

## Varied response of marigold (*Tagetes* spp.) genotypes to inoculation with different arbuscular mycorrhizal fungi

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### Abstract

Different genotypes of marigolds, representing different vegetative and flowering habits, were evaluated for colonization and responsiveness to inoculation by different arbuscular mycorrhizal (AM) fungi under low soil P conditions. Cultivars included were *Tagetes erecta* ‘Jubilee’, ‘Perfection’, ‘Antigua’, ‘Inca’, and ‘Discovery’ as tall and compact forms of the African-type; *Tagetes patula* ‘Aurora’, ‘Disco’, ‘Nema-gone’, and ‘Lemon Drop’ as French-types with single or double flowers; and *Tagetes tenuifolia* ‘Ursula’ as a fine-leaf Mexican-type. Considerable variation occurred in shoot and root biomass response depending on the cultivar and AM fungus used. Most cultivars responded minimally, but positively to inoculation with AM fungi (1–22% increase in total plant biomass compared to the non-AM controls); however, some responded in reduced growth (1–12% decrease compared to the non-AM controls). Cultivars varied in their pattern of partitioning biomass into roots or shoots, with some partitioning more into roots than others with similar shoot biomass. Flower number or plant height did not vary between AM and non-AM plants. Stem diameter, measured only on ‘Jubilee’ and ‘Nema-gone’, was increased only on ‘Jubilee’, compared to the non-AM controls.

There was also great variation in the extent of AM colonization, ranging from under 10% in ‘Ursula’, ‘Antigua’, ‘Inca’, and ‘Perfection’, to over 70% in ‘Jubilee’ and ‘Aurora’, by *Glomus intraradices*, *Glomus mosseae*, and *Glomus deserticola*; and notably, *Gigaspora albida* did not colonize any marigold cultivar. Intraradical colonization intensity ratings (arbuscules, vesicles, and internal hyphae) varied significantly among cultivars and AM fungal inoculants, as did extraradical hyphal development. These results document the potential variation in responsiveness that can occur depending on host genotype and AM fungal endophyte combination, and indicates the need for caution in predicting

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benefit from inoculating with mycorrhizal fungi under any set of conditions when different genotypes are grown.

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## 1. Introduction

Mycorrhizae are a symbiotic relationship between specific fungi and roots of most plants that results in benefits to plant growth, health, and survival. Benefits to the plant are derived when the relationship is fully established with intraradical colonization and extraradical colonization of the soil. Such colonization may not always result in measureable benefits to plant growth, however. The literature commonly contains reports that internal colonization of roots by arbuscular mycorrhizal (AM) fungi is not always indicative of a mutualistic association. Variation in the amount of extraradical hyphal development, on the other hand, is more directly related to enhanced plant growth response of highly responsive plants under low or deficient soil P conditions (Graham et al., 1982; Yao et al., 2001b). Variability in the amount of colonization that occurs among plant species is widely known (Brundrett, 1991), as is variability in the nature of colonization by different fungal isolates within a single plant host (Janson and Linderman, 1993; Gao et al., 2001; Graham and Abbott, 2000; Parke and Kaepler, 2000).

Of increasing interest and economic importance is the variation in mycorrhizal responsiveness to a specific fungus by cultivars of the same host. This concept has been reported for field-grown crops, including peanut (Kesava Rao et al., 1990), basil (Gupta et al., 2000), citrus (Graham and Eissenstat, 1994; Graham et al., 1997), grapes (Karagiannidis et al., 1995; Linderman and Davis, 2001), millet (Krishna et al., 1985), onions (Sharma and Adholeya, 2000; Tawaraya et al., 2001), alfalfa (Lackie et al., 1988), cowpea (Mercy et al., 1990), peas (Estaun et al., 1987), tomato (Gao et al., 2001), soybean (Heckman and Angle, 1987), corn (Kaepler et al., 2000; Khalil et al., 1994; Liu et al., 2000; Toth et al., 1990), triticale (Young et al., 1985), barley (Baon et al., 1993), rice (Dhillion, 1992), and wheat (Azcon and Ocampo, 1981; Hetrick et al., 1993; Xavier and Germida, 1998; Yao et al., 2001a,b; Young et al., 1985; Zhu et al., 2001), with most results indicating that there may be a strong genetic influence affecting differences in mycorrhizal colonization and host plant responsiveness, and that increased crop plant hybridization may have resulted in reduced symbiotic effectiveness. However, there has been little or no research documenting genetic variation in responsiveness to mycorrhizal inoculation of greenhouse or other ornamental plants where extensive breeding has occurred and where there are multitudes of cultivars and varieties selected for their aesthetic or landscape traits. In such cases, it may not be enough to say that because one selection is responsive to mycorrhizal inoculation that all selections would/should be.

Intense plant hybridization has occurred widely in the nursery industry, with desired traits such as plant size and uniformity, early flowering, flower color, and disease resistance guiding the search for new cultivars or varieties, particularly in bedding plant production.

Marigolds are annuals that generally become mycorrhizal but do not always exhibit significant responsiveness under P-limiting conditions (Graw, 1979; Ezawa and Yoshida, 1994; Aboul-Nasr, 1996; Koide et al., 1999). However, plants of this nature may still benefit from the symbiosis, if not by enhanced growth, then in enhanced disease resistance (Caron et al., 1986; Linderman, 2000; St.-Arnaud et al., 1994), increased environmental stress tolerance (Cantrell and Linderman, 2001), or other physiological changes (Koide, 2000; Davies et al., 1992; Ianson and Linderman, 1993).

