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Peter Moutoglis · Paul Widden

Vesicular-arbuscular mycorrhizal spore populations in sugar maple (*Acer saccharum* marsh. L.) forests

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Abstract The numbers and types of spores of vesicular-arbuscular mycorrhizal fungi occurring in the top 15 cm of the soil in three maple forests in Eastern Canada were investigated using traditional wet-sieving/decanting methods. In the most acid site, at St. Hippolyte, Québec, where the soil had been amended with base cations, after 1 year there was no effect on the numbers of spores present. Vesicular-arbuscular mycorrhizal spores present at St. Hippolyte consisted of *Glomus rubiforme,* other *Glomus* spp. and *Acaulospora* spp. Although the sporocarpic species, *G. aggregatum, G. macrocarpum* and *G. rubiforme* occurred at St. Hippolyte, they were not found at the two less acid sites (Waterloo, Ontario and Lacolle, Québec) . Spores of *Acaulospora* spp. were found at all three sites, but were most abundant at St. Hippolyte. At St. Hippolyte the total number of spores was much higher than at the other two sites; at Waterloo numbers were an order of magnitude lower than at St. Hippolyte. It is suggested that *G. rubiforme* and *Acaulospora* species may be adapted to acid conditions. Seasonal patterns of spore abundance suggested that *Acaulospora* spp. may sporulate during the spring, whereas *G. rubiforme* may sporulate during the fall.

Key words Vesicular-arbuscular mycorrhizae · *Acer saccharum* · Spore counts · Acid soil · Seasonality

Introduction

This century has seen many declines in sugar maple and other trees. Increased dieback of sugar maples has been monitored in Québec since 1982 (Hendershot and Jones 1989). In many situations the causal factor or factors of this decline are not known. In Québec, maple stands that show the most decline are located at high elevations and on shallow, droughty soils. One such site is that at St. Hippolyte, where the trees were seen to be in a moderate state of decline during 1988–89. Two predisposing stress factors are the poor soil conditions and the marginal climate (Hendershot and Jones 1989).

Sugar maples are colonized by vesicular-arbuscular mycorrhizal fungi (VAMf; Yawney and Schultz 1990; Cooke et al. 1992). Mycorrhizal fungi have been shown to be effective in reducing plant stresses caused by nutrient deficiency, drought and soil disturbances (Jasper et al. 1979, 1991; Allen and Boosalis 1983; Augé et al. 1986; Evans and Miller 1988, 1990). It is now widely accepted that mycorrhizae are important components of the root and rhizosphere flora and play a key role in root function and the establishment and maintenance of microbial populations in the soil around them, the mycorrhizosphere (Linderman 1988).

The great interest in vesicular-arbuscular mycorrhizae (VAM) in recent years has resulted in a number of surveys enumerating the species (or genera) of VAMF in a particular region (Morton 1986; Sylvia 1986; Koske 1987; Warner et al. 1987). VAMF are known to play an important role in the mineral nutrition of many deciduous trees (Kormanik 1981), but there have been few studies of VAM in natural forest ecosystems. Because VAM are important in the mineral nutrition of trees, and because one possible consequence of acid precipitation is the depletion of nutrients, particularly alkaline metals (Johnson and Taylor 1989), it is important to know how the fungi respond to soil conditions. The interaction between VAMF and higher plants may be affected by changing conditions in a number of ways. Changes in the environment may change colonization rates, either indirectly through changes in host physiology or by directly affecting the fungus. Furthermore, differing conditions may affect the numbers and types of VA mycorrhizal spores in the soil available to colonize the roots.

P. Moutoglis[1] · P. Widden (✉)
Biology Department, Concordia University,
1455 de Maisonneuve West, Montréal, Québec,
Canada H3G 1M8
e-mail: WIDDENP@VAX.2.CONCORDIA.CA

Present address:
[1] Institut en biologie végétale, Université de Montréal,
4101 est rue Sherbrooke, Montréal, Québec, Canada H1X 2B2

In this study we examined the effect of the environment on VA mycorrhizal spore populations in the soil in two ways. First, we conducted a detailed survey of VA mycorrhizal spores in a maple forest growing on an acid, nutrient-poor soil in the Laurentians (St. Hippolyte) where half of the plots had been amended with a base-cation mixture. Second, we performed surveys of VA mycorrhizal spore populations in soils of two other maple forests which were less acidic (Lacolle and Waterloo). These data were used to answer the following questions: (1) Will soil amendments, applied to replace cations lost due to acidification, change the relative abundances of the spores of VA mycorrhizal taxa in the soil? (2) Does the pool of VA mycorrhizal spores present in the soil under maple trees differ qualitatively and quantitatively with respect to geographic location and season?

