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Effects of arbuscular mycorrhizal infection on the growth and reproduction of the annual legume *Kummerowia striata* growing in a nutrient-poor alluvial soil

TAKAYUKI NAKATSUBO

Department of Environmental Studies, Faculty of Integrated Arts and Sciences, Hiroshima University, Kagamiyama 1-7-1, Higashi-Hiroshima 739, Japan

A culture experiment was conducted to examine the effects of arbuscular mycorrhizal (AM) fungi on the growth and reproduction of *Kummerowia striata*, a common annual legume of river floodplains of Japan. The plants were grown from seeds in pots with nutrient-poor sandy soil collected from a fluvial bar. Arbuscular mycorrhizal infection increased the aboveground biomass, nodule weight, leaf nitrogen concentration and seed production. However, flowering occurred earlier in plants without AM fungi. These effects of AM fungi were insignificant in plants supplied with phosphate. These results suggest that AM fungi may influence the establishment of *K. striata* in nutrient-poor, disturbed habitats.

Key words: arbuscular mycorrhizas; floodplain; *Kummerowia striata*; phosphorus; reproduction.

INTRODUCTION

Over the last two decades, a number of studies have indicated that arbuscular mycorrhizal (AM) fungi have a significant influence on the growth of plants in many natural ecosystems (e.g. Allen 1991). Arbuscular mycorrhizal fungi are known to have low host specificity and promote nutrient (especially phosphorus) absorption of the host plants. Thus, it is expected that the impact of AM fungi is important, especially in nutrient-poor conditions such as the pioneer stages of succession following disturbances.

Severe disturbances are known to reduce propagule density of AM fungi. Several authors have reported that the propagule density of AM fungi is much smaller in severely disturbed sites than in undisturbed sites (e.g. Allen & Allen 1980; Powell 1980). These facts suggest that AM fungi can be a limiting factor of plant colonization in severely disturbed habitats.

Floodplains along a river are exposed to frequent and severe disturbances. Previous studies have indicated that the distribution of plants in river floodplains was largely limited by disturbance and/or soil moisture conditions (e.g. Naohara 1936–1937; Walker *et al.* 1986; Nakatsubo 1995). However, some authors (e.g. Naohara 1936–1937) suggested that nutrient availability is also important in determining plant distribution. Nakatsubo *et al.* (1994) reported that the spore density and infection level of AM fungi tended to be lower at severely eroded sites in a river floodplain. It can be hypothesized that the abundance of AM fungi could be a limiting factor of plant distribution in river floodplains. Alternatively, it is possible that plants in this habitat are able to grow and reproduce independently of AM fungi. At present, however, the impact of AM fungi on plant populations of river floodplains is poorly defined.

In this study, the potential impact of AM fungi on the growth and reproduction of *Kummerowia striata* (Thunb.) Schindler, a common annual legume of the river floodplains in Japan, is examined.

METHODS

The soils for culture experiment were collected from a fluvial bar situated in the middle of the Ohta River in Hiroshima Prefecture. There were various

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habitat types in this bar (e.g. *Salix* shrubs, grassland and gravelly areas with sparse vegetation). A detailed description of this site appeared in Nakatsubo *et al.* (1994) and Nakatsubo (1995).

The sandy soil collected from an open area without vegetation cover was used in the culture experiment, as the previous study (Nakatsubo *et al.* 1994) showed that the density of AM fungi was especially low in these open sites. Carbon and nitrogen concentrations of this soil, determined with a CN-corder (Yanaco MT-500, Yanagimoto Co. Ltd, Kyoto, Japan), were 0.6 mg C g^{-1} and 0.02 mg N g^{-1}, respectively. Bicarbonate-extractable P (Olsen & Sommers 1982) of the soil was 0.6 μg P g^{-1}. The soil was autoclaved at 125°C for 30 min twice prior to the experiment (Soil A).

