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Predictive Models for the Growth Response of Eight *Azolla* Accessions to Climatic Variables[1]

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ABSTRACT

Although the freefloating aquatic fern *Azolla pinnata* has been grown for centuries in northern Vietnam and southeastern China as a nitrogen-fixing green manure for rice and as a fodder for pigs, little is known of the environmental tolerance of the seven known *Azolla* species. In this study, the relative growth rates (RGR) of *A. caroliniana*, *A. filiculoides*, *A. mexicana*, *A. microphylla*, *A. nilotica*, *A. pinnata* var. *pinnata*, *A. pinnata* var. *imbricata*, and *A. rubra* were evaluated over a wide range of climatic conditions. The species were grown on nitrogen-free nutrient solution under a glass roof at a site where air temperatures ranged from −7°C in the winter to 38°C in the summer. The RGR was calculated from increases in dry weight over 26 growth cycles of one week to 1 month. The RGRs of individual species were most closely related to minimum water temperature during spring and fall (R^2 = 0.67 to 0.99); minimum water temperature and daylength for spring plus fall (R^2 = 0.59 to 0.92); and minimum water temperature, solar radiation, and relative humidity during summer (R^2 = 0.66 to 0.85). This study suggests that temperature, daylength, solar radiation and relative humidity can be used for estimating the RGR of *Azolla*.

Additional index words: ***Azolla caroliniana*, *Azolla filiculoides*, *Azolla mexicana*, *Azolla microphylla*, *Azolla nilotica*, *Azolla pinnata*, *Azolla rubra*, Relative growth rate, Regression.**

THE nitrogen-fixing fern-alga symbiosis, *Azolla Anabaena*, is used in certain parts of the world as an aquatic green manure and fodder (Moore, 1969; Lumpkin and Plucknett, 1980, 1982; Li et al, 1982). Two of the seven known species of *Azolla*, *A. pinnata* var. *imbricata* and *A. filiculoides*, are presently cultivated on over 2 million ha in Vietnam and China. Cultivation is primarily limited to winter and spring because of low productivity during summer and early autumn.

Studies have been carried out on the effects of single climatic variables, usually temperature, on the relative growth rate of *Azolla* (Lu et al., 1963; Tran and Dao, 1973; Ashton, 1977; Peters et al., 1980; Talley and Rains, 1980; Watanabe and Berja, 1983), but none of the studies examined the interactive effects of more than one climatic variable on the relative growth rates (RGR) of all known *Azolla* species.

In growth chamber and glasshouse studies, Peters et al. (1980) found that the relative tolerance of four species to a constant temperature of 40°C was *A. mexicana* >*A. pinnata* >*A. caroliniana* >*A. filiculoides*. Maximum growth rates at a photon flux density of 400 μmol m^{-2} s^{-1} were measured at either 25 or 30°C. In phytotron studies, Watanabe et al. (1981) reported that under 30 klux light for 12 h per day, maximum growth rates for *A. mexicana*, *A. pinnata*, and *A. caroliniana* occurred at 37/29°C (day/night temperatures). *Azolla filiculoides* did not survive such high temperatures.

The performance of an accession, as indicated by its RGR in a particular environment, is probably the most important criterion for the selection of a cultivar for use as a green manure. The RGR is important because very little time is available to accumulate *Azolla* biomass in most rice cropping systems. Maximum RGRs reported for the known species differ considerably. In a review of the literature by Becking (1979), maximum RGRs (g kg^{-1} day^{-1}) were: *A. caroliniana*, 413; *A. filiculoides*, 239; *A. mexicana*, 248; and *A. pinnata* var. *imbricata*, 334. No data on growth rates of *A. microphylla*, *A. nilotica*, and *A. rubra* were found in the literature. Also, no studies were found where all the species were compared simultaneously.

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The need for information about the adaptability of *Azolla* species to different environments caused us to conduct a study of the growth rates of all known species in response to a wide range of climatic conditions (Lumpkin, 1983). Data generated from this research were used to develop models for predicting RGR in response to climate (Splinter, 1974; Monteith, 1981).

MATERIAL AND METHODS

Plant Material

Eight accessions of *Azolla*, including the seven known species of the genus (Fowler and Stennett-Willson, 1978; Lumpkin and Plucknett, 1982), were used for the study (Table 1). *Azolla pinnata* was represented by two of its recognized varieties. The accessions were obtained from collections of vegetative material maintained on N-free nutrient solution in Hawaii and China.

