Fatty Acid Methyl Ester Profiles for Characterization of Glomalean Fungi and Their Endomycorrhizae†

JAMES H. GRAHAM,^{1*} NANCY C. HODGE,² AND JOSEPH B. MORTON³

Citrus Research and Education Center and Department of Plant Pathology, University of Florida, IFAS, Lake Alfred, Florida 33850¹; and 1453 Fifield Building, Gainesville, Florida 32611²; and Division of Plant and Soil Sciences, West Virginia University, Morgantown, West Virginia 26506-6057³

Received 6 June 1994/Accepted 24 October 1994

Arbuscule-forming fungi in the order Glomales form obligate endomycorrhizal associations with plants that make them difficult to quantify, and taxonomy of the group is only beginning to be objectively understood. Fatty acid methyl ester (FAME) profiles were analyzed to assess the diversity and quantity of fatty acids in 53 isolates of 24 glomalean species. Spores and endomycorrhizal roots of sudan grass (*Sorghum sudanense*) and the citrus rootstock Carrizo citrange (*Poncirus trifoliata* × *Citrus sinensis*) were examined. Spores yielded reproducible FAME profiles from replicate spore collections extracted from soil pot cultures despite being grown in association with a host plant and with contaminating microorganisms present. Unweighted pair group analysis revealed relatively tight clusters of groups at the intraspecific, specific, and generic levels; however, lipid profiles at the family level were convergent. Thus, FAME profile comparisons provided a robust measure of similarity below the family level. FAME profiles in sudan grass roots containing vesicles and/or spores of *Glomus intraradices* were more similar to spore profiles than to profiles from nonmycorrhizal roots. The FAME profiles for *Gigaspora* species, which do not form vesicles or spores in roots, were less distinct from nonmycorrhizal roots. *G. intraradices* and *G. rosea* produced fatty acids in roots that were distinguishable from each other as well as from the host root. Production in citrus roots of the fatty acid 16:1_{ω5} cis by two *Glomus* species was correlated with the development of mycorrhizal colonization as measured by clearing and staining procedures and by estimates of total incidence and vesicle intensity. FAME analysis of roots not only provided a measure of colonization development but also served as an index of carbon allocated to intraradical fungal growth and lipid storage.

Fungi in the order Glomales (Zygomycotina) pose unique problems for study because they form obligate associations and must be propagated in association with intact plants or root explants (17). Therefore, methods for the objective measurement of mycorrhizal colonization in roots rely either on differential staining of roots (27) and estimation of the percentage or length of the root colonized by the fungus (8) or on determination of fungal biomass in roots by biochemical means, such as cell wall chitin analysis (5, 11).

Currently, classification and identification of glomalean fungi are based mostly on morphological characters of spores (17, 19). Comparative taxonomic analyses have been hindered by inadequately defined characters and ambiguous distinctions between morphologically similar species (18). Thus, molecular and biochemical characters are an important supplement to morphological characters used to define and relate taxa in the order Glomales (3, 20).

The abundance of lipids in spores and vesicles of colonized roots is a potentially useful biochemical character for taxonomic purposes and quantification of glomalean fungi in host root tissue. Recent advances in gas-liquid chromatography of lipids have generated widening interest in the use of fatty acid methyl ester (FAME) profiles for the purpose of identification and elucidation of relationships among organisms (33). The diversity of structure and quantity of fatty acids of 9 to 20

carbon atoms in length has provided characteristic profiles among species and genera of bacteria and yeasts (31, 33). Characterization of groups of filamentous fungi has recently been exploited to assess interspecific variation among *Ganoderma* isolates (15) and intraspecific differences among isolates of *Rhizoctonia solani* (32).

The lipid composition of glomalean fungi in mycorrhizal roots was first determined by Cooper and Lösel (7). Substantial information has been reported on the composition of fatty acids in endomycorrhizal citrus and soybean roots (22–24), fungal spores (1, 2, 23), and vesicles isolated from roots (14). The potential of lipids and fatty acids as taxonomic characters for glomalean fungi was first pointed out by Jabaji-Hare (13), who examined FAMES in three *Glomus* species and one *Gigaspora* species. Recently, Sancholle and Dalpé (28) characterized FAMES in seven *Glomus* species, one *Acaulospora* species, and one *Scutellospora* species. Neither study presented a comparative analysis of FAME profiles.