Because of the many species and cultivars within groups of ornamental plants that are the result of intense breeding and selection, largely for growth and flowering traits, there can be mixed results from inoculations with mycorrhizal fungi as a result of genetic variation in mycorrhizal colonization and plant growth responsiveness. Therefore, it was the purpose of this study to evaluate and document the genetic influence of the host plant, using marigold as a representative indicator, on response to mycorrhizal inoculation. Thus, we examined the relative responsiveness to inoculation with isolates of a range of AM fungal species of representatives of several different cultivars of marigolds grown in low-P soil conditions. The cultivars were selected to represent various vegetative and flowering habits found among the extensive array of marigolds currently produced.

## 2. Materials and methods

### 2.1. Soil preparation

The mineral base component of the soil mix consisted of a Willamette Valley alluvial loam mixed 1:1 (v:v) with river sand, with the following chemical properties: pH, 7.1; total N and C, 0.10 and 0.2  $\mu\text{g g}^{-1}$ ; available P, 8  $\text{mg kg}^{-1}$ ; K, Ca, Mg, and Na, 70, 5.0, 3.0, and 0.25  $\text{cmol kg}^{-1}$ , respectively. Prior to the study, the mix was steam-pasteurized at 75 °C for 1 h and was cooled and stored for at least a week prior to use. The potting soil mix prepared for the study consisted of the sandy loam base combined with 20% of a 1:1 mixture of horticultural grade perlite (Supreme<sup>®</sup>) and Lakeland<sup>®</sup> sphagnum peat moss (all combinations by volume). Plastic pots were filled with 0.6 l of this mix.

### 2.2. Mycorrhizal fungal inoculum preparation

Four AM fungal species isolates and one non-mycorrhizal control were used in this study. *Glomus intraradices* Schenck & Smith and *Glomus deserticola* Trappe, Bloss & Menge were originally obtained from Native Plants Incorporated, Salt Lake City, UT, and maintained in pot cultures at the USDA–ARS–Horticultural Crops Research Laboratory in Corvallis, Oregon. *Glomus mosseae* Gerdemann & Trappe was originally isolated by G.J. Bethlenfalvay (USDA–ARS) and cultured in-house, and *Gigaspora albida* Schenck & Smith was originally obtained from the INVAM collection in West Virginia.

All fungi were propagated in pot culture on roots of bunching onion (*Allium cepa* L. ‘White Lisbon’) grown in loam:sand (1:1) for 5 months. The non-mycorrhizal control was a similar culture without the AM fungi. Inocula consisted of a mixture of the soil medium, extraradical hyphae and spores, and colonized root segments ( $\leq 2$  mm in length).

Quantitative standardization of the AM inoculants was not attempted because of the inherent diversity in the infective characteristics of the different fungal isolates, i.e. spores of *G. intraradices* typically are formed intraradically and cannot be quantified in the same way as externally formed spores of other species. Pre-study population estimates by the MPN method (Woomer, 1994) of the different inoculants yielded the following propagules/g soil: *G. intraradices* = 10, *G. mosseae* = 8, *G. deserticola* = 14, and *G. albida* = 24.

Prior to transplant, 5 cm<sup>3</sup> of inoculum were placed in a depression in the middle of a pot, and plug transplants were placed on the inoculum so that seedling roots would grow directly into it. Root washings from pot cultures were applied to all pots after being passed through a 38 µm sieve and Whatman #1 filter paper. The combined filtrates of all mycorrhizal and non-mycorrhizal cultures were applied at 50 ml per pot to qualitatively standardize the rhizosphere microflora of the treatments. Each AM fungal species treatment did not receive filtrates from itself, but filtrates from the other species.

### 2.3. Seedling treatment

Marigold cultivars used in this study were *Tagetes erecta* ‘Jubilee’, ‘Discovery’, ‘Perfection’, ‘Antigua’, and ‘Inca’ representing tall and compact forms of the African-type; *Tagetes patula* ‘Aurora’, ‘Disco’, ‘Nema-gone’, and ‘Lemon Drop’ representing dwarf marigold habits including single- and double-flowers; and *Tagetes tenuifolia* ‘Ursula’ representing the finer-leaved Mexican-type.

Marigold seeds were germinated in 72-cell plug flats containing a seedling germination mix of 50% fine peat moss, 50% vermiculite, and trace elements but no claimed fertilizer macro elements obtained from OBC Northwest, Canby, OR. Seedlings were maintained in a greenhouse at 23/15 °C (day/night) and misted twice daily until transplanted. After 7–10 days seedlings had developed one set of true leaves and 2–3 roots, and were individually transplanted into pots containing the pasteurized soil with different AM fungal inoculants or non-inoculated controls.

### 2.4. Experimental design and plant maintenance

Pots were arranged on a greenhouse bench in a randomized complete block design with six replications per treatment. Greenhouse temperatures were maintained at 27/18 °C day/night temperatures and supplemental lighting with high-pressure sodium vapor lamps provided plants with an average of 750 µmol m<sup>-2</sup> s<sup>-1</sup> at canopy level for 14 h daylengths for the duration of the study. Plants were fertilized twice weekly with 13N–0.9P–10.8K soluble fertilizer (Plant Marvel<sup>®</sup>) prepared to supply N and K at approximately 200 mg kg<sup>-1</sup> N and P at 16 mg kg<sup>-1</sup>. Each pot received approximately 100 ml of solution to ensure thorough saturation of the medium. As plants matured and exhibited diverse moisture requirements, they were watered as needed relative to their size.

