

Vesicular-arbuscular mycorrhizae of Easter lily in the northwestern United States¹

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The vesicular-arbuscular (VA) mycorrhizal fungi of commercially grown Easter lily (*Lilium longiflorum* Thunb.) were studied. Soil and root samples were collected monthly from March through September 1975 from five fields in the coastal area of southern Oregon and northern California. Soil seivings were inoculated onto clover, onion, and lily to cause infections resulting in the production of many new mycorrhizal spores facilitating identification. Four VA mycorrhizal species were found: *Acaulospora trappei*, *A. elegans*, *Glomus monosporus*, and *G. fasciculatus*. All four VA species infected Easter lily, clover, and onion. *Acaulospora trappei* and *G. fasciculatus* were the most commonly isolated species from all five fields.

Mycorrhizal infections in roots of field-grown lilies were sparse and presumably young in March and gradually increased in size and number until September when bulbs were harvested. Over 75% of each root system became infected with mycorrhizae in fields with all four fungal species, and those levels were reached by July. In fields with only two mycorrhizal species, usually 50% or less of each root system was infected, even by the end of the growing season.

Introduction

Vesicular-arbuscular (VA) mycorrhizal fungi can be found in most soils (9, 18), being more numerous and diverse in cultivated rather than noncultivated soils (17, 20). These phycomycetous fungi belong to the family Endogonaceae (12) and colonize the roots of a large number of agricultural crops (9, 10). Ames (1) and Vanderploeg (27) have shown that lily seedlings benefit from a VA mycorrhizal association in greenhouse studies. However, there is no information on which fungi form VA mycorrhizae with Easter lilies (*Lilium longiflorum* Thunb.) in field production of bulbs. About 75% of the Easter lily bulbs grown in the United States for greenhouse forcing of potted plants are produced along the coast of southern Oregon and northern California (25). The purpose of this study was to determine which mycorrhizal fungi occur in these lily fields and their distribution, and how infection progresses through the growing season.

Materials and Methods

Area of Study

Plant and soil samples came from five fields located

along U.S. Highway 101, between Brookings, Oregon, and Smith River, California. Individual fields are referred to as A, B, C, D, and E.

Field Collection and Observation of Mycorrhizae

Soil and root samples were collected at monthly intervals from March through September from each of the five fields during the 1975 growing season. Samples from each field consisted of 10 adjacent plants in a single row. The roots were cleared and stained by a modification of the technique of Phillips and Hayman (24) and were observed for the presence of vesicles and arbuscules (2). The total percentage of the root systems colonized by mycorrhizal fungi was estimated by observing each cleared and stained root system under the dissecting microscope (7.5 ×). The soil collected from around the roots of all 10 plants was combined, mixed, and passed through a screen to remove large rocks and plant debris. Subsamples of about 50 cm³ were wet-sieved using a method similar to the one of Gerdemann and Nicolson (11), developed to recover VA spores.

Increasing Spore Numbers for Species Identification

To determine which VA fungi infected field-grown lilies, we used the method of Daft and Nicolson (6). Whole plants were removed from each field in March and September 1976. The plants were washed and replanted in the greenhouse in a pasteurized soil mix (1 soil : 1 hemlock bark : 3 sand) to allow spores to form in the soil on mycelium from the infected roots. The soil was then sieved to retrieve spores for identification.

Spores from soil sievings are often imperfect and difficult to identify. Mosse and Bowen (20) claimed that "the only confirmative evidence therefore is the repro-

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duction of a uniform and distinctive spore population in the rhizosphere of inoculated test plants." To do this, trap (test) plants were planted so that the roots passed through a layer of inoculum placed in small tubular plastic growing containers measuring 3 × 16 cm (Fig. 1). This method was similar to Gerdemann's (7) funnel technique, in that the roots were directed through a narrow area where they contact the mycorrhizal inoculum. Inoculum was collected by mixing a 100-cm³ soil sample in tap water, allowing it to settle for 1 min, and decanting it through nested screens with 355-, 250-, 125-, 90-, 63-, and 45- μ m openings.

