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Influence of Arbuscular Mycorrhizae Indigenous to Peru and a Flavonoid on Growth, Yield, and Leaf Elemental Concentration of 'Yungay' Potatoes

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***Abstract.* The influence of arbuscular mycorrhizal fungi (AMF) [two Peruvian mixed isolates, a pure isolate of *Glomus intraradices*] and the flavonoid, formononetin, were tested on growth, yield, and leaf elemental concentration of the Peruvian potato (*Solanum tuberosum* L.) 'Yungay'. Plants started from tissue culture-produced prenuclear minitubers of 'Yungay' were subjected to seven treatments, which included noncolonized (non-AMF) plants fertilized with Long Ashton nutrient solution modified to supply P at 11 and 44 µg·mL^{-1}. All AMF plants received low P (11 µg·mL^{-1}) and were inoculated with either a sierra-maize mixed isolate, sierra-papa mixed isolate, pure isolate of *G. intraradices*, sierra-maize mixed isolate + formononetin, or a sierra-papa mixed isolate + formononetin. Plants were grown in 3-L containers under shade house conditions in Lima, Peru. Non-AMF plants at low P had the poorest growth, while high P plants had the greatest overall growth. All AMF plants had greater growth, including a higher root to shoot ratio, higher phosphorus use efficiency [(g tuber)/(g P/kg tissue)], and a lower leaf to tuber ratio (indicating greater leaf efficiency in producing tuber dry matter), compared to non-AMF plants at low P. The mycorrhizal inoculation effect (MIE) ranged from +44% to +57%, indicating that 'Yungay' was moderately to highly mycorrhizal dependent. Plants colonized with the sierra-papa isolate + formononetin had the same tuber development and leaf to tuber ratio, compared to high P, non-AMF plants. Formononetin increased extraradical hyphae formation. Mycorrhizal enhancement was in part due to greater P, Fe, and Mg uptake, a higher phosphorus-use efficiency and greater extraradical hyphae formation.**

Potatoes (*Solanum tuberosum* L.) are grown worldwide under a wider range of altitude, latitude, and climatic conditions than any other major food crop—from sea level to >4000 m elevation. No other crop can match the potato in its production of food energy and food value per unit area (Sieczka and Thronton, 1992). It is also high in Vitamin C, niacin, and vitamin B$_6$. Yet, the potato plant has one of the heaviest production demands for fertilizer inputs of all vegetable crops, i.e., its N, P, and K requirements are respectively 100%, 100%, and 33% greater than that required for tomato or pepper plant production (Maynard and Hochmuth, 1997). Normal fertilizer applications are around 1000 kg·ha^{-1} of 10–30–10 (10N–12.9P–8.3K). Subsistence growers may not have access or be able to afford suitable organic or inorganic fertilizers. Modern sustainable agriculture systems are increasingly using reduced fertility inputs. Hence, there are excellent opportunities to incorporate arbuscular mycorrhizal fungi (AMF) as biofertilizers to enhance crop productivity and reduce fertilizer inputs.

AMF increase nutrient and water uptake, alleviate cultural and environmental stresses and enhance disease resistance and plant health (Bethlenfalvay and Linderman, 1992; Davies, et al., 1993, 1996; Pfleger and Linderman, 1994). AMF can enhance productivity of potatoes (Graham et al., 1976; Niemira et al., 1995). In part this may be due to enhanced nutrient uptake of potato plants, particularly P (Black and Tinker, 1977; McArther and Knowles, 1993), as well as enhanced disease resistance (Niemira et al., 1996). In Columbia, experiments show that a considerable amount of P fertilizer can be saved when potatoes are inoculated with effective AMF (Sieverding, 1991).

The highlands of Peru and Bolivia are the center of origin and diversity of the cultivated potato. Potato is the main staple crop in the highlands and accounts for 63% gross value of all crop production (Devaux et al., 1997). Average potato yields are very low (from 5 to 8 t·ha^{-1}) and limited by low fertility. While small producers apply organic N as animal or green manure, P is applied via chemical fertilizers which are costly and not always available. For subsistence and modern sustainable potato production in Peru it is important that native AMF isolates be selected. A limitation of mycorrhiza utilization is its commercial availability and the added production cost.

