

Mycorrhizae Increase Arsenic Uptake by the Hyperaccumulator Chinese Brake Fern (*Pteris vittata* L.)

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ABSTRACT

Chinese brake fern (*Pteris vittata* L.) is a hyperaccumulator of arsenic (As) that grows naturally on soils in the southern United States. It is reasonable to expect that mycorrhizal symbiosis may be involved in As uptake by this fern. This is because arbuscular mycorrhizal (AM) fungi have a well-documented role in increasing plant phosphorus (P) uptake, P and As have similar chemical properties, and ferns are known to be colonized by AM fungi. We conducted a factorial greenhouse experiment with three levels of As (0, 50, and 100 mg kg⁻¹) and P (0, 25, and 50 mg kg⁻¹) and with and without Chinese brake fern colonized by a community of AM fungi from an As-contaminated site. We found that the AM fungi not only tolerated As amendment, but their presence increased frond dry mass at the highest As application rate. Furthermore, the AM fungi increased As uptake across a range of P levels, while P uptake was generally increased only when there was no As amendment. These data indicate that AM fungi have an important role in arsenic accumulation by Chinese brake fern. Therefore, to effectively phytoremediate As-contaminated soils, the mycorrhizal status of ferns needs to be taken into account.

ARSENIC IS EXTREMELY TOXIC yet Chinese brake fern (*Pteris vittata* L.; Ma et al., 2001) and several other fern species (Raab et al., 2004; Meharg, 2003; Zhao et al., 2002) are known to be active hyperaccumulators of this element. The mechanism of As accumulation and tolerance in these plants is not fully known, but it is reasonable to expect that mycorrhizal symbiosis is involved. Arbuscular mycorrhizal (AM) fungi have a well-documented role in increasing plant uptake of phosphorus (P) and other poorly mobile elements (Smith and Read, 1997), and are recognized as important components of phytoremediation strategies for heavy metals (Khan et al., 2000). Both As and P belong to the same chemical group (V_A elements) and thus have similar geochemical behavior (Meharg et al., 1994; Adriano, 2001).

The fundamental explanation for improved uptake of poorly mobile elements by AM plants is that they have a better distributed absorbing surface area in the soil than do nonmycorrhizal plants (Sanders and Tinker, 1971). Additional mechanisms to account for improved uptake by mycorrhizal roots may include small fungal hyphae radii, greater total absorptive surface area, dif-

ferent uptake kinetics, faster extension rate, chemical alteration of the rhizosphere–hyphosphere, increased functional longevity, mineralization of organic forms, exploration of smaller pore spaces, greater carbon-use efficiency, and differences in associated rhizosphere populations (O’Keefe and Sylvia, 1991).

Ferns are known to be colonized by AM fungi (Berch and Kendrick, 1982; Iqbal et al., 1981; LaFerriere and Koske, 1981; Ponton et al., 1990; Schmid and Oberwinkler, 1995; Sharma, 1998). Preliminary observations from an As-contaminated site in north-central Florida indicate that Chinese brake fern is well colonized by AM fungi (unpublished data). In the low fertility soils where Chinese brake fern grows (Chen et al., 2002), we hypothesized that AM fungi have a role in maintaining its productivity and may contribute significantly to plant arsenic uptake. Our objective was, therefore, to evaluate the effect of AM fungal colonization on As and P uptake by Chinese brake fern. We tested a community of AM fungi from an As-contaminated site in north-central Florida over a range As and P concentrations.

MATERIALS AND METHODS

The completely randomized factorial experiment had five replicates per treatment combination and consisted of three applied As levels (0, 50, and 100 mg kg⁻¹), three applied P levels (0, 25, and 50 mg kg⁻¹), and inoculation or no inoculation with a community of mycorrhizal fungi from an As-contaminated site. The study was conducted in a greenhouse from 25 May to 21 Aug. 2003 with mean maximum and minimum temperatures, respectively, of 32 and 18°C, and mean maximum photosynthetic photon flux density (PPFD) of 1235 μmol m⁻² s⁻¹.