Washed river sand, which was autoclaved for 1 hour and stored until needed, was used as the growing medium. A paper plug was placed in the bottom of each tube, and 20 cm³ of this sand was placed over the plug. The soil inoculum, caught on each of the six screens, was placed in six respective tubes as a layer on top of the sand. This procedure was repeated six times for each field, giving a total of 36 trap tubes per field each month. Another 20 cm³ of sand was added on top of the inoculum in each tube, and test plants were planted in each. Twelve of the 36 tubes were planted to red clover (*Trifolium pratense* L.), onion (*Allium cepa* L.), or lily (*L. longiflorum*). Seed was used for clover and onion, and bulbets were used for lilies. Ten noninoculated tubes for each trap-plant species were used for controls each month.

The tubes, held in racks in a growth chamber, were watered as needed by submerging their bottoms 2-3 cm in water. The growth chamber was set for a 14-h photoperiod (minimum 12 000 lx) with a day temperature of 23°C (75°F) and a night temperature of 18°C (65°F). Plants were given about 5 ml of half-strength Hoagland's solution once a month. Trap tubes were checked for mycorrhizal development after 4 to 5 months by sieving sand from the tubes to recover newly formed spores. The whole root system from each plant was washed and then cleared and stained (2) to reveal mycorrhizal infections.

Results

Mycorrhizal Species Found in Lily Fields

Four VA mycorrhizal species were identified (12). One of the species, not previously recorded, has now been described as *Acaulospora trappei* Ames & Linderman (2). *Glomus fasciculatus* (Thaxt.) Gerd. & Trappe and *A. trappei* occurred in all five fields. Two other species, *Glomus monosporus* Gerd. & Trappe and *Acaulospora elegans* Trappe & Gerd. were found only in fields A, B, and D. None of the fungi necessarily occurred consistently in the monthly samples from a field (Table 1). All four VA species infected the roots of onion, lily, and clover. None of the control plants became mycorrhizal. Spores of the four fungi are shown in Fig. 2. No spores were found in tubes in which the trap plant did not become mycorrhizal.

The cleared and stained roots of trap plants revealed no unusual structures. All mycorrhizal species produced vesicles and arbuscules in each infected host. Root infection by *A. trappei* was

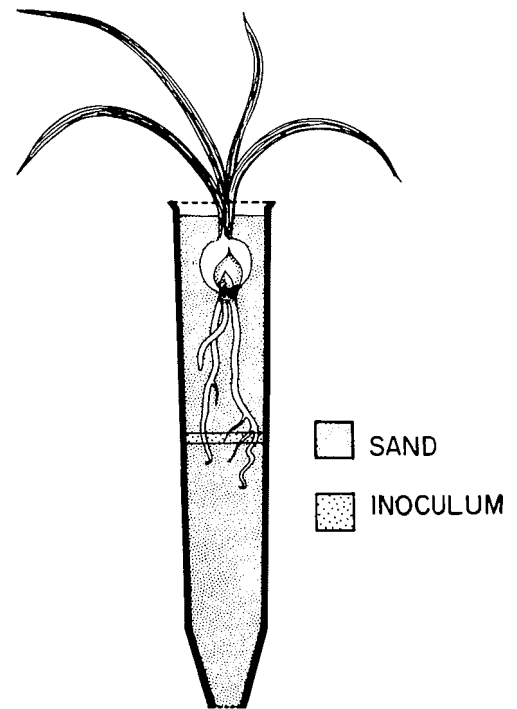


FIG. 1. Diagram of trap-tube system used to increase the number of VA mycorrhizal spores found in field soil sievings and used in the traps as inoculum.

particularly interesting, because spores still attached to vesicles were frequently observed inside the roots of trap plants. Because of this method of spore formation, *A. trappei* was relatively easy to distinguish from internally produced vesicles in a stained root. Identification of other VA species, especially in the genus *Glomus*, was more difficult. *Acaulospora trappei* spores, but not arbuscules, were also observed inside clover root nodules. Vevege and Bowen (3) suggested that infection of legume nodules by VA mycorrhizal fungi may occur only "with difficulty."

