

RESEARCH PAPER

Effects of *Azolla* species on weed emergence in a rice paddy ecosystem

MOLOY BISWAS,¹* SULTANA PARVEEN,¹ HIDEKI SHIMOZAWA² and NOBUKAZU NAKAGOSHI¹

¹Graduate School for International Development and Cooperation (IDEC), Hiroshima University, Kagamiyama, Higashi Hiroshima, Japan and ²Hiroshima Prefecture Agriculture Research Center, Hara, Hachihonmatsu, Higashi Hiroshima, Japan

The effects of *Azolla pinnata* R. Br. on weed emergence were evaluated in terms of plot area coverage by an *A. pinnata* mat, its biomass production and the amount of weed emergence, using fresh and dry weights, in a rice paddy field experiment. The experiment was conducted following a randomized block design with five combinations of fertilizer and *A. pinnata* treatments (control, *A. pinnata* + superphosphate, *A. pinnata* + urea, *A. pinnata* + compound fertilizer, *A. pinnata* + cow manure). The results revealed that after 18 days of inoculation, all superphosphate (T1) and cow manure (T4)-treated plots were fully covered by the *A. pinnata* mat. However, coverage of the urea (T2) and compound fertilizer (T3)-treated plots were only 80% and 70%, respectively. The full plot area coverage by the *A. pinnata* mat and the highest biomass production with superphosphate and cow manure-treated plots were able to completely inhibit two weed species (*Scirpus juncooides* Roxb. var. *hotarui* Ohwi and *Monochoria vaginalis* Burm. f. Presl var. *plantaginea* (Roxb.) Solms-Laub) and significantly suppressed four other weeds (*Cyperus serotinus* Rottb., *Echinochloa oryzicola* Vasing, and *Eclipta prostrata* L.). In all the treatments, the fresh weight of weeds significantly reduced to 13, 29, 34, and 9%, respectively, for treatments T1, T2, T3, and T4. The dry weights also were significantly reduced to 10.00, 16.00, 22.00, and 7.26%, respectively, for treatments T1, T2, T3, and T4 over the control. The results revealed that there was a significant correlation among plot area coverage by the *A. pinnata* mat, its biomass production and weed emergence in a rice paddy field over the control. *Azolla pinnata* did not have any detrimental effect on the growth of rice plants.

Keywords: *Azolla*, biomass, fertilizer, rice paddy, weed suppression.

INTRODUCTION

Weeds are an integral part of agricultural systems (Lovett & Knights 1996). In rice crops, weeds are the major constraint to high-yield production. Worldwide losses due to weeds are estimated at 10–15% (Smith 1983; Zoschke 1990; Baltazar & De Datta 1992). Without weed control, yield losses have been estimated to range from 16–86%, or even 100% (Zoschke 1990; Baltazar &

De Datta 1992; Kropff 1993). Thus, the control of weeds in rice paddy fields is crucial for optimum production (Jahromi *et al.* 2001). Weeds compete with crop plants for light, nutrients, water, and space (Glauning & Holzner 1982; Kropff 1993). The level of yield loss depends not only on the infestation, but also on the composition of weed flora. Furthermore, the land might be unsuitable for crop production if the perennial weeds become established (Oerke *et al.* 1994). The species that cause problems vary with the soil, temperature, latitude, altitude, rice culture, seeding method, water management, and weed control technology (Smith 1983; Hill *et al.* 1990).

Conventionally, herbicides are used to control weeds, particularly in developed countries. Recently, some developing countries have also progressed in herbicide use. Japanese rice farmers have been controlling paddy

*Correspondence to: Moley Biswas, Graduate School for International Development and Cooperation (IDEC), Hiroshima University, 1-5-1 Kagamiyama, Higashi Hiroshima 739-8529, Japan.
Email: mbiswas872003@yahoo.com

The authors have no commercial interest regarding the findings presented.

Received 5 August 2004; accepted 2 November 2004

weeds mainly by chemical methods, in which chemical herbicides have commonly been applied ≈ 1.8 times in one growing season of rice by sequential treatment. Fifty to 80% of the herbicides used in Japan are applied to rice cultivation (Shibayama 2001). As the herbicides are active substances, there are concerns about their effects on non-target organisms. Herbicide-resistant weeds and associated chemical pollution are serious environmental concerns. Prevention of the overuse of herbicides in rice paddy fields and the development of an alternative method without harming the agroecosystem are emerging issues.

The free-floating aquatic fern, *Azolla*, is distributed worldwide in tropical and temperate freshwater ecosystems. The *Azolla* spp. have been used extensively and effectively for green manure in rice fields instead of chemical fertilizers in Asia for centuries (Arora & Singh 2003), especially in Vietnam and China (Lumpkin & Plucknett 1980). The contribution of *Azolla* as a biofertilizer and its potential behavior in increasing fertilizer efficiency in rice paddy systems have been reported by many researchers (Lumpkin & Plucknett 1980; Wagner 1997; Macale *et al.* 2002). Besides biofertilizer, *Azolla* has multiple uses, such as biological herbicide, animal feed, and as a water purifier (Watanabe & Hove 1996).

