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Occurrence and infectivity of vesicular-arbuscular mycorrhizal fungi in north-western Italy vineyards

by

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Présence et infectivité d'endophytes à vesicules et arbuscules (VA) dans le sol de vignobles de l'Italie du nord-ouest

R e s u m é : 12 vignobles ont été echantillonés pendant un an, dans le but de mesurer l'infection VA et la quantité de spores dans le sol. 9 espèces fongiques ont été observées, dont 3 plus communes, qui se sont aussi montrées capables de réinfecter des plantes de vigne. Des sites proches des vignobles étudiés, mais plantés d'autres cultures, présentent souvent une différente distribution des espèces. Les sols à pH > 7,5 contiennent un nombre plus élevé d'espèces fongiques.

La fréquence des spores en général se réduit au printemps et augmente pendant l'été: dans les sols au pH plus élevé, contenant moins de phosphate, les spores sont plus abondantes. L'infection racinaire n'est pas correlée au nombre de spores dans le sol.

L'infectivité des sols au regard des plantes de vigne, mesurée comme nombre le plus probable de propagules (MPN), a été bien correlée avec le nombre de spores présentes au même temps, après enlèvement des racines. On peut conclure que la fréquence de spores peut exprimer la capacité du sol d'infecter la vigne et montrer, de cette façon, où l'inoculation artificielle peut être une perspective intéressante.

K e y w o r d s : mycorrhiza, infectivity, soil, acidity, phosphorus, host plant, Italy.

Introduction

Vesicular-arbuscular (VA) mycorrhizae are commonly associated with most cultivated species in almost any type of soil, as a general rule enhancing the growth of the host, mainly by increasing the uptake of the least mobile nutrients, e.g. P (HARLEY and SMITH 1983).

The species and hybrids of the genus *Vitis* used in agriculture have been repeatedly observed to be mycorrhizal in natural conditions; the amount of infection present in their roots is usually fairly large, and the plant seems strongly dependent on the presence of mycorrhiza for growth in many soils (POSSINGHAM and GROOT OBBINK 1971; GEBBING *et al.* 1977; MENGE *et al.* 1983).

Interest in the agricultural application of VA mycorrhizae, from the viewpoint of reducing fertilizer costs, is currently increasing (MENGE 1983): Woody fruit crops such as the grapevine have especial potential interest in this way, because they consist of a lower number of plants/ha (as compared with herbaceous crops) and because inoculation needs not be repeated annually, thus reducing inoculation costs; in addition, artificial VA mycorrhizal infection is strongly needed if soil fumigations are to be made in the vineyard (MENGE *et al.* 1983).

The common presence of natural VA endophytes in vineyard soils means that the prospect of artificial inoculation in the field needs knowledge of the mycorrhizal status of the crop and selection of endophytes more efficient than the natural ones.

The present work was aimed at: (1) determining the VA mycorrhizal fungi present in the vineyards and isolating them in order to be able to compare them singly with other endophytes; (2) measuring the amount of spores and infection present in the roots at different times of the year; (3) assessing the infectivity of the soils studied to get clues about the possibility of artificial inoculation in unsterile vineyard soils.

Materials and methods

1. Field survey

Replicate samples of soils containing roots were collected in the field at a depth between 10 and 35 cm and a distance of about 50 cm from the grapevine trunk. Replicates (up to 10) were bulked and 4 bulked samples were collected at each date in each sampling site.

The vineyards surveyed were located in the Piedmont and Aosta valley regions of NW Italy, and were chosen as representative of some of the different environmental conditions of the area. All vineyards were subjected to standard viticultural practices, including an NPK fertilization at the end of the winter; the soil was repeatedly tilled during the growing season (by hand, rototillage or shallow ploughing), with the exception of the Canelli (ploughed once in late winter) and Neive (chemically weeded, no tillage) sites.

Samples were collected at 4 dates during the year: March 30, June 15, October 10, November 20, which correspond broadly to the following phenological phases: budbreak, bloom, maturity and winter dormancy.

Soil originating from the 4 bulked replicates collected in June was analysed for texture and nutrient concentration; weed cover was measured as a percentage of ground surface at each sampling date. The sites, their soil analysis and average weed cover are listed in Table 1.