Whole plants transplanted from the field into containers of pasteurized soil in the greenhouse failed to produce any spores. In that *A. trappei* and *G. fasciculatus* were the most frequently isolated species from all five field sites, it is presumed that they were the predominant species to infect lilies in the field. Why spores failed to form is unknown.

Progress of Field Root Infections

Monthly root samples from each field were cleared and stained to evaluate the progress of VA mycorrhizal root colonization. Hyphae entered lily roots primarily through root hairs,

TABLE 1. Ratings of VA mycorrhizal infections on monthly soil and root samples of field-grown Easter lilies collected from five growers

Grower	Rating category ^{a,b,c}	Infection ratings						
		March	April	May	June	July	Aug.	Sept.
A	Age	Y	M	C	C	C	—	C
	Number	3	3	3	3	3	—	3
	VA species	At,Gf,Ae	At,Gf	At,Ae	At,Gf,Ae	At,Gf,Ae	—	At,Gf,Ae,Gm
B	Age	Y	Y	M	M	C	—	C
	Number	1	1	2	3	3	—	3
	VA species	At,Gf,Ae	At,Gf	Gf	At,Gf,Ae	At,Gm	—	At
C	Age	Y	Y	Y	C	C	—	C
	Number	1	1	1	1	1	—	1
	VA species	At	At,Gf	At	At,Gf	At	—	At
D	Age	Y	M	C	C	C	—	C
	Number	3	3	3	3	3	—	3
	VA species	Gf	Gf	Gf,Ae	At,Gf,Ae	At,Gf,Gm	—	At,Gf,Ae,Gm
E	Age	Y	Y	Y	C	C	—	C
	Number	1	1	1	1	1	—	1
	VA species	At,Gf	At,Gf	At,Gf	At,Gf	At,Gf	—	At,Gf

^aAges of infection units: based on an estimation of the number of young arbuscules per infection unit in early stages and on whether infection units had coalesced in later stages. Y = < 5 arbuscules/infection unit; M = 5–15 arbuscules/infection unit, but infection units not coalesced; C = > 15 arbuscules/infection unit and infection units coalesced.

^bNumber of infection units: relative number of infection units over the whole root system. 1 = few; 2 = moderate; 3 = many.

^cVA species present: At = *Acaulospora trappei*, Gf = *Glomus fasciculatus*, Ae = *Acaulospora elegans*, and Gm = *Glomus monosporus*.

as commonly observed in other hosts (6, 9). Vesicles and arbuscules abounded in stem roots and basal roots, but not in the large contractile roots of field-grown plants. In the March samples, only young, restricted infection units (IU) (5) were found, i.e., those with less than five arbuscules/IU. As the season progressed, the number of arbuscules/IU increased until adjacent IU's coalesced. In some fields, this coalescence was apparent in May, in others, not until June or July (Table 1). In fields A and D, where up to four VA species were found, there were many IU's in the first March samples. By the end of the season, more than 75% of each of these root systems was infected, as evidenced by abundant masses of arbuscules. In field B, which also had four VA species present, IU's were quite sparse in March but progressed to many by the end of the season. In fields C and E, which never had more than two VA species present, IU's were quite sparse in March and remained sparse throughout the season, although in certain areas the IU's did expand and coalesce. In those fields, the root systems on individual plants were never more than 50% infected.

Discussion

The number of VA mycorrhizal species in any field is determined by its agricultural uses and many other factors. In other studies only one or

two species (14, 15, 21), more frequently three or four (6, 8, 14), and occasionally five (15, 21) have been found. In our study four distinct species were found. Of these, *G. fasciculatus* and *A. trappei* have relatively small spores (35–100 μ m). Spores of *G. fasciculatus* usually occur in clusters that are caught on larger mesh screens (125–355 μ m). However, *A. trappei* spores occur individually and are usually caught on the 63- and 45- μ m mesh screens. Many workers fail to screen for mycorrhizal spores below the 100- μ m mesh screen (6, 14, 15, 21), and consequently many new species may have been overlooked. Nemeč (22) made this same observation and sieved soil down to 45 μ m. Gerdemann (8) sieved soil down to 44 μ m, but apparently neither he nor Nemeč encountered *A. trappei*. The small size of *A. trappei* spores and the fact that its spores remain suspended in water for relatively long periods of time may have delayed its discovery. Molina and Trappe (personal communication) have apparently found it with rangeland grasses, but its overall distribution is yet unknown.

