

## Decline of Vesicular-Arbuscular Mycorrhizae in Long Fallow Disorder of Field Crops and its Expression in Phosphorus Deficiency of Sunflower

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### Abstract

Poor growth of crops after long fallows (>12 months) in cracking clay soils of the northern areas of the Australian grain belt is known as 'long fallow disorder'. Various crop species, including wheat (*Triticum aestivum* L.), chickpea (*Cicer arietinum* L.), grain sorghum [*Sorghum bicolor* (L.) Moench], sudan grass [*Sorghum sudanense* (Piper) Stapf], sunflower (*Helianthus annuus* L.), soybean [*Glycine max* (L.) Merr.] and maize (*Zea mays* L.), had less root colonization with vesicular-arbuscular mycorrhizal (VAM) fungi and plant weight after long fallows than after short fallows.

An experiment was conducted with a phosphorus-deficient soil that had been either fallowed for 3 years or sequentially cropped to cotton, sorghum and sunflower. Cropped soil had more mycorrhizal propagules consisting of intact spores and colonized roots than long fallow soil. In the glasshouse, mycorrhizal colonization of sunflower (cv. Hysun 33) developed quickly in previously cropped soil to peak at 80% of root length at 72 days (flowering), but in long fallow soil it proceeded slowly, attaining 35% of root length at 72 days. Inoculation of long fallow soil with 20% w/w cropped soil resulted in extensive root colonization (89% at 72 days), eliminated P deficiency symptoms and more than doubled plant growth and final P uptake. Inoculation with similar soil treated with gamma radiation to kill propagules of mycorrhizal fungi had no effect on plant growth. Sunflower grew extremely poorly in irradiated soil with considerable leaf necrosis due to P deficiency. Reinoculation with cropped soil resulted in high levels of mycorrhizal colonization and good plant growth. It was concluded that long fallow disorder is caused by a decline in viable propagules of mycorrhizal fungi during fallowing, resulting in poor root colonization and symbiotic effectiveness of a subsequent crop.

Fertilizing with phosphorus (50 mg P/kg soil) delayed the development of mycorrhizal colonization, but increased final lengths of colonized roots at 72 days. Zinc fertilizer (15 mg Zn/kg soil) slightly improved mycorrhizal colonization, and basal fertilizer (N, K, S, Ca) substantially improved colonization in long fallow soil inoculated with cropped soil.

*Additional keyword:* Vertisols.

### Introduction

Fallows on cracking clay soils or vertisols in the northern Australian grain belt are usually cultivated to control weeds so as to conserve moisture and nitrate for subsequent crop growth. Longer fallows than normal (> 12 months instead of about 6 months) occur when changing between sequences of winter and summer crops or when insufficient rain falls for timely sowing. Conversely, if rainfall is sufficient, winter/summer crop sequences can be alternated by double cropping aided by higher rates of nitrogen fertilizer, and long fallow thus avoided. Poor growth of some crops sown immediately after long fallow has been noted since the 1940's (Leslie and Whitehouse 1965; Duncan 1967a) and is known as 'long fallow disorder'.

The disorder is seen intermittently but widespread throughout Queensland and northern New South Wales (Leslie and Whitehouse 1965; Duncan, 1967c). It affects many crop species, including wheat, sorghum, maize, linseed, cowpea, soybean and sunflower (Leslie and Whitehouse 1965). The problem intensifies with increasing length of preceding fallow (Leslie and Whitehouse 1966). Conversely, interrupting fallow by cropping or by uncontrolled weed growth alleviates the problem for a subsequent crop (Leslie and Whitehouse 1966; Duncan 1967a).

In many instances, correction of long fallow disorder has been obtained with zinc fertilizers (Hewitt 1962; Duncan 1966, 1967a, 1967b, 1967c; Leslie and Whitehouse, 1966, 1969, 1970, 1971), but not always (Leslie and Whitehouse 1967), and in the Central Highlands of Queensland long fallow disorder was expressed as an aggravated phosphorus deficiency (Leslie and Whitehouse 1968). In glasshouse trials, plants grown in soils disposed to long fallow disorder in the field often respond synergistically to both zinc and phosphorus fertilizers (Leslie and Whitehouse 1968). Although plants respond to these fertilizers, no marked, consistent differences have been obtained between long fallow and cropped soil in levels of 'available' zinc and phosphorus as assessed by usual extraction and chemical analysis methods (Whitehouse 1967) or bioassay by the *Azotobacter* plaque method (Thompson 1987). Neither should lower nutrient levels be expected in long fallow soil because inorganic nutrients usually increase during fallowing, as a result of continued microbial mineralization of soil organic matter without removal by plant growth. Many hypotheses involving chemical, physical and biological mechanisms have been advanced to explain the phenomenon (e.g. Leslie and Whitehouse 1966), but none has been found acceptable. Although plant growth corrected long fallow disorder for a subsequent crop, mere addition of plant tops or deep-frozen roots to fallow soil did not (J. K. Leslie, personal communication), suggesting that mechanisms like chelation of zinc by organic matter do not account for the double crop effect.

