Arbuscular mycorrhizal fungi and organic fertilizer influence photosynthesis, root phosphatase activity, nutrition, and growth of *Ipomoea carnea* ssp. *fistulosa*

L. AMAYA-CARPIO[*], F.T. DAVIES, Jr.[*,***], T. FOX[**], and C. HE[*]

Department of Horticultural Sciences and Faculty of Molecular and Environmental Plant Sciences (MEPS), Texas A&M University, College Station, TX 77843-2133, USA[*]
Department of Biology, University of West Florida, Pensacola, FL 34514-5751, USA[**]

Abstract

The effect of arbuscular mycorrhizal fungi (AMF) inoculation and organic slow release fertilizer (OSRF) on photosynthesis, root phosphatase activity, nutrient acquisition, and growth of *Ipomoea carnea* N. von Jacquin ssp. *fistulosa* (K. Von Martinus *ex* J. Choisy) D. Austin (bush morning glory) was determined in a greenhouse study. The AMF treatments consisted of a commercial isolate of *Glomus intraradices* and a non-colonized (NonAMF) control. The OSRF was applied at 10, 30, and 100 % of the manufacturer's recommended rate. AMF plants had a higher net photosynthetic rate (P_N), higher leaf elemental N, P, and K, and generally greater growth than NonAMF plants. Total colonization levels of AMF plants ranged from 27 % (100 % OSRF) to 79 % (30 % OSRF). Root acid phosphatase (ACP) and alkaline phosphatase (ALP) activities were generally higher in AMF than non-AMF plants. When compared to NonAMF at 100 % OSRF, AMF plants at 30 % OSRF had higher or comparable ACP and ALP activity, higher leaf elemental P, N, Fe, Cu, and Zn, and a greater P_N (at the end of the experiment), leading to generally greater growth parameters with the lower fertility in AMF plants. We suggest that AMF increased nutrient acquisition from an organic fertilizer source by enhancing ACP and ALP activity thus facilitating P acquisition, increasing photosynthesis, and improving plant growth.

Additional key words: chlorophyll; *Glomus intraradices*; leaf area; leaf area ratio; net photosynthetic rate; phosphatase; plant height; root; shoot; stomatal conductance.

Introduction

The trend towards organic production practices in agriculture/horticulture has increased in the last decade. Organic production is being considered by some U.S. nursery growers as a means of conforming to increased environmental regulation at the federal and state level (Greer 2000). Furthermore, plants produced organically in containerized ornamental systems utilizing organic slow release fertilizer (OSRF) can be marketed for their higher value in the landscape.

Arbuscular mycorrhizal fungi (AMF) improve the nutrient uptake of phosphorus (P), nitrogen (N), copper (Cu), zinc (Zn), iron (Fe), and other ions (Tobar *et al*. 1994, Smith and Read 1997, Douds *et al*. 2000), and thus improve nutrient acquisition from fertilizers. In addition, colonization of roots with AMF enhances plant water relations (Davies *et al*. 1992, 1996, Augé 2001, Augé *et al*. 2003, 2004), increases resistance to cultural and environmental stresses, and consequently improves plant growth (Linderman 1986, Smith and Read 1997, Amaya-Carpio *et al*. 2005).

Mechanisms by which AMF enhance nutrient uptake include increased surface area of roots with the development of an extensive extra-radical hyphae network (Marschner 1995, Smith and Read 1997, Augé *et al*.

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[***]Corresponding author; fax: +1 979-845-0627, e-mail: f-davies@tamu.edu
Abbreviations: ACP − root acid phosphatase; ALP − root alkaline phosphatase; AMF − arbuscular mycorrhizal fungi; Chl – chlorophyll; DM − dry mass; g_s − stomatal conductance; LAR − leaf area ratio; P_N − net photosynthetic rate; OSRF − organic slow release fertilizer; PPFD – photosynthetic photon flux density.
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2003). Extra-radical hyphae increase the absorptive surface area of the root system for greater nutrient acquisition. Extra-radical hyphae bypass the depletion zone that develops around plant roots when nutrients are removed more rapidly than they can be replaced either by diffusion from the surrounding soil or substrate solution (Bolan 1991, Marschner 1995, Clark and Zeto 2000). AMF also enhance nutrient acquisition through activation and excretion of various enzymes by AMF roots and/or hyphae (Smith and Read 1997).

Phosphorus exists in soil as inorganic orthophosphate, as inert complexes with iron phosphate ($FePO_4$), aluminum phosphate ($AlPO_4$), and in organic molecules such as lecithin and phytate. Phosphate is the form directly usable to plants. Plant roots colonized with intra- and extra-radical AMF hyphae can utilize sources of P in soil or container media that are not readily available to NonAMF roots. This is thought to involve an increase in the rate of solubilization of insoluble inorganic phosphorus (P_i) and/or hydrolysis of organic phosphorus (P_o) (Smith and Read 1997). Organic P is made available to plants largely after its mineralization or when hydrolyzed into P_i. Mineralization and hydrolysis of P_o is mediated by the enzymatic activity of phosphatase. Phosphatase activity in the rhizosphere of colonized plants originates from plant roots, AMF, and bacteria (Tarafdar and Marschner 1994). Koide and Kabir (2000) reported the release of phosphatase by AMF hyphae and the mineralization of P_o in a split-petri dish *in vitro* system utilizing *Glomus intraradices*-colonized carrots (*Daucus carota*). Greater enhancement of enzymatic acid phosphatase (ACP) and alkaline phosphatase (ALP) activity occurred with AMF roots compared to NonAMF roots (Tawaraya and Saito 1994, Fries *et al*. 1998). The activities of ACP and ALP are closely related to the level of AMF colonization in maize (*Zea mays*) roots (Fries *et al*. 1998). However, Gianinazzi-Pearson (1996) and Saito (2000) reported less ACP and ALP activity in AMF than NonAMF roots.