The AM fungal community inoculum was obtained from the abandoned copper–chromium–arsenic (CCA) wood treatment site in north-central Florida where the arsenic hyperaccumulation property of Chinese brake fern was first discovered (Ma et al., 2001). Soil samples were collected randomly from the surface 20-cm depth, sieved through a 1-cm pore-size screen, and mixed before use as inoculum in the experiment. A mycorrhizal inoculum percentage (MIP) bioassay (Sylvia, 1994) was established when the experiment was initiated, resulting in a mean of 43% of the root length colonized by AM fungi.

The potting medium for the experiment consisted of a 1:1:1 mixture of soil (from a noncontaminated location), acid washed sand, and coarse vermiculite that had the following chemical and physical properties: pH, 7.3 (1:1 soil to water ratio); cation exchange capacity, 4.4 cmol⁺ kg⁻¹ (Thomas, 1982); organic matter, 11.0 g kg⁻¹ (Nelson and Sommers, 1982); total As, 0.69 mg kg⁻¹ (USEPA, 1983); total P, 3.4 mg kg⁻¹ (Olsen and Sommers, 1982); and a particle size distribution of 94% sand, 3.6% silt, and 1.7% clay (Day, 1965). This mixture was pasteurized twice at 85°C for 8 h with 48 h between heating. After pasteuriza-

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Abbreviations: AM, arbuscular mycorrhizal fungi.

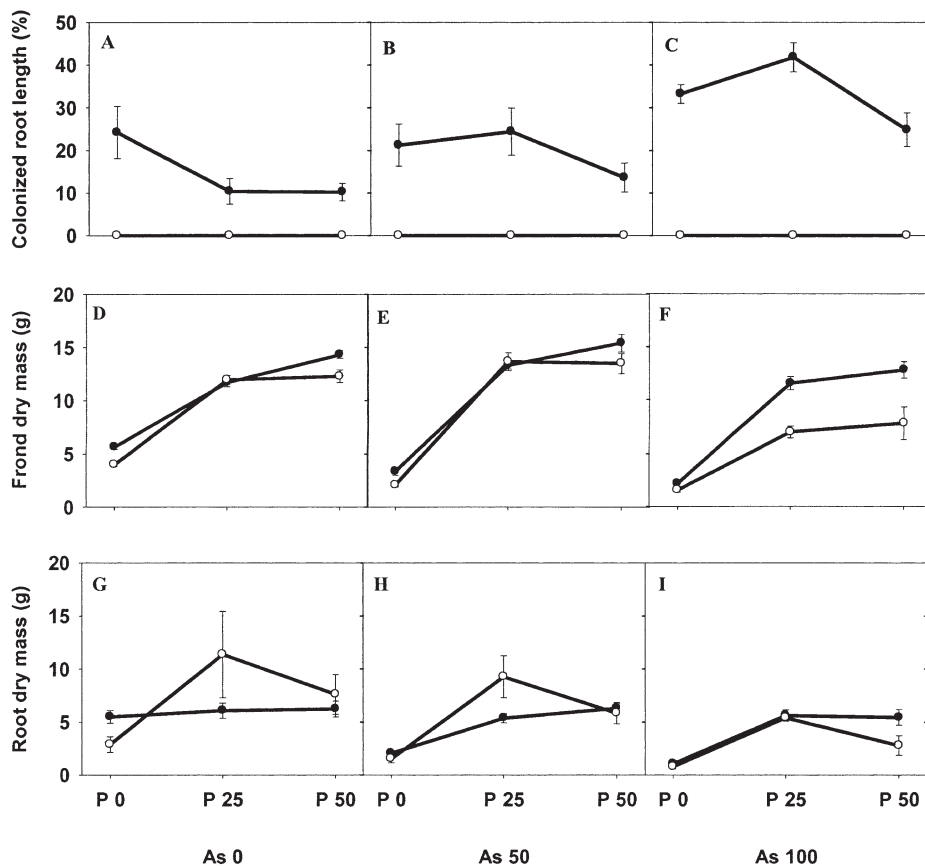


Fig. 1. Effect of As and P amendment (mg kg^{-1}) on percentage of root length colonized by arbuscular mycorrhizal (AM) fungi (A–C), and frond (D–F) and root (G–I) dry masses of Chinese brake fern (*Pteris vittata* L.) grown in the presence (●) or absence (○) of mycorrhizal inoculum. Data points represent means of five replicates \pm SEM.

tion, portions of the potting medium were amended with sodium arsenate ($\text{Na}_2\text{HAsO}_4 \cdot 7\text{H}_2\text{O}$) and potassium phosphate (KH_2PO_4) to achieve treatment concentrations and left to equilibrate for 1 wk.