The ability of *Azolla* to suppress other weeds has been mentioned in Philippino literature studies since 1927 (Moody & Janiya 1992). Weed growth is suppressed when *Azolla* forms a thick, virtually light-proof mat. There are probably two mechanisms for this suppression, the most effective being the light-starvation of young weed seedlings by the blockage of sunlight (Lumpkin & Plucknett 1980). The other mechanism is the physical resistance to weed seedling emergence created by a heavy, interlocking *Azolla* mat, which does not affect the growth of rice (Pons 1987). Bangun and Syam (1988) showed that an *Azolla* cover could significantly reduce weed infestation without harming the rice yield. Several studies have reported the suppressive effect of *Azolla* on rice weed species, such as *Utricularia flexuosa* Vanl., *Echinochloa crus galli* (L.) Beauv., *Sagittaria* spp., *Cyperus difformis* L. and *Polygonum* sp. (Nguyen 1930; Ngo 1973; Talley *et al.* 1977).

Worldwide concerns are growing regarding the prevention of herbicide overuse and the development of an alternative method that will be effective in controlling weeds without harming the agroecosystem and environment. In this situation, *Azolla* might be an effective bioherbicide that can reduce the use of herbicides in rice paddy fields in Japan. In addition, *Azolla* spp. have already been reported as endangered in rice paddy eco-

systems in Japan (Matsuo 2000). Therefore, as a source of bioherbicide, *Azolla* might get attention from a conservation perspective. However, in the modern literature, the information regarding the potential effects of *Azolla* on weeds in rice paddies is very limited. Therefore, the present study was conducted to discover the effects of different fertilizers on *Azolla* biomass production and *Azolla* on weed emergence in a rice paddy ecosystem.

MATERIALS AND METHODS

Field experiment

The experiment was conducted in the experiment farm of Hiroshima Prefecture Agriculture Research Center, Higashi Hiroshima, Japan, from May to October 2003. Nine soil samples (0–15 cm) of the experimental plots were taken in April 2003 and analysed in the Soil Science Laboratory of the Research Center. The soil characteristics of the experiment plots were: sandy loam; bulk density = 1.32 g mL^{-1} ; pH = 6.7; total carbon = 1.34%; total nitrogen (N) = 0.14%; cation exchange capacity = 9.3 me (milligram equivalent) 100 g soil^{-1} ; calcium oxide = $203 \text{ mg } 100 \text{ g soil}^{-1}$; magnesium oxide = $24 \text{ mg } 100 \text{ g soil}^{-1}$; sodium oxide = $7.8 \text{ mg } 100 \text{ g soil}^{-1}$; potassium oxide = $12.5 \text{ mg } 100 \text{ g soil}^{-1}$.

Experimental design

The experiment was set out following a completely randomized block design with five combinations of fertilizer and *Azolla* treatments. Each treatment had three replications in $1 \text{ m} \times 1 \text{ m}$ plots. Tilling and puddling was done during 7 May and 27 May 2003, respectively. Transplanting was done on 30 May 2003 using 20 day-aged seedlings (*Oryza sativa* L. "Hourei") with spacing of $30 \times 15 \text{ cm}$. In order to irrigate and to maintain water depth at 6–8 cm, each plot was connected to an individual underground pipe with a diameter of 5 cm and covered by a net. Again, each plot was carefully separated by plastic sheets to prevent water seepage.

Azolla pinnata R. Br. 103 was multiplied in a greenhouse two weeks prior to inoculation. Except for the control, 50 g fresh weight of *A. pinnata* was inoculated in all other treatments just after rice transplantation. The experimental treatments were: T0, the control (no *A. pinnata* or fertilizer); T1, *A. pinnata* 50 g at 0 days after transplanting (DAT) + superphosphate (containing 17.5% phosphorus [P]; dose = 41 kg ha^{-1}) at 7, 14, 21, and 28 DAT; T2, *A. pinnata* 50 g (0 DAT) + urea (46% N; dose = 29 kg ha^{-1}) at 15, 30, and 45 DAT; T3, *A. pinnata* 50 g (0 DAT) + compound fertilizer (17% N and 17% potassium [K]; dose = 150 kg ha^{-1}) at 0 and 28

DAT; T4, *A. pinnata* 50 g + cow manure (0.64% N, 0.67% P, and 0.8% K; dose = 10 t ha⁻¹) at 0 DAT. Based on the listed recommendations, the fertilizer treatments were applied on the plots.

The top-dressing of fertilizers was taken into consideration as four different treatments. The main objective of these four different fertilizer management practises was to know the plot area coverage and inhibition of weed emergence by the *Azolla* mat. Different fertilizer management practises were followed based on the different research findings. In treatment T1, P was applied at 7, 14, 21, and 28 DAT, considering that P is a limiting factor for *Azolla*'s growth and it should be applied in four equal doses at 7-day intervals (Tran & Dao 1973; Singh 1977; Talley *et al.* 1977; Lumpkin & Plucknet 1980).