Materials and methods

Study sites

Three deciduous forest sites were chosen in which sugar maples (*Acer saccharum* Marsh.) were the dominant trees, but where soil and climatic conditions varied. The first site, St. Hippolyte, is located in the Laurentian mountains, northwest of Montréal, at the University of Montréal's Biological Field Station near St. Hippolyte. The study area consists of six plots (30 m × 30 m) with 10-m buffer zones separating them, located in a 60- to 80-year-old stand which is predominantly sugar maple mixed with white birch (*Betula papyrifera* Marsh.) and striped maple (*A. pensylvanicum* L.). The soil, an Orthic Ferro-Humic Podzol with a thick (greater than 5.0 cm) but variable humus layer, is acidic (pH 4.2–4.3), and the rainfall has an average pH of about 4.3. The understorey consists mainly of saplings of *A. saccharum, A. pensylvanicum* and *Fagus grandifolia,* along with a number of forest herbs including *Clintonia borealis* (Ait.) Raf., *Maianthemum canadense* Desf., *Medeola virginiana* L., *Polygonatum pubescens* (Willd.) Pursh., *Smilacina racemosa* (L.) Desf., *Trillium grandiflorum* (Michx.) Salisb., *Trillium undulatum* Willd., *Trientalis borealis* Raf. and *Viola pallens* (Banks) Brainerd. Fertilization was carried out on 9 June 1989 in three of these plots using a base-cation mixture composed of 500 kg/ha K_2SO_4, 250 kg/ha $CaCO_3$ and 250 kg/ha $CaMg(CO_3)_2$ (Cooke et al. 1992).

The second site (Lacolle) is located in the Lower St. Lawrence floodplain, 8 km south of Lacolle, Québec, where the soil is a mull soil, less acidic (pH 6.0–6.3), and lacks a persistent humus layer. The major understorey species are sugar maple saplings along with forest herbs which include *Actaea pachypoda* Ell., *Actaea rubra* (Ait.) Willd., *Erythronium americanum* Ker-Gawl, *Smilacena racemosa, M. canadense, Trillium erectum* L. and *Trillium grandiflorum.* This site is described in more detail in Widden (1979).

The third site (Waterloo) is located 15 km west of Waterloo, Ontario, where the soil pH is slightly lower than that at Lacolle (pH 5.6–5.7). This soil is described in Brundrett and Kendrick (1988) as a Fox sandy loam, a Gray Brown Luvisol with low fertility and low moisture-holding capacity. The understorey vegetation at this site is very similar to that at Lacolle, *Erythronium americanum* being the predominant forest herb, along with *Arisaema atrorubens* (Ait.) Blume, *Smilacena racemosa* and *Trillium grandiflorum* (Brundrett and Kendrick 1988).

Soil sampling

In each plot at St. Hippolyte, five trees of similar age were chosen, based on trunk diameter. On each sample date, three soil cores were collected at random compass points 5 m from the base of the trunk of each tree in each plot, giving a total of 90 samples. The cores were taken with a 2.0-cm-diameter corer to a depth of 15 cm, as the majority of the maple roots were located in this region. Samples were bagged, returned to the laboratory in a cooler and stored in a cold room at 5 °C prior to processing. Samples at St. Hippolyte were taken before fertilization on 9 May 1989 and after fertilization on 15 July and 15 August 1989, and on 22 May, 10 July and 25 September 1990.

Based on experience at St. Hippolyte, we reduced the sample intensity at the other two sites. At both Lacolle and Waterloo, three trees were selected from just one plot, and 5 samples were taken at random compass points from each tree. Sampling was done in May, July, October, and February of 1991.