Our hypothesis was that inoculation with AMF in conjunction with an organic slow release fertilizer in container nursery production would enhance mineralization of organic nutrient sources in part, through increased ACP and ALP enzymatic activity of the colonized roots. Thus the objectives of this study were: (*1*) to evaluate the effectiveness of a selected commercial AMF inoculum on the physiology and growth of container produced *Ipomoea carnea* (bush morning glory) at different levels of an OSRF, (*2*) to determine the contribution of AMF and OSRF on root ACP and ALP enzymatic activity of *I. carnea*, and (*3*) to determine the relationship of root phosphatase activity in phosphorus acquisition of *I. carnea* as influenced by AMF and OSRF.

Materials and methods

Plants: This study was conducted in greenhouse at Texas A&M University, College Station, TX, USA, lat. 30º36'02''N and long. 96º18'44''W from 1 October to 29 November 2001. Uniform, single, three-node rooted liners of *I. carnea* (bush morning glory) were transplanted into 9 600 cm^3 containers (*Kord*, Bramalea, Ontario, Canada) with a substrate mixture of 1 sandy loam : 1 sand (v/v) with a textural analysis of 85 % sand, 10 % silt, 5 % clay, low organic matter (1.01 %), pH 6.8 and low nutrient contents [mg kg^{-1}]: 1 N, 3 P, 27 K, 547 Ca, 42 Mg, 3 Zn, 3.5 Mn, 0.07 Cu, 3.5 Na, and 5 S. The container substrate was steam-pasteurized (75 ^oC) for 6 h. Half the plants were inoculated with arbuscular mycorrhizal fungi (AMF) from a commercial inoculum of *G. intraradices* Schenck & Smith (*MycorisePro®*, *Premier Tech*, Rivière-du-Loup, Québec, Canada), and the remaining plants were non-inoculated (NonAMF). The inoculation rate for the experiment was 180 cm^3 per pot, which was 3× the commercially recommended rate. There was an estimated range of 135−915 propagules of AMF per plant, which were composed of spores, vegetative mycelia, and colonized root pieces. The control treatment (NonAMF) also contained the same commercial carrier (finely ground calcite clay, vermiculite, and perlite) without AMF propagules. The AMF inocula and NonAMF substrate carrier were introduced *via* a dibble hole, upon transplanting. Each NonAMF container received the same volume of carrier (180 cm^3) as the inoculated plants. Filtrate of AMF (50 cm^3) was added back to the NonAMF plants to reestablish any background microflora, *minus* mycorrhiza.

The three fertility rates of an OSRF [*Nitrell® 5-3-4* (5N-1.3P-3.3K) (*Fertrell Co*, Bainbridge, PA, USA)] were: 10 % (1.2 kg m^{-3}), 30 % (3.6 kg m^{-3}), and 100 % (12 kg m^{-3}) of the commercially recommended rate. The OSRF, *Nitrell®*, is a blend of natural organic materials including: hydrolyzed chicken manure, fishmeal, feather meal, peanut meal, cocoa meal, aragonite and blood meal, rock phosphate and sulfate of potash (*D. Mattocks*, *Fertrell Co.*, Bainbridge, PA, USA, personal communication). The OSRF was incorporated into the container substrate. Plants were watered as needed with de-ionized water and container leachate had an average pH of 7.3 by the end of the experiment, across all treatments.

A multi-channel *HOBO 8K* recorder for temperature and relative humidity (*Onset Computer Corp.*, Bourne, MA, USA) was used to monitor greenhouse environment. The average day/night temperature recorded was 30.4/21.5 ºC, average day/night relative humidity (RH) was 46.0/67.4 %, and the photoperiod was extended for 16 h with incandescent lights (100 W, *General Electric Lighting*, Cleveland, OH, USA). The maximum photosynthetic photon flux density (PPFD) at solar noon at plant level averaged 896 μmol m^{-2} s^{-1}, which was

measured with a photometer (model *LI-189*, *LI-COR*, Lincoln, NE, USA).

At the termination of the experiment (day 60), the plant growth index was calculated by the formula: [height×diameter$_1$×diameter$_2$] (Arnold and Lang 1996). Diameter$_2$ was measured perpendicular to diameter$_1$. At harvest, shoot and root dry mass (DM), and number of leaves were also determined ($n = 12$). Leaf area was determined with a leaf area meter (model *3000*, *LI-COR*, Lincoln, NE, USA). The leaf area ratio, LAR = leaf area [cm^2]/total plant DM [g] and the root to shoot ratio were also determined [kg kg^{-1}].

Leaf chlorophyll (Chl) content was measured non-destructively (Yadava 1986) using a portable Chl meter (*SPAD-502*, *Minolta Camera Co.*, Osaka, Japan) at day 60. The *SPAD-502* meter readings were correlated with a Chl content prediction equation: $y = -8.53 + 1.96\ x$, where y = Chl content [μmol cm^{-2}], and x = Chl meter reading ($r^2 = 0.85$). This equation was obtained by linear regression analysis between the *SPAD-502* readings obtained from the youngest pair of fully matured leaves of 4 different container grown plants of *I. carnea* and the total Chl content of the same pair of leaves at day 30. Leaf Chl was extracted using *N,N*-dimethylformamide and spectrophotometric measurements were made at 664 and 647 nm with a *Spectronic® 21* UVD spectrophotometer (*Bausch and Lomb*, Rochester, NY, USA). Total Chl content was calculated with the formula $Chl = 7.04\ A_{664} + 20.27\ A_{647}$ described by Moran (1982).