The response of a plant to AM infection depends largely on the fungal species (e.g. Allen 1991). Since this study aimed to find out the potential impact of AM fungi in the field, the soil for inoculation of AM fungi was collected from the grassland of the fluvial bar (Soil B). The density of spores of AM fungi in this soil, determined by the sucrose centrifugation method (Daniels & Skipper 1982) with some modification (cf. Nakatsubo *et al.* 1994), was about 30 per 4 g of dry soil.

The soil inoculated with AM fungi (AM+) and the control soil without AM fungi (AM–) were prepared as follows. About 300 g of air-dried soil for inoculation (Soil B) was suspended in 1.5 l of water and heavier particles were allowed to settle for about 20 s. The liquid was passed through a 500 μm-mesh sieve to remove large particles of organic matter. Then, the liquid was added to 8 kg of the autoclaved soil (Soil A) to inoculate soil microorganisms including AM fungi. The control soil without AM spores but with other soil microorganisms was prepared in the same manner except that the liquid was passed through a 38 μm-mesh sieve to remove spores of AM fungi. The particles retained in the 38 μm-mesh sieve were added to the AM+ soil. About 500 cm^3 of the soil was put in a culture pot, 8 cm in diameter and 10 cm in height.

The seeds of *K. striata* were collected from a community growing on the bank near the fluvial bar on 12 November 1994. They were stored in an air-dried state until the experiment. Just prior to the experiment, the surface of these seeds was sterilized by soaking in sodium hypochlorite solution (active Cl about 10%) for 5 min. Five seeds were sown in each pot on 18 April 1995. To prevent contamination of AM fungi, the pots with AM fungi and those without AM fungi were kept separately in translucent containers (35 cm width × 54 cm length × 30 cm height). The lid of the container was made of nylon mesh with an opening of 38 μm. These containers were placed in the greenhouse of Faculty of Integrated Arts and Sciences of Hiroshima University. From 12 May to the end of September 1995, the roof of the greenhouse was covered with shade cloth to prevent excess heating by solar radiation. The mean weekly maximum and minimum temperatures during the course of the experiment were 39 and 17°C, respectively. The plants were watered with 50 ml of deionized water once a week. When necessary, additional water (approximately 150 ml) was supplied to the pots in summer.

About 84% of the seeds germinated within a week after sowing, although a few seeds also germinated after that period. The percentage of germination determined at 5 weeks after sowing was about 93%.

During the culture experiment, the growth stage of each individual was recorded every week. The growth stages of the young seedlings of *K. striata* can be expressed in terms of the number of leaves (Nakatsubo 1995). In this study, a partly unfolded leaf was counted as 0.5. For example, a plant with three unfolded leaves and one partly unfolded leaf was expressed as growth stage 3.5.

At 5 weeks after sowing, all pots were thinned to contain three seedlings. Small individuals that showed delayed germination (see above) were removed from the pots at this time. One of the seedlings in each pot was sampled at 8 weeks after sowing. The second sampling was carried out at 15 weeks after sowing. The samples were divided into aboveground parts (leaves and stems) and belowground parts (roots and nodules). The aboveground parts were dried to a constant weight at 80°C to obtain the dry weight. For the second sampling, the weight of the nodules was also measured. The nitrogen concentration of the leaves was measured with a CHN/O elemental analyzer (Perkin Elmer 2400II, Norwalk, CT, USA).

The root samples were fixed and stored in 50% ethanol. They were cleared and stained according to Koske and Gemma (1989). The stained roots were observed under a light microscope for infection with AM fungi. This observation showed that one

pot without AM fungi (AM–) had been contaminated with AM fungi. The plants in this pot were eliminated from the data.

At 8 weeks, 50 ml of 6.5 mmol/l NaH_2PO_4 solution was added to half of the pots in each mycorrhizal treatment (AM+ or AM–). There were 24 pots in all (two mycorrhizal treatments × two levels of phosphate × six replicates).

In the course of the experiment, a few seedlings died at the end of July as a result of extreme heating. These seedlings were eliminated from the data.