Plants were harvested 6 weeks after transplant. Shoots were severed at the soil line and flowers counted before being oven-dried at 65 °C for 48 h. Shoot weights included flowers because no visible flower size or count differences occurred. Roots were washed free of soil and dried briefly with paper towels before being weighed. A subsample was weighed and used for subsequent microscopic evaluation of AM colonization. Remaining roots were then

re-weighed and oven-dried. Total root dry weight was adjusted for weight of the subsample used for AM assessment. AM colonization of marigold roots was assessed on 1 cm sections after clearing and staining by modified procedures of Phillips and Hayman (1970), replacing lacto-phenol with lacto-glycerin. Using the grid line-intersect method (Giovannetti and Mosse, 1980), 100 intersections per sample were examined for the presence or absence of vesicles, internal hyphae, and/or arbuscules. Colonization intensity was estimated and rated visually for both intraradical structures and extraradical hyphae.

### 2.5. Statistical analysis

This factorial study consisted of a combination of five AM treatments (four fungal isolates plus non-inoculated control) and 10 marigold cultivars, with six replications per treatment and replication used as the blocking unit. Log-transformed shoot and root biomass data, and arcsin-transformed root colonization data were analyzed by analysis of variance using Systat® (SPSS, Inc.). Where appropriate, Fisher's protected LSD tests were used to separate treatment means (Petersen, 1985). Mycorrhizal responsiveness (MR) in terms of shoot and root growth for each series was calculated as follows:  $MR\% = [(dry\ weight\ inoculated / dry\ weight\ non-inoculated) \times 100]$ . Within each marigold cultivar, the MR% was calculated for each VAM fungal isolate tested. Actual data are presented in the paper.

## 3. Results

Under low-P growing conditions, plant biomass, AM colonization, and mycorrhiza responsiveness (MR) of the marigold shoots and roots showed significant differences (Tables 1 and 2). Individual cultivars exhibited different growth responses to the various AM fungal inoculants (Tables 1 and 2).

Shoot mass of AM plants was not always significantly greater than that of non-AM plants, and was sometimes significantly lower, depending upon the fungal isolate. Likewise, root dry weights also varied greatly. Within a marigold cultivar, neither flower number or plant height differed between the AM treatments (data not shown). Stem diameter, measured only on 'Jubilee' and 'Nema-gone', only increased significantly ( $P = 0.001$ ) for 'Jubilee' in all AM treatments compared to the controls (5.90–6.50 mm vs. 5.70 mm for AM and non-AM plants, respectively).

Cultivars varied in their tendency to partition biomass into roots in the absence of mycorrhizae, even when shoot masses were very similar. Further, different AM fungal species influenced the partitioning process as indicated by changes in the root mass (Tables 1 and 2).

The range of AM root colonization levels across genotypes was great, from 4% in 'Inca' and 'Antigua' to over 70% in 'Jubilee' and 'Aurora' (Tables 4 and 5). Non-inoculated plants remained free of colonization. Noteworthy is the fact that in no case did *G. albida* colonize marigold roots, regardless of host cultivar. The fact that the inoculum contained 24 propagules/g (as determined by MPN assay on onion) and approximately 20 viable spores/g verified that the inoculum potential was high and suggests some host specificity or preference.

Table 1

Growth, mycorrhizal responsiveness (MR), and root colonization of *T. erecta* marigold cultivars inoculated with different arbuscular mycorrhizal (AM) fungi and grown under low P conditions, compared to non-inoculated controls

Cultivar	AM fungal treatment <sup>a</sup>	Shoot dry weight (g) <sup>d</sup>	Root dry weight (g) <sup>d</sup>	MR shoot <sup>b</sup> (%) <sup>d</sup>	MR root <sup>c</sup> (%) <sup>d</sup>	Colonization (%) <sup>d</sup>	Colonization intensity			
							V <sup>e</sup>	A <sup>e</sup>	IH <sup>e</sup>	EH <sup>f</sup>
Jubilee	Control	1.68 ab	0.83 bc	–	–	–	–	–	–	–
	G. m.	1.84 bc	0.85 bc	110 b	103 c	70 b	2	2	2	3
	G. int.	1.62 a	0.70 a	96 a	84 a	59 a	1	3	2	1
	G. alb.	1.94 c	0.77 ab	116 c	107 c	0	–	–	–	–
	G. des.	1.89 c	0.89 c	112 b	92 b	71 b	1	1	2	2
	MSE	0.001	0.001	0.007	0.010	0.001				
	<i>P</i> value	0.004	0.002	0.008	0.004	0.001				
Perfection	Control	5.84 a	4.27 abc	–	–	–	–	–	–	–
	G. m.	5.66 a	3.90 ab	97 a	91 a	17 b	1	2	2	3
	G. int.	5.77 a	4.89 c	99 a	115 c	9 a	1	1	2	1
	G. alb.	5.61 a	4.35 bcc	96 a	102 b	0 c	–	–	–	–
	G. des.	5.26 a	3.57 a	90 a	84 a	33 c	2	1	2	2
	MSE	0.001	0.002	0.004	0.019	0.001				
	<i>P</i> value	0.103	0.005	0.148	0.008	0.001				
Antigua	Control	4.10 abc	5.28 bc	–	–	–	–	–	–	–
	G. m.	3.95 a	4.55 a	96 a	86 a	35 b	2	1	2	2
	G. int.	4.17 bc	4.76 ab	102 b	91 a	4 a	1	1	1	1
	G. alb.	4.04 ab	5.82 c	98 a	110 b	0	–	–	–	–
	G. des.	4.29 c	5.91 c	105 c	112 b	43 c	2	1	2	2
	MSE	0.001	0.001	0.002	0.014	0.001				
	<i>P</i> value	0.037	0.001	0.041	0.002	0.001				
Inca	Control	6.03 a	4.23 a	–	–	–	–	–	–	–
	G. m.	5.99 a	4.67 ab	99 a	112 a	7 a	1	1	1	0
	G. int.	6.27 a	5.91 c	104 a	140 c	4 a	1	1	1	0
	G. alb.	6.25 a	5.04 b	104 a	119 b	0	–	–	–	–
	G. des.	6.27 a	4.92 ab	104 a	116 ab	31 b	1	1	2	2
	MSE	0.001	0.002	0.002	0.014	0.001				
	<i>P</i> value	0.347	0.002	0.290	0.004	0.001				
Discovery	Control	2.41 a	1.86 c	–	–	–	–	–	–	–
	G. m.	2.50 ab	1.51 a	104 a	81 a	27 b	1	1	2	2
	G. int.	2.67 b	1.58 ab	111 a	85 a	23 b	1	0	1	1
	G. alb.	2.58 ab	1.73 bc	107 a	93 b	0	–	–	–	–
	G. des.	2.66 b	1.80 c	110 a	97 b	17 a	1	1	1	1
	MSE	0.001	0.001	0.003	0.008	0.001				
	<i>P</i> value	0.030	0.003	0.149	0.031	0.001				