The flavonoid, formononetin, has been reported to enhance mycorrhizal effectivity of mycorrhizal plants (Nair et al., 1997; Davies et al., 1999; Koide et al., 1999). Formononetin could be utilized with introduced AMF to permit lower, more cost-effective levels of AMF inocula, or applied to stimulate indigenous AMF. The objectives of this research were to evaluate Peruvian AMF isolates as biofertilizers that enhance potato crop yield of the important Peruvian cultivar 'Yungay', while reducing fertility inputs required. A second objective was to determine if nutrient uptake efficiency differs among selected AMF isolates, including phosphorus use efficiency (PUE). The effect of formononetin on stimulating native mycorrhizal inoculum was also determined. A long-term goal of this research is to utilize formononetin or other effective flavonoids in stimulating native populations of mycorrhiza to enhance potato crop productivity.

Materials and Methods

Cultural conditions. This study was conducted under shade house conditions at the Universidad Nacional Agraria La Molina

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(UNALM) in Lima, Peru. Tissue culture-produced prenuclear minitubers (about 2.5 cm in diameter) of 'Yungay' potatoes were transplanted into 3.0-L pots containing 1 coarse sand : 1 low-P sandy loam soil (franco arenoso) (v/v) with a textural analysis of 75% sand, 8% clay and 17% silt. The soil medium had an EC of 0.51 dS·m^{-1}, pH 7.9, 0.33% $CaCO_3$, 1.2% MO, 5.8 µg·g^{-1} P and 299 kg·ha^{-1} K_2O. The medium was previously steam autoclaved at 110 °C. Pots were irrigated as needed and fertilized weekly after shoots emerged using 500 mL per pot of modified Long Ashton nutrient solution [LANS (Hewitt, 1966)] to supply P at 11or 44 µg·mL^{-1}.

The 7 treatments were 1) noninoculated (non-AMF) at low P (11 µg·mL^{-1}), 2) noninoculated (non-AMF) at high P (44 µg·mL^{-1}), 3) mixed AMF inoculum isolated form a corn field (sierra-maize) at low P, 4) mixed AMF inoculum isolated from a potato field (sierra-papa) at low P, 5) pure isolate of *Glomus intraradicies* (Schenck and Smith) at low P, 6) sierra-maize mixed AMF inoculum + formononetin, at low P, and 7) sierra-papa mixed AMF inoculum + formononetin at low P.

Selected indigenous Peruvian AMF isolates were collected from the highlands of Oyolo, Departamento de Ayacucho, Peru. Mixed inoculum containing *Acaulospora* spp., *Glomus* spp., *Scutellospora* spp., *Gigaspora* spp. *and Sclerocystis* spp. was harvested from 1) a sierra-maize (corn) production site at 3300 meters [sandy clay soil with textural analysis of 46% sand, 28% clay and 26% silt; the soil had 1.9% organic matter, an EC of 0.24 dS·m^{-1}, pH 5.9, 0.33% $CaCO_3$, 1.2% MO, 3.3 µg·g^{-1} P, 600 kg·ha^{-1} K_2O]; and 2) a sierra-papa (potato) production site at 3600 m [sandy loam soil with textural analysis of 59% sand, 16% clay and 25% silt; the soil had 2.6% organic matter, an EC of 0.35 dS·m^{-1}, pH 6.6, 0.33% $CaCO_3$, 1.2% MO, 26.6 µg·g^{-1} P, 2242 kg/ha K_2O]. These AMF were multiplied in a trap culture of Italian rye grass (*Lolium perenne* L.) and onion for four months. About 400 g of soil containing 2800 spores [7 spores/g] (sierra-maize AMF isolate) or 5600 spores [14 spores/g] (sierra-papa mixed AMF isolate), plus vegetative mycelium and roots of the trap culture plants were applied as a band at the bottom third of the pot, then covered with soil, and the roots of the potato tuber were allowed to grow into the band and become colonized. About 21.5 g. of commercial inoculum of *Glomus intraradices* (Reforestation Tech International, Salinas, Calif.) containing 3000 to 5400 spores [most probable number (MPN)] was also banded. To reestablish background microflora, 100 mL of filtrate of the three inocula were screened through a 45-µm sieve and Whatman filter paper #4 and added back to the soil of noncolonized plants. Half of the Peruvian mixed isolates also received two 250-mL soil drench applications containing 15 mg·L^{-1}Myconate (VAMTech L.L.C., Lansing, Mich.), which is a water-soluble form of the flavonoid, formononetin. The drenches were applied at 3 and 4 weeks after emergence of shoots.

