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Notes on the occurrence of endogonaceous spores and vesicular-arbuscular mycorrhizal associations in woodland sites in the middle valley of the Tiber (Italy)

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SEZIONE III

(Botanica, zoologia, fisiologia e patologia)

Botanica. — *Notes on the occurrence of endogonaceous spores and vesicular-arbuscular mycorrhizal associations in woodland sites in the middle valley of the Tiber (Italy).* Nota di GIGLIOLA PUPPI e SABINE RIESS, presentata (*) dal Corrisp. G. B. MARINI BETTOLO.

RIASSUNTO. — Tutte le piante erbacee raccolte in tre luoghi boschivi lungo la media valle del Tevere sono risultate infettate da funghi micorrizici. I livelli di infezione andavano dal 10,3 all'80%. Le associazioni micorriziche furono divise in tre tipi sulla base di caratteristiche morfologiche quali arbuscoli, vescicole, avvolgimenti ifali e connessioni tra le ife. Le spore di Endogonacee nel suolo variavano tra 0 e 486/100 g di suolo (peso secco). Sono stati identificati cinque tipi di endofiti fungini, i più comuni essendo: *Glomus macrocarpus* var. *macrocarpus*, *G. macrocarpus* var. *geosporus*, *G. fasciculatus*.

INTRODUCTION

Vesicular-arbuscular mycorrhizal associations occur in most plants, angiosperms and gymnosperms, and in many pteridophytes and bryophytes. The fungi which form VA mycorrhizae are members of the family Endogonaceae (Phycomycetes), and are so widespread that is nearly impossible to find natural soils anywhere in the world that do not contain them.

This paper describes the results of field studies designed to: 1) determine the types and abundance of Endogonaceous spores in natural soils from woodland sites in the middle valley of the Tiber, and 2) determine the intensity and morphological characteristics of VA mycorrhizal infection in members of different families of herbaceous angiosperms.

METHODS AND MATERIALS

Plant roots and soil samples were collected from three different sites (near Baschi, Penna and Stimigliano, respectively) on three collection dates: February 7–9, April 10 and June 2. Soil properties were: Baschi: pH 7.8, organic matter 13.5%, P (Truog) 28.8; Penna: pH 6.0, organic matter 12.4%, P 20.4; Stimigliano: pH 6.8, organic matter 14.5, P 35.4. The standing vegetation was a mixed wood, mainly *Quercus pubescens* Willd. In February soil samples only were collected, while in April and June plant roots were collected, together with the surrounding soil.

The percentage mycorrhizal infection was evaluated by the gridline intersect method (Giovannetti and Mosse, 1980) after staining with trypan blue (Philips and Hayman, 1970). Morphological characteristics were observed for both stained and uncoloured roots maintained in a solution of glutaraldehyde at pH

(*) Nella seduta dell'8 maggio 1982.

7.2 (Riess and Rambelli, 1980). Endogonaceous spores were recovered from 100 g soil subsamples by wet sieving and decanting (Gerdemann and Nicolson, 1963); 2 mm, 500, 250, and 100 μ sieves were used. Total counts were done on the entire sievings (500, 250 and 100 μ) by picking out spores under a dissecting microscope at ×20 or ×40 magnification. Identification of spore types (mounted in lactic acid) was according to the keys of Gerdemann and Trappe (1974), Nicolson and Schenck (1979), Hall and Fish (1979) and Walker and Trappe (1981).

Results and discussion

Table I records data on spore type and number observed in the three sites on the three collection dates. Spore number varies between 0 and 486/100 g soil (dry weight). This value agrees with the one obtained from tuffaceous soils in Latium (Puppi and Riess, 1980) and is comparable with those reported by Giovannetti (1980) from gullies in Tuscany, and by Abbott and Robson (1977) and Sward, Hallam and Holland (1978) from natural soils in Australia. In two sites, B and S, average spore number is lower in June, that is the warmer period.