Gas exchange measurements: Net photosynthetic rate (P_N) and stomatal conductance (g_s) of individual leaves were measured between 08:00 and 11:00 h on days 30 and 60. Four random plants per treatment ($n = 4$) were selected and analysed with a portable photosynthesis system (model *LI-6400*, *LI-COR*, Lincoln, NE, USA). The *LI-6400* was programmed with a constant leaf chamber block temperature at 25 ºC. A fixed substrate level of 360 mm^3 m^{-1} CO_2 was provided with a 12-g cartridge, and the light source was an *LED 6400 R/B* at 300 μmol m^{-2} s^{-1}.

Macro- and micro-nutrient analysis: Leaf tissue of physiologically mature leaves was harvested at the experiment termination, after 60-d; from 12 plants per treatment, a pool of three plants was combined so there were four replications per treatment ($n = 4$). Leaf elemental status of plants was then determined on a dry mass (DM) basis. Dry leaves were ground in a *Wiley* mill and part of the leaves used for N analysis and the remainder for other macro- and micro-elemental analysis. Nitrogen content was determined utilizing the Kjeldahl procedure (Rund 1984). The remaining samples were digested in wet acid (Jones *et al*. 1991), and macro- and micro-elemental determinations were made with an inductively coupled plasma atomic emission spectrophotometer (*3510 ICP*) at a commercial lab, J.R. Peters/ Scott's Testing Laboratory, Allentown, Penn, USA using the procedures of Munter and Grande (1981).

AMF colonization was assessed in four randomly selected plants per treatment and assayed on 15, 30, and 60 d after treatment. Sub-samples of the root system were harvested and roots were cleared with 2.5 % KOH and stained with 0.05 % trypan blue in acidic glycerol to identify the fungal structures in the root cortex as described by Koske and Gemma (1989). There were ten 1-cm stained root segments per slide, which were examined at 40× under a compound microscope (*Aphaphot YS. Nikon*, Melville, NY, USA). There was a total of 200 (1-cm) root segments per treatment (ten 1-cm roots per slide, 5 slides per plant at 4 plants per treatment). Three observations (top, middle, and bottom) were made of each 1-cm root piece, hence there were 600 root observations per treatment; $n = 4$. Total AMF colonization was reported as % of root colonized with any AMF structures, including arbuscules, vesicles, and/or intraradical hyphae in root cortical cells, which were determined as described by Biermann and Linderman (1981).

Root phosphatase activity: The activities of root acid phosphatase (ACP) and alkaline phosphatase (ALP) (soluble and wall bound) were determined in samples of four plants per treatment at the same time that mycorrhizal colonization samples were taken at 30 and 60 d after inoculation. Sub-samples of the root system were harvested. The procedure was a modification of that described by Tabatabai (1994), based on the hydrolysis of *p*-nitrophenyl phosphate substrate to yield *p*-nitrophenol and inorganic phosphate.

Roots were washed and root tips harvested and frozen in liquid nitrogen and then macerated with a porcelain mortar and pestle. This procedure was performed to stop the activity of the root enzymes at the time of sampling. After maceration, the root powder was placed in a micro-centrifuge tube, labeled, and stored in a freezer (−80 ºC) for later analysis. Root tissue samples (250−300 mg) were weighted out and transferred into a micro-centrifuge tube. A 250−300 mm^3 (1 : 1, m/v) of extraction modified universal buffer (MUB) [100 mM Tris, 100 mM maleic acid, 5 mM citric acid, and 100 mM boric acid], pH 5.5 for acid phosphatase and pH 8.5 for alkaline phosphatase, was added to each tube. The tissue was macerated with a micro-pestle and centrifuged at 13 000×*g* for 15 min at 4 ºC. The reaction mixture consisted of 0.3 cm^3 of supernatant with 4 cm^3 of MUB, and 1 cm^3 of 0.003 M *p*-nitrophenyl phosphate. The mixture was then incubated at 37 ºC for 10 min for ACP and 180 min for ALP, and the reaction stopped by the addition of 1 cm^3 0.5 M $CaCl_2$ and 4 cm^3 0.5 M NaOH. The precipitate was recovered by centrifugation at 12 000×*g* for 15 min. The *p*-nitrophenol content of the supernatant was quantified

spectrophotometrically (*DU® 640* spectrophotometer, *Beckman Coulter*, Fullerton, CA, USA) at 400 nm, and compared to a standard concentration series of authentic *p*-nitrophenol.

Statistical design: For plant growth and gas exchange, and ACP and ALP activity, the factorial experiment included 2 AMF levels: [AMF inoculated and NonAMF]×3 OSRF fertility levels = 6 treatments in a completely randomized design with each plant as an experimental unit. The data were analyzed separately for each sample time using analysis of variance (ANOVA) (*SAS Institute* 2000). Difference between means was determined by ±SE. For determination of AMF, the factorial experiment was 2 AMF levels×3 OSRF fertility levels×3 time levels. For growth and Chl data, $n = 12$; for gas exchange, leaf elemental analysis, root phosphatase activity, and mycorrhizal analysis, $n = 4$.

Results

Plant growth: There was a main treatment effect of the commercial AMF, *G. intraradices*, which increased root DM, height, stem caliper, growth index (height and diameter measurements), leaf number, leaf area, and LAR of *I. carnea* (Tables 1 and 2). The main treatment effect of fertilization significantly increased all growth parameters, except root DM (Tables 1 and 2). Leaf Chl content was only enhanced with increasing fertilization; AMF had no effect, nor was there an interaction (Table 2). Neither AMF nor fertility affected the root : shoot ratio [kg kg^{-1}] (data not shown). There was a significant interaction with root DM; AMF plants had greater root DM than NonAMF plants at 10 and 100 % OSRF (Table 1). There was also an interaction with shoot DM, which was greater in 100 % OSRF AMF than NonAMF plants, but no shoot DM differences occurred between AMF and NonAMF plants at 10 and 30 % OSRF (Table 1).