Spore extraction

Each sample was sieved according to the sieving and decanting procedure of Gerdemann and Nicolson (1963), using a high pressure water hose to wash the spores onto the final sieve (45 μm), where they were ultimately collected. To further clean the spores, centrifugation using sucrose (Ohms 1957; Daniels and Skipper 1962; Ross and Harper 1970) was used, modified as follows: First the material collected on the sieve was suspended in 50 ml H_2O. This suspension was equally divided between two 50-ml centrifuge tubes and layered on top of a 60% sucrose cushion. This was then centrifuged at 400 *g* for 20 min using an IEC universal model, swinging bucket, table-top centrifuge. The water portion was collected with a Pasteur pipette and passed through a gridded nitrocellulose Millipore filter (37 mm, 1.2 μm pore size). The spores collected on the filter were washed with distilled water to remove excess sucrose and to distribute the spores evenly over the surface. The filters were stored in the refrigerator in a small plastic Petri dish on a moist Whatman no. 1 filter paper for subsequent quantification.

Spore quantification

All the spores present on 10 squares in a predetermined pattern covering the entire surface of the gridded filters were counted. The average number per square was used to estimate the total number of spores on the filter. This number was then used to estimate the total number of spores m^{-2} to a depth of 15 cm. The number of spores in a sporocarp was determined by gently crushing the sporocarp so that all the spores could be enumerated. A subsample, consisting of all the spores from a single well-populated square, was chosen for the identification and counting of taxa. Using fine needles, the spores were mounted on a glass slide and mounted in polyvinyl-alcohol mounting medium (Omar et al. 1979). Identification was done using a Nikon Optiphot microscope equipped with differential interference optics along with the key and descriptions to the VAMF by Berch (1988). From this, the proportion of spores belonging to each taxon was estimated. For quantification, only easily recognizable taxa were identified. Identification of other species was carried out by Dr. Y. Dalpé on samples of spores in good condition collected from the filters.

Statistical analysis

Descriptive statistics were obtained using the SPSS-X computer program (Norusis 1990). An analysis of variance (ANOVA) was performed to examine seasonal trends. A nested ANOVA [spore samples nested within base points (trees), nested within plots then within treatments] was used to test for fertilization effects. Scheffé post-hoc comparisons ($P < 0.01$ and $P < 0.05$) were used to determine individual differences between sampling periods.

Results

Preliminary analysis showed that, after 1 year, fertilization had no effect on the number of spores present at St. Hippolyte (Table 1). Since no treatment effects were observed, the data were pooled to examine the relative abundance and seasonal trends of VA mycorrhizal taxa at St. Hippolyte. The only species that were easy enough to quantify were *Glomus geosporum* Nicolson & Gerdemann and *G. rubiforme* Gerdemann & Trappe. Other *Glomus* and *Acaulospora* species were identified to the genus. In 1989 (Fig. 1a) , there was no significant difference among seasons with regards to the abundance of *Glomus* spp.; however, *G. rubiforme* was more abundant in the fall than the spring, whereas the abundance of *Acaulospora* spp. decreased throughout the 1989 growing season (Table 2). In 1990, however, *Glomus* spp. differed in abundance ($P<0.05$) between the summer and fall sampling seasons (Table 2). In 1990 *G. rubiforme* showed a similar trend to that of 1989, but there was no significant seasonal effect for *Acaulospora* spp. (Table 2). *G. rubiforme,* which made up 20–40% of the spores in 1989 and 30–50% in 1990, was probably the single most abundant species at St. Hippolyte. These percentages are probably underestimates, since single spores of this species may have been grouped with other *Glomus* spp. Although *G. geosporum* was present at St. Hippolyte (Table 3), numbers were too low to be graphed, so they were pooled with *Glomus* spp.

VA mycorrhizal spores at St. Hippolyte were more abundant than at the other two sites by at least a factor of two (Fig. 2). No spores of *G. rubiforme* were found at Lacolle or Waterloo, nor were any other sporocarpic forms observed at either of these two sites (Table 3). The number of *Acaulospora* spores was roughly an order of magnitude higher at St. Hippolyte than at Lacolle or Waterloo (Fig. 3). Soil from the two Québec