At the end of the growing season (at 23 weeks after sowing), the plants were harvested and the number of seeds per plant was counted. In order to find out the germination capacity of these seeds, they were stored under air-dried conditions for about 6 months. Then, they were surface-sterilized in the same manner as described above. Five seeds were placed on filter paper wetted with deionized water in a 6 cm Petri dish with five replicates. They were allowed to germinate in a growth chamber at 20°C.

The difference in the mean aboveground weight between AM+ and AM– plants at 8 weeks was analyzed using a *t*-test. Variances were tested by *F*-test. The effects of AM infection and phosphorus application on the aboveground weight, nodule weight, N concentration and seed production were analyzed by a two-way ANOVA. For the aboveground weight, nodule weight and seed production, the data were logarithmically transformed. The effect of AM infection on the number of flowering plants was analyzed by a Chi-squared test.

RESULTS

Figure 1 shows the growth of seedlings growing on mycorrhizal and non-mycorrhizal soil before phosphorus application. There was no significant difference in the growth stage between the two mycorrhizal treatments from 1 to 6 weeks after sowing. After 7 weeks, the plants with AM fungi showed a higher growth rate than those without AM fungi. At 8 weeks, the average dry weight of the aboveground parts of the mycorrhizal plants (23.9 mg) was significantly larger than that of the non-mycorrhizal plants (17.5 mg; *t*-test; $P < 0.05$). The leaf life span was longer in the mycorrhizal plants than in the non-mycorrhizal plants. For example, cotyledons in

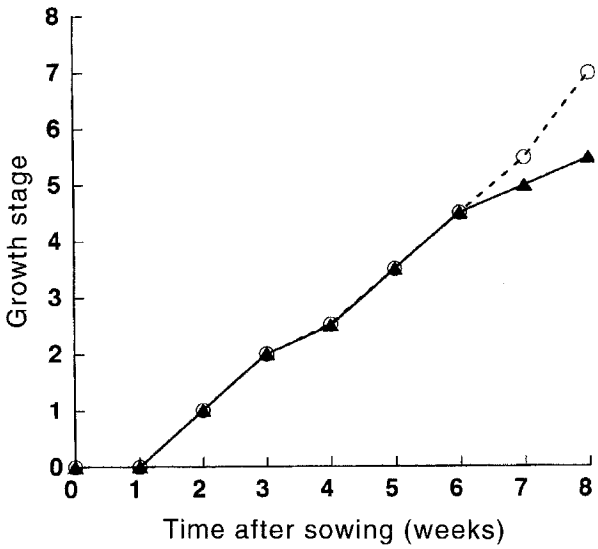

Fig. 1. The growth of *Kummerowia striata* seedlings inoculated with arbuscular mycorrhizal (AM) fungi (---○---) and those without AM fungi (—▲—) before phosphorus application. Growth stages were determined based on the number of leaves (see text). The median of the growth stage in each mycorrhizal treatment is shown (n = 33–56).

most of the mycorrhizal plants remained green at 8 weeks, while those in the non-mycorrhizal plants had turned yellow by that time.

The application of phosphate solution initially caused the shedding of some leaves both in mycorrhizal and non-mycorrhizal plants. However, they soon recovered their growth. Microscopic investigation revealed that plants in pots with AM fungi (AM+) were infected with fungi irrespective of P application.

The effects of AM infection and phosphorus application on plant growth were significant at 15 weeks after sowing (7 weeks after phosphorus addition; Table 1). The aboveground weight of the non-mycorrhizal plants without phosphorus application was less than one-third of those in the mycorrhizal infected or phosphorus applied plants (Fig. 2a). There was a significant interaction between AM infection and phosphorus application (Table 1); the aboveground weight of the phosphorus-applied non-mycorrhizal plants was similar to those in the mycorrhizal plants (Fig. 2a). The aboveground weight could not be determined at 23 weeks after sowing since part of the leaves had been shed in the non-mycorrhizal plants without phosphorus application. However, AM infection and phosphorus application had a significant effect on the stem