Gas exchange: AMF colonization significantly increased P_N of *I. carnea* at days 30 and 60 of the experiment (Table 3). Compared to NonAMF, P_N of AMF plants was on average 12 % higher at day 30 and 14 % higher at day 60. Among all treatments, plants at 100 % OSRF had higher P_N than the 10 % OSRF (Table 3). The g_s was higher at increased fertility rates at day 30, but not on day 60. AMF colonization did not affect g_s.

Leaf elemental analysis: AMF colonization enhanced the leaf elemental contents of N, P, and K when compared to NonAMF plants (Table 4). AMF colonization increased N leaf tissue content by 6, 14, and 7 %, respectively, at the 10, 30, and 100 % OSRF rates (Table 4). AMF colonization enhanced leaf P tissue content by 17, 32, and 14 %, respectively, at the 10, 30, and 100 % OSRF rates, respectively. In terms of total P content in leaves, AMF plants had 26, 36, and 31 % more total P, respectively, than NonAMF plants at increasing fertility levels (Table 4). There was no significant AMF effect on Mg, Ca (data not shown), or on Zn. There were significant interactions with N, P, K, Fe, Cu, and Zn. Leaves of AMF plants at 10 and 30 % OSRF had greater Cu content than NonAMF plants (Table 4). Compared with NonAMF at 100 % OSRF, AMF plants at 30 % OSRF had higher leaf elemental contents of P, N, Fe, Cu, and Zn (Table 4). Plants at 100 % OSRF had higher leaf contents of N, P, K, and Fe and lower content of Mg than plants at 10 % OSRF.

Table 1. Growth [dry matter (DM), height, caliper, and growth index) of *Ipomoea arnea* grown for 60 d with different rates of organic slower release fertilizer [(OSRF) *Nitrell® 5-3-4* (5N-1.3P-3.3K) applied at 10 %, 30 % and 100 % of the recommended rates] and inoculated with arbuscular mycorrhizal fungi [(AMF) *Glomus intraradices* (*MycorisePro®*, *Premier Tech*, Rivière-du-Loup, Québec, Canada)] or not [(NonAMF) non-inoculated controls]. The growth index = (height×$diameter_1$×$diameter_2$/3). *Significance* was determined by ANOVA and was NS, *, **, *** = non-significant or significant at $p<0.05$, 0.01, 0.001, respectively. Means ± SE; $n = 12$.

AMF	OSRF [%]	Root DM [g]	Leaf DM [g]	Shoot DM [g]	Total plant DM [g]	Plant height [cm]	Stem caliper [cm]	Growth index [cm^3]
NonAMF	10	2.3 ± 0.2	1.3 ± 0.1	10.1 ± 0.4	12.5 ± 0.5	54.9 ± 1.0	8.6 ± 0.2	6168 ± 206
	30	2.8 ± 0.1	1.6 ± 0.1	10.7 ± 0.4	13.6 ± 0.4	54.1 ± 1.8	8.3 ± 0.2	6720 ± 446
	100	2.7 ± 0.1	2.3 ± 0.2	11.0 ± 0.3	13.7 ± 0.3	54.3 ± 1.3	8.1 ± 0.2	9347 ± 443
AMF	10	2.9 ± 0.2	1.5 ± 0.1	10.1 ± 0.3	13.0 ± 0.4	62.6 ± 1.5	8.8 ± 0.2	7965 ± 755
	30	2.8 ± 0.1	1.7 ± 0.1	11.1 ± 0.4	13.9 ± 0.4	60.3 ± 1.6	9.1 ± 0.2	10805 ± 1263
	100	3.0 ± 0.1	2.8 ± 0.2	12.6 ± 0.6	15.6 ± 0.7	66.0 ± 1.8	9.2 ± 0.5	11912 ± 1010
Significance (p<F)								
AMF		*	**	**	*	***	***	***
Fertility		NS	***	***	***	***	***	***
AMF×Fertility		*	NS	*	NS	NS	NS	***

Table 2. Effect of arbuscular mycorrhizal fungi (AMF) and an organic slow release fertilizer [(OSRF) *Nitrell® 5-3-4* (5N-1.3P-3.3K) applied at 10, 30, and 100 % of the recommended rates] and inoculated with arbuscular mycorrhizal fungi [(AMF) *Glomus intraradices* (*MycorisePro®*, *Premier Tech*, Rivière-du-Loup, Québec, Canada)] or not [(NonAMF) non-inoculated controls] on leaf number, leaf area, leaf area ratio (LAR), and chlorophyll content of *Ipomoea carnea* during a 60-d containerized study. Significance was determined by ANOVA and was NS, *, **, ***, *i.e.* non-significant or significant at $p<0.05$, 0.01, 0.001, respectively. Means ± SE; $n = 12$.