Urea was applied at 15, 30, and 45 DAT in treatment T2, considering that urea in this medium considerably accelerates the growth of *A. pinnata* and a significant input of N is required to sustain its vigorous growth (Kitoh & Shiomi 1991; Cary & Weerts 1992). Moreover, a high floodwater pH is conducive to ammonia (NH₃) volatilization losses. Studies showed an increase of more than 10-fold in the NH₃ volatilization rate with a change of pH from 8–10 (Freney & Denmead 1992). Inoculating *Azolla* prior to urea application markedly suppressed the rise in daytime pH. Therefore, based on those reports, urea was applied in three splits so as to reduce the volatilization loss and to increase urea efficiency at the maximal vegetative growth stage of the rice plants.

Azolla is efficient in uptaking K from the soil and making it available for the rice plant. As a result of its slow-releasing nature, and to enhance the long-term growth of *Azolla*, K was applied at 0 DAT and 28 DAT in treatment T3.

Cow manure is widely considered to be a source of macronutrients and micronutrients. Many researchers recommended that for successful and quick growth of *Azolla*, macronutrient and micronutrient (e.g. molybdenum and iron) supplies also are important (Singh 1977; Talley *et al.* 1977). Considering *Azolla*'s slow-releasing nature and in order to provide initial support of the other nutrients for the long-term growth of *Azolla*, cow manure was applied at 0 DAT in treatment T4.

Soil, water, air temperature, and water pH in the experiment plots were recorded on a weekly basis. Based on the initial plot coverage (\approx 100%) by the *Azolla* mat under treatments T1 and T4, the *Azolla* biomass was started to be measured on a weekly basis using a 25 cm \times 25 cm quadrat at 18 DAT (18 June) and con-

tinued until 85 DAT (25 August), based on the water remaining on the soil surface. The amount of weed emergence was recorded every week but samples were collected for biomass measurement and oven-dried weight from above ground level at 85 DAT. In addition, the rice plant heights and tiller numbers also were measured on the same date.

Statistical analysis of data

All treatments were conducted in a completely randomized block design with three replications. Data were analysed by ANOVA with SPSS 11.0 for Windows (SPSS, Chicago, USA).

RESULTS AND DISCUSSION

Physicochemical and environmental conditions of the experimental plots

During June, July, and August, the mean daytime (08.00 hours–17.30 hours) air temperatures were 24, 33, and 38°C, respectively. The soil temperatures were recorded as 22.3, 27.0, and 33.0°C, respectively, and the water temperatures were 25.8, 35.0, and 38.8°C, respectively. The mean water pH in treatments T0, T1, T2, T3, and T4 were recorded as 7.2, 6.8, 6.5, 6.8, and 6.8, respectively. In June, July, and August, the daytime mean relative humidity measures were recorded as 65, 70, and 75%, respectively. During June and July, the mean water depth was 7 cm, but in August it decreased to 2 cm. These conditions indicate that the favorable conditions for the growth of *Azolla* spp. were during June and July. A high temperature, high humidity, and the low water depth in August might not be favorable conditions for the optimum growth and biomass production of *Azolla*. A mean air temperature of >30.0°C often has a direct negative impact on the growth and survival of *Azolla* (Peters *et al.* 1980; Tung & Watanabe 1983; Watanabe & Berja 1983; Debusk & Reddy 1987).

Plot area coverage by the *Azolla* mat

Figure 1 shows the plot area coverage by the *Azolla* mat of four fertilizer treatments in dual cropping with the rice paddy during 0 DAT–18 DAT. At 0 DAT, 50 g *A. pinnata* was inoculated in each treatment and the initial coverage was calculated at \approx 7% of the plots. After one week of inoculation, the mean plot area coverage in the T1, T2, T3, and T4 plots was increased to 45, 35, 25, and 50%, respectively. The mean plot area coverage increased to 80, 70, 50, and 90%, respectively, in T1, T2, T3, and T4 at 14 DAT, reaching 100% in T1 and T4 at

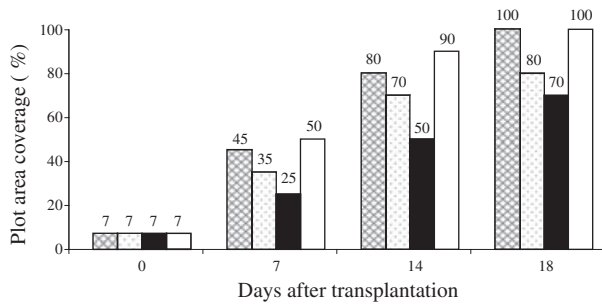


Fig. 1. The plot area coverage by *Azolla pinnata* with four fertilizer treatments in a rice paddy. (▨), T1: *A. pinnata* 50 g at 0 days after transplanting (DAT) + superphosphate (containing 17.5% phosphorus [P]; dose = 41 kg ha⁻¹) at 7, 14, 21, and 28 DAT; (▩), T2: *A. pinnata* 50 g (0 DAT) + urea (containing 46% nitrogen [N]; dose = 29 kg ha⁻¹) at 15, 30, and 45 DAT; (■), T3: *A. pinnata* 50 g (0 DAT) + compound fertilizer (17% N and 17% potassium [K]; dose = 150 kg ha⁻¹) at 0 and 28 DAT; (□), T4: *A. pinnata* 50 g + cow manure (0.64% N, 0.67% P, and 0.8% K; dose = 10 t ha⁻¹) at 0 DAT.