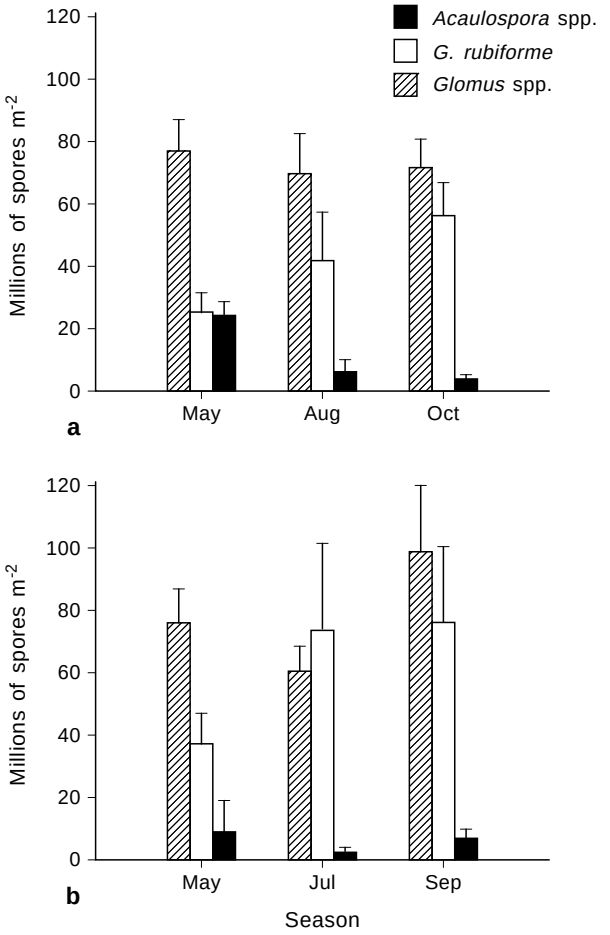

Fig. 1 Bar graph of the total abundance of VAM spores at St. Hippolyte during **a** the 1989 and **b** the 1990 sampling season. *Error bars* respresent the 95% confidence limits for the mean

Table 1 ANOVA table with probability values for the effect of fertilization (at St. Hippolyte) for identified taxa on each sample date

Taxon	Month	Degrees of freedom	*F* ratio	Probability for *F*
Glomus spp.	May 1989	24	1.28	.217
	Aug.1989	24	0.53	.955
	Oct. 1989	24	0.93	.563
	May 1990	24	0.88	.626
	Aug.1990	24	0.45	.982
	Oct. 1990	24	1.06	.414
Glomus rubiforme	May 1989	24	1.53	.093
	Aug.1989	24	0.95	.540
	Oct. 1989	24	0.50	.578
	May 1990	24	0.63	.894
	Aug.1990	24	0.97	.510
	Oct. 1990	24	1.03	.450
Acaulospora spp.	May 1989	24	0.88	.630
	Aug.1989	24	0.84	.669
	Oct. 1989	24	0.50	.965
	May 1990	24	1.07	.405
	Aug.1990	24	0.57	.933
	Oct. 1990	24	0.66	.869

Table 2 ANOVA table with probably values for the effect of sample date for identified taxa from St. Hippolyte

Taxon	Year	Degrees of freedom	*F* ratio	Probability for *F*
Glomus spp.	1989	2	0.43	.650
Glomus rubiforme	1989	2	7.14	.001
Acaulospora spp.	1989	2	52.47	.000
Glomus spp.	1990	2	5.09	.007
Glomus rubiforme	1990	2	3.52	.031
Acaulospora spp.	1990	2	1.51	.223

Table 3 Mean number of spores m^{-2} of counted taxa and the presence (+) or absence (−) of other species of VAM spores at the three sampling sites (+ species present at the site but not quantified, − species not detected at the site)

Taxon	Mean number of spores m^{-2}			
	St. Hippolyte		Lacolle (1991)	Waterloo (1991)
	(1989)	(1990)		
Glomus spp.	7.22×10^7	7.84×10^7	5.78×10^7	4.32×10^6
G. geosporum	+	+	6.08×10^5	1.17×10^5
G. clarum	+	+	−	−
G. hoi	+	+	−	+
G. macrocarpum	+	+	+	+
G. aggregatum	+	+	−	−
G. microaggregatum	+	+	−	−
G. mosseae	+	+	+	+
G. rubiforme	4.10×10^7	6.25×10^7	−	−
Acaulospora spp.	1.16×10^7	6.43×10^6	3.33×10^5	3.63×10^5