Table 1

Soil analysis and average weed cover of the vineyards studied throughout the year

Analyse du sol et couverture moyenne en mauvaises herbes des vignobles échantillonés pendant un an

Site	Natl. grid ref. nr. (zone 32T)	Soil texture	pH	Organic matter %	N %	Olsen P ppm	Weed cover %
Arvier	LR576632	Sandy loam	7.4	1.70	0.098	34	10
Brusnengo	MR419506	Sandy loam	5.0	5.07	0.213	63	5
Canelli	MQ437514	Loamy sand	7.8	0.97	0.050	6	70
Carema	MR073486	Loamy sand	6.4	2.87	0.150	70	25
Costigliole	LQ798357	Sandy loam	6.2	1.19	0.067	46	20
Cuccaro	MQ569823	Clay	7.5	1.54	0.099	46	15
Loazzolo	MQ406484	Sandy loam	7.9	0.66	0.044	14	10
Neive	MQ317534	Clay loam	7.7	1.55	0.114	3	0
Refrancore	MQ484768	Sandy loam	6.6	0.82	0.059	20	30
Roasio	MR442497	Sandy loam	5.3	0.88	0.540	26	10
Sostegno	MR451542	Loamy sand	4.6	1.19	0.055	46	5
Vezzolano	MQ177927	Clay	7.5	1.27	0.079	12	10

In addition to the sites listed in Table 1, samples were collected in 29 more locations. These were either vineyards (sampled only once a year), or fields supporting other crops, but located in the vicinity of the vineyards of Table 1, in order to see the influence of crop species on the population of VA endophytes; in the latter cases only 1 bulked sample was collected in the month of June.

2. Observation of spores and assessment of infection

30 g of soil from each bulked sample were sieved and decanted according to GERDEMANN and NICOLSON (1963); the spores thus isolated were mounted in lactic acid and observed at the light microscope, either intact or crushed. Spores were counted on a stereomicroscope. Soil dry weight was measured to express spore numbers on this base.

Root infection was assessed after staining with trypan blue (PHILLIPS and HAYMAN 1970) using the root-intersect method (GIOVANNETTI and MOSSE 1980). The whole amount of roots present in the bulked sample was examined; a longer time (up to 6 h) in diluted HCl was necessary to clear the roots than indicated in the standard method and often a passage in H_2O_2 was also required. Percent infection was expressed using only the young, unsuberized roots; older roots were neglected as, although they sometimes stained blue, arbuscules were never visible in them.

To enable determination of the endophytes isolated, onion plants in pots were inoculated and new spores and sporocarps formed were observed after 4 months. Grapevine seedlings (*V. berlandieri* × *V. riparia* 420 A) were inoculated with these spores to prove the direct association between the fungal species isolated and the plant.

3. Soil infectivity

Soil infectivity was measured using the most probable number (MPN) method (PORTER 1979). Seedlings of 420 A were planted in plastic vials containing 25 ml soil; 5 dilutions of the original soil were made with γ-irradiated soil at a factor of ¼, each dilution being replicated 5 times; they were grown in a growth cabinet, at 23 °C, 80 % rel. humidity and 110 $\mu E \cdot m^{-2} \cdot sec^{-1}$ for 16 h a day. Plants were harvested after 6 weeks' growth and infection assessed as described above; the MPN of mycorrhizal propagules was calculated using the tables of FISHER and YATES (1973).

Results

1. Fungal species observed in the soils sampled

Spore characters allowed identification of 9 species belonging to the genera *Acaulospora, Gigaspora* and *Glomus*. Species found in each site sampled are listed in Table 2.

It can be observed that some strains were more widespread *(Glomus fasciculatum, G. microcarpum, G. monosporum, G. occultum)* whereas the others were found sporadically; this was true both in individual vineyards and in the total of the sites sampled. Spores were absent in some samples originating from fields supporting herbaceous crops (maize, wheat) or poplar, whereas they were always recovered from vineyard soil.

More than 2 fungal species together were only found in soils moderately alkaline (pH 7.4—7.9), containing less than 1.7 % organic matter; in these soils *G. fasciculatum, G. monosporum* and *G. occultum* were best represented, the second being less abundant than the others.