In the present study, we evaluated FAME profiles in 53 isolates of 24 species of glomalean fungi as a measure of fungal diversity that might have taxonomic and diagnostic applications. We also examined FAMES in mycorrhizae of citrus and sudan grass to quantify and identify glomalean fungi in roots and possibly to study allocation of carbon to mycorrhizae. The Microbial Identification System (Microbial ID, Inc., Newark, Del.) was employed as a quick and accurate technique for FAME profile characterization. Using this approach, Ben-tivenga and Morton (4) have demonstrated that growth and storage conditions of cultures, as well as effects of culture host plant species, do not affect FAME profiles of representative fungi in all families of the order Glomales.

* Corresponding author. Phone: 813-956-1151. Fax: 813-956-4631. Electronic mail address: jhg@gnv.ifas.ufl.edu.

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TABLE 1. Fungal isolates used in this study for FAME analysis

Isolate (no. referred to in Fig. 1 to 3)	INVAM ^a	Origin	Spore no. extracted
<i>Acaulospora laevigatum</i> nom. ined. (1)	WV736-3	West Virginia	400
<i>Acaulospora longula</i> Spain and Schenck (2)	AZ661-1	Arizona	500
<i>Acaulospora mellea</i> Spain and Schenck (3)	CL551-1	Colombia	550
<i>Acaulospora mellea</i> (4)	FL266-1	Florida	500
<i>Acaulospora morrowiae</i> Spain and Schenck (5)	CL358-2	Colombia	500
<i>Acaulospora spinosa</i> Walker and Trappe (6)	WV860	West Virginia	400
<i>Entrophospora colombiana</i> Spain and Schenck (7)	GA101	Georgia	500
<i>Entrophospora contigua</i> nom. ined. (8)	WV201-1	West Virginia	500
<i>Glomus claroideum</i> Schenck and Smith (9)	UT171-1	Utah	500
<i>Glomus clarum</i> Nicolson and Schenck (10)	BR143-1	Brazil	360
<i>G. clarum</i> (11)	CL976-1	Colombia	500
<i>G. clarum</i> (12)	FL239-2	Florida	500
<i>G. clarum</i> (13)	CL883	Colombia	500
<i>G. clarum</i> (14)	WV979-1	West Virginia	500
<i>Glomus diaphanum</i> Morton and Walker (15)	WV579	West Virginia	350
<i>Glomus etunicatum</i> Becker and Gerdemann (16)	FL980A-1	Florida	500
<i>G. etunicatum</i> (17)	MD108-1	Maryland	500
<i>G. etunicatum</i> (18)	NE108A-2	Nebraska	500
<i>G. etunicatum</i> (19)	UT316-1	Utah	500
<i>G. etunicatum</i> (20)	WV998-1	West Virginia	500
<i>Glomus intraradices</i> Schenck and Smith (21)	AU109-1	Australia	500
<i>G. intraradices</i> (22)	FL208-2	Florida	500
<i>G. intraradices</i> (23)	SW101-1	Switzerland	500
<i>G. intraradices</i> (24)	SW103-1	Switzerland	500
<i>G. intraradices</i> (25)	UT118-2	Utah	500
<i>G. intraradices</i> (26)	UT126-1	Utah	500
<i>G. intraradices</i> (27)	UT143	Utah	350
<i>G. intraradices</i> (28)	UT176	Utah	500
<i>G. intraradices</i> (29)	WV994-1	West Virginia	500
<i>Glomus leptotichum</i> Schenck and Smith (30)	WV954-1	West Virginia	400
<i>Glomus occultum</i> Walker (31)	CL383-1	Colombia	400
<i>G. occultum</i> (32)	CL699-1	Colombia	500
<i>G. occultum</i> (33)	IA702	Iowa	500
<i>G. occultum</i> (34)	WV771	West Virginia	400
<i>Gigaspora albida</i> Schenck and Smith (35)	BR201	Brazil	250
<i>Gigaspora albida</i> (36)	FL927-2	Florida	130
<i>Gigaspora gigantea</i> (Nicol. and Gerd.) Gerdemann and Trappe (37)	HA953-2	Hawaii	150
<i>Gigaspora gigantea</i> (38)	MD105-1	Maryland	150
<i>G. gigantea</i> (39)	MN922	Minnesota	150
<i>Gigaspora rosea</i> Nicolson and Schenck (40)	BR151A-1	Brazil	350
<i>Gigaspora margarita</i> Becker and Hall (41)	BR444-1	Brazil	350
<i>Gigaspora rosea</i> (42)	FL105-2	Florida	250
<i>G. rosea</i> (43)	FL105-3	Florida	350
<i>G. rosea</i> (44)	FL219-1	Florida	350
<i>G. rosea</i> (45)	UT102	Utah	350
<i>G. margarita</i> (46)	WV205	West Virginia	350
<i>Scutellospora heterogama</i> (Nicolson and Schenck) Walker and Sanders (47)	BR154-1	Brazil	350
<i>Scutellospora dipurpurascens</i> Morton and Koske (48)	WV930-1	West Virginia	400
<i>Scutellospora fulgida</i> Koske and Walker (49)	VA105A	Virginia	240
<i>Scutellospora pellucida</i> (Nicolson and Schenck) Walker and Sanders (50)	WV935	West Virginia	250
<i>Scutellospora persica</i> (Koske and Walker) Walker and Sanders (51)	VA102-2	Virginia	250
<i>Scutellospora verrucosa</i> (Koske and Walker) Walker and Sanders (52)	HA150A	Hawaii	250
<i>Scutellospora verrucosa</i> (53)	VA105B	Virginia	260