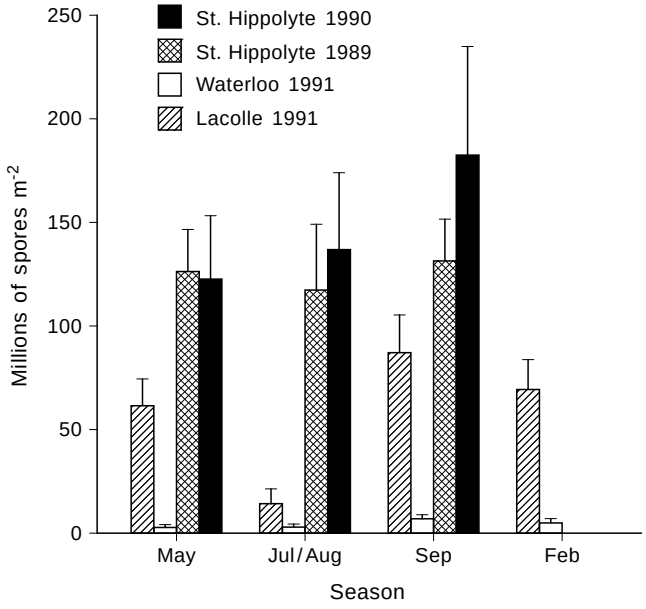

Fig. 2 Bar graph of the seasonal abundances for total spore numbers for the three sampling sites. *Error bars* represent the 95% confidence limits for the mean

sites had similar numbers of *Glomus* spores other than *G. rubiforme* and *G. geosporum.* The Lacolle soil contained more *G. geosporum* spores than the Waterloo soil, but both soils had similar numbers of *Acaulospora* spores (Fig. 3). However, the soil at Waterloo had an order of magnitude fewer *Glomus* spores than the Québec sites.

The species of *Glomus* that could be identified from these soils included *G. rubiforme, G. clarum* Nicolson & Schenck, *G. aggregatum* Schenck & Smith emend. Koske, *G. geosporum, G. hoi* Berch & Trappe, *G. macrocarpum* Tulasne & Tulasne, *G. microaggregatum* Koske, Gemma & Olexia and *G. mosseae* Nicolson & Gerdemann (Table 3). However, the sporocarpic forms were found only at St. Hippolyte, and *G. geosporum* was apparently much rarer at St. Hippolyte than at the other two sites.

Discussion

The population survey at St. Hippolyte has shown that VA mycorrhial spore numbers in this natural maple stand vary over a growing season and can differ from year to year, probably due to local environmental variations. The addition of the cation fertilizer to the soil had no effect on the VA mycorrhizal spore populations 1 year after the initial application. However, because the identifications were mostly limited to the genus level, it is possible that undetected effects occurred at the species level.

Different VAMF have different sporulation phenologies (Gemma and Koske 1988; Gemma et al. 1989). The tendency for *G. rubiforme* to have higher numbers of spores during the fall may reflect its timing of sporulation. Using a direct observation method (Moutoglis et al. 1995), we have seen *G. rubiforme* sporulating on maple roots only in the fall, whereas some non-sporocarpic species were found sporulating throughout the growing season. It is possible that *Acaulospora* spp. tend to sporulate in the spring. The activity of the VAMF over a growing season may be synchronous with the intermittent growth of the maple feeder roots; this growth occurs in response to moisture changes in the soil, giving the roots their characteristic beaded appearance (Brundrett and Kendrick 1988). VAMF have also been shown to be affected directly by moisture availability (Hetrick 1986), so it is possible that VA mycorrhizal hyphal activity (expansion) and subsequent sporulation are correlated with new root growth. In order to better understand the seasonal dynamics of VAMF, biotic and abiotic variables need to be measured simultaneously with sporulation rates and colonization rates.

Comparing the spore abundances found in this study with other surveys is rather difficulty, because experimental designs differ and very few surveys have been done in this type of ecosystem. For agricultural soils, it has been determined that varying numbers of VA mycorrhizal spores are present, and Sutton and Barron (1972) found 70 ± 17 spores g^{-1} dry soil under maize,