In this paper, evidence is provided from field samples that the phenomenon of long fallow disorder results from poor root colonization with the symbiotic fungi forming vesicular-arbuscular mycorrhizae (VAM). These fungi colonize roots of a wide range of plant species and greatly aid nutrition through enhanced uptake of poorly mobile nutrients like phosphorus (Mosse 1973a; Abbott and Robson 1982) and zinc (Gilmore 1971). The fungal partner cannot multiply independently of living plant roots, and consequently could decline during weed-free fallow resulting in poor root colonization and symbiotic effectiveness of the next crop. Further evidence to support this hypothesis was obtained in a comprehensive glasshouse experiment by tracing mycorrhizal colonization and its effects on growth of sunflower (*Helianthus annuus* L.) in a phosphorus-deficient vertisol either long fallowed or cropped in the field. Prior to cropping in the glasshouse, the soils were subjected to 30 treatments involving gamma irradiation to kill VAM, soil admixtures to enrich with VAM, and to provide other controls, and fertilizer additions to assess the mode of action of VAM.

## Materials and Methods

### *Effects of Long Fallow on Mycorrhizal Colonization, Plant Weight and Nutrient Contents of Field-grown Crops*

Soil and plant samples were taken from farmers' fields where paired crops existed, one growing poorly after long fallow and the other growing better after shorter fallow, but treated similarly in other respects.

Soil, plant root and top samples were obtained from three to five positions within each crop and treated separately through all analyses. Soil with roots was excavated from around the plants in a block about 30 cm deep. On return to the laboratory, roots were recovered by soaking and wet-sieving through a 500  $\mu\text{m}$  mesh sieve. Weighed subsamples of roots were cleared in KOH and stained with trypan blue (Phillips and Hayman 1970), and root length and per cent colonization with mycorrhizae were determined by the grid-intersect method (Giovannetti and Mosse 1980). Plants were counted in 1 m lengths of row and tops removed for dry weight determination by drying at 70°C for 3 days. The dried plant material was ground and analysed for nitrogen and phosphorus by an automated colorimetric procedure (Murphy and Riley 1962) and for zinc by atomic absorption spectroscopy. Soil (0–15 cm layer) was sampled with a 5 cm corer from between rows, air-dried, and analysed for nitrate nitrogen (Best 1976) in 1 M KCl extracts, bicarbonate-extractable phosphate (extraction in 0.5 M NaHCO<sub>3</sub> for 16 h with a soil : solution ratio of 1 : 100; Colwell 1963), acid-extractable phosphate (extraction in 0.005 M H<sub>2</sub>SO<sub>4</sub> for 16 h with a soil : solution ratio of 1 : 200; Kerr and von Steiglitz 1938), diethylenetriaminepentaacetic acid (DTPA) extractable zinc (Lindsay and Norvell 1978) and pH (1 : 5 soil : water suspension).

*Effects of Gamma Irradiation, Soil Admixture and Nutrient Addition Treatments of Long Fallow and Cropped Soils on Mycorrhizal Colonization and Growth of Sunflower in the Glasshouse*

*Soils*

Soil was collected from Emerald (lat. 23° 32' S.; long. 148° 10' E.) in the Central Highlands of Queensland in July 1981 from a site where both long fallow and cropped soils were available. At this site, sunflower had responded to phosphorus fertilizer on long fallow in 1979 but not on short fallow after sunflower or sorghum in 1980 (D. Hibberd, personal communication). Mycorrhizal colonization of the sunflower on short fallow after sorghum was determined, by methods described above, as 57% of root length colonized at flowering. The soil was type B<sub>Ug</sub>-2 of McDonald (1978) developed on decomposing basalt and classified as a black earth (Stace *et al.* 1968), Mollic Torrert (Soil Survey Staff 1975), or Principal Profile Form U<sub>g</sub> 5.12 (Northcote 1979). The long fallow soil had been fallowed for 3 years with weed control as necessary by cultivation with tined implements. During this period the cropped soil had sequentially grown cotton, sorghum and sunflower harvested in June 1981. Soil was collected to a depth of 25 cm from both the long fallow and cropped areas, with the latter from along the rows of harvested sunflower. The soil was broken manually to <2 cm, mixed thoroughly and stored in polythene bags at an average of 15°C until use.