<sup>a</sup> AM fungal isolates—G. m.: *Glomus mosseae*; G. int.: *G. intraradices*; G. alb.: *Gigaspora albida*; G. des.: *G. deserticola*.

<sup>b</sup> Mycorrhizal responsiveness (MR) for shoot growth expressed as (+AM shoot dry wt./–AM shoot dry wt.) × 100.

<sup>c</sup> Mycorrhizal responsiveness (MR) for root growth expressed as (+AM root dry wt./–AM root dry wt.) × 100.

<sup>d</sup> Means within a column for each cultivar followed by the same letter are not significantly different at  $P = 0.05$  for untransformed data. ANOVA probability value for AM factor is indicated below mean square error (MSE) using transformed data. Dashes indicate unapplicable data.

<sup>e</sup> Intensity rating system for internal structures (V: vesicles, A: arbuscules, IH: internal hyphae)—0: structures absent; 1: present but scarce; 2: abundant throughout root piece; 3: densely packed throughout root piece.

<sup>f</sup> Extraradical hyphae (EH) rating—0: absent, not apparent on root surface; 1: present, mainly confined to root surface; 2: hyphae more abundant and adherence to root surface and extending away from root; 3: prolific hyphal extension from root surface, sometimes with attached spores.

Table 2

Growth, mycorrhizal responsiveness (MR), and root colonization of *T. patula* and *T. tenuifolia* ('Ursula') marigold cultivars inoculated with different arbuscular mycorrhizal (AM) fungi under low P conditions compared to the non-inoculated controls

Cultivar	AM fungal treatment <sup>a</sup>	Shoot dry weight (g) <sup>d</sup>	Root dry weight (g) <sup>d</sup>	MR shoot <sup>b</sup> (%) <sup>d</sup>	MR root <sup>c</sup> (%) <sup>d</sup>	Colonization (%) <sup>d</sup>	Colonization intensity			
							V <sup>e</sup>	A <sup>e</sup>	IH <sup>e</sup>	EH <sup>f</sup>
Aurora	Control	2.39 a	0.59 a	–	–	–	–	–	–	–
	G. m.	2.38 a	0.55 a	100 a	93 a	78 c	1	3	2	3
	G. int.	2.42 a	0.60 a	101 a	102 ab	46 a	1	3	3	1
	G. alb.	2.32 a	0.64 a	97 a	108 b	0	–	–	–	–
	G. des.	2.74 b	0.76 a	115 b	128 c	71 b	1	2	3	1
	MSE	0.001	0.001	0.008	0.040	0.001				
	<i>P</i> value	0.007	0.072	0.018	0.004	0.001				
Disco	Control	2.94 a	0.59 c	–	–	–	–	–	–	–
	G. m.	2.68 a	0.38 a	94 a	64 a	71 c	1	1	2	3
	G. int.	2.88 a	0.42 ab	103 a	71 a	56 b	1	1	1	1
	G. alb.	2.68 a	0.49 ab	96 a	79 a	0	–	–	–	–
	G. des.	3.10 a	0.46 b	110 a	83 a	23 a	1	1	1	1
	MSE	0.006	0.001	0.025	0.016	0.001				
	<i>P</i> value	0.524	0.001	0.336	0.085	0.001				
Lemon Drop	Control	3.09 b	1.17 abc	–	–	–	–	–	–	–
	G. m.	2.81 a	1.01 a	91 b	87 a	19 a	1	1	2	1
	G. int.	2.70 a	1.09 ab	87 a	94 a	18 a	1	0	1	0
	G. alb.	2.75 a	1.25 bc	89 b	108 b	0	–	–	–	–
	G. des.	3.23 b	1.28 c	105 c	110 b	26 b	1	1	1	0
	MSE	0.001	0.001	0.002	0.013	0.001				
	<i>P</i> value	0.001	0.019	0.001	0.009	0.001				
Nema-gone	Control	6.08 c	3.55 b	–	–	–	–	–	–	–
	G. m.	5.43 ab	2.30 a	89 b	65 a	21 b	1	1	2	2
	G. int.	5.49 b	3.44 b	90 b	97 b	16 a	1	1	1	0
	G. alb.	5.10 a	3.23 b	84 a	91 b	0	–	–	–	–
	G. des.	6.43 c	3.28 b	106 c	93 b	36 c	2	2	2	2
	MSE	0.001	0.003	0.003	0.024	0.001				
	<i>P</i> value	0.001	0.002	0.001	0.011	0.001				
Ursula	Control	1.58 b	0.24 a	–	–	–	–	–	–	–
	G. m.	1.22 a	0.34 bc	86 a	142 a	8 a	3	1	2	1
	G. int.	1.57 b	0.30 ab	99 b	122 a	0	–	–	–	–
	G. alb.	1.81 b	0.32 bc	114 c	133 a	0	–	–	–	–
	G. des.	1.79 b	0.38 c	113 c	156 a	9 a	1	2	2	1
	MSE	0.003	0.001	0.013	0.061	0.001				
	<i>P</i> value	0.011	0.006	0.001	0.164	0.001				