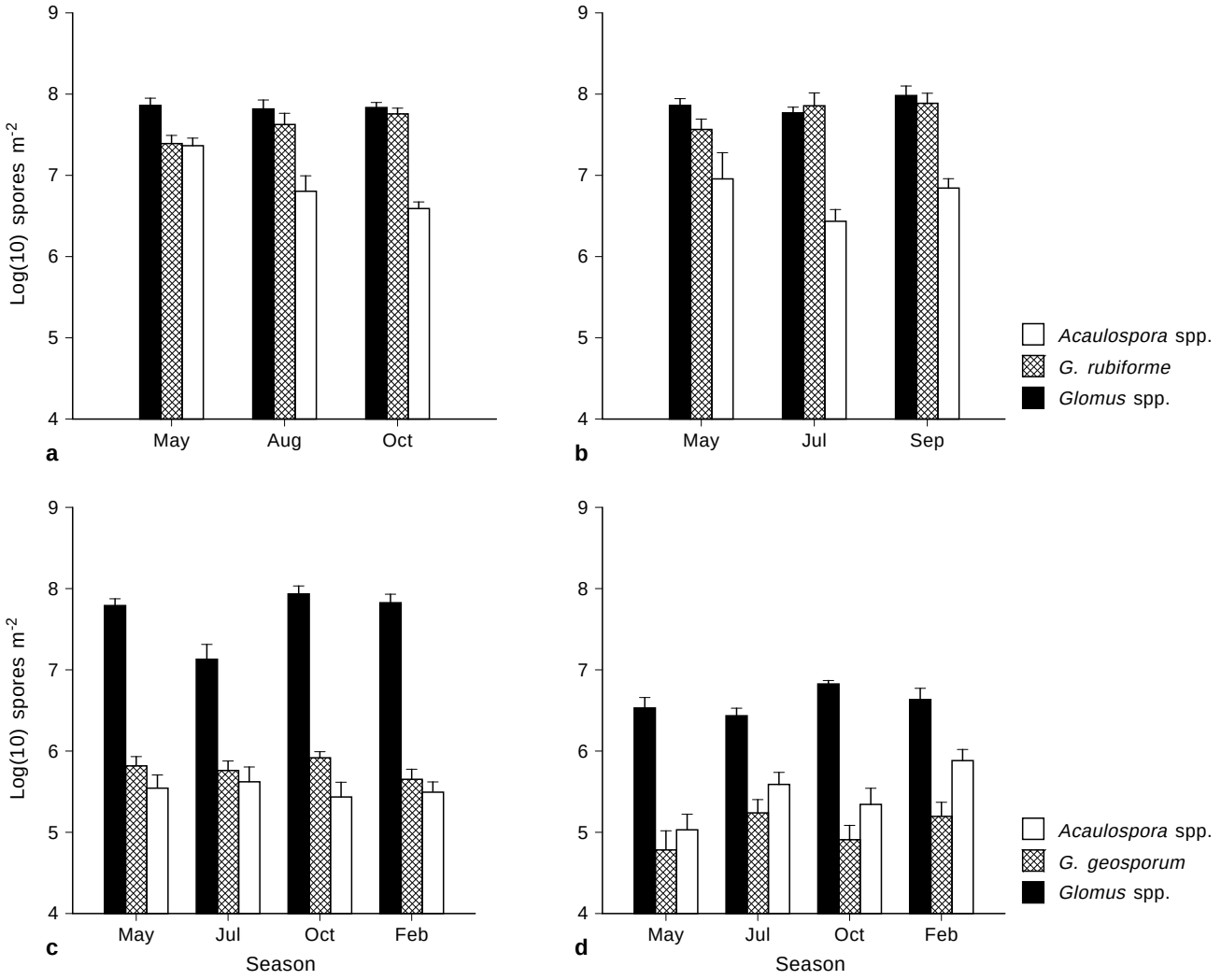

Fig. 3 Log scale bar graphos of the seasonal spore abundances at the three sampling sites: **a** St. Hippolyte (1989); **b** St. Hippolyte (1990); **c** Lacolle (1991); **d** Waterloo (1991). *Error bars* represent the 95% confidence limits for the mean

and up to 86 ± 11 spores under strawberry, all in the top 24 cm of soil. Recalculation of our data gives spore numbers g^{-1} of soil of 1000–1600 at St. Hippolyte, 133–782 at Lacolle and 27–65 at Waterloo. Clearly the spore numbers at St. Hippolyte were very high, whereas at Lacolle the numbers were comparable to those from the agricultural soils studied by Sutton and Barron. Kessler and Blank (1972) estimated that the upper 10 cm of soil in a Michigan hardwood forest dominated by maple contained nearly 7 million VA mycorrhizal sporocarps (*G. rubiforme*) per hectare, which equals 700 sporocarps per m^{-2} in the top 10 cm of soil. For the sake of comparison, this is the equivalent of 1050 sporocarps m^{-2} in 15 cm of soil. Counts of *G. rubiforme* sporocarps from St. Hippolyte, showed approximately 800000 sporocarps m^{-2} in the top 15 cm of soil. Therefore it is evident that a significantly larger number of sporocarps are present at the St. Hippolyte site than were found at the Michigan site. Our data, therefore, indicate that sporocarp numbers at St. Hippolyte, an acid forest site, are much higher than those in the less acid sites for which data are available.