*Mycorrhizal and chemical characterization of soil*

Levels of potentially infective propagules of VAM fungi in the long fallow and cropped soils were assessed on triplicate 50-g subsamples by wet sieving and decanting (Gerdemann and Nicolson 1963) using a 1 min sedimentation time to remove fine microaggregates of soil. Spores collected on 63, 106 and 250  $\mu\text{m}$  mesh sieves were counted using a Doncaster dish and stereomicroscope. Roots remaining from previous crops were collected on a 500  $\mu\text{m}$  mesh sieve and mycorrhizal colonization was determined by methods described above. Five subsamples of each soil were analysed as before for nitrate, bicarbonate-extractable phosphate, and DTPA-extractable zinc.

*Experimental design*

An experiment with 30 treatments (Table 4) and three replications was designed to test growth of sunflower in long fallow and cropped soils in relation to the development of VA-mycorrhizal colonization in the root system and the supply of phosphorus and zinc. The basis of the treatment design was as follows. Five nutrient treatments (nil control, and Zn and P applied as a 2×2 factorial in the presence of a basal nutrient treatment containing N, S, K and Ca) were applied to long fallow (treatments 1–5) or cropped soil (treatments 6–10). A similar set of nutrient treatments was applied to long fallow soil mixed with 20% (w/w) of cropped soil as an enriched source of mycorrhizal inoculum (treatments 11–15). Another set of nutrient treatments was applied to cropped soil that had been irradiated with 10 kGy of gamma radiation (Australian Atomic Energy Commission Establishment, Lucas Heights) to kill mycorrhizal propagules (treatments 16–20). This level of irradiation is known to kill mycorrhizal fungi (Mosse 1973b) but leaves a residual population of bacteria (Skyring and Thompson 1966) to carry on a general decomposer function. Treatments 21 and 22 (Table 4) tested effects of reintroduction of mycorrhizal fungi from cropped soil into irradiated cropped soil both in the absence and presence of basal nutrients. Treatments 23 and 24 tested effects of irradiating long fallow soil, while treatments 25 and 26 tested effects of adding cropped soil containing mycorrhizal propagules to irradiated long fallow

soil. Treatments 27 and 28 checked irradiated long fallow soil as inoculum, and treatments 29 and 30 tested effects of irradiated, cropped soil instead of untreated, cropped soil as inoculum.

#### *Pot culture methods*

Polythene pots without drainage holes were filled with appropriately treated (Table 4), undried soil to give the equivalent of 2 kg oven-dry soil per pot. Soil inoculum, as specified in column 3 of Table 4, replaced 20% w/w of soil as specified in column 2, and all was thoroughly mixed, together prior to potting. A quantity of soil was kept aside for later covering of the seed to a depth of about 2 cm. Appropriate nutrients were added in solution to the base layer followed by enough deionized water to bring the whole of the soil to 45% moisture content, the equivalent of pF 2 in this soil, and to carry the nutrients deeper into the soil. The basal nutrient solutions (Table 4) consisting of  $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ ,  $\text{KNO}_3$ ,  $(\text{NH}_4)_2\text{SO}_4$  and  $\text{K}_2\text{SO}_4$  were primarily used to supply nitrogen (100 mg N/kg soil with a  $\text{NO}_3\text{-N} : \text{NH}_4\text{-N}$  ratio of 95 : 5), but also contained potassium (144 mg K/kg soil), sulfur (78 mg  $\text{SO}_4\text{-S/kg}$  soil), and calcium (107 mg Ca/kg) in a suitable K/Ca ratio (Hewitt 1966). Phosphorus (50 mg P/kg soil) was supplied as  $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$  and zinc (15 mg Zn/kg soil) as  $\text{ZnCl}_2$  (treatments applied as specified in column 4 of Table 4). The cropped soil was given an additional 12 mg  $\text{NO}_3\text{-N/kg}$  soil as  $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$  to raise available nitrogen to the level in long fallow soil as determined by chemical analysis. Washed sunflower seeds (cv. Hysun 33) were placed 6 per pot on the basal layer then covered with the top layer of soil. Appropriate aseptic techniques were followed during all stages of preparation and potting to avoid cross contamination of the variously treated soils.