Cultivation Site

The experiment was conducted at the Zhejiang Academy of Agricultural Sciences in Hangzhou, China (30.19°N 120.12°E). The Academy is located on an alluvial plain at sea level, and has a continental climate characterized by hot summers and cold winters. Mean temperatures at the Academy are approximately 27.9°C in July and 4.1°C in January and extremes of 42.1°C and −10.1°C have been recorded (Chu, 1937; Wu, 1980).

Culture Conditions

The experiment consisted of 26 growth cycles over a 1 year period. Each treatment (*Azolla* accession) was replicated six times in separate pots and the individual pots were arranged in a completely randomized design. The pots were made of a glazed ceramic with a volume of 6.6 L (20 cm diam, 21 cm high) and were placed under a glass-roofed structure with open sides. The roof was approximately 2.5 m high and allowed for near ambient light and air conditions but excluded precipitation.

All accessions were preconditioned by growing them under the glass roof in N-free culture solution for 2 months before RGR studies began. A second culture of all accessions was maintained at the experiment site with sufficient protection to assure a source of inoculum when climatic extremes eliminated any accessions from a growth cycle. Protective measures included partial shading during summer and heating during winter to prevent loss of an accession from the reserve culture.

Each pot was inoculated with 5 g of fresh *Azolla*, an amount equivalent to 160 g m^{-2} or 1.6 Mg ha^{-1} over the 314 cm^2 pot surface. Growth cycles from inoculation to harvest varied from one week during summer to one month during winter. These periods allowed *Azolla* biomass to approach but not exceed one-third of the maximum fresh weight biomass attained by species which form relatively thin mats. Since, biomass at harvest did not exceed 800 g m^{-2} or 8 Mg ha^{-1}, it was assumed that growth of the accessions was potentially exponential and not limited by competition.

After measuring fresh weight at the end of each growth cycle, 5 g were used as inoculum for the next growth cycle to eliminate the need for pre-conditioning of inoculum. When accessions perished during hot or cold weather, the reserve cultures were used as a source of inoculum. Six liters of fresh nitrogen-free nutrient solution were used at the beginning of each cycle. This nutrient solution contained all essential plant nutrients plus molybdenum, cobalt (Becking, 1979), and sodium (Olsen, 1972) for the cyanobacterium, and was maintained at a pH in the range of 5 to 6.

Table 1. Sources of *Azolla* accessions used for the growth study.

Section Species Variety	Collection site Lat.	Long.	Source	Country
Azolla				
A. caroliniana	39.47°N	83.54°W	G. A. Peters	USA
A. filiculoides	52.30°N	13.30°E	Li Z. X.	East Germany
A. mexicana	38.20°N	121.30°W	S. N. Talley	USA
A. microphylla	0.38°S	90.20°W	T. A. Lumpkin	Galapagos Islands
A. rubra	34.00°N	136.00°E	K. Seto	Japan
Rhizosperma				
A. nilotica	13.09°N	32.39°E	T. A. Lumpkin	Sudan
A. pinnata				
var. *pinnata*	8.00°N	6.00°W	C. VanHove	Ivory Coast
var. *imbricata*	30.19°N	120.12°E	Li Z. X.	China

The harvested *Azolla* was placed under shade between two thick layers of blotting paper for approximately 1 h before measuring fresh weight. Relative growth rate (g kg^{-1} day^{-1} dry wt.) was calculated by the formula:

$$RGR = (\ln W_2 - \ln W_1)(t \cdot 10^3)^{-1}$$

where W_1 = initial weight, W_2 = final weight and t = number of days of growth (Evans, 1972; Mitchell, 1974).

Air temperature and percent relative humidity were measured with a hygrothermograph and pot water temperatures were recorded with maximum-minimum thermometers. Light intensities were measured with a lux meter and cloud cover was estimated daily at 0900, 1200, and 1500 h. Irradiance was calculated by methods proposed by Thompson (1976), from clear sky solar radiation for 30°N latitude and the measured light intensities and estimated cloud cover. Daily precipitation data were obtained from the Zhejiang Province Meterological Station at Hangzhou.

Data Analysis

Each growth cycle was analyzed for ANOV using all replicates of each surviving accession. The RGRs of individual accessions across growth cycles were used in regression to develop predictive models for individual accessions. Combined RGRs of all accessions within growth cycles were used to calculate correlation with climatic variables.