To prepare the mycorrhizal inoculation treatments, the potting medium was amended with fresh or autoclaved CCA soil in a ratio of 15 to 1. The autoclaved soil was further amended with a microbial filtrate ($<20\text{-}\mu\text{m}$ pore size) from the fresh CCA soil to reintroduce a portion of the microbial community other than AM fungi (Ames et al., 1987). The materials were mixed thoroughly and 1.5 kg of each medium was placed in sterilized 1.5-L experimental potting units. To ensure nonmycorrhizal plant production the ferns were initiated from spores that were placed on sterilized sandy soil at room temperature and incubated in diffuse natural light for several weeks. Uniform plants consisting of five to six fronds were selected and one plant was placed in each experimental unit. A dilute (10% strength) Hoagland's solution (Hoagland and Arnon, 1938) was applied to the pots on a weekly basis and plants were watered with deionized water as needed.

At harvest, percentage root length colonized by AM fungi, frond and root dry masses, and As and P concentrations were determined. Fronds were cut at the soil surface and dried at 65°C for 48 h. Roots were removed from the potting medium by washing the root ball over a coarse sieve, and fresh masses were determined. Subsamples of 0.5 g were removed from the roots, cleared and stained for mycorrhizal observation, and quantified by a gridline-intersect method (Sylvia, 1994), while the remainder of the root samples were dried. The fresh to

dry root ratio was used to estimate the total dry mass of the root sample.

Ground fronds and roots (20-mesh) were digested with concentrated HNO_3 and deionized H_2O (1:1, v/v), followed by 30% H_2O_2 for As determination (USEPA, 1983) or with HNO_3 , H_2SO_4 , and 30% H_2O_2 for P determination (Olsen and Sommers, 1982). The As concentration was determined using a graphite furnace atomic absorption spectrophotometer (SIMMA 6000; PerkinElmer, Wellesley, MA) and P concentration was determined using a colorimetric assay (Murphy and Riley, 1962) adapted for a microplate reader (Model 550; Bio-Rad, Hercules, CA). In both analyses, blanks and internal standards were included for quality assurance. Translocation and bioconcentration factors of As in Chinese brake fern were calculated based on the arsenic concentration ratio of fronds to roots and the arsenic concentration ratio of plant to soil (native soil As concentration plus amended value), respectively.