The plants were grown in a glasshouse at Toowoomba (lat.  $27^\circ 33' \text{S}$ , long.  $151^\circ 57' \text{E}$ ) from November to January when average diurnal temperature of the soil within the pots was  $15\text{--}30^\circ\text{C}$ . The pots were watered to weight with deionized water to return the soil to 45% moisture content twice weekly, then every second day, then daily as the requirements of the sunflower increased. After 17 days, plants were destructively sampled to leave two plants per pot. Another plant was sampled at 27 days and the last plant was sampled at 72 days. Two additional pots of treatments 1 to 10 were sampled at 41 days instead of 72 days. At each sampling time the plant top was removed for determinations of dry weight, and phosphorus, zinc and nitrogen concentrations. The root system of the sampled plant was recovered by excavation. Adhering soil was washed back into the pot with deionized water and roots were collected on a  $425 \mu\text{m}$  mesh stainless steel sieve. Extracted roots were cleaned in 0.1% NaCl solution to disperse any adhering clay, and subsampled by fresh weight for determination of root length and mycorrhizal colonization by the grid intersect method, dry weight and concentrations of phosphorus and nitrogen as described above. In addition, per cent cortex colonized by VAM was determined under a compound microscope by the method of Ocampo *et al.* (1980) on microscopic slide preparations consisting of 20 1-cm stained root pieces selected from predetermined intersections of lines in the grid dish.

Non-destructive assessments of plant growth were made at 56 and 70 days. Variables determined were plant height, stem diameter measured with vernier calipers at the first and fourth internodes, total number of leaves, and number of leaves with partial and complete necrosis from phosphorus deficiency. Leaf area was determined at 56 and 70 days by the non-destructive method of Trehan *et al.* (1975) using their form factor of 0.6798. The number of days to flower, defined as all ray florets open, was recorded. At day 72, plants were subdivided into head, stem and leaves for separate dry matter determinations.

#### *Statistical analyses*

An analysis of variance (ANOVA) was done for each variable, and where the *F* ratio was significant the least significant difference (l.s.d.) was calculated for  $P < 0.05$  and  $P < 0.01$ . Data on percentage root colonization with VAM were transformed by  $\arcsin \sqrt{(\text{percentage})}$  prior to ANOVA.

#### *Relative mycorrhizal dependency*

Relative mycorrhizal dependency (RMD) for sunflower was calculated after the method of Plenchette *et al.* (1983) by which values range from 0 to 100% as plant species range from fully independent to fully dependent on mycorrhizae for growth. Due account is taken of the available phosphorus status of the test soil. Thus RMD was calculated at three levels of soil phosphorus occurring in this study, on the basis of plant weights at 72 days in treatments receiving basal nutrients, with the formula:

$$\text{RMD} = 100 \times (\text{dry weight of mycorrhizal plant} - \text{dry weight of non-mycorrhizal plant}) / \text{dry weight of mycorrhizal plant}.$$

## Results

### *Effects of Long Fallow on Mycorrhizal Colonization, Plant Weight and Nutrient Contents of Field-grown Crops*

The results of determinations made on paired crops, one poor and sown after long fallow (LF), and the other healthier and sown after short fallow (SF), are given in Table 1. Usually dry weight per plant was substantially less after LF than after SF (Table 1). Sometimes where sampling was delayed after the problem was first noticed by the farmer, differences in dry weight were not as marked, e.g. wheat from Dirranbandi. Generally, per cent root length colonized with VAM and length of VAM-colonized root per plant were markedly less for LF than for SF crops (Table 1). No marked, consistent differences were apparent in P and Zn status of LF and SF soils or in their pH. Generally, in both LF and SF soil, available P or Zn or both could be considered marginal for crop growth. The LF soil usually contained more nitrate-N than the SF soil, probably partly from poor extraction by long fallow disordered crops and partly from higher levels before sowing. Plant concentrations of P and Zn were usually less in LF than in SF plants. All sites showed marked differences between LF and SF crops in P, Zn and N yields per plant (Table 1).