<sup>a</sup> AM fungal isolates—G. m.: *Glomus mosseae*; G. int.: *G. intraradices*; G. alb.: *Gigaspora albida*; G. des.: *G. deserticola*.

<sup>b</sup> Mycorrhizal responsiveness (MR) for shoot growth expressed as (+AM shoot dry wt./–AM shoot dry wt.) × 100.

<sup>c</sup> Mycorrhizal responsiveness (MR) for root growth expressed as (+AM root dry wt./–AM root dry wt.) × 100.

<sup>d</sup> Means within a column for each cultivar followed by the same letter are not significantly different at *P* = 0.05 for untransformed data. ANOVA probability value for AM factor is indicated below mean square error (MSE) using transformed data. Dashes indicate unapplicable data.

<sup>e</sup> Intensity rating system for internal structures (V: vesicles, A: arbuscules, IH: internal hyphae)—0: structures absent; 1: present but scarce; 2: abundant throughout root piece; 3: densely packed throughout root piece.

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Intensity of root colonization varied considerably with the cultivar–AM fungal combinations. Intraradical production of arbuscules, vesicles, and hyphae was very high for some combinations and low for others. Similarly, some combinations resulted in extensive extraradical hypha development, while others had very little (Tables 1 and 2).

#### 4. Discussion

There are numerous reports on the genetic variation in plant responses to inoculation with mycorrhizal fungi (Parke and Kaeppler, 2000). Most of those studies involved field crops, whereas ours addressed a nursery/greenhouse/landscape plant under greenhouse conditions, and compared responses to several different AM fungal species as contrasted to the usual evaluation of response to only one fungus or a mixture of fungi. Our results, like those of others, indicate that there can be significant variation in responsiveness of plant species or cultivars within a species to inoculation with AM fungi under a given set of environmental and edaphic conditions. The basis for the variation could not be pin-pointed in this study with marigold genotypes, but variation in the level of vegetative hyphal growth, both intraradical and extraradical, as well as the genetic variation of the host morphology and physiology appear to interact, resulting in a given level of response.

In this study, the responsiveness of marigolds grown under low-P soil conditions was not great in terms of plant biomass, ranging from +22 to –12%, compared to non-inoculated control plants, and there is no clear correlation between growth enhancement and AM colonization level and/or intensity. A high level of intraradical colonization did not necessarily result in a high level of growth enhancement; and a low level did not necessarily reduce the plant response. An anomaly in this regard was the lack of colonization of any marigold by *G. albida*, yet in some cases growth was enhanced compared to the non-inoculated controls. These results suggest that host specificity was involved because the same inoculum did colonize onions in the MPN tests. One could question how this AM fungus could enhance growth without colonizing the host plant. Perhaps the answer lies in the probability that the inoculum carried with it, from the previous pot culture, other organisms (non-AM fungi and bacteria) that induced some growth response independent of colonization of marigold roots (Linderman et al., 1996; Andrade et al., 1997).

Regarding the genetic variation of marigold genotypes, or other plants reported in the literature for that matter, there must be variation in P requirements and the morphological and physiological capacity of the plant to acquire P independent of AM colonization. As discussed by Parke and Kaeppler (2000), P efficiency is reflected in the plant's ability to produce dry matter without the addition of P to the soil or growth medium in the absence of mycorrhizae. We report here large variation between cultivars of marigold in the size of root systems, many times without variation in shoot biomass. For example, similar shoot biomasses occurred with the cultivars of *T. patula* 'Aurora', 'Disco', 'Lemon Drop', and 'Nema-gone', but root biomasses varied greatly, in the absence of AM inoculation, with 'Nema-gone' having the greatest. Inoculation of 'Nema-gone' with any of the AM fungal species, however, had little or no effect on growth, yet colonization was significant. These results indicate that there was no benefit to having mycorrhizae in terms of P acquisition; the large root system itself could acquire the P needed to sustain growth within the available

P levels we provided. Had we developed P-response data, we might have learned that we did not actually have P-limiting growth conditions for marigolds. In other experiments (unpublished data), we determined that the deficiency threshold for marigold would be approximately  $20 \text{ mg l}^{-1}$  available P.