The experiment was initiated on 17 Sept. 1999 and terminated on 17 Dec. 1999. Average shade house minimum/maximum temperature ranged from 13.6/28.8 °C, and the maximum mean irradiance was 822.2 µmol·m^{-2}·s^{-1}.

Assessment of plant growth and development and leaf tissue elemental analysis. Final growth measurements of 15 plants per treatment (n = 15) were taken at day 90 after transplanting, and included leaf, shoot, root, stolon, tuber and total plant dry matter, tuber number, root to shoot ratio (g·g^{-1}), leaf to tuber ratio, (g·g^{-1}), phosphorus use efficiency [{PUE} (g tuber)/(g P/kg)]. Leaf area was based on a regression of leaf dry matter and area. The mycorrhizal inoculation effect (MIE) was determined by MIE (%) = (dry matter of inoculated plant – dry matter of noninoculated plant) × 100 × (dry matter noninoculated/plant)$^{-1}$ (Bagyaraj et al., 1988).

Leaf elemental analysis was done with an inductively coupled atomic emission spectrophotometer (3510 ICP; J.R Peters Laboratory, Allentown, Pa.). From fifteen plants per treatment, newly matured leaves from 5 plants were pooled into a single sample for analysis (n = 3).

Assessment of mycorrhizal development. Soil aggregation, as a measure for extraradical hyphae development, was assessed by allowing the soil of five potted plants from each treatment to dry at room temperature (25 °C), screening the root system, and shaking it gently in order to obtain pieces of soil attached to roots and bound by fungal hyphae (Graham et al., 1982, Kough and Linderman, 1986). The attached soil was then removed by washing the roots, collecting the remnant soil and drying it in a forced air oven. The soil dry weight divided by root dry weight was utilized to calculate the extraradical hyphae index (ERH index).

For spore counts, samples consisting of 100 g of soil from five plants per treatment (n = 5) were processed through glycerol floatation and spore extraction methods (Furlan and Fortin, 1975; Schenck, 1982). The supernatant was resuspended in 20 mL of distilled water and three replicates of 1.0 mL were taken in order to perform spore counts. The results were recorded as number of spores per g soil.

For AMF analysis of roots, 1-cm root segments from 15 plants per treatment were sampled at harvest and pooled to assess colonization percentage through clearing and staining of the root samples (Phillips and Hyman, 1970). Ten 1-cm stained root pieces were placed on each slide and three microscopic observations per 1-cm root piece at 40× was made at the top, the middle and the bottom of each root piece. There were 15 slides per treatment (n = 450 per treatment). The presence of arbuscules, vesicles and/or hyphae were recorded as total root colonization.

Statistical design. The experiment was composed of seven treatments in a completely randomized design. There was one prenuclear minituber per pot with each pot as a single replication. All data were analyzed using Analysis of Variance (ANOVA) (SAS Institute Inc., 2000).

Results

Plant growth and development. Non-AMF plants at low P had poorest growth, while non-AMF high P plants had among the greatest overall growth in terms of leaf area, leaf, shoot, tuber and total plant dry matter, and tuber number (Table 1). High P, non-AMF plants also had among the lowest leaf to tuber ratio, indicating greater efficiency in producing tuber mass, while low P, non-AMF plants had the lowest efficiency (Table 1). All AMF plants had greater growth, including a higher root to shoot ratio, and a greater P use efficiency (PUE) than non-AMF

Table 1. Effect of arbuscular mycorrhizal fungi (AMF) native to Peru, a pure isolate of *Glomus intraradices*, the flavonoid, formononetin and phosphorus on growth and development of 'Yungay' potatoes.