Relatively few spore types are observed. *Glomus macrocarpus* var. *macrocarpus* Tul. & Tul., *G. macrocarpus* var. *geosporus* (Nicol. & Gerd) Gerdemann & Trappe and *G. fasciculatus* (Thaxter sensu Gerdemann) Gerdemann & Trappe, are the dominant species, as was to be expected from woodland sites (Gerdemann and Trappe, 1974) (Plate I, Figs. 1–4). *G. fasciculatus* is more common in the February samples (coldest period), while *G. macrocarpus* var. *macrocarpus* is more frequent in the April samples. The only other genus represented is *Acaulospora*, with the species *Acaulospora spinosa* Walker & Trappe.

Table II records spore number and type, percentage of mycorrhizal infection and main morphological characteristics of mycorrhizal associations on different plant species. Mycorrhizal associations were observed in all plants checked for the same. The percentage of mycorrhizal infection is similar for each species, the only exception being *Trifolium pratense* L., for the different sites and sampling dates. As for the morphology, no consistent differences were observed between the April and June samples. Descriptions of the morphological characteristics of mycorrhizal associations are, as far as possible, such as to be comparable with those of Abbott and Robson (1978, 1979). On the basis of presence/absence of arbuscules, vesicles, hyphal loops or coils, and junctions between hyphae, it was possible to ascribe the individual associations to three morphological types (Plate II, Figs. 5–8):

Type I.

Recorded on *Ajuga reptans* L., *Anemone* spp. *Muscari racemosum* Mill. The mycelium is irregular; hyphal diameter ranges between 5.4 and 9 μ. Arbuscules at all stages of development are observed. Vesicles are rarely found and typically associated with lysing arbuscules; may be round, 21.6–39.6 μ in diameter, or oval, 25.2–39.8×41.4–72 μ, and intercellular as well as intracellular. Hyphae and vesicles may have granular contents.

TABLE I.

Spore types and number in the three sites.

SITE (*a*)	SAMPLING PERIOD	SAMPLES EXAMINED	SPORES / 100 g DRY WEIGHT							% HUMIDITY
			M (*b*)	G (*b*)	F (*b*)	sp (*b*)	A (*b*)	Total	Range	
B	9.2	5	—	3.4± 1.7	16.4± 8.2	—	—	19.8± 9.9	0 - 54.4	21
B	10.4	18	27.4± 8.5	26.8± 8.5	3.5± 0.9	0.6±0.5	0.3±0.1	58.8±14.5	1.2–218.3	21.3
B	2.6	16	6.2± 1.5	7.3± 3	1.7± 0.3	0.9±0.6	—	15.2± 3.6	2.4– 60.1	20.2
P	9.2	5	—	71.4±21.9	26.2±11.9	—	—	97.8±34	16.6–209.4	19
P	10.4	21	39 ±21.6	44 ± 8.4	2 ± 0.8	—	—	85.4±24	0 –486	20
P	2.6	28	8.9± 3.5	64.6± 9.2	—	—	—	73.5± 9.2	11.5–230.8	15.4
S	7.2	5	—	18.8± 9.7	7.1± 7.1	—	—	26 ±17	5.7– 93.1	15
S	10.4	12	13.8± 5	19.8± 9	4.4± 2.9	—	—	38.2±10.2	0 –108.2	27.3
S	2.6	18	6.6± 1.3	1.6± 0.8	0.5± 0.2	1.7±0.9	—	10.7± 1.9	1 - 39.2	8.9

(*a*) B = Baschi; P = Penna; S = Stimigliano.
(*b*) M = Glomus macrocarpus var. macrocarpus.
G = Glomus macrocarpus var. geosporus.
F = Glomus fasciculatus.
sp = Glomus sp.
A = Acaulospora spinosa.

TABLE II.

Spore number and type, percentage mycorrhizal infection and main morphological characteristics of mycorrhizal associations on different plant species.