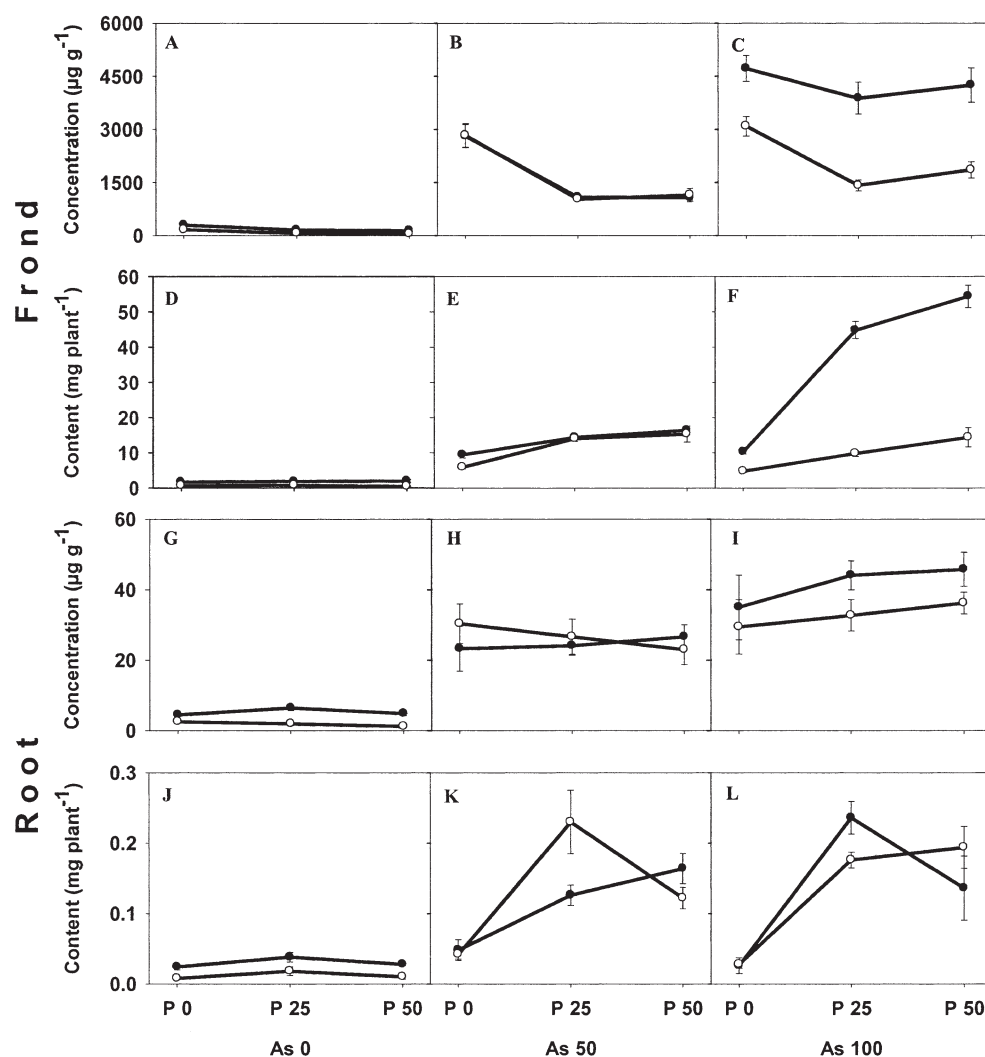
A general linear model was applied to the data to test for the main effects and their interactions on all response variables using PROC GLM (SAS Institute, 2003). Population normality was tested for each variable before using parametric statistics for comparisons and testing.

RESULTS AND DISCUSSION

Root length colonized by AM fungi ranged from 10 to 42% in treatments receiving the community inoculum (Fig. 1A–1C). Increasing P concentration generally reduced AM colonization, while increasing As concentra-

Table 1. Analysis of variance (ANOVA) summary (*P* values) for the effects of mycorrhizae, As, P, and their interactions on biomass and uptake of As and P by Chinese brake fern (*Pteris vittata* L.).

Variable	Treatment						
	Mycorrhizae (Myc)	Arsenic (As)	Phosphorus (P)	Myc × As	Myc × P	As × P	Myc × As × P
FronD dry mass (g)	<0.0001	<0.0001	<0.0001	0.0016	0.0265	0.0001	0.0102
Root dry mass (g)	0.4770	0.0004	<0.0001	0.2219	0.0136	0.9310	0.2528
FronD/root	0.8742	0.0141	0.0011	0.4675	0.0716	0.0561	0.1030
Colonization (%)	<0.0001	<0.0001	0.0053	<0.0001	0.0053	0.0808	0.0808
FronD As concentration ($\mu\text{g g}^{-1}$)	<0.0001	<0.0001	<0.0001	<0.0001	0.5344	<0.0001	0.6030
FronD As content (mg plant^{-1})	<0.0001	<0.0001	<0.0001	0.0052	0.3481	<0.0001	0.0199
Root As concentration ($\mu\text{g g}^{-1}$)	0.1047	<0.0001	<0.0001	0.1102	0.5264	0.4570	0.9433
Root As content (mg plant^{-1})	0.9351	<0.0001	0.6712	0.2441	0.8031	<0.0001	0.0003
FronD P concentration ($\mu\text{g g}^{-1}$)	0.0064	<0.0001	0.0382	0.0021	0.0594	0.0061	0.0281
FronD P content (mg plant^{-1})	<0.0001	0.0009	<0.0001	0.0095	0.0001	0.0776	0.0586
Root P concentration ($\mu\text{g g}^{-1}$)	<0.0001	<0.0001	0.0004	0.0437	0.8459	0.1287	0.2732
Root P content (mg plant^{-1})	0.0007	0.4127	<0.0001	0.0042	0.0992	0.573	0.6349
As translocation	0.0294	<0.0001	<0.0001	0.0449	0.553	0.1852	0.7316
As bioconcentration	<0.0001	<0.0001	<0.0001	<0.0001	0.6831	<0.0001	0.6775