18 DAT. This indicates that after 18 days of inoculation, all superphosphate and cow manure-treated plots were fully covered by the *Azolla* mat. Coverage of the urea and compound fertilizer-treated plots were 80 and 70%, respectively.

Phosphorus is the most common limiting factor for the growth of *Azolla*. As P diffusion from soil to water is slow, the field population of floating *Azolla* suffers from P deficiency. In that case, P fertilizer application is effective to enhance its growth and it is recommended that it be applied on top of the *Azolla* mat (Lumpkin & Plucknett 1980). Therefore, the 100% plot area coverage at 18 DAT resulting from superphosphate and cow manure treatments might be the effect of P.

In urea-treated plots, 80% coverage by the *Azolla* mat might be seen as the adverse effect of N fertilizer on the growth of *Azolla* (Le Van & Sobochkin 1963; Anonymous 1975; Singh 1977). According to Lu *et al.* (1963) and Anonymous (1975), N fertilizer is required if *Azolla* is grown in low temperatures, but in our experimental plots the mean air temperature varied from 20–24°C at 0–18 DAT. Therefore, urea might have a negative effect on *Azolla* growth. However, the compound fertilizer contained N and K, but lacked P, which might have affected the growth of *Azolla* in the T3 treatment. In this regard, 70% coverage of compound fertilizer-treated plots could be seen as the P-limiting effect.

Trends of *Azolla* biomass production

The trends of biomass production and the growth rate of *Azolla* from 18 June to 25 August 2003 in dual cropping with the rice paddy are presented in Fig. 2. This shows that, among the four treatments, the biomass production was the highest after the application of cow manure, followed by superphosphate, urea, and compound fertilizer. At the beginning of the sampling on 18 June, the mean biomass production was 161, 155, 110, and 85 g 25 cm⁻² for treatments T4, T1, T2, and T3, respectively; these differences were statistically significant ($P < 0.04$). For the superphosphate (243 g) and urea (181 g)-treated plots, the biomass production showed a peak at the end of July (56 and 63 DAT, respectively) and started to decline at the first week of August, but it was still considerable in the last week of August (210 g and 149 g, respectively). For the compound fertilizer and cow manure-treated plots, the sampled biomass production reached a peak of 150 and 268 g 25 cm⁻² at the first week of August. In the case of the compound fertilizer, the biomass production started to decline in the second week of August (70 DAT) but for cow manure, it declined one week later (77 DAT). At 85 DAT (25 August), the biomass was recorded as 210, 149, 121, and 260 g 25 cm⁻² in T1, T2, T3, and T4, respectively ($P < 0.03$). The maximum biomass production (fresh weight) was 4.2 kg m⁻² in T4 at 63 DAT, followed by T1 at 56 DAT (3.9 kg m⁻²), T2 at 56 DAT (2.9 kg m⁻²), and in T3 at 63 DAT (2.4 kg m⁻²).

Azolla pinnata doubles its biomass in 3–5 days in the laboratory but, under field conditions, it ranges from ≥5–10 days, which is considered to be normal (Van Hove *et al.* 1983). Arora and Singh (2003) reported that the doubling time of *A. pinnata* biomass was 14 days in a vinyl house at 30 ± 2°C. Under Asian rice field conditions, *A. pinnata* realistically produces 8–10 t ha⁻¹ fresh weight (Lumpkin & Plucknett 1980). Our result shows that the biomass of superphosphate and cow manure-treated plots tripled in three weeks, while it was more than double for the urea treatment. The biomass was less than double in compound fertilizers only. The declining rate of P-treated plots was the highest and the lowest in cow manure-treated plots (Fig. 2). The application of organic manure leads to a slow release to flood water and provides initial support for other nutrients, resulting in a long-term benefit (Lumpkin & Plucknett 1980).

Weed emergence

Table 1 represents the types and amounts of weed emergence from June to August 2003 in the rice paddy field experiment. Five types of weeds species were identified