RESULTS AND DISCUSSION

Weather During the Study Period

The study period extended from December 1979 through November 1980. January 1980 was the coldest month with a mean air temperature of 4.4°C and a low extreme of −7.3°C. The mean July 1980 air temperature fell exactly on the 10-year mean of 27.9°C and had a high extreme of 37.7°C. Water temperatures in the *Azolla* root zone followed a similar pattern (Fig. 1). Mean relative humidity ranged from 55 to 83% and the site had 207 days with precipitation which totaled 1534 mm during the study period.

RGR Over the 1-Year Study Period

Relative growth rates of the eight *Azolla* accessions were calculated after each of the 26 growth cycles during the period December 1979 to November 1980. The coefficients of variation for these growth cycles varied between 4.0 and 20.1% with an average of 9.2%. Only three accessions (*A. caroliniana*, *A. filicu-*

loides, A. pinnata var. *imbricata*) had sufficiently wide climatic adaptation to survive most of the year (Fig. 1a and 1b). The other five accessions either did not survive the colder months (day 0 to about day 120, Fig. 1c and 1d), or the hotter months (approximately day 200 to day 260, Fig. 1b and 1d). The RGR of all accessions showed two peaks during the year, once in the spring and again in the fall (Fig. 1). For seven of the eight accessions, these peaks occurred at about day 175 and day 270.

The RGRs of all accessions were poorly correlated with any single climatic variable or combination of variables over the entire 1-year study period. No single climatic factor could account for greater than a 61% of the variation in RGR. Also, no combination of up to three climatic variables and their interactions could satisfactorily account for the variability in the RGRs of the eight accessions over the 1-year study period. For the whole year, RGR was most highly correlated with minimum water temperature ($r=0.61$, significant at $P=0.01$).

Most of the data used in the combined analysis for the whole year were collected during cool to cold weather during spring and fall when RGRs were low. The RGR was positively correlated with minimum water temperature, presumably because growth increased in the spring as water temperature increased toward optimum and decreased in the fall as water temperatures decreased away from optimum. In contrast to spring and fall, RGRs during the agriculturally important summer season were high and negatively correlated with minimum water temperature (Fig. 1, Table 2). We believe that this negative correlation resulted from temperatures being either at or above the optimum for growth during most of the summer.

Because of the poor correlation of climatic variables with RGRs for the whole year, the year was di-

Table 2. Coefficients of correlation (r) between *Azolla* relative growth rates (Y) and mean minimum water temperatures (X). The calculations are based on the mean of six replications of each accession from each growth cycle (n).

	Year		Spring		Fall		Cool		Summer	
Azolla accession	r	n†	r	n	r	n	r	n	r	n
A. caroliniana	0.76	26	0.97	7	0.89	6	0.93	13	−0.70	11
A. filiculoides	0.57	26	0.95	7	0.96	6	0.66	13	−0.81	11
A. mexicana	0.48	22	0.94	6	0.99	5	0.71	11	−0.57	11
A. microphylla	0.62	22	0.89	6	0.88	5	0.79	11	−0.20	11
A. nilotica	0.17	21	0.83	6	0.82	5	0.49	11	−0.75	10
A. pinnata var. *pinnata*	0.58	19	0.83	6	0.96	6	0.72	12	−0.72	7
A. pinnata var. *imbricata*	0.85	26	0.94	7	0.97	6	0.89	13	−0.22	11
A. rubra	0.52	17	0.97‡	6	0.91	5	0.49‡	11	--	--

† Number of samples. Samples are mean RGRs from individual growth cycles.
‡ Observation of 27 June deleted from model.