It is also believed by some that where there is no obvious growth enhancement from AM inoculation, the cost of maintaining the mycorrhizal status could be offsetting the benefits derived from the association (Graham and Eissenstat, 1994; Koide and Elliot, 1989; Parke and Kaeppler, 2000). Parke and Kaeppler (2000) also caution that modern plant breeding indirectly and inadvertently may be selecting plants with less genetic capacity to respond positively to the mycorrhizal association, at least in terms of biomass production. Contrary to that approach, however, was the report by Krishna et al. (1985) where selections of pearl millet were made on the basis of their ability to respond to AM under P-limiting soil conditions that would be expected in low-input agriculture. Some currently grown cultivars could have been developed that could benefit from mycorrhizae, but not be dependent on mycorrhizae for maximum productivity. They might also have lost gene alleles that affect colonization and responsiveness to AM (Hetrick et al., 1992, 1993, 1995). That apparently was the case reported by Kaeppler et al. (2000) where 28 inbred lines of maize showed a range of mycorrhizal growth response ranging from 66 to 653% under low-P soil conditions. Colonization of these lines by a mixture of five AM fungal species varied twofold without any correlation between colonization level and responsiveness. Parke and Kaeppler (2000) also expressed concern that breeding for resistance to diseases might inadvertently result in “resistance” to mycorrhiza formation, as reported by Toth et al. (1990).

It is safe to say, we believe, that plant breeders breeding and selecting flowering crops like marigolds have not considered whether or not released cultivars have reduced responsiveness to mycorrhizae; selection is based on horticultural traits like flower color and overall plant morphology and response to usual greenhouse cultural practices of fertilization and irrigation. Furthermore, there has been no genetic analysis of the genes that might control colonization and responsiveness as has occurred with food crops like corn (Hetrick et al., 1993, 1995; Kaeppler et al., 2000). Horticultural variety is the major goal of breeders of ornamental plants, not how they respond to inoculation with mycorrhizal fungi. Thus, we have variation in genotype responsiveness that results from breeding and selection, and we must consider that factor in predictions and expectations of growth benefit.

In addition to genetic factors that may influence the time and extent of mycorrhizal colonization as well as those that might influence responsiveness, we must consider variation in the fungal symbiont in the relationship. In our study, we considered the effectiveness of several fungi, in contrast to most studies where plants were either inoculated with a single AM fungus or a mixture of fungi under controlled conditions or in the field. Variation with the AM fungi could result from genes that determine species morphology and ecological behavior, as well as response to factors in the environment and molecular signals from the host plants. AM fungi have long been considered to be relatively not host-specific regarding the ability to colonize roots of appropriate host plants to some extent. In this study, we determined that *G. albida* was not able to colonize any marigold cultivar, yet could readily colonize onions. This response is uncommon, based on the literature to date. In addition, *G. intraradices* readily colonized most of the marigold cultivars but did not colonize ‘Ursula’; other AM fungi also colonized ‘Ursula’ at very low levels compared to

other cultivars. These results indicate that host specificity by some AM fungi does exist, and that some cultivars appear to limit AM colonization.

Plant response to inoculation with AM fungi appears to be also a function of how early in the growth cycle significant colonization occurs. In the present study, we inoculated plug transplants of marigolds by placing inoculum under the plug at the time of transplant. Growth enhancement resulting from those inoculations was minimal compared to that we observed by direct seeding or transplanting seedlings into the final growth medium where P availability was low. The plug mix used may have provided sufficient P fertility to sustain early plant growth for days or weeks, and at the same time suppressed AM colonization until the P was depleted. Biermann and Linderman (1983) reported that soilless plug mixes could provide enough P to inhibit root colonization by AM fungi. In our marigold study, that delay in colonization could have affected the magnitude of the growth response.

## 5. Conclusions

The conclusions to be drawn from this study are that varied growth response should be expected from inoculation with AM fungi on different genotypes of marigolds, or any other plant species, and that different AM fungi can affect plants differently, both in terms of their level of colonization and their capacity to enhance plant growth. Variation in growth response is the result of the confluence of multiple factors of fungus and host plant genetics and the environmental conditions of the tests. One cannot predict what the plant growth response to inoculation will be, only that it will be varied depending on the genotypes of both the plant and the fungal symbiont. The net response is a function of host preference as well as the time and extent of fungal colonization, host plant genes that influence or control colonization and responsiveness, and the relative efficiency of the root system to acquire needed nutrients in the absence of mycorrhizae.

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## References

- Aboul-Nasr, A., 1996. Effects of vesicular–arbuscular mycorrhiza on *Tagetes erecta* and *Zinnia elegans*. *Mycorrhiza* 6, 61–64.
- Andrade, G., Mihara, K.L., Linderman, R.G., Bethlenfalvay, G.J., 1997. Bacteria from rhizosphere and hyphosphere soils of different arbuscular-mycorrhizal fungi. *Plant Soil* 192, 71–79.
- Azcon, R., Ocampo, J.A., 1981. Factors affecting the vesicular–arbuscular infection and mycorrhizal dependency of thirteen wheat cultivars. *New Phytol.* 87, 677–685.
- Baon, J.B., Smith, S.E., Alston, M.A., 1993. Mycorrhizal responses of barley cultivars differing in P efficiency. *Plant Soil* 157, 97–105.
- Biermann, B., Linderman, R.G., 1983. Effect of container plant growth medium and fertilizer phosphorus on establishment and host growth response to vesicular–arbuscular mycorrhizae. *J. Am. Soc. Hortic. Sci.* 108 (6), 972–976.