Table 2

VA endophytes species found in the sites studied

Espèces d'endophytes VA observées dans les sites étudiés

Site[1])	Crop	Fungal species identified[2])								
		AL	GC	GF	GMA	GMI	GMN	GM	GO	GIG
Arvier (AO)	Grapevine			×		×	×		×	
	Meadow			×					×	
Brusnengo (VC)	Grapevine					×				
Caluso (TO)	Clover						×			
	Grapevine						×			
	Maize									
	Wheat									
Canelli (AT)	Grapevine			×		×	×	×	×	
Carema (TO)	Grapevine							×		
Chieri (TO)	Apricot						×		×	
	Grapevine	×	×	×					×	
Costigliole (CN)	Grapevine					×	×			
Cuccaro (AL)	Grapevine				×		×			
Guarene (CN)	Celery						×			
	Grapevine								×	
	Grapevine nurs.			×			×		×	
	Maize						×			
Loazzolo (AT)	Grapevine			×					×	×
	Maize						×	×		
Neive (CN)	Grapevine			×			×		×	×
	Maize									
	Meadow						×			
Pinerolo (TO)	Grapevine								×	
Quarto (AT)	Grapevine nurs.			×						
	Maize						×			
	Poplar									
Refrancore (AT)	Grapevine					×			×	
	Maize									
	Peach			×			×			
Roasio (VC)	Grapevine					×				
S. Damiano (AT)	Grapevine			×						
	Plum			×						
Sostegno (VC)	Grapevine			×						
Superga (TO)	Grapevine			×					×	
Vezzolano (AT)	Grapevine						×		×	
	Meadow						×			
	Pear						×			

[1]) AL = Alessandria; AO = Aosta; AT = Asti; CN = Cuneo; NO = Novara; TO = Torino; VC = Vercelli.

[2]) AL = *Acaulospora leavis* GERD. et TRAPPE; GC = *Glomus constrictum* TRAPPE; GF = *Glomus fasciculatum* (THAXTER sensu GERD.) GERD et TRAPPE; GMA = *Glomus macrocarpum* TUL. et TUL.; GMI = *Glomus microcarpum* TUL. et TUL.; GMN = *Glomus monosporum* GERD. et TRAPPE; GM = *Glomus mosseae* (NIC. et GERD.) GERD. et TRAPPE; GO = *Glomus occultum* WALKER; GIG = *Gigaspora gigantea* (NICOL. et GERD.) GERD. et TRAPPE.

When soils of lower pH (< 6.6) were sampled a smaller number of fungal species were observed, and among these the most common were *G. monosporum* and *G. occultum* again and a strain similar to *G. microcarpum.*

Reinoculation of grapevine seedlings in pots was successful with 3 endophytes *(G. fasciculatum, G. monosporum* and *G. occultum);* it was not attempted with the other species as there were insufficient spores.

2. Spore frequency throughout the year

The number of spores found in our samples (up to 635/100 g soil) is observed in the same range on other crops in previous works (SUTTON and BARRON 1972).

Spore counts during the year followed different patterns in the sites sampled (Table 3). As spore counts were done using the stereomicroscope it proved difficult to

Table 3

Spore frequency at the 4 sampling times (number of spores/100 g soil dry wt)

Nombre de spores (par 100 g de sol sec) aux 4 dates d'echantillonage

Site	Type	Spore sampling times			
		March	June	Oct.	Nov.
Arvier	GF-GO	[1])	2 ± 2	3 ± 3	0
	GMN	[1])	1 ± 1	0	0
	Others	[1])	1 ± 1	0	0
Brusnengo	Others	1 ± 1	0	0	0
Canelli	GF-GO	223 ± 73	125 ± 19	103 ± 10	171 ± 41
	GMN	1	0	19 ± 9	23 ± 5
	Others	0	0	1 ± 1	0
Carema	Others	**	2 ± 1	0	0
Costigliole	GMN	7 ± 4	0	0	6 ± 5
	Others	10 ± 5	0	0	4 ± 2
Cuccaro	GF-GO	19 ± 10	49 ± 23	71 ± 28	60 ± 26
	Others	0	0	0	3 ± 2
Loazzolo	GF-GO	107 ± 51	231 ± 91	200 ± 50	238 ± 36
	Others	0	3 ± 1	0	0
Neive	GF-GO	635 ± 241	84 ± 30	62 ± 8	207 ± 36
	GMN	0	0	2 ± 1	4 ± 2
	Others	1 ± 1	0	1 ± 1	1 ± 1
Refrancore	GF-GO	0	8 ± 5	32 ± 11	7 ± 6
	GMN	0	0	4 ± 3	0
Roasio	Others	0	0	0	1 ± 1
Sostegno	GF-GO	3 ± 2	0	0	0
Vezzolano	GF-GO	243 ± 50	431 ± 65	243 ± 47	223 ± 62
	GMN	3 ± 3	0	0	0

GF-GO = *G. fasciculatum* and *G. occultum* (see text); GMN = *G. monosporum.*
Data listed are averages of 4 replicates ± standard error.
[1]) Samples not collected.

separate spores of *G. fasciculatum* from those of *G. occultum* because of their similar size and, often, colour. For this reason they were counted together when both were present.

A general trend towards an increase in spore numbers from the beginning of the summer to the autumn was observed; in some cases however this was not true, e.g. the Vezzolano site where the peak spore frequency was in June. A decrease in spore numbers in spring was observed in some sites (Canelli, Neive).