AMF	OSRF [%]	Leaves [no.]	Leaf area [cm^2]	LAR [$cm^2\ g^{-1}$]	Leaf chlorophyll [$mg\ m^{-2}$]
NonAMF	10	7.6 ± 0.4	257.9 ± 42.4	21.5 ± 0.6	8.10 ± 0.08
	30	7.7 ± 0.5	279.3 ± 28.6	21.5 ± 1.2	8.41 ± 0.10
	100	9.6 ± 0.5	442.2 ± 28.9	34.4 ± 0.9	8.97 ± 0.06
AMF	10	8.7 ± 0.2	289.9 ± 14.6	23.4 ± 0.8	8.44 ± 0.05
	30	9.0 ± 0.6	332.3 ± 18.1	31.4 ± 1.7	8.42 ± 0.06
	100	10.5 ± 0.7	525.3 ± 42.4	35.4 ± 0.9	8.92 ± 0.03
Significance (p<F)					
AMF		**	**	*	NS
Fertility		***	***	***	***
AMF×Fertility		NS	NS	NS	NS

Table 3. Effect of an organic slow release fertilizer [(OSRF) *Nitrell® 5-3-4* (5N-1.3P-3.3K) applied at 10, 30, and 100 % of the recommended rates] and inoculated with arbuscular mycorrhizal fungi [(AMF) *Glomus intraradices* (*MycorisePro®*, *Premier Tech*, Rivière-du-Loup, Québec, Canada)] or not [(NonAMF) non-inoculated controls] on stomatal conductance (g_s) and photosynthetic rate (P_N) of *Ipomoea carnea* at 30 and 60 d after inoculation. Significance was determined by ANOVA and was NS, *, **, *** = non-significant or significant at $p<0.05$, 0.01, 0.001, respectively. Means ± SE; $n = 4$.

Mycorrhiza	OSRF [%]	g_s [$mol\ m^{-2}\ s^{-1}$] 30 d	P_N [$\mu mol\ m^{-2}\ s^{-1}$]	g_s [$mol\ m^{-2}\ s^{-1}$] 60 d	P_N [$\mu mol\ m^{-2}\ s^{-1}$]
NonAMF	10	0.4 ± 0.2	11.5 ± 0.8	0.2 ± 0.0	11.0 ± 0.0
	30	0.2 ± 0.1	10.7 ± 0.6	0.1 ± 0.1	12.7 ± 0.2
	100	0.5 ± 0.1	14.1 ± 0.8	0.2 ± 0.0	12.9 ± 0.1
AMF	10	0.3 ± 0.0	13.4 ± 0.1	0.2 ± 0.1	13.9 ± 0.0
	30	0.3 ± 0.1	12.1 ± 0.6	0.3 ± 0.1	13.4 ± 0.0
	100	0.6 ± 0.1	15.1 ± 0.4	0.2 ± 0.1	14.5 ± 0.1
Signicance (p<F)					
AMF		NS	*	NS	*
Fertility		*	***	NS	*
AMF×Fertility		NS	NS	NS	NS

AMF colonization occurred at all fertility levels (Fig. 1). The main treatment effects of AMF, fertility, and sampling days and their interactions were highly significant ($p<0.001$). No colonization occurred with the NonAMF plants (data not shown). Within 15 d after inoculation, AMF plants had total colonization rates from 9 to 26 % among the different OSRF rates (Fig. 1). At day 15, a significantly higher initial colonization of 26 % and arbuscule formation (15 %) was obtained at the 100 % OSRF rate than 10 % or 30 % OSRF plants; the level of total colonization remained constant for the high fertility (100 % OSRF) plants for the duration of the experiment. Total colonization of AMF plants at 10 % OSRF plants peaked at day 30 at 59 % and then plateaued, while the 30 % OSRF reached 79 % total colonization at the end of the experiment (day 60), which was the highest level obtained. None the less, total colonization levels of all treatments ranged from 26 to 79 %.

Arbuscule formation and vesicle/endospore formation followed the same general pattern as total colonization. Arbuscule formation and vesicle/endospore formation at day 30 was higher with AMF plants at 10 and 30 % OSRF, and lowest at 100 % OSRF (Fig. 1). By the experiment termination, highest colonization, presence of arbuscules, and vesicles/endospores were found in plants at 30 % OSRF rate and the lowest with plants at 100 % OSRF.

Table 4. Effect of an organic slow release fertilizer [(OSRF) *Nitrell® 5-3-4* (5N-1.3P-3.3K) applied at 10, 30, and 100 % of the recommended rates] and inoculated with arbuscular mycorrhizal fungi [(AMF) *Glomus intraradices* (*MycorisePro®*, *Premier Tech*, Rivière-du-Loup, Québec, Canada)] or not [(NonAMF) non-inoculated controls] on leaf tissue macro-elemental and micro-elemental concentration, and total leaf phosphorus (P) of *Ipomoea carnea* during a 60-d study. Significance was determined by ANOVA and was NS, *, **, *** = non-significant or significant at $p<0.05$, 0.01, 0.001, respectively. Means ± SE; $n = 4$.

Mycorrhiza	OSRF [%]	N [g.kg^{-1}]	P [g.kg^{-1}]	K [g.kg^{-1}]	Mg [g.kg^{-1}]	Fe [g.kg^{-1}]	Cu [µg.kg^{-1}]	Zn [µg.kg^{-1}]	Total leaf P [mg]
NonAMF	10	33.5 ± 0.4	1.9 ± 0.1	27.2 ± 0.5	3.2 ± 0.1	50 ± 3	8 ± 0	52 ± 3	2.6 ± 0.1
	30	35.1 ± 0.9	2.1 ± 0.1	29.3 ± 0.3	2.9 ± 0.1	58 ± 4	8 ± 0	59 ± 1	3.4 ± 0.1
	100	38.4 ± 0.2	2.4 ± 0.1	31.1 ± 0.4	2.7 ± 0.1	60 ± 1	10 ± 0	55 ± 3	5.5 ± 0.3
AMF	10	35.6 ± 0.8	2.3 ± 0.1	28.7 ± 0.5	3.3 ± 0.1	51 ± 2	10 ± 0	54 ± 1	3.5 ± 0.2
	30	41.0 ± 0.6	3.1 ± 0.1	30.8 ± 0.1	3.1 ± 0.1	67 ± 3	13 ± 0	74 ± 4	5.3 ± 0.2
	100	41.2 ± 0.5	2.8 ± 0.1	32.6 ± 0.3	2.8 ± 0.1	63 ± 2	9 ± 1	54 ± 4	8.0 ± 0.2
Significance (p<F)									
AMF		**	***	*	NS	NS	***	NS	***
Fertility		***	***	***	***	***	***	***	***
AMF×Fertility		***	***	***	NS	*	***	*	***

Root phosphatase activities were measured 30 (ACP) and 60 (ALP) d after AMF inoculation. For ACP at 30 d, the main treatment effects of AMF and fertility and their interactions were highly significant ($p<0.001$), whereas at day 60 only the effect of AMF was significant ($p<0.05$) (Fig. 2).