- Brundrett, M., 1991. Mycorrhizas in natural ecosystems. *Adv. Ecol. Res.* 21, 171–313.
- Cantrell, I.C., Linderman, R.G., 2001. Preinoculation of lettuce and onion with VA mycorrhizal fungi reduces deleterious effects of soil salinity. *Plant Soil* 233, 269–281.
- Caron, M., Fortin, J.A., Richard, C., 1986. Effect of phosphorus concentration and *Glomus intraradices* on Fusarium crown and root rot of tomatoes. *Phytopathology* 76, 942–946.
- Davies, F.T., Potter, J.R., Linderman, R.G., 1992. Mycorrhiza and repeated drought exposure affect drought resistance and extraradical hyphae development of pepper plants independent of plant size and nutrient content. *J. Plant Physiol.* 139, 289–294.
- Dhillion, S., 1992. Host-endophyte specificity of vesicular–arbuscular mycorrhizal colonization of *Oryza sativa* L. at the pre-transplant stage in low or high phosphorus soil. *Soil Biol. Biochem.* 24, 405–411.
- Estaun, V., Calvet, C., Hayman, D.S., 1987. Influence of plant genotype on mycorrhizal infection: response of three pea cultivars. *Plant Soil* 103, 296–298.
- Ezawa, T., Yoshida, Y., 1994. Acid phosphatase specific to arbuscular mycorrhizal infection in marigold and possible role in symbiosis. *Soil Sci. Plant Nutr.* 40, 655–665.
- Gao, L.-L., Delp, G., Smith, S.E., 2001. Colonization patterns in a mycorrhiza-defective mutant tomato vary with different arbuscular-mycorrhizal fungi. *New Phytol.* 151, 477–491.
- Giovannetti, M., Mosse, B., 1980. An evaluation of techniques for measuring vesicular–arbuscular mycorrhizal infection in roots. *New Phytol.* 84, 489–500.
- Graham, J.H., Abbott, L.K., 2000. Wheat responses to aggressive and non-aggressive arbuscular mycorrhizal fungi. *Plant Soil* 220, 207–218.
- Graham, J.H., Eissenstat, D.M., 1994. Host genotype and the formation and function of VA mycorrhizae. *Plant Soil* 159, 179–185.
- Graham, J.H., Linderman, R.G., Menge, J.A., 1982. Development of external hyphae by different isolates of mycorrhizal *Glomus* spp. in relation to colonization and growth of Troyer citrange. *New Phytol.* 91, 183–189.
- Graham, J.H., Duncan, L.W., Eissenstat, D.M., 1997. Carbohydrate allocation patterns in citrus genotypes as affected by phosphorus nutrition, mycorrhizal colonization and mycorrhizal dependency. *New Phytol.* 135, 335–343.
- Graw, D., 1979. The influence of soil pH on the efficiency of vesicular–arbuscular mycorrhiza. *New Phytol.* 82, 687–695.
- Gupta, M.L., Khaliq, A., Pandey, R., Shukla, R.S., Singh, H.K., Kumar, S., 2000. Vesicular–arbuscular mycorrhizal fungi associated with *Ocimum* spp. *J. Herbs Spices Med. Plant* 7, 57–63.
- Heckman, J.R., Angle, J.S., 1987. Variation between soybean cultivars in vesicular–arbuscular mycorrhiza fungi colonization. *Agron. J.* 79, 428–430.
- Hetrick, B.A.D., Wilson, G.W.T., Cox, T.S., 1992. Mycorrhizal dependency of modern wheat varieties, landraces, and ancestors. *Can. J. Bot.* 70, 2032–2040.
- Hetrick, B.A.D., Wilson, G.W.T., Cox, T.S., 1993. Mycorrhizal dependence of modern wheat cultivars and ancestors: a synthesis. *Can. J. Bot.* 71, 512–518.
- Hetrick, B.A.D., Wilson, G.W.T., Gill, B.S., Cox, T.S., 1995. Chromosomal location of mycorrhizal responsiveness genes in wheat. *Can. J. Bot.* 74, 19–25.
- Ianson, D.C., Linderman, R.G., 1993. Variation in the response of nodulating pigeonpea (*Cajanus cajan*) to different isolates of mycorrhizal fungi. *Symbiosis* 15, 105–119.
- Kaeppler, S.M., Parke, J.L., Mueller, S.M., Senior, L., Stuber, C., Tracey, W.F., 2000. Variation among maize inbred lines and detection of quantitative trait loci for growth at low phosphorus and responsiveness to arbuscular mycorrhizal fungi. *Crop Sci.* 40, 358–364.
- Karagiannidis, N., Nikolaou, N., Matteou, A., 1995. Influence of three VA-mycorrhiza species on the growth and nutrient uptake of three grapevine rootstocks and one table grape cultivar. *Vitis* 34, 85–89.
- Kesava Rao, P.S., Tilak, K.V.B., Arunachalam, V., 1990. Genetic variation for VA mycorrhiza-dependent phosphate mobilization in groundnut (*Arachis hypogaea* L.). *Plant Soil* 122, 137–142.
- Khalil, S., Loynachan, T.E., Tabatai, M.T., 1994. Mycorrhizal dependency and nutrient uptake by improved and unimproved corn and soybean cultivars. *Agron. J.* 86, 949–958.
- Koide, R.T., 2000. Mycorrhizal symbiosis and plant reproduction. In: Kapulnik, Y., Douds, D.D. (Eds.), *Arbuscular Mycorrhizas: Physiology and Function*. Kluwer Academic Publishers, Dordrecht, pp. 19–46.
- Koide, R.T., Elliot, G., 1989. Cost, benefit and efficiency of the vesicular–arbuscular mycorrhizal symbiosis. *Funct. Ecol.* 3, 252–255.