Cultural practices undoubtedly influence the mycorrhizal species found in any field and the degree to which plant roots become infected. In a study of lily growth in the Oregon-California area, Roberts *et al.* (26) stated that soil management is similar in all fields but that fertilizer use varies considerably. Adverse effects of high

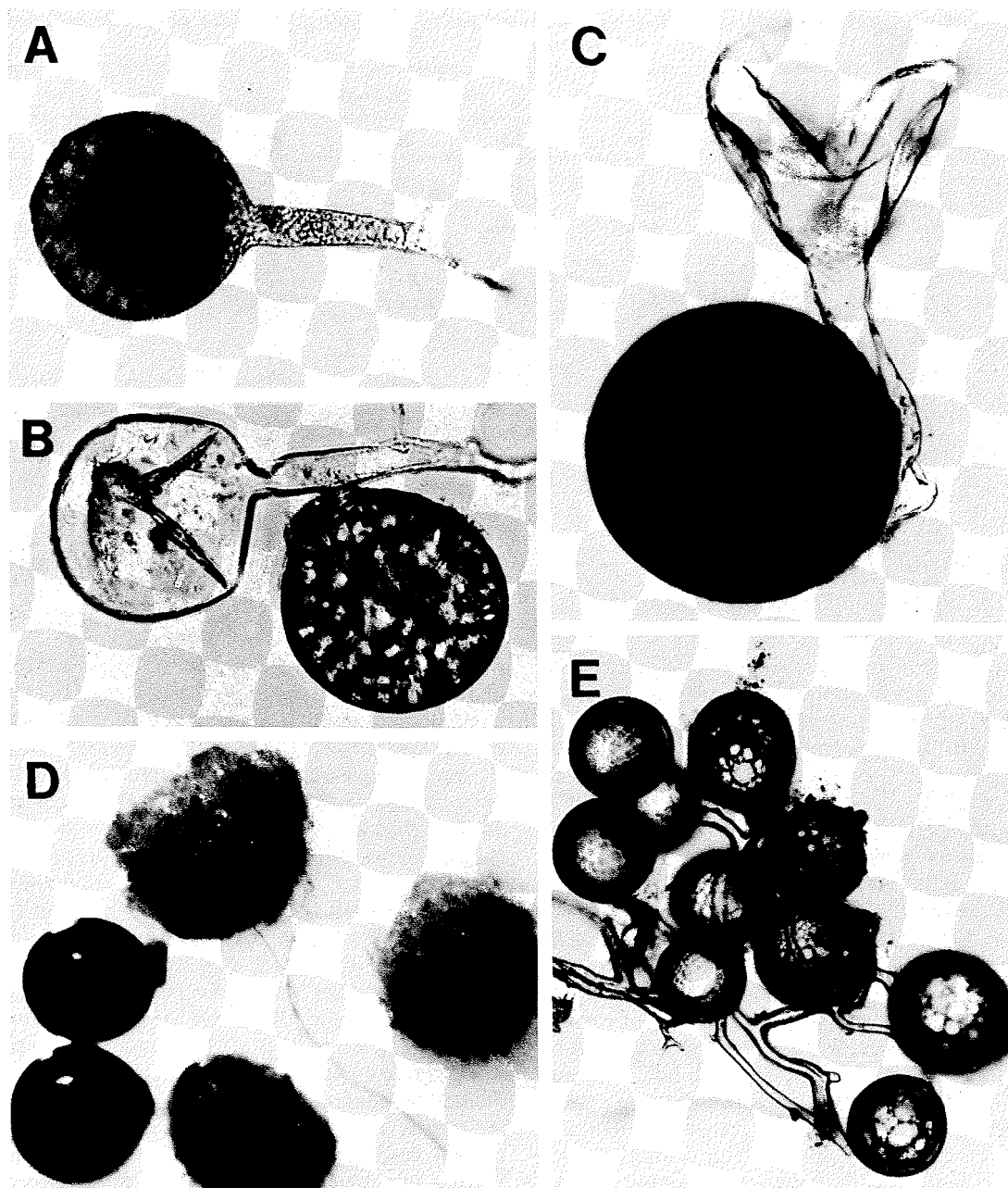


FIG. 2. Species of VA mycorrhizal fungi found in Easter lily fields. (A-B) *Acaulospora trappei* ($\times 250$). (A) Young vesicle before spore formation. (B) Mature spore with empty vesicle attached. (C) *Acaulospora elegans* with empty vesicle attached ($\times 125$). (D) *Glomus monosporus* showing spores with and without hyphal mantle ($\times 31.2$). (E) *Glomus fasciculatus* spore cluster ($\times 125$).

levels of fertilizer on mycorrhizae have been reported, and we have observed the same effect on Easter lilies grown in the greenhouse (Ames and Linderman, unpublished data).

Treatments to control pathogens may also

affect lily mycorrhizae. Fumigation with nematocides enhances mycorrhiza formation (4). Pentachloronitrobenzene (PCNB), a fungicide used as a bulb treatment to inhibit some fungal pathogens, is detrimental to mycorrhizal de-

velopment on corn (23). Menge *et al.* (17) demonstrated that PCNB can reduce spore production of *G. fasciculatus* from 70 to 90% depending on the time of fungicide application. PCNB can also reduce phosphorus accumulation by mycorrhizal onion plants (13).