There was also a major difference between the sites regarding the types of fungi found. The most obvious difference was that, at St. Hippolyte, spores of *G. rubiforme* and other sporocarpic forms (*G. aggregatum, G. macrocarpum*) were a significant component of the VA mycorrhizal flora, whereas these forms were not seen at the two less acid sites. Their absence at the Waterloo site is confirmed by a more intensive study by Klironomos (1994). Another major difference between sites was that the numbers of *Acaulospora* spores at St. Hippolyte were much higher than at the other two sites. Excluding *G. rubiforme,* the two Québec sites did not differ greatly from one another with respect to the numbers of spores of *Glomus,* but had significantly higher populations of these spores than the Ontario site.

It is possible that *G. rubiforme* is adapted to soils of greater acidity, as it was only found at St. Hippolyte.

Others collecting from acid sites have also found this species, behaving in a similar seasonal fashion (Y. Dalpé, personal communication). Specific VAMF have been previously shown to prefer specific pH conditions (Powell 1975). *Acaulospora laevis* has been associated with acid soils (Porter et al. 1987a, b; Johnson et al. 1991b), and sporulation by *Acaulospora foveata* has been shown to be negatively correlated with soil pH (Klironomos et. al. 1993). Our data, therefore, support other studies indicating that *Acaulospora* species may have a preference for acid sites. These seemingly acid-tolerant VAMF may prove to be useful where agricultural crops or nursery trees are being transplanted to acid soils.

In soils, assessing species composition of VA mycorrhizal fungal communities by direct counts of spores has an advantage over the most-probable number (MPN) method, in that no assumptions concerning host specificity by VAMF are made. Our data illustrate the importance of this, as *G. rubiforme* was the most abundant VA mycorrhizal spore at St. Hippolyte soil but was absent from the other two sites. This fungus has not been successfully cultured, and has never been previously detected on maple roots. Use of trap plants would probably, therefore, have failed to reveal its presence. Only by means of spore counts or direct observation of washed roots (Moutoglis et al. 1995) could it be detected.

Our study and other surveys of VA mycorrhizal spores provide information on the mycorrhizal species present in particular plant communities. Such surveys show that the soil in any location may support a wide diversity of VAMF. These surveys may be deceptive, because spore viability is unknown and species that do not produce recognizable spores may be present (Johnson 1977; Morton 1988; McGee 1989). However, the presence of spores of many VAMF in these soils suggests that both interspecific and intraspecific competition between them is possible. Koske (1981) was unable to prove that any of the VAMF present in a coastal ecosystem were better competitors than others; rather, he suggested that environmental factors and host plants were more important factors influencing their distribution. Contrary to this, Gemma et al. (1989) found seasonal differences in spore production between co-existing species and suggested that the correlation between the abundant sporulation of one VAMF and reduced sporulation by others may have been due to antagonism between species. Clearly, however, there is the potential for competition to result in host specificity in nature, even when such host specificity is not apparent from pot-culture data. This question of host specificity in nature has yet to be addressed, though direct observation methods and molecular-based methods (Simon et al. 1992) give some hope that it can now be examined in natural communities. Studies in experimental gardens and in agricultural systems have, however, shown clearly that not only abiotic soil factors, but also the available host plants affect the community composition of VAMF, based on spore counts (Johnson et al. 1991a, 1992).

In summary, use of spore counts has demonstrated that, after 1 year, fertilization had no effect on numbers or taxa of spores in the soil, that there were major differences between sites, and that there were seasonal patterns for some taxa. VA mycorrhizal fungal taxa apparently exist that are adapted to acid soil conditions, and these could prove to be of great interest, given that VAMF in agricultural systems may not be very acid tolerant (Wang vet al. 1985; Medeiros et al. 1994). Major differences in numbers of VA mycorrhizal spores at different sites also indicate that VAMF may employ different strategies when colonizing roots. Depending on local conditions, extramatrical hyphae and/or spores may serve as the major source of inoculum, a possibility suggested in a study by Klironomos et al. (1993). A better knowledge of the ecology of VAMF in natural systems is essential if we are to effectively use VAM to improve crops, particularly in marginal habitats.

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