PLANT GROWTH RESPONSES TO VESICULAR–ARBUSCULAR MYCORRHIZA

XIII. SPREAD OF AN INTRODUCED VA ENDOPHYTE IN THE FIELD AND RESIDUAL GROWTH EFFECTS OF INOCULATION IN THE SECOND YEAR

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Summary

Spread of *Glomus caledonius* and *Glomus mosseae* was monitored 3, 15 and 21 months after inoculation in the field. The occurrence of their characteristic spores or sporocarps was used to establish their presence. Thirteen weeks after inoculation, *G. caledonius* had spread an average of 7·5 cm and a maximum of 22·5 cm from the point of inoculation. After 15 months its spores were irregularly distributed all over the plot irrespective of the initial inoculation points and the fungus had bridged a gap of 4·5 m between inoculation points. After 21 months *G. caledonius* spores throughout the plot had multiplied about fivefold since the previous survey and many *G. mosseae* sporocarps were also present. Spores and sporocarp numbers were more similar to those in pot cultures than in normal field situations.

Inoculation had residual effects within the plot on the growth of lucerne sown 12 months after inoculation and cut 3 months later.

Introduction

There has so far been no study of the spread of an introduced endophyte under field conditions, for which two methods are available. Anatomy of the infection in the root can sometimes be used to identify endophytes, but the technique is laborious, and only practicable if the inoculant has some very characteristic feature distinguishing it from infection by indigenous endophytes. It is generally easier to depend on recognizing resting spores of the inoculant in the soil. If its spores are initially lacking from the soil, their subsequent occurrence at a particular point may be taken as evidence of spread to that point, although absence of the particular spores does not necessarily prove the reverse.

Based on the presence of spores *and* fructifications in soil samples taken at particular positions in relation to inoculation points, the spread of two introduced endophytes was monitored 3, 15 and 21 months after inoculation.

Materials and Methods

The complete cropping sequence and sampling dates are given in Table 1. In 1978, a field experiment to assess the response of three hosts to inoculation with VA endophytes was set up on Sawyers field at Rothamsted. A more detailed description of the site and growth results recorded for the 1978 crop are given in Owusu-Bennoah and Mosse (1979). The plot (22·5 × 1·8 m) was divided into 12 subplots

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Table 1. *Cropping sequence and sampling dates*

Date	Treatment	Harvest: time after sowing	Soil sampled: time after inoculation
	First crop		
June 1978	Onion, lucerne + barley sown and inoculated	—	—
September 1978	Harvested and soil sampled	13 weeks	13 weeks
	Second crop		
June 1979	Plot re-sown with lucerne	—	—
August 1979	Harvested (shoots only) and soil sampled	9 weeks	15 months
	Lucerne allowed to re-grow		
February 1980	Soil sampled	—	21 months
June 1980	Single rows harvested	12 months	—

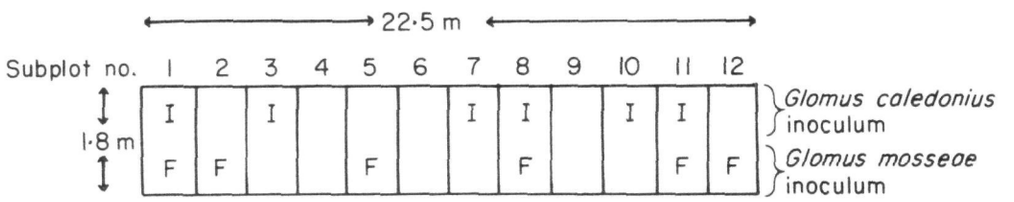

Fig. 1. Layout of 1978 field plot showing treatments applied: I, inoculated; F, formalin.

(1·5 × 1·8 m). There were four treatments, with or without mycorrhizal inoculum and with or without formalin (300 ml of 38 % formalin diluted to 4·81 m^{-2}). A randomized block design with three replicates was used (Fig. 1). Each subplot contained a row of barley (cv. Ark Royal), onion (cv. Ailsa Craig) and lucerne (cv. European, inoculated with *Rhizobium* RCR 2001) sown 45 cm apart. In inoculated subplots, half of each row received *G. caledonius* (Gerdemann and Trappe, 1974) inoculum which had been grown in association with *Nardus stricta*, and half received a mixed inoculum, the main component of which was *G. mosseae* which had been grown in association with *Zea mays*. Uninoculated control subplots received similar amounts of uninoculated soil and sand. Inoculum was placed 3 cm below the seeds which were sown at 7·5 cm intervals along the rows. The plants were harvested 13 weeks after sowing. Care was taken to minimize soil disturbance when harvesting and most of the roots were left in the soil. Immediately before harvest, a detailed record of the position and size of the plants in each row was made.