The VA species that infect lilies in the field may be those that are carried along on the roots of the planting stock; however, we found no mycorrhizae in root fragments remaining on 40 untreated bulbs we examined after harvest. These fragments were mainly portions of large, contractile roots, which we found to be non-mycorrhizal in our examination of roots of field-grown lilies.

The cover and rotation crops, as well as weed hosts, may influence the levels of mycorrhizae before lily planting. Kruckelmann (16) reported that different crops greatly influenced the total population of mycorrhizal spores. Some crops may not become heavily infected and therefore do not produce many spores. Fields A, B, and D, which had periods of 2 or 3 years between lily plantings, maintained a more diverse spore population and greater incidences of VA root infections than did fields C and E with 1 and 5 years of rotation time, respectively. Kruckelmann (16) stated that crop rotation "had no effect on the frequency of spore types in the soil." However, many other factors in addition to rotation period may have influenced the relative amounts of mycorrhizae in these fields (19).

This report identified the VA mycorrhizal fungi that occurred in some Easter lily fields in the northwestern United States and described how their infections in lily roots progressed throughout the growing season. Future studies are needed to identify the cultural practices that most influence VA mycorrhizal spore populations and infection rates. Once these factors are known, growers can begin to manage Easter lily fields to enhance the beneficial activities of these fungi.