^a INVAM, International Culture Collection of Arbuscular and Vesicular-Arbuscular Fungi.

MATERIALS AND METHODS

Identification of spore culture conditions. Fungi in the Glomales that form endomycorrhizal associations with plants were obtained from living cultures in the International Culture Collection of Arbuscular and Vesicular-Arbuscular Fungi at West Virginia University. Procedures for culture development on plants were reported in detail by Morton et al. (21). Briefly, cultures of fungi listed in Table 1 were established from single isolates in 150-cm³ containers (Stuewe and

Sons, Inc., Corvallis, Oreg.) in Lily sandy loam (Typic Hapludult; 8 mg of P kg⁻¹ of soil and <1% organic matter) mixed 1:2 (vol/vol) with sand and limed to pH 6.2. Cultures with sudan grass (*Sorghum sudanense* (Staph.) Piper) as the host were maintained for 4 months, after which spores were extracted from air-dried soil containing chopped roots. Spores of similar morphologies (size, color, and microscopic features) were collected, rinsed with distilled H₂O, and air dried to the bottom surfaces of screw-capped test tubes (13 by 100 mm) for storage prior

FIG. 1. The most abundant 16-carbon-length FAMES in 53 isolates of 23 species in the genera *Acaulospora*, *Entrophospora*, *Glomus*, *Gigaspora*, and *Scutellospora* (order Glomales). Abundance is measured as the percentage of the total FAMES of 9 to 20 carbon atoms in chain length. Numbers on the x axis refer to species names in Table 1.

to fatty acid analysis. For some isolates, air-dried mycorrhizal roots were separated from the soil and assayed for fatty acid composition.

Spore extraction and profile analysis. Fatty acids were analyzed for 53 isolates of 24 glomalean species. Between 130 and 500 spores (approximately 10 to 20 mg) of each fungal isolate (Table 1), depending on spore volume, were extracted in the screw-capped test tubes according to procedures used for bacterial cells and derivatized to methyl esters (31). The Microbial Identification System was used to identify FAMES of 9 to 20 carbon atoms in length. The gas chromatograph (Hewlett-Packard 5890A) was fitted with a flame ionization detector and a capillary column (Ultra 2; 25 m by 0.22 mm; cross-linked 5% phenylmethyl silicone) with hydrogen as the carrier gas. FAME peaks were measured by a Hewlett-Packard 3392 integrator and named using the MIDI Microbial Identification Software (TSBA Library version 3.60; Microbial ID, Inc.). The software calculated a quantity, called equivalent carbon length, by comparison of retention times with standards (straight-chain FAMES from C-9 to C-20). Structural confirmation of most of the FAMES in the peak library data base has been accomplished by mass spectrometry (29).

A library was created from the resulting profiles, and library entries were analyzed by a MIDI cluster analysis program. Unweighted pair groups were portrayed as dendrograms to determine similarity among the profiles.

Comparison of spore and root profiles. Root colonization by six isolates of *Glomus intraradices*, two isolates of *Gigaspora rosea*, and one isolate of *Gigaspora margarita* was measured from 0.2 g (fresh weight) of roots from three replicate containers of sudan grass. Modified procedures from Phillips and Hayman (27) for clearing and staining roots (8) and quantification by the line-intersect method (9) were used to estimate percentages of root length colonized or percentages of spores per root length of sudan grass.