- Koide, R.T., Landherr, L.L., Besmer, Y.L., Detweiler, D.M., Holcomb, E.J., 1999. Strategies for mycorrhizal inoculation of six annual bedding plant species. *HortScience* 34, 1217–1220.
- Krishna, K.R., Shetty, K.G., Dart, P.J., Andrews, D.J., 1985. Genotype dependent variation in mycorrhizal colonization and response to inoculation of pearl millet. *Plant Soil* 86, 113–125.
- Lackie, S.M., Bowley, S.R., Peterson, R.L., 1988. Comparison of colonization among half-sib families of *Medicago sativa* L. by *Glomus versiforme* (Daniels and Trappe) Berch. *New Phytol.* 108, 477–482.
- Linderman, R.G., 2000. Effects of mycorrhizas on plant tolerance to diseases. In: Kapulnik, Y., Douds, D.D. (Eds.), *Arbuscular Mycorrhizas: Physiology and Function*. Kluwer Academic Publishers, Dordrecht, pp. 345–365.
- Linderman, R.G., Davis, E.A., 2001. Comparative response of selected grapevine rootstocks and cultivars to inoculation with different VA mycorrhizal fungi. *Am. J. Enol. Viticult.* 52, 8–12.
- Linderman, R.G., Marlow, J.L., Davis, E.A., 1996. Contribution of microbial associates of VA mycorrhizae to mycorrhiza effects on plant growth and health. In: Szaro, T.M., Bruns, T.D. (Eds.), *Proceedings of the First International Conference on Mycorrhizae*, Berkeley, CA, p. 77.
- Liu, A., Hamel, C., Hamilton, R.L., Smith, D.L., 2000. Mycorrhizae formation and nutrient uptake of new corn (*Zea mays* L.) hybrids with extreme canopy and leaf architecture as influenced by soil N and P levels. *Plant Soil* 221, 157–166.
- Mercy, M.A., Shivashankar, G., Bagyaraj, D.J., 1990. Mycorrhizal colonization in cowpea is host dependent and heritable. *Plant Soil* 121, 292–294.
- Parke, J.L., Kaeppler, S.A., 2000. Effects of genetic differences among crop species and cultivars upon the arbuscular mycorrhizal symbiosis. In: Kapulnik, Y., Douds, D.D. (Eds.), *Arbuscular Mycorrhizas: Physiology and Function*. Kluwer Academic Publishers, Dordrecht, pp. 131–146.
- Petersen, R.G., 1985. *Design and Analysis of Experiments*. Marcel Dekker, New York.
- Phillips, J.M., Hayman, D.S., 1970. Improved procedures for clearing roots and staining parasitic and vesicular–arbuscular mycorrhizal fungi for rapid assessment of infection. *Trans. Brit. Mycol. Soc.* 55, 158–161.
- Sharma, M.P., Adholeya, A., 2000. Enhanced growth and productivity following inoculation with indigenous AM fungi in four varieties of onion (*Allium cepa* L.) in an alfisol. *Biol. Agric. Hortic.* 18, 1–14.
- St.-Arnaud, M., Hamel, C., Fortin, J.A., 1994. Inhibition of *Pythium ultimum* in roots and growth substrate of mycorrhizal *Tagetes patula* colonized with *Glomus intraradices*. *Can. J. Plant Pathol.* 16, 187–194.
- Tawaraya, K., Tokairin, K., Wagatsuma, T., 2001. Dependence of *Allium fistulosum* cultivars on the arbuscular mycorrhizal fungus, *Glomus fasciculatum*. *Appl. Soil Ecol.* 17, 119–124.
- Toth, R., Toth, D., Starke, D., 1990. Vesicular–arbuscular mycorrhizal colonization in *Zea mays* affected by breeding for resistance to fungal pathogens. *Can. J. Bot.* 68, 1039–1044.
- Woomer, P.L., 1994. Most probable number counts, In: Weaver, R.W. (Ed.), *Methods of Soil Analysis, Part 2*. Soil Science Society of America, Madison, WI, pp. 59–79.
- Xavier, L.J.C., Germida, J.J., 1998. Response of spring wheat cultivars to *Glomus clarum* NT4 in a P-deficient soil containing arbuscular mycorrhizal fungi. *Can. J. Soil Sci.* 78, 481–484.
- Yao, Q., Li, X., Christie, P., 2001a. Factors affecting arbuscular mycorrhizal dependency of wheat genotypes with different phosphorus efficiencies. *J. Plant Nutr.* 24, 1409–1419.
- Yao, Q., Li, X., Feng, G., Christie, P., 2001b. Influence of extramatrical hyphae on mycorrhizal dependency of wheat genotypes. *Commun. Soil Sci. Plant Anal.* 32, 3307–3317.
- Young, J.L., Davis, E.A., Rose, S.L., 1985. Endomycorrhizal fungi in breeder wheats and triticale cultivars field-grown on fertile soil. *Agron. J.* 77, 219–223.
- Zhu, Y.G., Smith, S.E., Barritt, A.R., Smith, F.A., 2001. Phosphorus (P) efficiencies and mycorrhizal responsiveness of old and modern wheat cultivars. *Plant Soil* 237, 249–255.