Table 1 ANOVA table on the effects of arbuscular mycorrhizal infection (AM) and phosphorus application (P) on the aboveground weight, nodule weight, leaf nitrogen concentration and seed production of *Kummerowia striata*

	Effects	d.f.	SS	*F*	*P*
Aboveground weight*					
	AM	1	0.645	34.0	< 0.0001
	P	1	0.271	14.3	0.0020
	AM × P	1	0.401	21.2	0.0004
Nodule weight*					
	AM	1	1.131	30.9	< 0.0001
	P	1	1.803	49.3	< 0.0001
	AM × P	1	0.842	23.0	0.0003
Leaf N concentration*					
	AM	1	2.057	90.0	< 0.0001
	P	1	4.201	183.8	< 0.0001
	AM × P	1	2.296	100.4	< 0.0001
Seed production**					
	AM	1	0.923	37.8	< 0.0001
	P	1	0.553	22.7	0.0002
	AM × P	1	0.520	21.3	0.0003

*Determined 15 weeks after sowing; **determined 23 weeks after sowing.
SS, sum of squares.

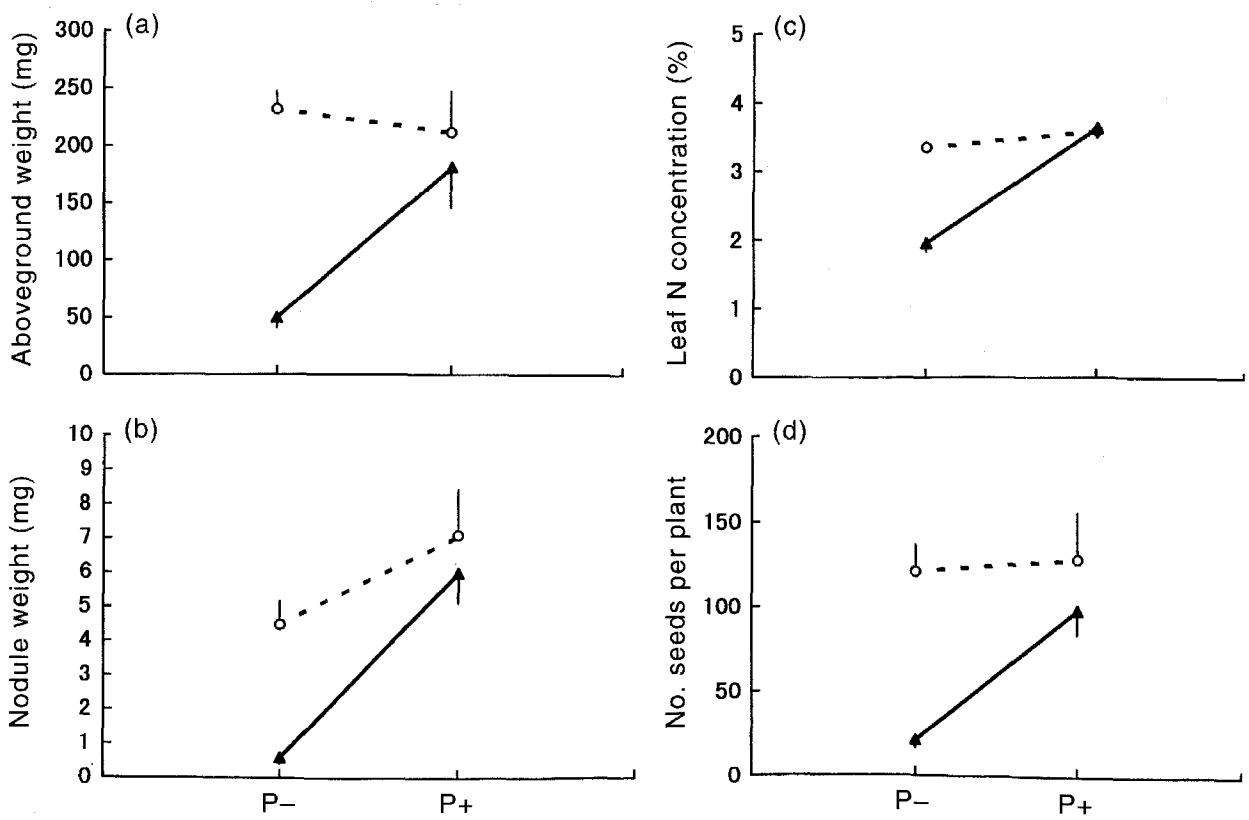

Fig. 2. The effects of arbuscular mycorrhizal (AM) infection and phosphorus application (P) on (a) the aboveground weight, (b) the nodule weight per plant, (c) the leaf nitrogen concentration and (d) the seed production of *Kummerowia striata* (n = 4–6): ---○--- , with AM fungi; —▲—, without AM fungi. Seed production was determined at 23 weeks after sowing; other data were obtained at 15 weeks. Vertical bars represent SE.

weight at 23 weeks; the mean stem weight of the non-mycorrhizal plants without phosphorus application was less than 12% of those in the mycorrhizal or phosphorus-applied plants.

The effect of AM infection and phosphorus application on nodule weight was also significant (Table 1). The mean nodule weight of the non-mycorrhizal plants without phosphorus application was less than 14% of those in the mycorrhizal plants (Fig. 2b). As in the aboveground biomass, there was a significant interaction between AM infection and phosphorus application (Table 1); the effect of AM infection was insignificant in the phosphorus-applied plants. The effects of AM infection and phosphorus application on leaf nitrogen concentration were essentially the same as in the aboveground weight (Table 1, Fig. 2c).

The branching pattern was also affected by the treatments; the non-mycorrhizal plants without phosphorus application rarely branched while the mycorrhizal and/or phosphorus-applied plants branched frequently (data not shown).