Fatty acids were extracted from 15 to 30 mg of the oven-dried roots of two replicate containers from each mycorrhizal isolate on sudan grass and four replicate containers of nonmycorrhizal sudan grass. The same extraction and chromatographic separation procedures that were used for spores were performed for roots. An unweighted pair group comparison was used to generate

dendrograms to evaluate relative similarities of spore and mycorrhizal and non-mycorrhizal root profiles. Euclidian distance units (EDU), the distance in two-dimensional array between two entries, were used to portray relatedness among FAME profiles (30).

Quantification of mycorrhizae in citrus. Plant culture and inoculation. Seeds of the citrus rootstock cultivar Carrizo citrange (*Poncirus trifoliata* (L.) Raf. × *Citrus sinensis* (L.)) were germinated in soilless medium, and seedlings were grown to the 2- to 4-true-leaf stage. One-month-old seedlings were transplanted in 150-cm³ containers filled with autoclaved Candler fine sand soil (3.8 mg of P kg⁻¹, <1% organic matter, pH 6.8). For mycorrhizal treatments, a pad of air-dried sudan grass roots (about 100 mg) containing 1.2 propagules per mg of root of *G. intraradices* FL208 or 3.4 propagules per mg of root of *Glomus* sp. strain FL906 was placed in the middle of the soil profile in the container during transplanting. Nonmycorrhizal plants received an extract of inocula passed through a 38-μm-pore-size sieve to remove the mycorrhizal fungal propagules to establish a similar soil microflora.

Seedlings were fertilized weekly with Hoagland's solution [1 mM KH₂PO₄, 5 mM KNO₃, 5 mM Ca(NO₃)₂, 2 mM MgSO₄, micronutrients] and were grown under greenhouse conditions (photosynthetic photon flux density, >600 μm m⁻² s⁻¹; >14-h photoperiod) from May through August 1991. At 14, 28, 56, and 84 days after transplantation, five seedlings from each treatment were harvested by removal of soil from the roots and measurement of total plant dry weight (70°C for 3 days).

Quantification of colonization in citrus roots. A subsample of dried fibrous roots (diameter, >2 mm) of Carrizo citrange was cleared and stained from each plant by modifications of the procedures described by Phillips and Hayman (27) and Bevege (6) as previously described (10). Twenty individual 1-cm root segments for each subsample were mounted on slides for examination at a magnification of ×200. The incidence of colonization was determined as the percentage of 20 segments (scored individually) that contained vesicles, arbuscules, or hyphae. The intensity of vesicular development was estimated for each 1-cm root segment on a scale of 0 to 5 (0, no vesicles; 1, 2, 3, 4, and 5, 0 to 5%, 6 to 25%,

FIG. 2. The most abundant 18-carbon-length FAMES in 53 isolates of 23 species in the genera *Acaulospora*, *Entrophospora*, *Glomus*, *Gigaspora*, and *Scutellospora* (order Glomales). Abundance is measured as the percentage of the total FAMES of 9 to 20 carbon atoms in chain length. Numbers on the x axis refer to species names in Table 1.

26 to 50%, 51 to 75%, and 76 to 100% of the root length with vesicles present, respectively) and was averaged for root segments on each slide ($n = 20$).

Fungal colonization was also estimated by FAME analysis of citrus roots. The extraction procedures that were performed for citrus roots were performed as described above for sudan grass (30 mg of root per plant). As previously reported (26), concentrations of individual fatty acids were expressed relative to the highest value in samples using the integrated areas from the FAME profiles.

RESULTS

Identification of spore fatty acid composition. Eight fatty acids were identified that accounted for >1.0% of the profile in one or more of the isolates (Fig. 1 to 3). In spores, 16:0 was present in all isolates and constituted 10 to 38% of the total profile (Fig. 1A). One unsaturated fatty acid, 16:1_{ω5} *cis*, accounted for more than 50% of the total fatty acids in all isolates of *Acaulospora* and *Entrophospora* and most isolates of *Glomus* and *Scutellospora* (Table 1) (Fig. 1B). This fatty acid was absent or was detected at <1% in *Glomus* isolates whose mycorrhizae fail to stain histochemically with trypan blue (i.e., *Glomus leptotichum*, *Glomus occultum*) and in all *Gigaspora* isolates.