**Fig. 2.** Effect of As and P amendment (mg kg^{-1}) on As accumulation by Chinese brake fern (*Pteris vittata* L.) grown in the presence (●) or absence (○) of mycorrhizal inoculum; frond concentration (A–C) and content (D–F) and root concentration (G–I) and content (J–L). Data points represent means of five replicates \pm SEM.

tion tended to increase percentage colonization. The systems of noninoculated plants had no evidence of AM fungal colonization.

Mycorrhizal colonization had a significant and posi-

tive effect on frond dry mass at the highest level of As amendment and with addition of phosphorus (Fig. 1D–1F, Table 1). In contrast, mycorrhizal treatments did not significantly affect root mass in the presence of increas-

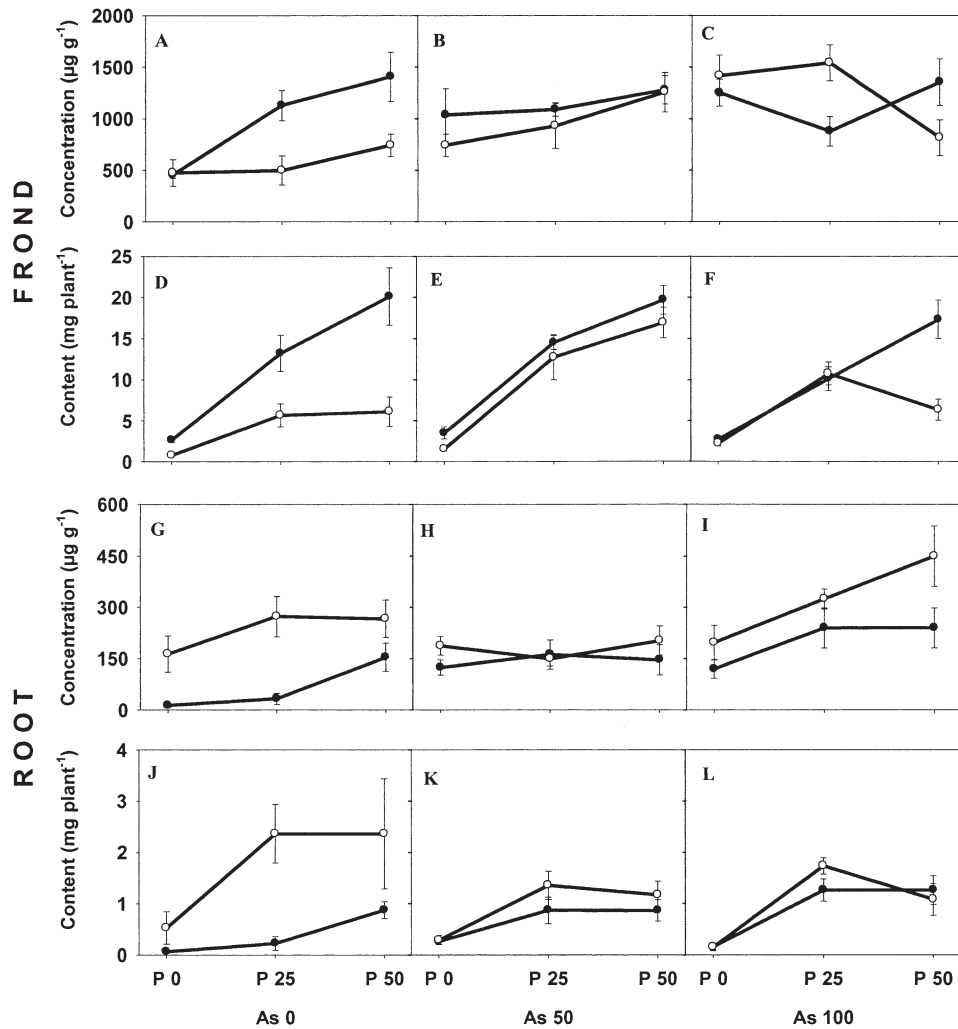


Fig. 3. Effect of As and P amendment (mg kg^{-1}) on the P accumulation by Chinese brake fern (*Pteris vittata* L.) grown in the presence (●) or absence (○) of mycorrhizal inoculum; frond concentration (A–C) and content (D–F) and root concentration (G–I) and content (J–L). Data points represent means of five replicates \pm SEM.

ing levels of As and P (Fig. 1G–1I). Sufficient biomass production is essential for successful phytoremediation employing hyperaccumulator plants (Kramer and Charadonnens, 2001). The fact that mycorrhizal colonization significantly increased the biomass of Chinese brake fern at high As levels suggests that inoculation with mycorrhizae may be a viable technology to improve the efficiency of plant arsenic uptake.