Spread of VA mycorrhizal inoculum was monitored in that part of the experiment inoculated with *G. caledonius* as its characteristic spores could be distinguished with certainty from any spores formed by the indigenous endophytes. In two adjacent inoculated subplots, ± formalin, one large and one small plant were selected from each row. Soil samples were taken 7 to 10 cm below the soil surface at 7·5 cm intervals from each plant along a line crossing the row at right angles, and at the point of inoculation [Fig. 2(a)]. One hundred grams of soil from each sample was wet sieved (Gerdemann, 1961) through a series of sieves with 750, 250 and 106 μm apertures. All spores collected on the 106 and 250 μm sieves were counted using a nematode counting dish (Doncaster, 1962).

To assess the effects of host species and sterilization treatment on establishment

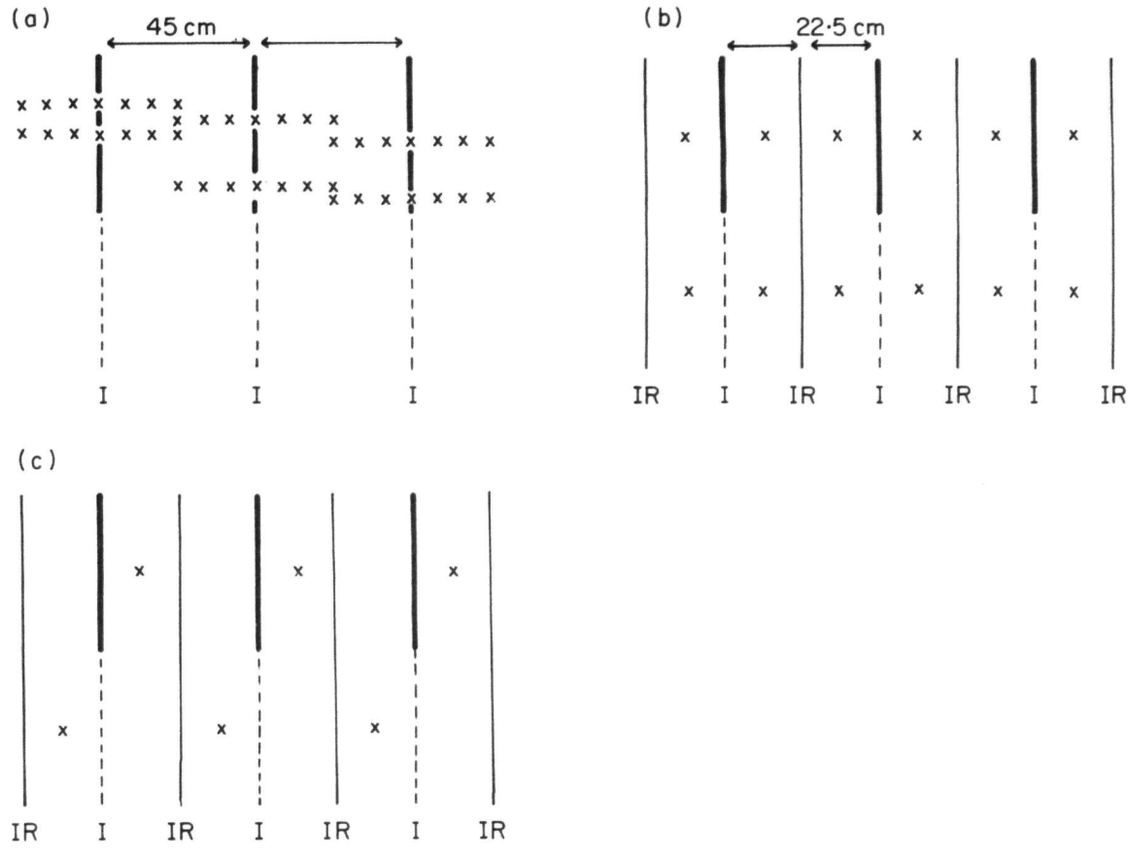

Fig. 2. Sampling positions (a) 13 weeks, (b) 15 months and (c) 21 months after inoculation. (b) and (c) refer also to uninoculated plots. ——, *Glomus caledonius* inoculum; – – –, *Glomus mosseae* inoculum; I, inoculated row; IR, uninoculated inter-row.

and sporulation of the fungus, a composite soil sample consisting of three subsamples was collected at intervals along each inoculated row. Spore numbers in 100 g of the composite sample were determined as previously described. Soil samples were also taken from uninoculated rows to determine the indigenous spore population. The plot was left undisturbed until the following year.

By June 1979 a sparse weed cover consisting mainly of *Polygonum* sp. and *Senecio vulgaris* had grown over the plot. These were removed at ground level by hoeing. Rows of lucerne inoculated with *Rhizobium* RCR 2001 were sown in the position of the previous year's rows, and also between rows, therefore the inter-row distance was now 22·5 cm [Fig. 2(b)].