Conversely, 18:1_{ω9} *cis* accounted for 38 to 48% of the total fatty acids in all *Gigaspora* isolates tested (Fig. 2A). A high proportion of 18:1_{ω9} *cis* also occurred in *G. leptotichum* and *Scutellospora persica*. Isolates of *G. leptotichum* and *G. occultum* were unique in possessing 16:1_{ω7} *cis* (Fig. 1C). In a previous study (26), 16:1_{ω7} *cis* was erroneously reported to be the principal fatty acid present in citrus roots infected by *G. intraradices* FL208; the fatty acid identified was actually 16:1_{ω5} *cis*, as reported here.

The unsaturated fatty acid 18:1_{ω5} *cis* was present at low levels (<1.7%) in about 50% of the isolates assayed, but it was widely distributed among species of *Glomus*, *Gigaspora*, and *Scutellospora* (Fig. 2B). Another acid, 20:4_{ω6,9,12,15} *cis*, was also detected in all of the genera tested, but at levels below 2.9% of the total FAME profile (Fig. 3C).

Other 20 C fatty acids appeared to have a more restricted distribution at the genus and species levels. For example, 20:

1_{ω9} *cis* was found only in *Gigaspora* species at levels ranging from 8 to 15% (Fig. 3B). In all other fungi, this acid was absent or was detected at a level of <1%. The 20:0 iso acid, ranging from 10 to 18%, was present only in the five isolates of *Glomus etunicatum* and the one isolate of *Glomus claroideum* (Fig. 3A). This acid occurred at very low levels (<0.5%) in only two other fungi, *G. intraradices* UT126 and *Scutellospora heterogama* BR154-1.

Comparison of spore and root profiles. Spore profiles of *G. intraradices* isolates from diverse geographic locations were clustered within 14 EDU (Fig. 4). Colonization levels for *G. intraradices* in sudan grass roots ranged from 17 to 41%. Fatty acid profiles of these mycorrhizae were clustered similarly at ≤20 EDU. Spore and mycorrhiza profiles for *G. intraradices* were further separated (36 EDU); however, they were more closely related to each other than to profiles of *G. rosea* and *G. margarita* spores, their mycorrhizae, and nonmycorrhizal roots.

Spore profiles of *G. rosea* and *G. margarita* were clustered within 14 EDU but separated from root profiles by 40 EDU (Fig. 4). Roots were heavily colonized by *Gigaspora* isolates, with levels varying from 35 to 48% of the root lengths. FAME profiles of *G. rosea* and *G. margarita* mycorrhizae were as similar to those of nonmycorrhizal roots as they were to each other. Profiles of *G. margarita* mycorrhizae were more similar to those of nonmycorrhizal roots than to those of *G. rosea* mycorrhizae. Roots infected with *G. margarita* contained no FAMES that were different from those of the nonmycorrhizal roots. However, roots colonized by *G. rosea* contained 20:1_{ω9} *cis* not found in noninfected sudan grass roots.

Quantification of mycorrhizae in citrus. Colonization incidence in Carrizo citrange roots increased more rapidly for *G. intraradices* FL208 than for *Glomus* sp. strain FL906 (Fig. 5A). There was a potential bias toward detection of the larger vesicles in root pieces for two reasons: (i) suberized hypodermis and multiple layers (8 to 10) of small cortical cells (diameter, 10 to 15 μm) made hyphae and arbuscules difficult to visualize, and (ii) mycorrhizal structures were reduced in size when they

FIG. 3. The most abundant 20-carbon-length FAMES in 53 isolates of 23 species in the genera *Acaulospora*, *Entrophospora*, *Glomus*, *Gigaspora*, and *Scutellospora* (order Glomales). Abundance is measured as the percentage of the total FAMES of 9 to 20 carbon atoms in chain length. Numbers on the x axis refer to species names in Table 1.

were constrained by small cortical cells and intercellular spaces. Estimation of vesicle intensity confirmed that *G. intraradices* FL208 formed vesicles sooner after inoculation, and the rate of vesicle development was greater than that for *Glomus* sp. strain FL906 (Fig. 5B).