At day 30, AMF plants at 10 and 100 % OSRF rates had higher ACP activity than NonAMF plants and the lowest ACP occurred with NonAMF plants at the 100 % OSRF (Fig. 2). ACP of plants at 10 % OSRF was greater than 100 % OSRP at day 30. At day 60, AMF plants had higher ACP than NonAMF plants; there was no significant fertility effect nor interaction.

While assays for ALP were also performed on days 30 and 60 (Fig. 3), the magnitude was only 10 % that of ACP (Fig. 2). At day 30 for ALP activity, only the main treatment effect of AMF was significant ($p<0.01$), whereas at day 60, both AMF and fertility were significant ($p<0.01$), with no significant interactions (Fig. 3). AMF plants had higher ALP than NonAMF plants at days 30 and 60. While fertility had no effect on ALP at day 30, at day 60, increasing fertility significantly increased ALP among AMF and Non-AMF plants (Fig. 3).

Discussion

This is the first study to show that AMF colonization enhanced root ACP and ALP activity of *I. carnea* at recommended and lower rates of OSRF. AMF inoculation with *G. intraradices* enhanced selected leaf macro- and micro-elemental and P contents, had greater P_N, and selected plant growth than NonAMF plants. In a container study of *I. carnea* under high temperature field production conditions (compared to the moderate temperature conditions of the current greenhouse study), AMF enhanced growth and leaf elemental N, P, and K compared to NonAMF plants (Amaya-Carpio *et al*. 2005).

At the 100 % OSRF rate, inoculation with AMF generally produced the greatest shoot, leaf, and total plant DM. Inoculation with AMF also generally enhanced height, stem caliper, growth index, leaf number, LAR, and total leaf area with increasing fertility level. In our experiment, P was supplied from the mineralization of OSRF; the increase in leaf number, leaf area, and LAR were subsequently related in part to total P content and higher leaf tissue P content. Increased leaf area and leaf expansion rate were correlated with greater leaf P in *Helianthus annuus* L. (sunflower) inoculated with AMF (Koide and Li 1989, Koide 1991). A comparable P-mediated growth response also occurred with AMF *Azadirachta indica* (neem trees) (Phavaphutanon 1996).

While AMF colonization had no effect on leaf Chl content, increasing OSRF rates enhanced it. Hence, there was a close relationship between plant nutritional status and Chl synthesis. The effect of the leaf mineral status, particularly N, Cu, and Fe on Chl synthesis, in part, may account for increased leaf Chl content at higher OSRF rates. The increase in plant Chl content in plants at the 100 % OSRF was associated with higher P_N and greater plant growth.

AMF enhanced P_N of *I. carnea*. In other plant species such as *Citrus aurantium*, AMF enhanced P_N was correlated with increased tissue P and Chl content, and ribulose-1,5-bisphosphate carboxylase/oxygenase activity, RuBPCO (Nemec and Vu 1990). The significant AMF enhancement of overall nutritional status, particularly P, accounts in part for the increased in P_N of AMF *I. carnea*. The higher P_N in AMF plants found in this study contributed to enhanced growth, and helped compensate for the higher photosynthate demand of AMF

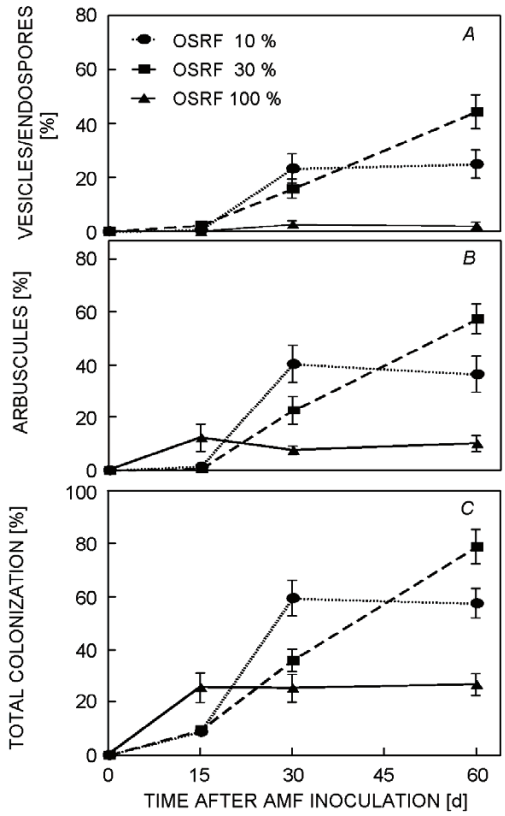

Fig. 1. Root colonization by arbuscular mycorrhizal fungi (AMF) in *Ipomoea carnea* grown with different rates of organic slow release fertilizer (OSRF). Percentage of root sections observed with (*A*) vesicles and/or endospores, (*B*) arbuscules, or (*C*) any AMF structure (total colonization). Colonization was determined for 600 observations per treatment at 15, 30, and 60 d after inoculation with AMF (*MycorisePro*, *Premier Tech*, Rivière-du-Loup, Québec, Canada, *Glomus intraradices*). OSRF was applied at 10, 30, and 100 % of the recommended rate for *Nitrel 5-3-4* (5N-1.3P-3.3K) (*Fertrell Co*, Bainbridge, PA, USA). Means (n = 4) ± SE. ANOVA results indicated the main treatment effects of AMF, Fertility, and Time, as well as their interactions were significant (p<0.001). Any ± SE bar not visible falls within the mean of given data point.