Three weeks after emergence, seedling growth was assessed visually in five categories. Plants were harvested 9 weeks after germination and shoot fresh and dry wt recorded for every row in the plot. Three replicate soil samples were collected within 10 to 15 cm of the positions midway between each row as shown in Fig. 2(b). Fifty grams of soil from each sample was wet sieved and *G. caledonius* spores counted. The plot was left undisturbed until February 1980, by which time the lucerne had regrown and shoots were approximately 7 cm high.

In February 1980, 21 months after inoculation, the soil was again sampled and the spread of both *G. caledonius* and *G. mosseae* was monitored because the latter had by this time formed many, identifiable sporocarps in the soil. Samples were collected from positions midway between each row [Fig. 2(c)]. The six samples from each subplot were bulked and a 50 g aliquot taken to assess spore numbers. Four individual samples were also taken for comparison with bulked samples.

In June 1980, the central row of plants and immediately adjacent inter-rows in each of the 12 subplots were harvested, weighed and the number of plants in each row counted.

RESULTS

Spread of G. caledonius *in the first year*

Soil sievings from the uninoculated rows yielded few spores, and those recovered did not include *G. caledonius*. Presence of this spore type in soil samples taken from either side of the rows originally inoculated with *G. caledonius* therefore showed that the endophyte had spread from the point of inoculation. The maximum distance from inoculated rows over which *G. caledonius* had spread was 22·5 cm, but generally the distance was 7 to 15 cm in 13 weeks (Table 2). Spread was not related to plant size. It was greater in subplots not treated with formalin and with lucerne as a host plant rather than with onion, as found by Warner (1980) in pot trials comparing five host species. In those trials spread was greatest in two legumes, followed by lettuce, a graminaceous host and then onion.

The total number of spores from the soil samples taken at right angles to each plant (Table 2) was not related to plant size, nor to the distance spread or formalin treatment. Spore numbers recorded within each inoculated row (Table 3) were also too variable to show any statistically significant difference between host or treatment.

Table 2. *Maximum distance (cm) from point of inoculation at which* Glomus caledonius *spores were found and total number of spores in seven 100 g soil samples collected at the point of inoculation and at 7·5 cm intervals from this point*

Soil treatment	Plant size	Host		
		Onion	Barley	Lucerne
		Maximum spread (cm)		
Formalin	Large	7·5	7·5	15
	Small	7·5	7·5	7·5
No formalin	Large	7·5	15	15
	Small	15	15	22·5
		Total no. of spores in 7 soil samples of 100 g each		
Formalin	Large	178	52	142
	Small	208	64	42
No formalin	Large	121	44	88
	Small	99	46	420

Table 3. *Mean number of* Glomus caledonius *spores in 100 g of soil* in inoculated rows planted with onion, barley and lucerne in plots with and without formalin treatment*

Treatment	Host		
	Onion	Barley	Lucerne
Formalin	172	69	86
No formalin	63	91	56

* Mean of three replicates. L.S.D. = 67·6, $P = 0·05$.

Spread of G. caledonius *in the second year*

The extreme variability of spore numbers of *G. caledonius* was apparent not only within subplots, but even between replicate samples taken within 10 to 15 cm of each other. To ensure that such variability was not due to soil sieving and counting technique, spore numbers in replicate aliquots of the same soil sample were counted and found to be similar (Table 4). The extreme variablity between samples was therefore attributed to an uneven distribution of spores within the plot. Such variability within a site is no greater than that reported by Redhead (1974), who studied variation in spore numbers in 12 samples taken at 2 m intervals from undisturbed soil. Numbers of the commonest spore type varied from 1 to 66, and of others from 1 to 13 and 14 to 23 per 25 g of soil. Redhead concluded that at least 12 subsamples were necessary to adequately sample a site.

There was no consistent difference between spore numbers in originally inoculated and uninoculated subplots, and *G. caledonius* spores were as common in that half of the subplot inoculated with *G. mosseae*. The largest gap between inoculated rows was 4·5 m (see subplots 3 to 7, Fig. 1). Figure 3 shows the

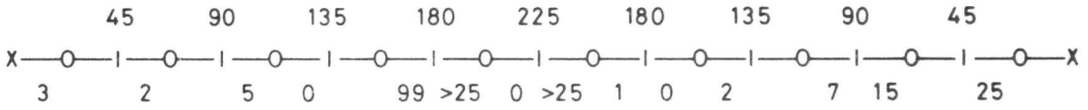

Fig. 3. Distribution of *Glomus caledonius* spores over 4·5 m gap between rows inoculated 15 months earlier. Position of rows: over previously inoculated (X), uninoculated (I), or inter-row (O) positions. Numbers above line give the distance (cm) from the nearest inoculated row. Numbers below line give number of spores recovered from 50 g soil.

distribution of spores in this region. In spite of the erratic distribution there was a concentration of spores near the mid point 2·25 m from the nearest inoculated row, representing a weekly rate of spread of 3·5 cm.