The increase in colonization incidence and vesicle development by both fungi (Fig. 5A to C) was paralleled by an increase in 16:1_{ω5} *cis*, a fungal fatty acid not found in nonmycorrhizal citrus roots. Incidence and vesicle intensity of colonization by *G. intraradices* FL208 were highly correlated ($r = 0.92$; $P \leq 0.001$). Fatty acid accumulation in roots was correlated slightly better with vesicle intensity ($r = 0.57$; $P \leq 0.01$) than with colonization incidence ($r = 0.52$; $P \leq 0.05$). For *Glomus* sp. strain FL906, incidence and intensity were less well-correlated with each other ($r = 0.69$; $P \leq 0.001$). The presence of 16:1_{ω5} *cis* fatty acid was more highly correlated with incidence ($r = 0.71$; $P \leq 0.001$) than with vesicle intensity ($r = 0.58$; $P \leq 0.01$).

Although colonization by *G. intraradices* FL208 was more rapid, *Glomus* sp. strain FL906 provided a growth response in low-P soil greater than those of nonmycorrhizal control plants (Fig. 5D).

DISCUSSION

Glomalean fungal spores contained abundant fatty acids, as previously reported (1, 13), with between 130 and 500 spores required for extraction to obtain a definitive profile (Table 1). Spore size was an important factor; the smallest spored fungus, *G. occultum*, yielded the fewest total FAMES per spore, and one of the largest, *G. gigantea*, yielded the most (data not

shown). Despite being grown in association with a host plant over a 4-month period and in the presence of contaminating microorganisms, an unweighted numerical analysis of the FAME profiles revealed relatively tight clusters at intraspecific, specific, and generic levels (data not shown). Replicate

FIG. 4. Pair group comparison of FAME profiles from nonmycorrhizal roots of sudan grass as well as mycorrhizal roots (myc) and spores of *G. intraradices*, *G. rosea*, and *G. margarita* isolates.

FIG. 5. Colonization of citrus roots by two *Glomus* isolates evaluated as the percentage incidence of hyphae, vesicles and arbuscules (A) intensity of vesicle development (0 = no vesicles; 1, 2, 3, 4, and 5 = 0 to 5%, 6 to 25%, 26 to 50%, 51 to 75%, and 76 to 100% of the root length with vesicles present, respectively) (B), accumulation of 16:1 ω_5 cis in roots (C), and growth response of mycorrhizal and nonmycorrhizal Carrizo citrange (*Poncirus trifoliata* $\times$ *Citrus sinensis*) at low-phosphorus supply (D).

spore samples from individual pot cultures of sudan grass were consistently closely linked at less than 5 EDU. Nevertheless, contaminating microorganisms in cultures may alter profiles. When spores of *Glomus* sp. strain FL312 from an aeroponic culture (12) were visibly contaminated with *Microdochium bolleyi* (Sprague) de Hoog and Hermanides-Nijhoj, the FAME profile varied by 48 EDU from that of noncontaminated spores of the same isolate from a soil pot culture (10a). Thus, each spore population must be examined closely for visible contaminating microorganisms and hyperparasites in spores.

Twenty-seven different FAMES were identified among the fungi surveyed, of which eight accounted for >90% of the profiles. The fatty acid 16:1 ω_5 cis accounted for more than 50% of the total composition in all *Acaulospora* and *Entrophospora* isolates and in most *Glomus* isolates. The fatty acid 18:1 ω_9 cis was shared by all *Gigaspora* isolates and was in proportion similar to that in fungi from two other genera: *G. leptotichum* WV954-1 and *Scutellospora verrucosa* HA150A. This distribution suggests that convergent evolution of fatty acid biosynthesis occurred at the family level.

Profiles of *G. etunicatum* were distinct in that 20:0 iso was unique to these isolates and the one isolate of *G. claroideum* studied. *G. leptotichum* and *G. occultum* isolates were distinguished from all other glomalean fungi by the high levels of 16:1 ω_7 cis (almost 50% in two *G. occultum* isolates) and the trace amounts of 16:1 ω_5 cis. Interestingly, *G. leptotichum* and *G. occultum* were the only species sampled whose mycorrhizae do not stain in trypan blue (16). The significance of these observations remains unclear.