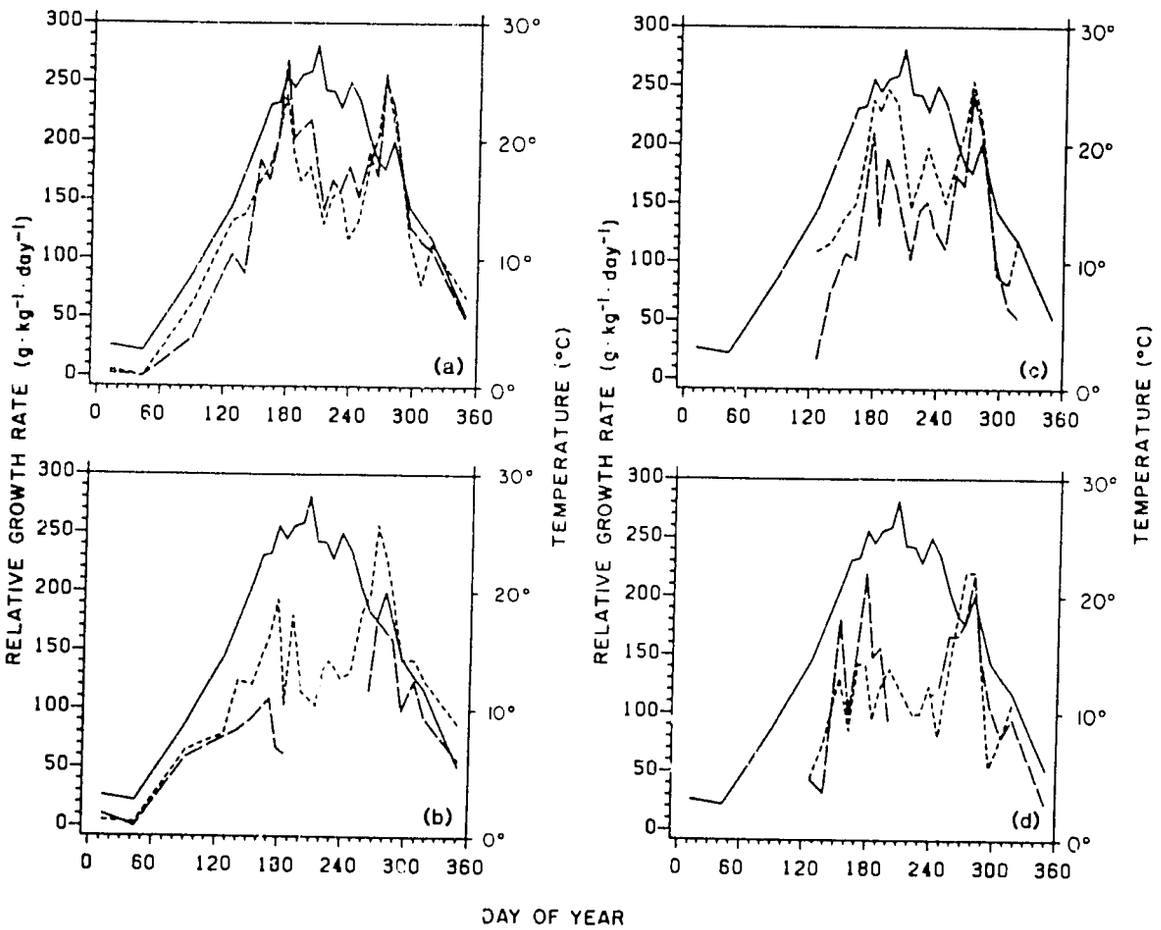

Fig. 1. Minimum water temperature (solid line) and relative growth rates of (a) *A. caroliniana* (short dashes) and *A. pinnata* var. *imbricata* (long dashes), (b) *A. filiculoides* (short dashes) and *A. rubra* (long dashes), (c) *A. microphylla* (short dashes) and *A. mexicana* (long dashes), and (d) *A. nilotica* (short dashes) and *A. pinnata* var. *pinnata* (long dashes) during the whole year. Lines connect averages from each growth period.

vided into two periods to evaluate the seasonal influence of climatic variables on RGR. These two periods were defined as a COOL period of increasing or decreasing temperature and increasing or decreasing RGR (spring and fall) from March to June and October to December, and a summer season of high temperatures and fluctuating RGR from July through September.

RGR During the Cool Period (Spring plus Fall)

Similarities in the r values between RGR and climatic variables for the spring and fall suggested the possibility of combining the results into a single data set (Table 2). However, combining the spring and fall data (cool) generally reduced the R^2 values of a minimum water temperature only model relative to that obtained for the same model in spring and fall. This problem was overcome by using a multiple regression model where temperature and daylength accounted for the majority of the variation in RGR, and resulted in relatively high R^2 values for the COOL season data (Table 3). The RGR was positively correlated with daylength during spring and fall, and its inclusion in the multiple regression analysis accounted for variation in RGR not explained by temperature alone. Daylength apparently corrected for the fact that spring days of a given length are generally cooler than fall days of the same length due to the thermal buffering capacity of the earth.