Table 5 shows the residual effect of inoculation on early growth and on shoot dry wt of lucerne 9 weeks after sowing. At the 3 week assessment, plants in previously inoculated rows (I) were significantly larger (5 % significance level, χ^2 analysis) than those in uninoculated rows (NI) or inter-row positions. Nine weeks after sowing, shoot weight in initially inoculated rows was 17 % more than in uninoculated rows ($P < 0{\cdot}5$) and was also significantly greater ($P < 0{\cdot}01$) than that of plants in the inter-row positions. The residual growth effects of row versus inter-row might be due to nutrient accumulation in roots of plants grown in 1978 left behind at harvest and made available when the roots decayed.

Spread of G. caledonius *in the third year*

Twenty-one months after inoculation, a remarkable build up of spores of *G. caledonius* and sporocarps of *G. mosseae* had occurred throughout the plot (Table 4). Again the extreme variability in spore and sporocarp numbers was apparent. Numbers of *G. caledonius* spores recorded were more comparable to those usually found in pot cultures in sterile soil than to those normally found in agricultural soil, and the soil could well be used as inoculum in further field tests. There was no longer a visible effect of inoculation within the plot and no significant difference between mean shoot dry wt of inoculated, uninoculated and inter-row plants was found (Table 5).

Table 4. *Numbers of* Glomus caledonius *spores and* Glomus mosseae *sporocarps in 50 g soil*

Subplot	Treatment with (+) or without (−) inoculum	15 months after inoculation *G. caledonius*	21 months after inoculation			
			G. caledonius		*G. mosseae* (sporocarps)	
			Bulked samples	Individual samples	Bulked samples	Individual samples
1	+	(4, 6)*; 34*; 14; 65; 22; 7; 32	47	—	47	—
2	−	42	486	—	18	—
3	+	(1, 1)*; 65*; 12; 17; 3	97	—	60	—
4	−	(0, 0); (99, 103)*; 26*; 2; 5	168	—	0	—
5	−	(0, 0); (0, 0); 25; 27; 13	468	278*; 500*	11	0*, 146*
6	−	7; 17; 15; 25; 30	286	110	7	4
7	+	35	285	—	60	—
8	+	19	204	232	157	86
9	−	32	254	—	50	—
10	+	7	106	271	26	21
11	+	16	307	—	50	—
12	—	90	140	—	13	—

Figures in brackets are spore numbers in replicate aliquots of the same soil samples. Starred pairs of figures represent replicate soil samples taken within 10 to 15 cm of each other.

Table 5. *Mean growth category (1 to 5) and dry wt (g per 1·8 m row) of lucerne shoots at two harvests*

Age	Measurement	No. replicates	Previous treatment*			
			I	NI	I+½	NI+½
3 weeks	Growth category	18	2·9	2·3	1·7	1·8
9 weeks	Dry wt	18	75·6	64·3	58·8	59·1
12 months	g cm^{-1} row	6	301	264	265	240

* I, inoculated row; NI, uninoculated row; I+½ and NI+½, respective inter-rows.

Discussion

Sawyers field soil has a large indigenous endophyte population (Owusu-Bennoah and Mosse, 1979); nevertheless both introduced endophytes *G. mosseae* and *G. caledonius* established and competed successfully with this population. The spread of *G. caledonius* observed in this experiment is comparable to annual spread rates calculated by Powell (1979) for *Glomus tenuis* in a soil already containing VA endophytes. On the basis of such figures, inoculum placed 4 m apart might be expected to affect the following year's crop. However, the spread observed could have been aided by contamination either man-made or due to soil fauna. *Endogone* spores have been recovered from the guts of rodents (Bakerspiegel, 1958) and are ingested inadvertently if not intentionally by earthworms (McIlveen and Cole, 1976). Transfer of small amounts of soil during weeding and even by wind, rain or drainage water cannot be excluded in the field situation.

The large build up of spore numbers of both inoculants between the 15 and 21 month assessment was unexpected. No comparable data are available for the build up of an introduced endophyte over two cropping seasons. Numbers of *G. caledonius* spores from a wheat field on the same farm varied seasonally, but were never above 125 in 50 g of soil (Hayman, 1970). Whether such a build up of spores can be regarded as normal or is related to the continuous presence of a host plant over winter, or to a special effect of a legume host is uncertain. In eight Australian soils where lucerne was growing, total spore numbers did not exceed 829 in 50 g soil and only one of the soils contained *G. caledonius* (Hayman and Stovold, 1979) arguing against a specific affinity between lucerne and this endophyte.

Good establishment and residual growth effects of the introduced inoculants have important implications for the economics of field inoulation with VA endophytes.

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