probability values (PR>F) for F values of azolla main effects (Chapter 4, Experiment 3).

Parameter	Species	Biomass	Light
Fresh weight 10 DAP	0.001	0.001	0.001
Fresh weight 20 DAP	0.001	0.001	0.032
Dry weight 10 DAP	0.001	0.001	0.001
Dry weight 20 DAP	0.265	0.001	0.001
Nitrogen content 10 DAP	0.002	0.096	0.509
Nitrogen content 20 DAP	0.001	0.087	0.687

APPENDIX E. Sample ANOVA of nested-plot analysis for dry matter content at 10 DAP.

Source	df	SS	MS	F	P>F
Temperature	1	1.690	1.690 <—	4.27	[a]ns
Replicate	2	0.488	0.244		
Temp x Rep	2	1.098	0.549		
Error a [Rep+(Temp x Rep)]	4	1.586	0.396 —		
Light	2	3.987	1.994 <—	9.02	.001
Light x Temperature	2	2.165	1.082 <—	4.90	.05
Error b (Rep x Temp x Light)	8	1.768	0.221 —		
Species	1	4.950	4.95 <—	19.64	.001
Species x Light	2	0.001	.0005 <—	.0001	ns
Species x Temperature	1	0.251	0.251 <—	1.00	ns
Species x Temp x Light	2	0.250	0.125 <—	0.50	ns
Error C (Residual)	12	3.024	0.252 —		
Total	35	19.672			

[a]Not significant.

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