On the other hand, the time taken to initiate flowering was earlier in the non-mycorrhizal plants than in the mycorrhizal plants. Arbuscular mycorrhizal fungal infection significantly decreased the number of flowering plants at 15 weeks after sowing (χ^2 test, $P < 0.001$); all of the non-mycorrhizal plants without phosphorus application had initiated flowering by 15 weeks, while only one individual among the eighteen mycorrhizal plants bore flowers (Table 2). The percentage of flowering plants increased gradually in August, but two individuals among the nine mycorrhizal plants had no flower even at 17 weeks after sowing.

All of the plants produced seeds by the end of the experiment irrespective of mycorrhizal infection. However, AM infection and phosphorus application significantly increased the number of seeds per plant (Table 1, Fig. 2d). Again, there was a significant interaction between the two treatments; the effect of AM infection on seed production was not significant in the phosphorus-applied plants. The number of seeds in the mycorrhizal and/or phosphorus-applied plants might be underestimated because most of the seeds were still immature when harvested (at 23 weeks). On the other hand, most of the seeds appeared to be mature in the non-mycorrhizal plants without phosphorus-application. The germination capacity of these apparently mature seeds was tested in a laboratory at 20°C. The percentage of germination of these seeds for 35 days was 92%, which is similar to the value in the natural population of *K. striata* (Nakatsubo 1995). The percentage of germination was not determined for the mycorrhizal and/or phosphorus-applied plants because of the limited number of mature seeds.

DISCUSSION

Although growth responses of plants to AM fungal infection are known to vary widely among plant species, ranging from virtually no response to nearly obligatory dependence on AM fungi (e.g. Allen 1991), a number of studies have shown that AM infection significantly enhanced the growth of many legume species (e.g. Hall & Armstrong 1979; Fitter 1988). In this study, the aboveground biomass, nodule weight and seed number per plant in the mycorrhizal plants of *K. striata* were more than three times as large as those in the non-mycorrhizal plants, confirming earlier observations.