Mycorrhizal colonization did not affect frond As concentration in Chinese brake fern at treatments levels of 0 or 50 mg As kg^{-1} . However, As concentration was consistently increased at As concentration of 100 mg As kg^{-1} (Fig. 2A–2C), resulting in an average increase in the fronds of nearly 100% over the nonmycorrhizal controls across the range of P amendment. The total content of As in the fronds followed a similar trend with little effect at lower As levels, but a significant increase at the highest As amendment (Fig. 2D–2F). Furthermore, As accumulation increased with increasing P levels. For example, the As content was approximately five times greater with mycorrhizae than without mycorrhizae in the soils amended with 100 mg As kg^{-1}

and 50 mg P kg^{-1} (Fig. 2F). This implies an important role for mycorrhizae in enhancing plant As accumulation in soils with high As contamination.

Arsenic concentration and content in the roots were not significantly affected by mycorrhizal colonization (Table 1) and were either positively affected by As amendment (Fig. 2G–2I) or more variable (Fig. 2J, 2L), reflecting the results observed for root mass. It has been well documented that frond concentration of As in Chinese brake fern is substantially greater than root concentration (Ma et al., 2001), and our experiment confirms these results with a 100-fold greater concentration in the aboveground portion than the roots of the plant.

In the absence of applied As, P concentrations and contents in the fronds and roots increased significantly with increasing P amendment (Fig. 3A, 3D, 3G, and 3J). Furthermore, the P concentrations and contents in the fronds were greater in mycorrhizal plants, while the opposite was found for the roots. Mycorrhizae apparently enhanced P transport from the roots to fronds.

In treatments amended with As, the effects of mycorrhizae on plant P concentrations as well as contents