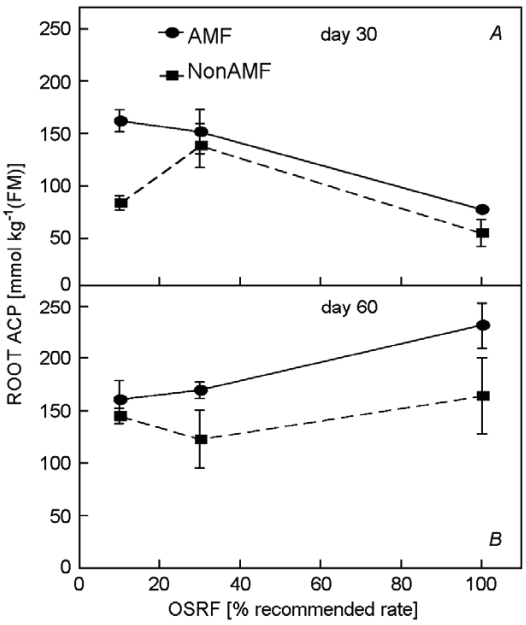

Fig. 2. Root acid phosphatase activity (ACP) in *Ipomoea carnea* grown with different rates of organic slower release fertilizer (OSRF) and inoculated (AMF) or not (NonAMF) with arbuscular mycorrhizal fungi. ACP was assessed at (*A*) 30 and (B) 60 d after inoculation with AMF. OSRF was applied at 10, 30, (3.6 kg m^{-3}) and 100 % of the recommended rate for a *5-3-4* (5N-1.3P-3.3K) product. Means (n = 4) ± SE. ANOVA results indicated the main treatment effects of AMF and Fertility and their interaction were significant (p<0.001) at 30 d, and only the main effect of AMF was significant at 60 d (p<0.05). Any ± SE bar not visible falls within the mean of given data point.

colonization (Smith and Read 1997). The majority of carbon to support the metabolism of AMF originates directly from host plant photosynthesis (Douds *et al*. 2000). Consequently, AMF plants often have higher P_N than NonAMF plants (Davies *et al*. 1993, Koide 1993, Aguilera-Gomez *et al*. 1999, Augé 2001). Besides biochemical factors such as the amount and activity of RuBPCO and improved nutrition, increased carbon sink strength can occur with AMF roots which enhances P_N (Koide 1993). AMF inoculation did not affect g_s or transpiration rate (data not shown) of *I. carnea*. While AMF sometimes have higher g_s than NonAMF plants, it is generally during drought stress that higher g_s in AMF plants is apparent (Augé 2001, Davies *et al*. 1993). Hence the greater P_N of *I. carnea* is not associated with higher g_s, but rather could be due to improved nutrition, in part *via* greater ACP and ALP activity. MF plants had higher leaf contents of N, P, K, and Cu. In general, enhanced nutrition of AMF plants resulted in a larger plant biomass. In a container study of *I. carnea* under high temperature field conditions, AMF enhanced growth and leaf N, P, and K contents compared to NonAMF *I. carnea* plants (Amaya-Carpio *et al*. 2005). AMF colonization enhances the availability and uptake of both macro and micronutrients (Smith and Read 1997, Davies *et al*. 2000).

As previously mentioned, the OSRF *Nitrell*® is a blend of natural organic materials. These organic materials require the action of different microorganisms naturally present in the fertilizer to slowly break down (mineralize) and release mineral nutrients in inorganic form, which can be taken up by plant roots (Linderman and Davis 2004). Hence, the presence of AMF in colonized plants increased the availability and uptake of the OSRF. This was possibly due to increased mineralization rate of the OSRF, and the increased absorptive surface area of extra-radical hyphae (Davies *et al*. 1992, Augé *et al*. 2003). Extra-radical hyphae greatly increase the absorbing surface area of roots (Marschner 1995, Smith and Read 1997), and enhance the absorption of ions with low diffusion coefficient such as P, Cu, Fe,

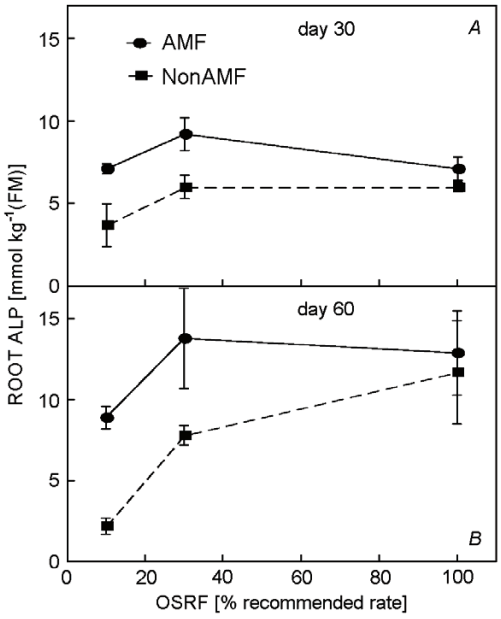

Fig. 3. Root alkaline phosphatase activity (ALP) in *Ipomoea carnea* grown with different rates of organic slower release fertilizer (OSRF) and inoculated (AMF) or not (NonAMF) with arbuscular mycorrhizal fungi. ALP was assessed at (*A*) 30 and (*B*) 60 d after inoculation with AMF. OSRF was applied at 10, 30, and 100 % of the recommended rate for a *5-3-4* (5N-1.3P-3.3K) product. Means ($n = 4$) ± SE. ANOVA results indicated only the main treatment effect of AMF was significant ($p<0.01$) at 30 d, and the main treatments effect of AMF and fertility were significant at 60 d ($p<0.01$). Any ± SE bar not visible falls within the mean of given data point.

and Zn (Marschner and Dell 1994, Smith and Read 1997). AMF mycelia can also mineralize and enhance utilization of organic sources of P by red clover (Feng *et al*. 2003). Increased contents of tissue P can also indirectly increase uptake of other ions such as N, Cu, Fe, and Zn (Marschner and Dell 1994).