Phosphorus concentration of the plants could not be determined in this study because of the small size of the non-mycorrhizal plants without phosphorus

Table 2 The effects of arbuscular mycorrhizal (AM) fungal infection and phosphorus application on the flowering of *Kummerowia striata* at 15 weeks after sowing

Treatments*		No. plants with flowers (A)**	No. plants without flowers (B)	A/(A+B) × 100 (%)
AM+	P–	1	9	10
AM+	P+	0	8	0
AM–	P–	9	0	100
AM–	P+	4	7	36

*AM+, plants inoculated with AM fungi; AM–, plants without AM fungi; P+, plants with phosphorus application; P–, plants without phosphorus application.

**Plants with cleistogamous flowers and those with wilting flowers are also included.

application. However, it appeared that AM fungi enhanced plant growth by promoting phosphorus absorption, since AM infection and phosphorus application had almost the same influence on the plants.

It has been reported that AM infection increases the nitrogen-fixing activity of nodules in legume species (e.g. Eom *et al.* 1994). In this study, the nodule weight of the non-mycorrhizal plants without phosphorus application was less than 14% of the mycorrhizal plants. In addition, the nitrogen concentration of leaves was much smaller in the non-mycorrhizal plants without phosphorus application than in the mycorrhizal and/or phosphorus-applied plants. These facts suggest that the restricted phosphorus absorption in the non-mycorrhizal plants caused a reduction in nitrogen-fixing activity which resulted in nitrogen deficiency in the plants.

It is noteworthy that the phosphorus application had little effect on the growth and reproduction of mycorrhizal plants. This indicates that the mycorrhizal plants absorbed a sufficient amount of phosphorus from the nutrient-poor soil. The level of bicarbonate-extractable phosphorus in the soil of this study (< 1 μg g^{-1}) was much lower than those of previous studies in which the effects of AM infection on plant growth were examined (e.g. Carpenter & Allen 1988; Fitter 1988; Lu & Koide 1994). Olsen and Sommers (1982) noted that many crops show some response to phosphorus fertilizer if the soil phosphorus level is lower than 5 μg g^{-1}. The low phosphate demand of mycorrhizal *K. striata* may partly explain the abundance of this species in nutrient-poor disturbed habitats.

Despite slower vegetative growth in the non-mycorrhizal plants, the time taken to initiate flowering was significantly earlier in the non-mycorrhizal plants than in the mycorrhizal plants. Similar differences in flowering phenology between mycorrhizal and non-mycorrhizal plants have been reported for some xeric grasses (Allen & Allen 1986). In contrast, Lu and Koide (1994) reported that infection with AM fungi decreased the time taken to initiate flowering in *Abutilon theophrasti* Medic. (Malvaceae).

The early flowering of the non-mycorrhizal *K. striata* may be a compensatory response to phosphorus deficiency. Theoretical studies have predicted that the switch from vegetative to reproductive growth in annual plants is timed to maximize reproductive yield (e.g. King & Roughgarden 1983). It seems risky for a nutrient-limited annual plant to continue vegetative growth until late in the growing season because it may fail to reproduce. In such cases, early flowering would enhance the chance of reproduction even if the seed number per plant may be small.

The non-mycorrhizal plants without phosphorus application survived through the growing season and produced seeds which had a high germination capacity. Therefore, it seems unlikely that the abundance of AM fungi directly limits the colonization of *K. striata* in river floodplains. However, mycorrhizal infection may affect the distribution pattern of the offspring generation through its effect on fecundity, since seed availability is one of the most important factors that limits the distribution of *K. striata* in a fluvial bar (Nakatsubo 1995).

In addition, AM fungi may confer competitive advantages to mycorrhizal species in the presence of competition from non-mycotrophic species. In the fluvial bar of the Ohta River, non-mycotrophic species such as *Polygonum hidropiper* L., *Lepidium virginicum* L. and *Cyperus rotundus* L. coexist with mycorrhizal species (Nakatsubo *et al.* 1994).

The results suggest that AM fungi profoundly influence the growth and reproduction of *K. striata* in nutrient-poor, disturbed habitats. The role of AM fungi in determining the community structure of river floodplains merits further investigation.

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