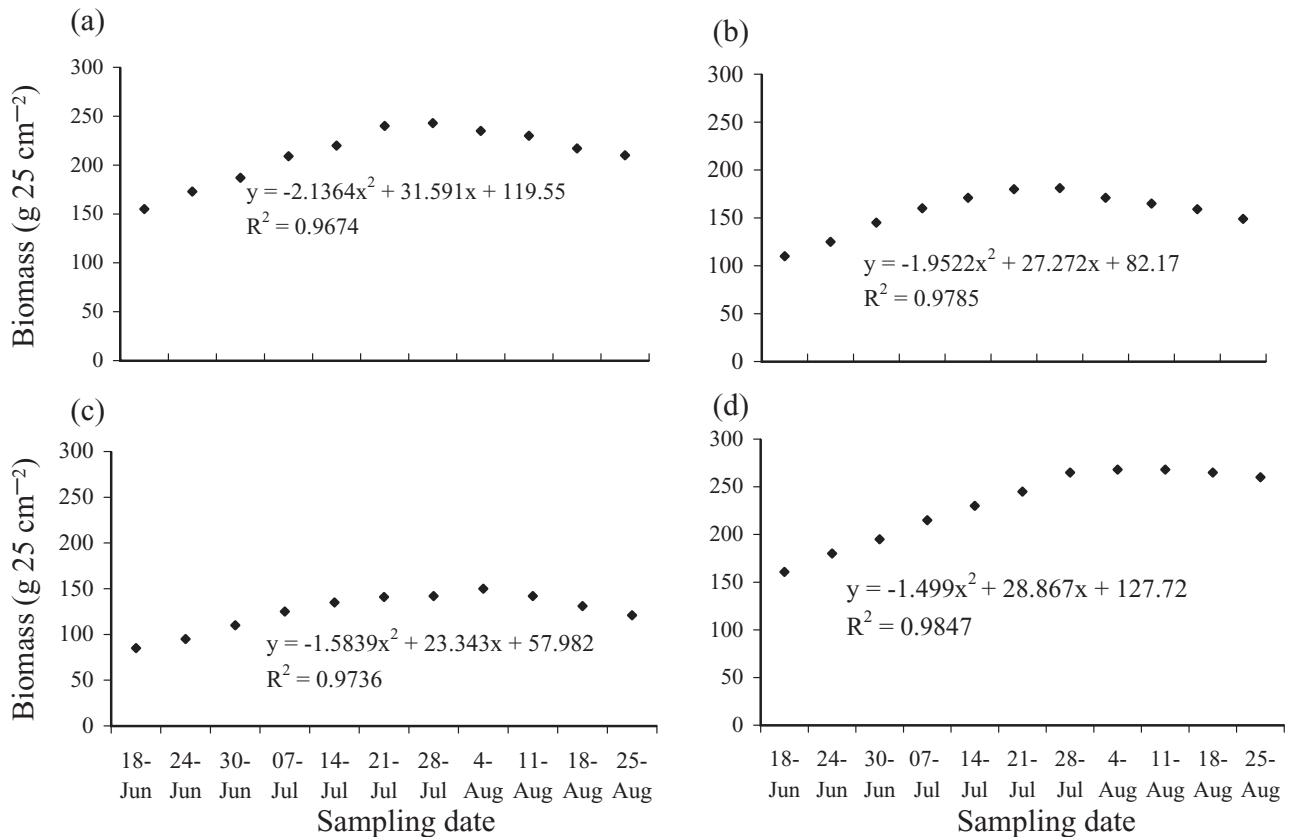


Fig. 2. The growth rate of *Azolla pinnata* after three weeks of inoculation in a rice paddy with different fertilizer treatments. (a) T1: *A. pinnata* 50 g at 0 days after transplanting (DAT) + superphosphate (containing 17.5% phosphorus [P]; dose = 41 kg ha⁻¹) at 7, 14, 21, and 28 DAT, (b) T2: *A. pinnata* 50 g (0 DAT) + urea (containing 46% nitrogen [N]; dose = 29 kg ha⁻¹) at 15, 30, and 45 DAT, (c) T3: *A. pinnata* 50 g (0 DAT) + compound fertilizer (17% N and 17% potassium [K]; dose = 150 kg ha⁻¹) at 0 and 28 DAT and (d) T4: *A. pinnata* 50 g + cow manure (0.64% N, 0.67% P, and 0.8% K; dose = 10 t ha⁻¹) at 0 DAT.

in the control plots: namely, *Cyperus serotinus* Rottb., *Scirpus juncooides* Roxb. var. *hotarui* Ohwi, *Echinochloa oryzicola* Vasing, *Eclipta prostrata* (L) L., *Monochoria vaginalis* (Brum. f) Presl var. *plantaginea* (Roxb.) Solms-Laub, totalling 21 weeds. The number of weeds in control plots was fewer than expected. However, there is a congruence in the number of weed species found in the control plots with the findings of Xuan *et al.* (2001), who conducted an experiment in a paddy field at Miyazaki University, Japan, from June to October 2000 and reported a total of 99 weed emergences in the control plot (3 × 12 m). In an another paddy field experiment by Xuan *et al.* (2003) from June to October 2001 at Miyazaki University, they reported a total of 46 weed emergences in the control plot (4 × 4 m). Moody and Janiya (1992) also mentioned that keeping the field flooded after planting will kill some weeds and will slow the growth of others. The water depth of the experi-

mental plots was 7 cm until July, which was effective in reducing the weed emergence.

In the superphosphate and cow manure-treated plots, two weed species (*S. juncooides* var. *hotarui* and *M. vaginalis* var. *plantaginea*) were completely inhibited by the *Azolla* mat and weed emergence reduced to 81 and 86%, respectively, over the control. Meanwhile, in the urea and compound fertilizer-treated plots, one weed species (*M. vaginalis* var. *plantaginea*) was completely inhibited by the *Azolla* mat but the other four types (as in T0) were recorded. These results suggest that full plot coverage by the *Azolla* mat in superphosphate and cow manure-treated plots was able to inhibit two rice weeds (*S. juncooides* var. *hotarui* and *M. vaginalis* var. *plantaginea*) and could reduce the other three weeds by 42 and 52%, respectively, over the control (Pons 1987; Moody & Janiya 1992).