AMF treatment	P (µg·mL^{-1})	Leaf area[z] (cm^2)	Leaf dry matter (g)	Shoot dry matter (g)	Root dry matter (g)	Tuber dry matter (g)	Tuber no.	Solon dry matter (g)	Total plant dry matter (g)	Root to shoot ratio (g·g^{-1})	Leaf to tuber ratio (g·g^{-1})	PUE[y] (g/g P/kg)	MIE[x] (%)
Control	11	619 ± 33[w]	2.9 ± 0.2	3.3 ± 0.2	1.8 ± 0.1	2.5 ± 0.6	2.9 ± 0.6	1.1	11.5 ± 0.7	0.55 ± 0.03	4.3 ± 1.8	1.0 ± 0.3	---
Control	44	952 ± 59	4.5 ± 0.3	4.8 ± 0.2	2.3 ± 0.2	7.0 ± 0.7	6.5 ± 0.6	1.2	19.8 ± 0.7	0.50 ± 0.05	0.8 ± 0.1	2.0 ± 0.2	---
S-Maize	11	842 ± 43	4.0 ± 0.2	4.3 ± 0.1	2.8 ± 0.1	4.1 ± 0.6	4.5 ± 0.7	1.6	16.6 ± 0.6	0.64 ± 0.03	1.6 ± 0.4	1.6 ± 0.2	44
S-Papa	11	852 ± 36	4.0 ± 0.2	4.4 ± 0.1	2.8 ± 0.2	4.4 ± 0.7	4.6 ± 0.5	1.8	17.4 ± 0.6	0.62 ± 0.03	1.8 ± 0.5	1.7 ± 0.3	51
Glomus intraradices	11	779 ± 34	3.7 ± 0.2	3.6 ± 0.1	2.7 ± 0.1	5.4 ± 0.8	5.1 ± 0.5	1.2	16.7 ± 0.9	0.76 ± 0.03	0.9 ± 0.1	1.9 ± 0.3	45
S-Maize+ formononetin	11	783 ± 49	3.7 ± 0.2	4.3 ± 0.1	2.9 ± 0.2	5.3 ± 0.8	4.6 ± 0.8	1.6	17.8 ± 0.5	0.68 ± 0.04	1.2 ± 0.4	2.0 ± 0.3	55
S-Papa+formononetin	11	750 ± 35	3.5 ± 0.2	4.2 ± 0.1	3.1 ± 0.2	5.6 ± 0.9	5.1 ± 0.8	1.6	18.0 ± 0.7	0.74 ± 0.05	1.1 ± 0.2	2.2 ± 0.3	57
Significance		0.0006	0.0001	0.0001	0.0001	0.0038	0.0165	NS	0.0001	0.0001	0.0179	0.0511	

[z†] Leaf area based on a regression of leaf dry matter.

[y] Phosphorus use efficiency [{PUE} (tuber [g])/(g P/kg)].

[x] MIE (%) = (dm of AMF plant – dm 11 µg P/non-AMF plant) × 100 × (dry matter 11 µg P/non-AMF plant)$^{-1}$.

[w] Mean and ± SE, n = 15.

Table 2. Effect of arbuscular mycorrhizal fungi (AMF) native to Peru, a pure isolate of *Glomus intraradices*, the flavonoid, formononetin, and phosphorus on leaf elemental content of 'Yungay' potatoes (*Solanum tuberosum*).

AMF treatment	P (μg·mL^{-1})	N (g·kg^{-1})	P (g·kg^{-1})	K (g·kg^{-1})	Ca (g·kg^{-1})	Mg (g·kg^{-1})	B (g·kg^{-1})	Cu (μg·g^{-1})	Fe (μg·g^{-1})	Mn (μg·g^{-1})	Mo (μg·g^{-1})	Zn (μg·g^{-1})	Na (μg·g^{-1})	Al (μg·g^{-1})
Control	11	49.2	2.4 ± 0.1^{z}	42.8 ± 2.3	22.4	5.5 ± 0.1	88 ± 5	19	423 ± 34	226	3	83 ± 1	557 ± 27	561
Control	44	51.5	3.6 ± 0.2	31.5 ± 1.5	21.4	6.7 ± 0.3	93 ± 3	20	564 ± 54	243	4	69 ± 4	696 ± 60	723
S-Maize	11	49.3	2.7 ± 0.1	29.9 ± 0.6	22.2	6.5 ± 0	97 ± 4	25	644 ± 74	241	2	88 ± 1	607 ± 7	894
S-Papa	11	49.4	2.7 ± 0	31.1 ± 0.3	22.1	6.7 ± 0.2	98 ± 1	20	595 ± 25	249	2	80 ± 1	544 ± 28	808
Glomus intraradices	11	51.5	2.7 ± 0	35.0 ± 0.8	20.5	5.7 ± 0.2	108 ± 4	23	525 ± 10	250	3	80 ± 3	574 ± 27	702
S-Maize + formononetin	11	51.2	2.8 ± 0.1	32.7 ± 0.7	21.0	6.6 ± 0.1	93 ± 2	21	505 ± 14	211	3	81 ± 3	498 ± 18	666
S-Papa + formononetin	11	51.9	2.8 ± 0.1	32.2 ± 0.3	20.7	6.5 ± 0.1	93 ± 2	20	514 ± 44	236	2	80 ± 2	516 ± 34	680
Significance		NS	0.0001	0.0001	NS	0.0002	0.0112	NS	0.0494	NS	NS	0.0025	0.0136	NS