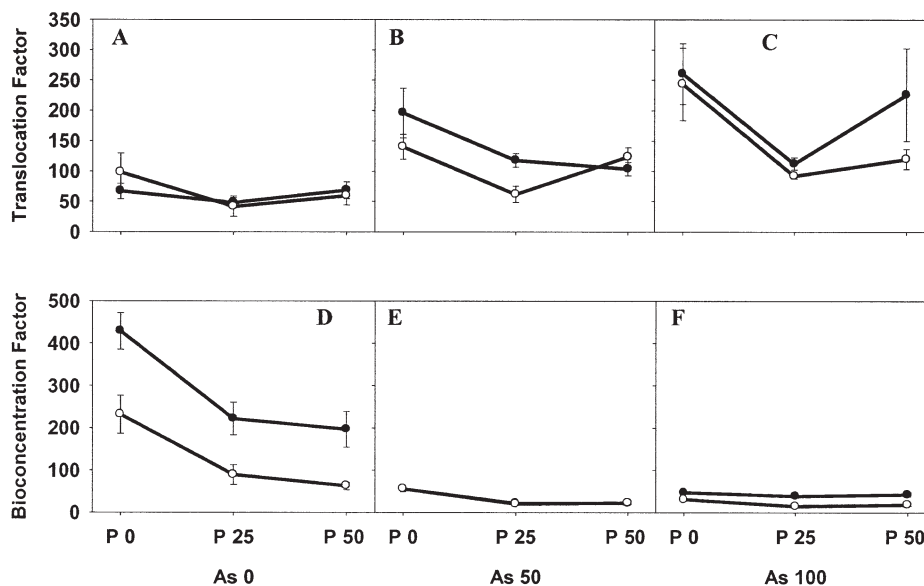


Fig. 4. Effect of As and P amendment (mg kg^{-1}) on translocation factors (the ratio of fronds As content to those in roots) (A–C) and bioconcentration factors (the ratio of fronds As concentration to those in soil) (D–F) of Chinese brake fern (*Pteris vittata* L.) grown in the presence (●) or absence (○) of mycorrhizal inoculum. Data points represent means of five replicates \pm SEM.

were more varied. In the medium amended with 50 mg As kg^{-1} , with increased P levels, P concentrations and contents in the fronds were increased whereas those in roots stayed nearly constant (Fig. 3B, 3E, 3H, and 3K). In the medium amended with $100 \text{ mg As kg}^{-1}$, the opposite was true with the exception of frond P content (Fig. 3C, 3F, 3I, and 3L).

Increasing P levels decreased As translocation from the roots to fronds (Fig. 4A–4C) and also decreased As bioconcentration factors (Fig. 4D–4F). Overall, mycorrhizae increased bioconcentration factors (Table 1), but the magnitude was greatest in the nonamended medium due to the low native As concentration. Bioconcentration factors of As in mycorrhizal compared to the non-mycorrhizal Chinese brake fern were increased threefold in treatments that received no As and twofold in treatments that received $100 \text{ mg As kg}^{-1}$.

Our research on Chinese brake fern supports the previous observations (Meharg and Cairney, 1999; Gonzalez-Chavez, 2000; Sharples et al., 2000a, 2000b) that plants growing in As contaminated soils are mycorrhizal. One of the principal roles for mycorrhizal fungi is to obtain P for their hosts (Smith and Read, 1997); however, this may become a problem when soil has high As concentrations. Our results are also consistent with those reported by Wang et al. (2002) on the interactions of As and P in plant uptake. We observed that addition of As to the soil affected plant P concentration while addition of P affected plant As concentration, thus supporting the evidence that arsenic uptake by Chinese brake fern is via P transport systems.

The hyperaccumulation status of Chinese brake fern has been extensively reported in the literature (Cao et al., 2004; Lombi et al., 2002; Ma et al., 2001; Tu and Ma, 2003). However, previous studies have not addressed the role of AM fungi in As uptake by ferns. Our data indicate that AM fungi have a significant role

in the increase of aboveground biomass and As accumulation, translocation, and bioconcentration by Chinese brake fern. Our results, however, differ markedly from studies of As–mycorrhizal interactions in other plants. For example, Knudson et al. (2003) found that AM fungal colonization of basin wildrye [*Leymus cinereus* (Scribn. & Merr.) Á. Löve] had no effect on As accumulation. In a very different system, Sharples et al. (2000a, 2000b) reported that an ericoid mycorrhizal associate of *Calluna* selectively accumulated P over As, acting as a filter to maintain low plant As levels.

Plentiful biomass production is critical for commercial application of Chinese brake fern because it will decrease the number of ferns required to complete the remediation of a given site. Our data support the first two steps described by McGrath and Zhao (2003) on hyperaccumulation processes, which include root uptake, root-to-shoot transport, complexation with chelating molecules, and compartmentalization into cell vacuole. The role of mycorrhizal fungi and other rhizosphere microorganism in As speciation as well as cell storage mechanisms of Chinese brake fern should be the objectives of future investigation.

Mycorrhizal fungi that significantly increase Chinese brake fern growth and As uptake may compete well with engineered bacteria for bioremediation because bacteria required the addition of sufficient nutrients to ensure their success (French and Rosser, 1999). Even though our research validates the role of mycorrhizae in enhancing plant growth and As uptake, we concur with Wang et al. (2002) that the mechanisms responsible for As hyperaccumulation in Chinese brake fern are still unclear. Based on the results of this study, it can be concluded that the mycorrhizal status of ferns used for phytoremediation of As-contaminated soils should be taken into account.

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