Table 1. The species and amounts of weed emergence (No. m⁻²) in different treatments from June to August in rice paddy experiment plots

Treatment	<i>Cyperus serotinus</i>		<i>Scirpus juncooides</i>		<i>Echinochloa oryzicola</i>		<i>Eclipta prostrata</i>		<i>Monochoria vaginalis</i> var. <i>plantaginea</i>		Total		
	June	July	Aug.	June	July	Aug.	June	July	Aug.	June		July	Aug.
Control (T0)	3	1	0	4	1	1	3	0	0	3	0	0	21
<i>Azolla pinnata</i> + superphosphate (T1)	1	0	0	0	0	0	2	0	0	1	0	0	4
<i>A. pinnata</i> + urea (T2)	1	0	0	2	1	0	2	0	0	2	0	0	8
<i>A. pinnata</i> + compound fertilizer (T3)	2	0	0	2	1	1	2	0	0	2	0	0	10
<i>A. pinnata</i> + cow manure (T4)	1	0	0	0	0	0	1	0	0	1	0	0	3

Growth of weeds

The effect of *Azolla* on weed growth was evaluated in terms of the number of weeds, their total fresh weight and total dry weight (Table 2). In the control plots, the total fresh weight (416.6 g m⁻²) and total dry weight (106.7 g m⁻²) of weeds were much higher than the fertilizer treatments. In the superphosphate, urea, compound fertilizer and cow manure-treated plots, the total fresh weights were 53.7, 120.4, 140.7, and 37.9 g m⁻², respectively. In the treatments T1, T2, T3, and T4, the fresh weight significantly reduced to 13, 29, 34, and 9%, respectively. The dry weights were also significantly reduced to 10, 16, 22, and 7%, respectively, over the control. Although the fertilizer application in the crop field tends to enhance the weed infestation, the use of *Azolla* could reduce the weed infestation in comparison to no fertilizer.

Growth of rice plants

The effects of *Azolla* on the growth of rice plants also was evaluated in terms of height and tiller numbers. In all the treatments, the plant height and tiller numbers were slightly increased over the control but not significant (Table 2). It indicates that *Azolla* did not have any detrimental effect on the growth of rice plants. As a result of more weed infestation (21 weeds m⁻²), the number, and fresh and dry weights of weeds were significantly higher in the control in comparison to the other four treatments (Table 2). However, because of the fertilizer application and the additional nutrient supplementation by *Azolla*, the plant height and tiller numbers of rice in the other treatments were not different from the control. Rice seedlings are not affected by *Azolla*'s weed suppression effect because, when transplanted, they stand above the *Azolla* mat (Lumpkin & Plucknett 1980).

In our paddy field experiment, the mean water temperature of the experimental plots was increased to 25.8°C from the mean air temperature of 24°C in June, when 100% of all treatment plots were initially covered by the *Azolla* mat. In July, the mean air and water temperatures rose to 33°C and 35°C, respectively. In August, the air and water temperatures rose to 38°C and 38.8°C, respectively. At the end of August, along with the decrease in water depths to 2 cm, the *Azolla* biomass started to decline, which was conducive to reducing the water temperature. Although, the water temperature was increased by the *Azolla* mat, it could not affect the rice plants.

Therefore, on the basis of the above findings and discussion, it is possible to say that there was a significant

Table 2. Correlations among *Azolla pinnata* biomass, weed emergence and the growth of rice plants

Treatment	No. of weeds	Total fresh weight of weeds (g m ⁻²)	Total dry weight of weeds (g m ⁻²)	Rice plant	
				Height (cm)	No. of tillers
T0	21 (100)	416.58 (100)	106.66 (100)	76.0 (100)	13.0 (100)
T1	4 (19)***	53.68 (13)***	10.73 (10)***	76.3 (100) [†]	13.7 (105) [†]
T2	8 (38)**	120.36 (29)***	17.30 (16)***	77.4 (102) [†]	13.3 (102) [†]
T3	10 (48)**	140.66 (34)**	23.19 (22)***	77.0 (101) [†]	13.3 (102) [†]
T4	3 (14)***	37.82 (9)***	7.75 (7)***	76.3 (100) [†]	13.7 (105) [†]

,* Significant at the 0.05 and 0.01 probability levels, respectively; [†] not significant. The numbers in the parentheses are the rates in comparison with the control. T0, control (no *Azolla pinnata* or fertilizer); T1, *A. pinnata* 50 g at 0 days after transplanting (DAT) + superphosphate (containing 17.5% phosphorus [P]; dose = 41 kg ha⁻¹) at 7, 14, 21, and 28 DAT; T2, *A. pinnata* 50 g (0 DAT) + urea (46% nitrogen [N]; dose = 29 kg ha⁻¹) at 15, 30, and 45 DAT; T3, *A. pinnata* 50 g (0 DAT) + compound fertilizer (17% N and 17% potassium [K]; dose = 150 kg ha⁻¹) at 0 and 28 DAT; T4, *A. pinnata* 50 g + cow manure (0.64% N, 0.67% P, and 0.8% K; dose = 10 t ha⁻¹) at 0 DAT.