PLANT SPECIES	Site (*a*)	Sampling Date	Samples Examined	Spores/100 g dry weight (Range)	Spore type (*b*)					% mycorrhizal Infection	Morphological characteristics (*c*)		
					M	G	F	sp	A		Type I	Type II	Type III
Labiatae:													
Ajuga reptans L.	B	10.4.81	2	64.3–139.5	+		+			78.5	Ad; V		
Ajuga reptans L.	S	10.4.81	3	19.1– 33.1	+	+				79	A; Ad; V		
Ranuncolaceae:													
Anemone apennina L. ...	S	10.4.81	3	11.4– 70	+	+				60	A; Ad; V	H; S; V	
Anemone hortensis L.	B	10.4.81	4	12.1–217.2	+	+	+			47	A; Ad; V	H; S; V	
Anemone hortensis L.	P	10.4.81	3	48 –129.6	+	+				60	Ad; V	H; V	
Euphorbiceae:													
Euphorbia cyparissias L...	B	10.4.81	3	71.6–168.6	+	+	+	+		10.3			C; G; V
Euphorbia cyparissias L...	B	2.6.81	3	7.2– 60.1	+	+	+			15.6			C; G;
Euphorbia cyparissias L...	P	10.4.81	3	22.8–120	+	+				20.3			G; V
Euphorbia cyparissias L...	P	2.6.81	3	46.2–230.8	+	+				26.3		H; V	G; V
Rosaceae:													
Fragaria vesca L.	P	10.4.81	3	42 –132	+	+	+			49.3		H; S; V	
Fragaria vesca L.	P	2.6.81	3	23.1– 98.1	+	+				43		H; V	
Rubiaceae:													
Galium vernum Scop. ...	B	10.4.81	3	14.6– 37.6	+	+	+			56			C; G; V
Galium vernum Scop. ...	P	10.4.81	3	42 –486	+	+	+			72.6		H; V	C; G; V
Galium vernum Scop. ...	P	2.6.81	3	30 –111.9	+	+				50.6			G
Geraniaceae:													
Geranium robertianum L.	B	2.6.81	2	3.6– 18	+		+	+		80		H; S	
Geranium robertianum L.	P	2.6.81	3	11.5–114.2	+	+				71.5		H; S; V	
Liliaceae:													
Muscari racemosum Mill.	B	10.4.81	3	15.7– 21.8	+	+	+		+	71	A; Ad; V	H	
Muscari racemosum Mill.	P	10.4.81	3	12 –258	+	+	+			78	Ad; V	H; V	
Leguminosae:													
Trifolium pratense L. ...	P	2.6.81	3	23.1– 75	+	+				59		H; S; V	
Trifolium pratense L. ...	S	2.6.81	3	1.1– 12	+	+	+	+		42.5		H; V	
Trifolium repens L.	S	2.6.81	3	5.4– 15.2	+	+	+	+		70		H; S; V	G

(*a*) B = Baschi; S = Stimigliano; P = Penna.
(*b*) M = *Glomus macrocarpus* var. *macrocarpus*; G = *Glomus macrocarpus* var. *geosporus*; F = *Glomus fasciculatus*; sp = *Glomus* sp.; A = *Acaulospora spinosa*.
(*c*) A = Arbuscule; Ad = lysing arbuscule; C = loops; G = coils; H, S = H- and S-connections; V = vesicle.

Type II.

Recorded on *Anemone* spp., *Euphorbia cyparissias* L. *Fragaria vesca* L., *Galium vernum* Scop., *Muscari racemosum* Mill., *Trifolium* spp. It is the commonest type, and is characterized by the absence of arbuscules; vesicles are observed in large numbers, and are similar to those of Type I. Mycelium regular, with H—and S—connections; hyphae are about 3.4 μ in diameter; mycelial contents may be granular.

Type III.