A comparison of the total number of spores retrieved in the June samples with the chemical characters of the soils collected at the same time shows an influence of the pH and of the available P concentration; more than 20 spores/100 g were found when pH was $\geqq 7.5$ or Olsen P was <20 ppm. Apart from this threshold limit, no significant correlations were observed.

3. Root infection

Percent infection of the young, non-suberized grapevine roots is reported in Table 4. In all sites, at least at some sampling times, an infection level >20 % was observed; fungal root colonization fluctuated strongly throughout the year, but no definite trend could be detected. The amount of infection appeared to be related neither to the spore frequency nor to any of the soil characters.

4. Soil infectivity

The inoculum potential of some soils was assessed in order to see how important the role of spores as inoculum would be in those conditions. Results show that the

Table 4

Percent root infection at the 4 sampling times

Pourcentage d'infection aux 4 dates d'échantillonage

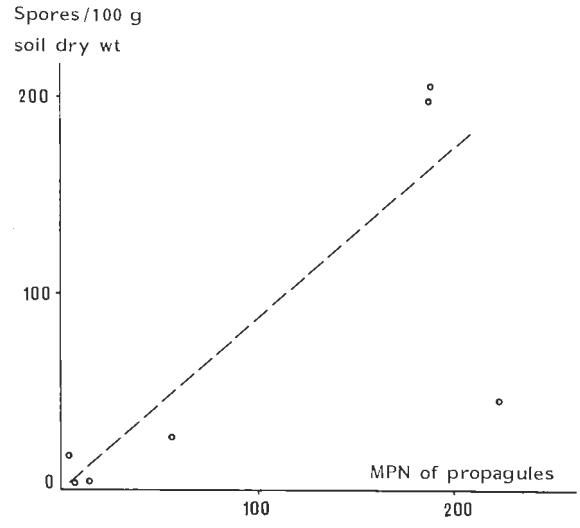
Site	Sampling times			
	March	June	Oct.	Nov.
Arvier	[1])	64 ± 6	74 ± 27	75 ± 5
Brusnengo	39 ± 16	60 ± 5	33 ± 12	90 ± 20
Canelli	51 ± 11	62 ± 22	90 ± 10	69 ± 7
Carema	[1])	39 ± 23	33 ± 15	72 ± 19
Costigliole	22 ± 13	3 ± 2	[2])	[2])
Cuccaro	30 ± 18	85 ± 8	57 ± 5	40 ± 13
Loazzolo	62 ± 19	69 ± 4	70 ± 23	94 ± 21
Neive	63 ± 4	80 ± 6	76 ± 20	80 ± 12
Refrancore	55 ± 7	44 ± 13	68 ± 17	[2])
Roasio	33 ± 19	44 ± 6	[2])	30 ± 10
Sostegno	32 ± 15	[2])	[2])	27 ± 18
Vezzolano	44 ± 15	57 ± 19	68 ± 6	89 ± 6

Data listed are averages of 4 replicates ± standard error.

[1]) Samples not collected.

[2]) No roots in the samples.

MPN values agreed well with the spore frequency at the same sampling date, with the exception of the Vezzolano site; for the other soils r = 0.986 and b = 0.86 (Fig.). This exception coincided, in the same site, with the unexpected decrease in spore numbers on the same date mentioned earlier; possibly an unusual factor affecting viability and germinability of spores, either of physiological or biotical nature, could explain this behaviour.



Total spore numbers and most probable number (MPN) of propagules at the November sampling.

Nombre total de spores et nombre le plus probable (MPN) de propagules à l'echantillonage de Novembre.

Discussion

Some of the fungal species observed seemed to be more frequent in some types of soils. A specific adaptation to pH or other related soil characters has been observed by GRAW (1979) for *G. macrocarpum* and by TAVARES and HAYMAN (1982) for several VA endophytes; in this case, however, direct comparisons with the mentioned results are not possible as fungal strains had different origin.

Results show that crops other than grapevine support a range of endophytes that is often different from that found in vineyards close to them (at a distance of less than 200 m). It is generally accepted that VA mycorrhizal endophytes have no strict specificity for their hosts, although SCHENCK and KINLOCH (1980) have observed that the frequency of spores of different fungal strains varied, after several years of monocropping in a newly cleared site, according to the host species. The different distribution of fungal species in the crops sampled in this work can possibly be explained by taking into account that these crops were subjected to very different cultural practices; in fact it has been repeatedly observed that e.g. tillage or fertilizing techniques can affect spore population and that this effect can be more or less important according to the endophyte strain considered (HAYMAN 1975; KRUCKELMANN 1975).