The ranking of RGRs for cold tolerant accessions during the cool period was *A. filiculoides* > *A. caroliniana* > *A. pinnata* var. *imbricata* > *A. rubra*; an order similar to separate rankings in spring and fall. *Azolla filiculoides* and *A. caroliniana* were ranked first and second because their productivity at low temperatures exceeded that of *A. pinnata* var. *imbricata*, and their productivity over the range of COOL temperatures far exceeded that of *A. rubra*.

RGR During the Summer Season

Relative growth rates during the summer season were not linearly related to one or a combination of two climatic variables as was the case during the cool period. Plots of RGRs for the year (Fig. 1) show that summer season RGRs were characterized by peaks and valleys that were unrelated to individual climatic variables.

The failure of simple regression analysis to account for the variability in RGR during summer (Table 4) prompted an analysis by multiple linear regression. All possible combinations of up to four independent variables were evaluated. *Azolla pinnata* var. *pinnata* and *A. rubra* were not included in this evaluation because of an insufficient number of observations. High R^2 values were obtained for every accession with the use of a model containing minimum water temperature, irradiance, and relative humidity (Table 5). Irradiance was positively correlated with maximum water temperature (0.65) and thus represented it through interaction (Table 4).

The RGRs measured in summer were highly variable due to the hot and humid conditions which were conducive to growth of a *Rhizoctonia* sp., a fungal pathogen. All eight accessions either died or stopped growing for three days at the end of the 20 July growth cycle when water temperatures averaged 36.6°C for the week with an extreme on 1 day of 39.7°C; low water temperatures averaged 28.1°C. The high temperature effect on the accessions was exacerbated by fungal attack.

The fungal pathogen, *Rhizoctonia* sp. has been found with *Azolla* everywhere in the world under hot, humid conditions (Shahjahan et al., 1980; Arunyanart et al., 1982; Venkataraman and Kannaiyan, 1984). benomyl, PCNB, Ceresan (ethylmercury chloride), and Nirit (2,4-dinitrophenyl thiocyanate) can be used to control *Rhizoctonia* (Lumpkin and Plucknett, 1982)

Table 3. Best fit multiple regression equations and coefficients of determination for RGR (Y) and environmental parameters during the COOL season. The model includes mean minimum water temperature (X_1) and daylength (X_2).

Azolla accessions	n	Regression equations R^2 and contribution of each variable
A. caroliniana		$Y = 97.5 + 10.5X_1 + (-0.168X_2)$
	13	$0.89 = 0.86 + 0.03$
A. filiculoides		$Y = 374 + 11.2X_1 + (-0.561X_2)$
	13	$0.91 = 0.44 + 0.47$
A. mexicana		$Y = 298 + 19.4X_1 + (-0.709X_2)$
	11	$0.88 = 0.56 + 0.32$
A. microphylla		$Y = 1035 + 17.0X_1 + (-2.88X_2)$
	11	$0.77 = 0.63 + 0.14$
A. nilotica		$Y = 241 + 14.0X_1 + (-0.465X_2)$
	11	$0.59 = 0.24 + 0.35$
A. pinnata var. *pinnata*		$Y = 325 + 16.7X_1 + (-0.661X_2)$
	12	$0.79 = 0.52 + 0.27$
A. pinnata var. *imbricata*		$Y = 229 + 15.0X_1 + (-0.446X_2)$
	13	$0.92 = 0.79 + 0.13$
A. rubra		$Y = 259 + 6.89X_1 + (-0.362X_2)$
	11	$0.73 = 0.24 + 0.49$

Table 4. Coefficients of correlation (r) between the RGRs of *Azolla* accessions and several climatic variables occurring during the SUMMER season. N = 11 for all species except 10 for *A. nilotica*.

Azolla accession	Minimum water temperature	Irradiance	Relative humidity	Maximum water temperature
A. caroliniana	−0.70	0.20	−0.27	−0.43
A. filiculoides	−0.81	0.19	−0.36	−0.46
A. mexicana	−0.58	0.43	−0.40	−0.17
A. microphylla	−0.21	0.34	−0.09	−0.01
A. nilotica	−0.75	0.26	−0.36	−0.41
A. pinnata var. *imbricata*	−0.23	0.62	−0.46	0.18
Minimum water temperature	–	0.08	0.31	0.79
Irradiance	–	–	−0.84	0.65
Relative humidity	–		–	−0.29

Table 5. A multiple linear regression model for predicting RGR (g kg^{-1} day^{-1}) of six *Azolla* accessions during the SUMMER. The model uses the following climatic variables: minimum water temperature (X_1), irradiance (X_2), and relative humidity (X_3). N = 11 for all species except 10 for *A. nilotica*.