Acknowledgements

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- AMES, R. N. 1977. Studies on the vesicular-arbuscular mycorrhizae of Easter lily in the Pacific Northwest. MS Thesis, Oregon State University, Corvallis, Oregon.
- AMES, R. N., and R. G. LINDERMAN. 1976. *Acaulospora trappei* sp. nov. Mycotaxon. 3(3): 565-569.
- BEVEGE, D. I., and G. D. BOWEN. 1975. *Endogone* strain and host plant differences in development of vesicular-arbuscular mycorrhizas. In *Endomycorrhizas*. Edited by F. E. Sanders *et al.* Academic Press, London. pp. 77-86.
- BIRD, G. W., J. R. RICH, and S. U. GLOVER. 1974. Increased endomycorrhizae of cotton roots in soil treated with nematocides. *Phytopathology*, 64: 48-51.
- COX, G., and F. SANDERS. 1974. Ultrastructure of the host-fungus interface in a vesicular-arbuscular mycorrhiza. *New Phytol.* 73: 901-912.
- DAFT, M. J., and T. H. NICOLSON. 1974. Arbuscular mycorrhizas in plants colonizing coal wastes in Scotland. *New Phytol.* 73: 1129-1138.
- GERDEMANN, J. W. 1955. Relation of a large soil-borne spore to phycocomycetous mycorrhizal infections. *Mycologia*, 47: 619-632.
- GERDEMANN, J. W. 1961. A species of *Endogone* from corn causing vesicular-arbuscular mycorrhiza. *Mycologia*, 53: 254-261.
- GERDEMANN, J. W. 1968. Vesicular-arbuscular mycorrhiza and plant growth. *Annu. Rev. Phytopathol.* 6: 397-418.
- GERDEMANN, J. W. 1975. Vesicular-arbuscular mycorrhizae. In *The development and function of roots*. Edited by J. G. Torrey and D. T. Clarkson. Academic Press Inc., London. pp. 575-591.
- GERDEMANN, J. W., and T. H. NICOLSON. 1963. Spores of mycorrhizal *Endogone* species extracted from soil by wet sieving and decanting. *Trans. Br. Mycol. Soc.* 46: 235-244.
- GERDEMANN, J. W., and J. M. TRAPPE. 1974. The *Endogonaceae* in the Pacific Northwest. *Mycol. Mem. No. 5*.
- GRAY, L. E., and J. W. GERDEMANN. 1969. Uptake of phosphorus-32 by vesicular-arbuscular mycorrhizae. *Plant Soil* 30: 415-422.
- HAYMAN, D. S. 1970. *Endogone* spore numbers in soil and vesicular-arbuscular mycorrhiza in wheat as influenced by season and soil treatment. *Trans. Br. Mycol. Soc.* 54: 53-63.
- KHAN, A. G. 1971. Occurrence of *Endogone* spores in West Pakistan soils. *Trans. Br. Mycol. Soc.* 56: 217-224.
- KRUCKELMANN, H. W. 1975. Effects of fertilizers, soils, soil tillage, and plant species on the frequency of *Endogone* chlamydospores and mycorrhizal infection in arable soils. In *Endomycorrhizas*. Edited by F. E. Sanders *et al.* Academic Press, London. pp. 511-525.
- MENGE, J. A., V. MINASSIAN, and E. L. V. JOHNSON. 1976. The effects of heat treatments and three pesticides upon inoculum of the vesicular-arbuscular mycorrhizal fungus *Glomus fasciculatus*. *Proc. Am. Phytopathol. Soc.* 3: 275. (Abstr.)
- MOSSE, B. 1973. Advances in the study of vesicular-arbuscular mycorrhiza. *Annu. Rev. Phytopathol.* 11: 171-196.
- MOSSE, B. 1975. Specificity in VA mycorrhizas. In *Endomycorrhizas*. Edited by F. E. Sanders *et al.* Academic Press, London. pp. 469-484.
- MOSSE, B., and G. D. BOWEN. 1968. A key to the recognition of some *Endogone* spore types. *Trans. Br. Mycol. Soc.* 51: 469-483.
- MOSSE, B., and G. D. BOWEN. 1968. The distribution of *Endogone* spores in some Australian and New Zea-

- land soils, and in an experimental field soil at Rothamsted. *Trans. Br. Mycol. Soc.* **51**: 485-492.
22. NEMEC, S. 1974. Populations of *Endogone* in strawberry fields in relation to root rot infection. *Trans. Br. Mycol. Soc.* **62**: 45-49.
23. NESHEIM, O. N., and M. B. LINN. 1969. Deleterious effect of certain fungitoxicants on the formation of mycorrhizae on corn by *Endogone fasciculata* and on corn root development. *Phytopathology*, **59**: 297-300.
24. PHILLIPS, J. M., and D. S. HAYMAN. 1970. Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. *Trans. Br. Mycol. Soc.* **55**: 158-161.
25. REES, A. R. 1972. The growth of bulbs. Academic Press, London.
26. ROBERTS, A. N., L. T. BLANEY, and O. C. COMPTON. 1964. Seasonal changes of certain nutrient elements in the leaves and bulbs of Croft lily, *Lilium longiflorum*, and their relation to bulb yield. *Am. Soc. Hort. Sci.* **85**: 611-630.
27. VANDERPLOEG, J. F. 1972. The mycorrhizal association between *Lilium* taxa and the phycomycete *Endogone fasciculata*. MS Thesis, University of Delaware, Newark, Delaware.