correlation among the plot area coverage by *Azolla*, its biomass production and weed emergence in a rice paddy field over the control. In the rice paddy field, when the plot area coverage reached 100% at 18 DAT and peaked after 56 DAT with a biomass production of ≈2.5 and 4.2 kg m⁻², respectively, then it was able to inhibit more weeds. The result indicates that *A. pinnata* can be regarded as a biological control agent of paddy weeds. Further study is needed to examine the details of the biological control effects of *Azolla* spp. on the rice paddy weeds.

ACKNOWLEDGMENTS

We gratefully acknowledge Fuji Xerox Setsutaro Kobayashi Memorial Fund for their grant support. The authors thank Dr Kishida Yoshirou, Okayama City, for providing *Azolla pinnata* 103 inocula, Mr Matsuura Kenkichi, Research Scientist, Hiroshima Prefecture Agriculture Research Center, Higashi Hiroshima, Japan, for providing facilities for soil sample analysis, and Dr Yuji Isagi, Associate Professor, Department of Environmental Studies, IDEC, Hiroshima University, Japan, for his valuable suggestions.

REFERENCES

- Anonymous. 1975. *Cultivation, Propagation and Utilization of Azolla*. Chekiang Agricultural Academy, Institute of Soils and Fertilizer, Zhejiang Sheng, China.
- Arora A. and Singh P.K. 2003. Comparison of biomass productivity and nitrogen fixing potential of *Azolla* spp. *Biomass Bioenerg.* **24**, 175–178.
- Baltazar A.M. and De Datta S.K. 1992. Weed management in rice. *Weed Abstr.* **41**, 495–507.
- Bangun P. and Syam M. 1988. Weed management in rice crops. *PADI* **2**, 579–599.
- Cary P.R. and Weerts P.G.J. 1992. Growth and nutrient composition of *Azolla pinnata* R. Brown and *Azolla filiculoides* Lamarck as affected by water temperature, nitrogen and phosphorus supply, light intensity and pH. *Aquat. Bot.* **43**, 163–180.
- Debusk W.F. and Reddy K.R. 1987. Growth and nutrient uptake potential of *Azolla caroliniana* Willd. and *Salvinia potundifolia* Willd. as a function of temperature. *Environ. Exp. Bot.* **27**, 215–221.
- De Macale M.A.R., Vlek P.L.G. and San Valetin G.O. 2002. The role of *Azolla* cover in improving the nitrogen use efficiency of lowland rice. In: Friederichsen J.R., Palmer I., eds. *Proceedings of the International Symposium on Sustaining Food Security and Managing Natural Resources in South Asia, Challenges for the 21st Century* (Chiang Mai, Thailand, 8–11 January 2002). Available from URL: http://www.uni-hohenheim.de/symposium2002/pa_full/Full-Pap-S3B-2_De_Macale.pdf. Accessed 15 July 2004.
- Freney J.R. and Denmead O.T. 1992. Factors controlling ammonia and nitrous oxide emissions from flooded rice fields in the Philippines. *Biol. Fertil. Soils* **9**, 31–36.
- Glauning J. and Holzner W. 1982. Interference between weeds and crops: a review of literature. In: *Biology and Ecology of Weeds* (ed. by Holzner W. and Numata N.). Junk W. Publisher, The Hague, 149–157.
- Hill J.E., De Datta S.K. and Real J.G. 1990. *Echinochloa* competition in rice: A comparison of studies from direct-seeded and transplanted flooded rice. In: Auld B.A., Umalay R.C., Tjitrosomo S.S., eds. *Proceedings of the Symposium on Weed Management* (Bogor, Indonesia). Seameo-Biotrop, Bogor, Indonesia, 115–129.
- Jahromi F., Cothier E. and Ash G. 2001. Weed control in rice crops. Suitability of *Rhynchosporium alismatis* as mycoherbicide for integrated management of *Damasonium minus* in rice fields. RIRDC, Australia. Available from URL: <http://www.rirdc.gov.au/reports/RIC/01-39.pdf>. Accessed 1 August 2004.
- Kitoh S. and Shiomi N. 1991. Effect of mineral nutrients and combined nitrogen sources in the medium on growth and nitrogen fixation of the *Azolla-Anabaena* association. *Soil Sci. Plant Nutr.* **37**, 419–426.
- Kropff M.J. 1993. Eco-physiological models for crop–weed competition. In: *Modelling Crop–Weed Interactions* (ed. by Kropff M.J. and Van Laar H.H.X.). Oxford University Press, the Philippines, 25–32.
- Le Van K. and Sobochkin A.A. 1963. The problems of the utilization of *Azolla* as a green manure in the Democratic Republic of Vietnam. *Timui. Moscow. Agric. Acad.* **94**, 93–97.
- Lovett J.V. and Knights S.E. 1996. Where in the world is weed science going? In: *Weed Science Society of Victoria, ed. Proceedings of the Eleventh Australian Weed Conference, R. C. H. Shepherd* (Melbourne, Australia, 30 September–3 October 1996). Weed Science Society of Victoria, Melbourne, 2–13.
- Lu S.K., Chen A.S. and Ge S. 1963. Rice paddy green manure studies on the biological characteristics of Red *Azolla*. *Zhong. Nong. Kex.* **11**, 35–40.