The matter is complicated by the presence of weeds in the vineyards many of which can in theory support VA infection, as we observed in some of them (*Setaria* sp., *Trifolium* spp.); also KRUCKELMANN (1975) related that almost all the weeds he inoculated in pots became easily infected.

The two sites that were free from weeds during the whole year because of chemical weeding (Rivetti and Superga) supported 4 fungal endophytes *(Glomus fasciculatum, G. monosporum, G. occultum* and *Gigaspora gigantea),* the first three of which were successfully inoculated on 420 A seedlings in pots. In a survey carried out in California, MENGE *et al.* (1983) obtained mycorrhizal infection on grapevine with, among others, *G. constrictum, G. fasciculatum* and *G. macrocarpum;* the question remains open whether the other species found in the present work (*G. microcarpum, G. mosseae, Gigaspora gigantea, Acaulospora* leavis) are actually symbionts of the grapevine.

In grassy plants it has been repeatedly reported that spore frequencies increase from plant germination to the period of senescence when roots decay and the fungus remains viable mainly in the form of resting spores (HAYMAN 1970; SUTTON and BARRON 1972). In case of woody crops, such relationships are likely to be less clearcut as, even if all the young, unsuberized roots (where the endophyte is present in the arbuscular form) die before winter, the fungus can survive in the form of hyphae and vesicles in old and suberized roots, and the need for extra-matrical spore production possibly becomes less important. We could actually observe many such roots containing clumps of mycelium and vesicles in the November samples.

As a consequence, although a decrease of spore numbers in spring and a subsequent increase in autumn was often observed in our samples and was reported for viticultural soils in Southern Tyrol (NAPPI *et al.* 1980/81), a maximum frequency of spores in autumn and winter may not necessarily be found in vineyards; in addition, spore production is highly variable, depending on characters related to the plant, the fungus and the environment (e.g. rate of root growth, photosynthesis rate, soil temperature). These factors may explain the deviations observed from the general rule, as that of the Vezzolano samples.

A fairly large amount of infection was regularly present in all soils, irrespective of their P content. Although in most pot experiments it has been observed (MOSSE and PHILLIPS 1972; MENGE *et al.* 1978) that the amount of P in the soil can be a limit for VA mycorrhizal infection in the field, and particularly on the grapevine, HAYMAN *et al.* (1976) have shown that factors other than nutrient content of soil can be more important in influencing infection levels.

The lack of correlation between spore frequency and root infection is in agreement with many previous observations (MOSSE 1973): the ability of the endophyte to survive the whole year in the host's roots can give a further explanation of this in the case of a perennial crop.

It remains to understand, however, what the factors are that inhibited spore production in the cases where infection was high but spores were nearly absent, as was found in some of the sites sampled during this survey (e.g. Carema, Roasio). The presence in these sites of an endophyte producing spores too small to be recovered by the method used in this work (*Glomus tenuis;* see HALL 1978) seems unlikely as the infective structures typical of this species were never seen in the roots. Recently SYLVIA and SCHENCK (1983) have observed that a superphosphate drench of the soil reduced sporulation (nr. of spores/length of infected root) of non P-tolerant VA mycorrhizal strains; as a consequence the hypothesis can be made that in these cases the endophytes found in the roots were unable to produce spores because the soil environmental conditions, e.g. high P concentration, were inhibitory.

In the soils tested, the number of fungal propagules was fairly close to the number of spores; this means that, under the conditions of this survey, the spore frequency of a soil where roots are absent (as would be a vineyard at planting) may give a good idea of the capability of that soil to infect new plants. For this reason, as fast initial development of the endophyte strongly favours an early growth response in the host (ABBOTT and ROBSON 1978), soils like those of the Fabiole and Gilodi sites, very poor in spores, could benefit in a larger extent from artificial VA mycorrhizal inoculation, provided that P-tolerant endophytes are used; in other soils, supporting a larger natural spore population, such a practice may be useful only if strains able to develop faster than the indigenous ones are selected.

Summary

12 vineyards were surveyed during one year to assess root VA infection and spore population in the soil. 9 fungal species were present; 3 of them were more common and could be reinoculated successfully on grapevine. Other crops, in sites close to the vineyard sampled, often contained a different population of species. More species were present in soils of pH > 7.5.

Spores were most abundant in autumn, their number decreasing in spring. Soils with least P and higher pH contained more spores. Root infection was unrelated to spore frequency or soil characters.

Infectivity of the soil on grapevine plants, assessed by means of the MPN method, was well related to the number of spores present in the soil at the same time, if roots were removed. It is concluded that spore numbers can express the ability of the soil to infect plants, thus showing where artificial inoculation could be potentially more effective.

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