Azolla accessions	Regression equation R^2 = contribution of each variable
A. caroliniana	$Y = (-)569 + (-)4.45X_1 + 1.31X_2 + 7.83X_3$
	$0.76 = 0.49 + 0.17 + 0.10$
A. filiculoides	$Y = (-)571 + (-)2.37X_1 + 1.54X_2 + 7.16X_3$
	$0.80 = 0.65 + 0.07 + 0.08$
A. mexicana	$Y = (-)1002 + 3.72X_1 + 1.97X_2 + 9.74X_3$
	$0.82 = 0.33 + 0.23 + 0.26$
A. microphylla	$Y = (-)912 + (-)3.05X_1 + 1.40X_2 + 11.0X_3$
	$0.72 = 0.04 + 0.13 + 0.55$
A. nilotica	$Y = 23.9 + (-)21.6X_1 + 0.254X_2 + 5.17X_3$
	$0.85 = 0.56 + 0.12 + 0.17$
A. pinnata var. *imbricata*	$Y = (-)762 + 5.83X_1 + 1.52X_2 + 7.13X_3$
	$0.66 = 0.05 + 0.42 + 0.19$

but were not used in this study since they would make the cost of *Azolla* production unprofitable and pose a serious environmental threat to fish if used on a large scale.

After the summer growth cycles, the accessions were ranked for their potential productivity during the summer on the basis of RGR and their susceptibility to disease and heat stress. *Azolla microphylla* and *A. pinnata* var. *imbricata* had the highest mean RGRs and lowest standard deviations during the summer. These two accessions were superior because they were relatively disease free and tolerated the high summer water temperatures. The RGRs of both *A. microphylla* and *A. pinnata* var. *imbricata* were poorly correlated with mean minimum and maximum water temperatures (Table 4), which may indicate heat tolerance. *Azolla microphylla* was also more resistant to fungal attack. This may account for the poor correlation between the RGR of *A. microphylla* and relative humidity (Table 4). The high correlation between RGR and irradiance for *A. pinnata* var. *imbricata* (Table 4) may indicate that it is able to utilize high irradiance and/or grow well at high temperature. Although *A. mexicana* had a relatively low mean RGR, correlation coefficients for summer were similar to those of *A. microphylla* and *A. pinnata* var. *imbricata*. Its lower mean RGR may be partially explained by its continuous production of spores, which consume photosynthate and increase maintenance respiration.

The lower productivity of *A. caroliniana*, *A. filiculoides*, and *A. nilotica* was assumed to be caused by the high temperatures which prevailed during summer. This assumption is supported by the high negative correlation between their RGRs and water temperature (Table 4).

CONCLUSIONS

The year-round pot experiment carried out at Hangzhou was of sufficient magnitude and scope to: i) define a range of RGRs for each species accession over a wide range of temperatures, ii) show that RGR is temperature driven or limited during cooler months of the year, and iii) show that high temperatures and high humidity increase risks associated with trying to grow a crop. Furthermore it is suggested that the resulting models to predict RGR have a broad application due to the wide range of climatic conditions under which the experiment was conducted.

At any location, RGRs must be determined and climatic variables measured before these models can be tested. The Hangzhou data was first analyzed using a model for *A. filiculoides* developed by Talley and Rains (1980), involving the log of maximum air temperature. The results proved helpful though inferior to the cool and summer period models developed from data collected at Hangzhou because their model was based on a narrower climatic range.

The applicability of individual models will be determined by season and location. In temperate locations, the cool period model will probably be limited to spring and fall. It may also be appropriate in subtropical locations, such as northern Vietnam where *Azolla* is usually grown during a winter cool period which is associated with a reversal of daylength. The summer model involving temperature, irradiance, and relative humidity may be applicable during most of the year in tropical locations and during summer in temperate and subtropical locations. This model may be the most important because of the previous unpredictability of *Azolla* RGR during hot weather.

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