Recorded on *Euphorbia cyparissias* and *Galium vernum.* Arbuscules are absent, vesicles very sparse. Hyphal loops and coils are formed. Hyphal diameter ranges from 1.8 to 3.4 μ.

There is no evident correlation between mycorrhizal infection and spore type and number. The unrelatedness of spore number in soil and percentage of mycorrhizal infection is widely recorded. The interrelationships between plant species, type of mycorrhizal endophyte and morphological characters of mycorrhizal associations is a topic which is still to be examined.

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REFERENCES

ABBOTT L. K. and ROBSON A. D. (1977) – *The distribution and abundance of vesicular-arbuscular endophytes in some western Australian soils.* « Aust. J. Bot »., *25*, 515–522.

ABBOTT L. K. and ROBSON A. D. (1978) – *Growth of subterranean clover in relation to the formation of endomycorrhizas by introduced and indigenous fungi in a field soil.* « New Phytol. », *81*, 575–585.

ABBOTT L. K. and ROBSON A. D. (1979) – *A quantitative study of the spores and anatomy of mycorrhizas formed by a species of* Glomus, *with reference to its taxonomy.* « Aust. J. Bot. », *27*, 363–375.

GERDEMANN J. W. and NICOLSON T. H. (1963) – *Spores of mycorrizal* Endogone *species extracted from soil by wet sieving and decanting.* « Trans. Br. mycol. Soc. », *46*, 235–244.

GERDEMANN J. W. and TRAPPE J. M. (1974) – *The Endogonaceae in the Pacific Northwest.* Mycologia Memoir n. 5.

GIOVANNETTI M. (1980) – *Spore di Endogonaceae presenti nei calanchi argillosi di origine pliocenica ed infezione micorrizica nelle piante pioniere.* « Congr. Naz. Soc. It. microbiol. », Catania, Italy.

GIOVANNETTI M. and MOSSE B. (1980) – *An evaluation of techniques for measuring vesicular-arbuscular mycorrhizal infection in roots.* « New Phytol. », *84*, 489–500.

HALL I. R. and FISCH B. J. (1979) – *A key of Endogonaceae.* « Trans. Br. mycol. Soc. », *73*, 261–270.

NICOLSON T. H. and SCHENCK N. C. (1979) – *Endogonaceous mycorrhizal endophytes in Florida.* « Mycologia », *71*, 178–198.

PHILIPS J. M. and HAYMAN D. S. (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.

PUPPI G. and RIESS S. (1980) – *Spore di Endogonaceae e associazioni micorriziche in terreni di tufo nel Lazio.* « I° Congr. Naz. S. IT. E. », Salsomagggiore, Italy, 21–24 ottobre.

RIESS S. and RAMBELLI A. (1980) – *Preliminary note on mycorrhizas in a natural tropical rainforest (Ivory Coast).* In: « Tropical mycorrhiza research » (Ed. by P. Mikola) pp. 143–145. Oxford University Press, New York.

SWARD R. J., HALLAM N. D. and HOLLAND A. A. (1978) – Endogone *spores in a heathland area of South-eastern Australia.* « Aust. J. Bot. », *26*, 29–43.

WALKER C. and TRAPPE J. M. (1981) – Acaulospora spinosa *sp. nov. with a key to the species of* Acaulospora. « Mycotaxon », *12*, 515–521.

EXPLANATION OF PLATES I–II

PLATE I

Fig. 1. – *Glomus macrocarpus* var. *macrocarpus.*

Fig. 2. – *Glomus macrocarpus* var. *geosporus.*

Fig. 3. – *Glomus fasciculatus.*

Fig. 4. – *Acaulospora spinosa.*

(The bar represents 30 μ).