- Lumpkin T.A. and Plucknett D.L. 1980. *Azolla*: botany, physiology and use as a green manure. *Econ. Bot.* **34**, 111–153.
- Matsuo K. 2000. The distribution of water plants and plant species diversity in ill-drained fields of Bei village. In: *Preservation of Bio-diversity in Paddy Field Under Asian Monsoon Climate and its Sustainable Use* (ed. by Hayashi K.). Chinese Academy of Agricultural Sciences/China National Rice Research Institute/The Ministry of Agriculture, Forestry and Fisheries of Japan/Mitsubishi Research Institute, Tokyo, 44–49.
- Moody K. and Janiya J.D. 1992. The role of *Azolla* in weed control in rice. *Philipp. J. Weed Sci.* **19**, 79–102.
- Ngo G.D. 1973. The effect of *Azolla Pinnata* R. Br. on rice growth. Biotrop, Bogor, Indonesia. No. 23.
- Nguyen C.T. 1930. L'*Azolle* cultivee comme engrais vert. *Bull. Econ. Indochine* **33**, 335–350.
- Oerke E.C., Weber A., Dehne H.W. and Schönbeck F. 1994. Conclusions and perspectives. In: *Crop Production and Crop Protection, Estimated Losses in Major Food and Cash Crops* (ed. by Oerke E.C., Dehne H.W., Schönbeck F. and Weber A.). Elsevier Science, Amsterdam, 742–770.
- Peters G.A., Toia R.E. Jr, Evans W.R., Crist D.K., Mayne B.C. and Poole R.E. 1980. Characterization and comparisons of five N-fixing *Azolla*–*Anabaena* associations. I. Optimization of growth conditions for biomass increase and N content in a controlled environment. *Plant Cell Environ.* **3**, 261–269.
- Pons T.L. 1987. Growth rates and competitiveness to rice of some annual weed species. In: *Studies on Weeds and Rice Competition* (ed. by Sorianegara I.). Biotrop, Bogor, Indonesia, 13–21.
- Shibayama H. 2001. Weeds and weed management in rice production in Japan. *Weed Biol. Manag.* **1**, 53–60.
- Singh P.K. 1977. Multiplication and utilization of fern '*Azolla*' containing nitrogen-fixing algal symbiont, a green manure in rice cultivation. *Rizo* **26**, 125–137.
- Smith R.J. Jr. 1983. Weeds of major economic importance in rice and yield losses due to weed competition. In: *Proceedings of the Conference on Weed Control in Rice* (Manila, the Philippines). International Rice Research Institute, Manila, 19–36.
- Talley S.N., Talley B.J. and Rains D.W. 1977. Nitrogen fixation by *Azolla* in rice fields. In: *Genetic Engineering for Nitrogen Fixation* (ed. by Hollaender A.). Plenum Press, New York, 259–281.
- Tran Q.T. and Dao T.T. 1973. *Azolla*: a green compost. Vietnamese studies 38. *Agric. Prob.* **4**, 119–127.
- Tung H.F. and Watanabe I. 1983. Differential response of *Azolla*–*Anabaena* associations to high temperature and minus phosphorus treatments. *New Phytol.* **93**, 423–431.
- Van Hove C., Diara H. and Godard P. 1983. *Azolla en Afrique de l'Quest in West Africa*. Food and Agriculture Organization, Rome.
- Wagner G.M. 1997. *Azolla*: a review of its biology and utilization. *Bot. Rev.* **63**, 1–26.
- Watanabe I. and Berja N.S. 1983. The growth of four species of *Azolla* as affected by temperature. *Aquat. Bot.* **15**, 175–185.
- Watanabe I. and Hove C.V. 1996. Phylogenetic, molecular and breeding aspects of *Azolla*–*Anabaena* symbiosis. In: *Pteridology in Perspective* (ed. by Camus J.M., Gibby M. and Johns R.J.). Royal Botanic Gardens, Kew, 611–619.
- Xuan T.D., Tsuzuki E., Terao H., Mitsuhiro M., Khanh T., Yama S.M. et al. 2003. Alfalfa, rice by-products and their incorporation for weed control in rice. *Weed Biol. Manag.* **3**, 137–144.
- Xuan T.D., Tsuzuki E., Uematsu H. and Terao H. 2001. Weed control with alfalfa pellets in transplanting rice. *Weed Biol. Manag.* **1**, 231–235.
- Zoschke A. 1990. Yield loss in tropical rice as influenced by the competition of weed flora and the timing of its elimination. In: *Pest Management in Rice* (ed. by Grayson B.T., Green M.B. and Copping L.G.). Elsevier Science, London, 301–313.