FAME profiles of spores of *G. intraradices* were more similar to their respective profiles in mycorrhizal sudan grass roots than were spore profiles of *G. margarita* and *G. rosea* to their mycorrhizae. FAME profiles of *G. intraradices* mycorrhizae

were nearly as tightly clustered as were profiles from their respective spores. Fatty acid profiles in roots containing vesicles and/or spores of *G. intraradices* were more similar to spore profiles than to profiles from nonmycorrhizal roots. Fatty acid profiles of mycorrhizae formed by *Gigaspora* species which do not form vesicles or spores in roots were more similar to those of nonmycorrhizal roots than to those of spores. The only major difference from nonmycorrhizal roots was the presence of one acid, 20:1 ω_9 cis in mycorrhizal roots of *G. rosea*. *G. margarita*-infected roots, which did not contain this fatty acid, had the FAME profile that was least distinguishable from those of nonmycorrhizal roots.

Since 20:1 ω_9 cis was not detected in sudan grass roots infected by *G. intraradices* isolates, the potential exists to identify and quantify *G. rosea* in roots coinfected by *G. intraradices*. Likewise, *G. intraradices* produced 16:1 ω_5 cis, a fatty acid not produced by *G. rosea* or host roots. Thus, the biosynthesis of the two fatty acids, unique to each fungus, may afford the opportunity to study competition of organisms at the intergeneric level during the root colonization process.

The limitation of this approach is the relative proportions of fatty acids in each fungus. The 20:1 ω_9 cis of *G. rosea* mycorrhizae constituted only 5% of the profile, and total fatty acid levels in roots were only about 1.5 times higher than those in nonmycorrhizal roots. In *G. intraradices*-colonized roots with vesicles and spores present, total fatty acid content was 2.8 times higher than that in nonmycorrhizal roots, and 30 to 50% of the total FAME profile in sudan grass was represented by 16:1 ω_5 cis. Unlike *Glomus* species, *Gigaspora* species do not develop lipid-rich vesicles in roots; therefore, colonization as hyphae and arbuscules may not be detectable until it reaches a high level.

The predominance of 16:1 ω_5 cis in *Glomus* species provided an index for the development of colonization in citrus roots. Despite improved clearing and staining procedures (10), colonization in citrus roots was impossible to accurately quantify by the standard grid-line intersect method because of a suberized hyperdermis and relatively small cortical cells that reduced the sizes of mycorrhizal structures (10). Vesicles were most easily visualized, and fatty acid levels in roots provided a biochemical measure of colonization that was correlated with estimates of vesicle intensity and colonization incidence. For the highly vesicular fungus *G. intraradices*, the relationship between 16:1 ω_5 cis and vesicle development was closer than that for colonization incidence, whereas for *Glomus* sp. strain FL906, a less vesicular fungus, the correlation was higher for colonization incidence. For both fungi, fatty acid analysis of mycorrhizal roots validated that the rate of development of colonization is reasonably well-estimated by visual clearing and staining and quantification methods.

While total fungal biomass cannot be directly estimated from the presence of 16:1 ω_5 cis, the relative amount of this fatty acid in roots may provide an index of the amount of carbon allocated for fungal growth and lipid storage in the root during the colonization process. In a previous study (26), total fatty acid levels in mycorrhizal and nonmycorrhizal citrus roots were correlated with root construction cost measured by the heat of combustion and by nitrogen and ash contents of root tissue (34). Peng et al. (26) estimated that about 10% of the increase in allocation of carbon to roots at 52 days after inoculation of *Citrus volkameriana* roots with *G. intraradices* FL208 could be attributed to fungal construction cost.

The correlation between 16:1 ω_5 cis fatty acid and vesicular development in citrus roots suggests that more fungal carbon was allocated to lipid storage in vesicles and/or spores of *G. intraradices* FL208. When fatty acid content and the rate and

magnitude of colonization and vesicle intensity were considered together, more fungal carbon appeared to be allocated for P acquisition by *Glomus* sp. strain FL906. These results indicate the usefulness of comparing allocation patterns among fungi; however, fatty acids cannot be used alone as an index of fungal effectiveness. The potentially large differences in the P uptake and translocation by different arbuscular mycorrhizal fungi are also important considerations (25).

The relationship between allocation of carbon to the fungus and the presence of fungal fatty acids in the root may be apparent only in mycorrhizae formed by highly vesicular fungi such as the two *Glomus* isolates studied here. Carbon cost of mycorrhizal roots would likely be underestimated for fungi in the family Gigasporaceae. These fungi produce large lipid-rich spores in the rhizosphere, as well as extraradical auxiliary cells which also contain abundant lipid (13). Analysis of root material would only exclude these potentially important sources of fungal carbon.

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