MycorisePro®, the commercial AMF inoculum utilized in this experiment, consisted of a single isolate of *G. intraradices,* which was effective in establishing colonization of the roots of *I. carnea.* A significant increase in total colonization with increasing rates of OSRF was observed at day 15. Total colonization at day 15 (hyphae, arbuscules, vesicles/endospores, and entry points) was greatest at the 100 % OSRF rate. The early colonization (26 %) obtained at 100 % OSRF (commercially recommended rate) suggests there was an initial nutrient threshold that promoted the development and infectivity of *G. intraradices*, which was initially more optimum than the lower OSRF rates.

Bolan *et al.* (1984) reported that low P availability inhibited colonization and suggested that small supplementary additions of P may result in increased colonization. Conversely, high P can reduce colonization (Marschner and Dell 1994, Davies *et al*. 2000, Linderman and Davis 2004). Abbott and Robson (1984) reported that small additions of P could increase extra-radical hyphae growth and total AMF colonization, when the initial soil P concentration was extremely low. The low soil P concentration of the substrate used in this study [3 mg(P) kg^{-1}] and the likely small amount of P_i mineralized from organic sources at the first sample date (day 15) could account for the higher total colonization reported at the recommended application rate (100 % OSRF rate).

Our understanding of the biochemical and biophysical process involved in P metabolism and phosphatase synthesis and activity continues to evolve (Tarafdar *et al.* 2001, van Aarle *et al*. 2002, Ezawa *et al*. 2005, Javot *et al.* 2007). While AMF mycelia can mineralize organic P (Feng *et al*. 2003), AMF can also enhance P acquisition *via* up-regulation of acid phosphatase gene expression and secretion in host plant roots (Ezawa *et al.* 2005). Due to the important role of root phosphatase enzymatic activity on the bioavailability of organic phosphorus (P_o) sources, we followed the changes of ACP and ALP during the growth of AMF and NonAMF *I. carnea* at different OSRF rates. Both root ACP and ALP increased during the course of the 60-d study. The initial container leachate had a pH of 6.8 at the experiment initiation, while the container leachate of combined treatments had a pH of 7.3 at the termination of the experiment (data not reported), so pH was neutral. By day 60, AMF colonization enhanced root ACP activity from 13 to 30 % and root ALP activity from 8 to 43 % compared to Non-AMF plants. Higher ACP activity occurred in AMF colonized *Glycine max* (soybean) roots than NonAMF plants (Pacovsky 1991). Goldstein *et al*. (1988) reported that ACP increased in AMF roots growing under P stress. When P is limited, plant roots and microorganisms can be induced to produce more ACP (Tabatabai 1994). ACP can enhance release of P_i from organophosphates; Fries *et al*. (1998) reported that AMF increased ACP activity in AMF maize plants, in part because of greater release of inorganic phosphorus (P_i) from mineralization of P_o, leading to increased nutrient availability and enhanced growth. ACP production in the NonAMF control plants occurred from roots, natural microbial populations present in the OSRF material, and/or background microflora from the AMF filtrate. None the less, ACP activity in AMF plants still exceeded that of NonAMF plants.

AMF enhancement in ALP activity in our study is consistent with other reports (Fries *et al.* 1998, Saito 2000, Tawaraya and Saito 1994). Higher plant roots are devoid of ALP (Tabatabai 1994), thus ALP activity is largely due to rhizosphere organisms (AMF, bacteria, *etc*.). The 10-fold greater difference in root ACP than ALP levels is likely the result of the higher contribution of plant roots to the overall ACP activity. While we did not test for bacterial levels, AMF colonization may indirectly stimulate greater bacterial populations that enhance the mineralization of P_o (Linderman 1986) *via* enhanced phosphatase activity, P-availability and plant

acquisition (Gryndler *et al*. 2002, Villegas and Fortin 2002). While the container medium of this study was pasteurized, there could have been microbes involved that derive their support from both root and AMF hyphal exudates and some of which could be phosphatase producers as well as P-solubilizers; Andrade *et al*. (1998) reported microbial effects on nutrient availability in the presence of AMF. The localization of ALP in the hyphae of *Glomus mosseae*, *Glomus etunicatum*, and *Gigaspora rosea* is associated with effective AMF colonization (Ezawa *et al*. 1995, Tisserant *et al*. 1993). Gianinazzi *et al*. (1979) reported that ALP production in mature intra-radical arbuscules is involved with polyphosphate breakdown. This is supported in our study where AMF plants at the 30 % OSRF rate, also had greatest arbuscule development (58 %) and among the highest ALP activity.

In conclusion, the enzymes ACP and ALF could be used as potential indicators of efficient AMF symbiosis. The time-course increase in both enzymes as plants became colonized by AMF affected the root physiology of colonized plants, increased P_o mineralization, and P_i availability. The greater P acquisition, in part, led to higher P_N and increased growth of AMF plants.

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