[z] Mean and standard error; leaves of five randomly sampled plants were pooled as one replication, n = 3.

plants at low P. The mycorrhizal inoculation effect (MIE) ranged from 44% to 57%. Root dry matter and the root to shoot ratio of AMF plants were also greater than high P, non-AMF plants. The sierra-papa AMF mix + formononetin was one of the best AMF treatments, based on tuber dry matter, PUE and MIE. Low P, AMF plants colonized with the sierra-papa AMF mix + formononetin had similar tuber number and tuber dry matter, and leaf to tuber (g·g^{-1}) ratio compared to high P, non-AMF plants.

Leaf tissue elemental analysis. High P, non-AMF plants had the highest leaf tissue P, Na and lowest Zn (Table 2). AMF plants had greater P, Mg, and Fe, and lower K than low P, non-AMF plants. No treatment differences occurred with N, Ca, Cu, Mn, Mo, or Al.

Mycorrhizal development. Colonization was high among all AMF inoculated plants (70% to 80%) (Table 3). Formononetin enhanced extraradical hyphae formation of the sierra-maize and sierra-papa AMF mixes and increased sporulation of the sierra-maize AMF mix (Table 3). The number of spores recovered from the *Glomus intraradicies* treatment was lowest, but root colonization by this isolate was one of the highest.

Discussion

This study shows the benefit of mycorrhiza as biofertilizers on growth, tuber yield, nutrient uptake and PUE of 'Yungay' potatoes. In low input, sustainable agriculture systems of the Peruvian highlands, it is important to use native mycorrhiza. While some limited studies have been done with nonnative mycorrhiza in Peru (Calderón, 1994; Moreno, 1988), this is the first successful report, to our knowledge, where native mycorrhiza were used as biofertilizers to enhance growth and yield of the important potato cultivar 'Yungay'. There were differences in plant growth, nutrient uptake and PUE among the AMF isolates. Formononetin also enhanced the growth effectiveness of the two Peruvian mixed isolates.

Plant growth and development. All of the AMF isolates enhanced growth of 'Yungay'. Mycorrhiza are known to enhance productivity of potatoes (Graham et al., 1976). AMF can also increase leaf surface area and plant growth by increasing P supply (Koide, 1998). The mycorrhizal inoculation effect (MIE) ranged from 44% to 57%, indicating that 'Yungay' was moderately to highly mycorrhizal dependent under P-limiting conditions (Bagyaraj et al., 1988).

One of the best AMF treatments was the sierra-papa mixed isolate + formononetin —based on total plant dry matter, tuber development, leaf to tuber ratio (g·g^{-1}) and phosphorus use efficiency [(PUE) {g tuber/g P/kg}]. Nutrient use efficiency estimation is used to measure plant capacity for uptake and use of nutrients for biomass production (Errebhi, et al. 1998). In horticulture production this can be measured by total biomass or economic yield produced by unit of mineral ions absorbed. At low P (11 μg·mL^{-1}), the PUE was greater in all AMF plants compared to non-AMF plants.

At low fertility, AMF enhanced tuber development. There were no statistical differences in tuber number, leaf to tuber ratio (g·g^{-1}) and tuber dry matter between the high P, non-AMF and low P, sierra-papa AMF mix + formononetin; the later had 2.2-fold greater tuber dry matter than low P, non-AMF plants.

Leaf tissue elemental analysis. The higher tissue P in AMF than low P, non-AMF plants was in part due to the ability of AMF to provide P to the plant, when P is less mobile in the soil because of decreased soil moisture content or with limited P supply, as in this study. Sometime leaves are very conservative in their P concentration. When more P becomes available, they produce greater leaf area and thus maintain the same tissue concentration levels (Smith and Read, 1997). However, at low P, all AMF plants had greater leaf area, leaf dry matter and leaf P than non-AMF plants. There was also greater total plant acquisition of P since the biomass of the AMF colonized plants was greater than non-AMF plants. In a nonmycorrhizal study, P-enhanced growth of potato plants was attributed to earlier canopy growth, increased radiation interception and subsequent greater biomass production (Jenkins and Ali, 1999).