PLATE II

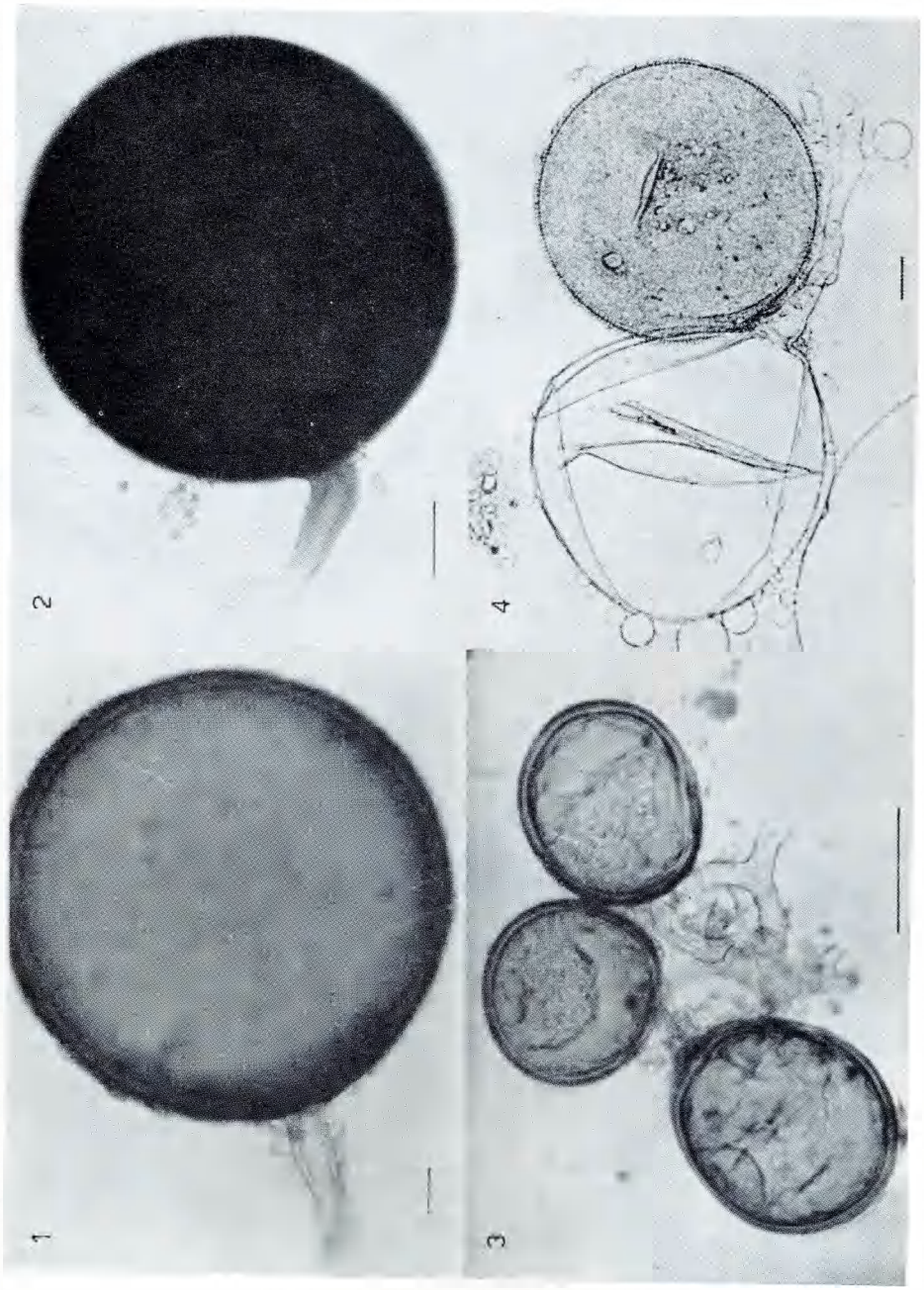
Fig. 5. – Mycorrhizal type I: arbuscule.

Fig. 6. – Mycorrhizal type II: vesicles.

Fig. 7. – Mycorrhizal type II: H-connections.

Fig. 8. – Mycorrhizal type III: loops.

(The bar represents 30 μ).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