AMF enhancement of plant growth may also be attributed to increased uptake of Fe and Mg. The higher uptake of selected nutrients may also be due to greater P uptake of AMF plants, i.e., the influence of higher P uptake on subsequent greater uptake of Fe is documented (Jones et al., 1991). AMF plants had reduced K. This is in part due to the dilution effect of AMF plants with a larger biomass (Johnson et al., 1980).

While all AMF 'Yungay' plants had extraradical hyphae development, the sierra-papa AMF mix had among the highest development. AMF increases P absorption for the most part by increasing the absorptive surface areas in contact with the soil solution (Koide, 1998). AMF hyphae are much longer than most root hairs, which allows them to explore pockets of soil that are unavailable to non-AMF roots. Extraradical hyphae can enhance plant water status (Davies et al., 1992 and 2002) and increase nutrient uptake in other crops (Bethlenfalvay and Linderman, 1992; Davies et al., 2000). AMF explore the soil volume in a manner analogous to increasing root density. Extraradical hyphae bridge gaps between the soil and roots as well as

Table 3. Effect of arbuscular mycorrhizal fungi (AMF) native to Peru, a pure isolate of *Glomus intraradices*, the flavonoid, formononetin, and phosphorus on vesicle and arbuscule formation, total colonization, extraradical hyphae development and soil AMF spore formation of 'Yungay' potatoes.

			Extraradical hyphae index	
AMF Treatment	P (μg·mL^{-1})	Total colonization (%)	Soil aggregate dry matter/ root (g·g^{-1})	Spores recovered/ g soil
Control	11	0 ± 0^{z,y}	24.9 ± 6.5^{z,x}	3.9 ± 0.4^{z,w}
Control	44	0 ± 0	27.1 ± 2.1	6.1 ± 0.4
S-Maize	11	71.3 ± 4.7	54.5 ± 8.7	18.7 ± 1.9
S-Papa	11	73.3 ± 4.2	70.7 ± 5.8	18.1 ± 2.4
Glomus intraradicies	11	79.9 ± 4.5	57.1 ± 12.3	4.9 ± 0.7
S-Maize+ formononetin	11	70.1 ± 3.9	79.4 ± 5.2	25.4 ± 2.3
S-Papa+ formononetin	11	78.6 ± 4.9	81.2 ± 4.9	18.1 ± 1.6
Significance		0.0001	0.0001	0.0001

[z] Mean and standard error.

[y] n = 150.

[x] n = 5.

[w] From five 100-g soil samples per treatment, three subsamples of 1 mL from 20 mL of resuspended supernatant per 100 g sample = 15 observations per treatment.

binding soil particles to each other (water stable aggregates) and to roots. This can be beneficial for enhancing nutrient uptake and minimizing water loss with diurnal fluctuations in soil water and subsequent soil shrinkage and gaps in the soil–root interface and between soil particles (Wright and Upadhyaya, 1998).

P nutrition is considered by many to be central to mycorrhizal enhancement of plant growth and its subsequent application to low input sustainable agricultural systems (Bethlenfalvay and Linderman, 1992). Phosphorus use efficiency (PUE) can also be measured by net photosynthesis (*A*) per tissue P concentration, since *A* is a key growth parameter, and P deficiency is a limiting factor to crop productivity. [In our study, PUE was based on tuber (g) development per g P/kg leaf dry matter]. Higher PUE is associated with less leaf mass needed to generate tuber mass, which suggests greater leaf and photosynthetic efficiency in AMF plants. The PUE of AMF treatments (all at low P) was similar to the high P, non-AMF plants—even though the later were given 4-fold greater P supply. In a study with *G. intraradices* (Aguilera-Gomez et al., 1999), highest PUE (*A* /P) occurred with AMF chile ancho plants at the lowest P level, whereas PUE was higher in non-AMF plants at moderate and high P. High PUE has been reported in mycorrhizal plants (Brown and Bethlenfalvay, 1988; Davies et al., 1993). Plants with optimum P concentration should be more vigorous with higher photosynthetic rates and stomatal conductance than plants with limiting P (Dietz and Foyer, 1986; Hensen et al., 1998). In a greenhouse, container study, formononetin stimulated greater extraradical hyphae development, net photosynthesis, stomatal conductance and shoot development in Russet Norkotah potatoes (Davies et al., 1999).

Mycorrhizal development. Potato plants are not known for high colonization levels, even though trace levels (0.4% colonization) are reported to enhance growth (Niemira, et al., 1995). With 'Yungay', high colonization levels from 70% to 80% occurred. Part of this may be the high initial inoculum levels utilized and the particular isolates selected. While it was beyond the scope of the current study to establish a minimum inoculation level for AFM effectivity, future studies should be conducted to determine economic levels of AMF inoculation. Formononetin enhanced extraradical hyphae formation of the sierra-papa AMF mix, but did not affect total root colonization. AMF colonization enhances soil aggregation through extraradical hyphae that are external to plant roots and by exuding the glycoprotein, glomalin, from extraradical hyphae that cements soil microaggregates into larger soil aggregate structures (Wright and Upadhyaya, 1998). The improved soil tilth that occurs enhances air and water percolation, and improves root system access to soil water and nutrients.

In a study with AMF maize, formononetin enhanced total root colonization from low to high soil P conditions (Fries et al., 1998). Phosphatases are secreted into the soil by roots and likely influenced by soil P status and AMF colonization. Acid phosphatase (ACP) and alkaline phosphatase (ALP) were closely related to root colonization. Soil P affects enzymes such as ACP, which are involved in the increase uptake of P from soil, while ALP has been linked to active phosphate assimilation and transport in roots (Fries et al., 1998).

All AMF spores, young and old, were counted in this study. Soil spore number is a function of AMF species, climate, soil characteristics, seasonality, etc. In another study conducted in a farmer's field at 3900 m in the Peruvian Highlands, the rhizosphere soil of control plants contained 6.3 ± 1.2 spores/g of soil (the land had been fallow for 5 years), whereas formononetin treatments had 19.4 ± 1.1 spores/g of soil (Davies et al., 2002). At higher altitudes in the Andean highlands, temperature is much lower, and sporulation of mycorrhiza and plant growth are reduced. There is only limited information on mycorrhizal species and soil spore levels in selected production sites in Peru (Calderon, 1994). Soil spore numbers have been reported from 50 to 800 spores/g soil (Nemec, 1974). In another study Sutton and Barron (1972) reported from 20 to 92 spores/g soil, and others researchers have reported from 0.1 to 37 spores/g soil (Hayman et al., 1975; Smith and Read, 1997). In the current containerized study, inoculated plants of 'Yungay' had from 5 to 25 spores/g soil. While formononetin enhanced spore number of the sierra-maize AMF mix, it did not affect sporulation of the sierra-papa mixed isolate. Both AMF mixed isolates contained *Acaulospora* spp., *Glomus* spp., *Scutellospora* spp., *Gigaspora* spp., and *Sclerocystis* spp. The low spore number of *Glomus intraradicies* was due to its sporulation characteristics. The *G. intraradicies* was used in comparison with the Peruvian isolates since it is a very aggressive isolate (Davies et al., 1999).

In subsistence agricultural systems, it is important to use indigenous AMF that are ecotypically adapted to the site. One of the strengths of this paper is that it shows potential benefits of applying formononetin or other effective flavonoids to stimulate the effectivity of native, ecotypically adapted mycorrhiza. Formononetin used in combination with indigenous isolates, could also allow for a lower, more cost-effective levels of AMF inocula to be utilized. The cost of formononetin would need to be considerably less than P-based fertilizers, which are estimated at $330 to $460 per hectare (J.C. Miller, Jr., personal communication). Formononetin has been reported to enhance effectivity of mycorrhizal plants (Koide et al., 1999; Nair et al., 1997). Hence, there are excellent opportunities to use and manipulate AMF to enhance crop productivity and reduce agricultural chemical inputs.

In summary, AMF enhancement was in part due to greater P, Fe, and Mg uptake, a higher PUE, and greater extraradical hyphae formation. One of the best AMF treatments was the Sierra-Papa AMF mix + formononetin based on tuber and total plant dry matter, mycorrhizal enhancement effect and PUE.

Beneficial AMF are one of the important cornerstones of sustainable agricultural systems. They can make plants more efficient in using available soil water and fertility; i.e., they serve as biofertilizers and increase drought resistance and plant productivity. A long-term goal of our research is to use formononetin in stimulating native populations of mycorrhiza to enhance potato crop productivity.

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