### *Effects of Gamma Irradiation, Soil Admixture and Nutrient Addition Treatments of Long Fallow and Cropped Soils on Mycorrhizal Colonization and Growth of Sunflower in the Glasshouse*

#### *Soil characterization*

The long fallow soil contained considerably fewer VAM propagules, both spores retained on a 63  $\mu\text{m}$  mesh sieve and colonized root pieces retained on a 500  $\mu\text{m}$  mesh sieve than the cropped soil (Table 2). Single-endophyte cultures made from yellow spores were identified as *Glomus etunicatum* Becker and Gerdemann and *Glomus c. mosseae* (Nicol. and Gerd.) Gerdemann and Trappe. Red-brown reticulate spores (Fig. 1) were similar to the species *Gigaspora reticulata* Koske, Miller and Walker and *Acaulospora bireticulata* Rothwell and Trappe, but spores recovered by wet-sieving had no hyphal attachments for generic identification and attempts to produce cultures were unsuccessful. Virtually no root material was recovered from fallow soil. This lower density of VAM propagules in LF soil was consistent with results from farmers' fields (Table 1) in which poorer VAM colonization occurred in crops sown after LF than after SF. Some chemical properties of the soils used in the glasshouse experiment are given in Table 3. The LF soil contained more nitrate-N and more bicarbonate-extractable P than the cropped soil. More nitrate in LF soil was an expected result from past experience and the results of samples from farmer's fields (Table 1), but substantially more phosphate in LF was not. A difference of this order is most likely due to natural variation in available P, which is within the range encountered in the BUg-2 soils (R. C. McDonald and D. E. Hibberd, personal communication).

#### *Mycorrhizal colonization of sunflower roots in the glasshouse*

Percentage mycorrhizal colonization of the root system (Table 4) proceeded more rapidly in cropped soil than in LF soil (compare treatments 6-10 with 1-5). Inoculation of LF soil with 20% cropped soil quite effectively increased

**Table 1. Comparison of poor crops grown after long fallow with healthier crops grown after short fallow in root colonization with vesicular-arbuscular mycorrhiza, plant weight and nutrient contents, and soil chemical properties**

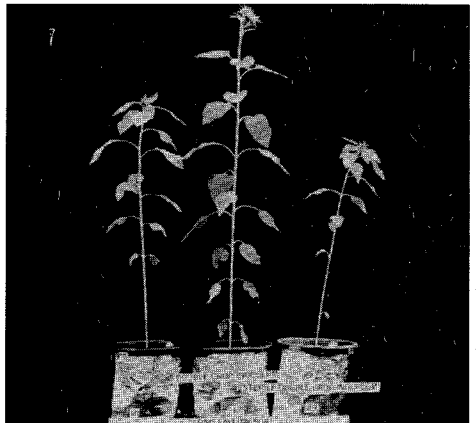
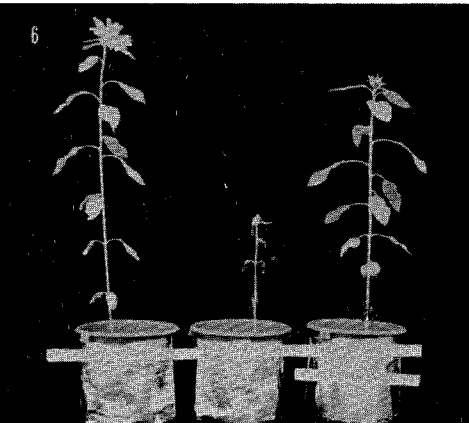
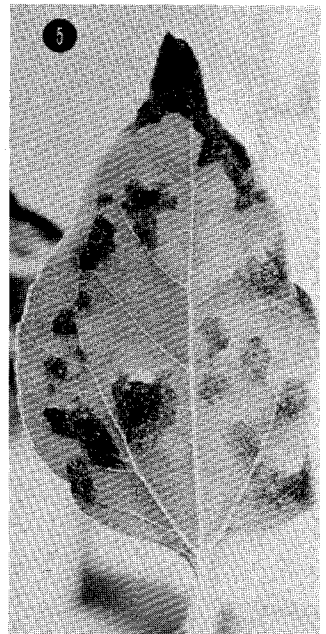
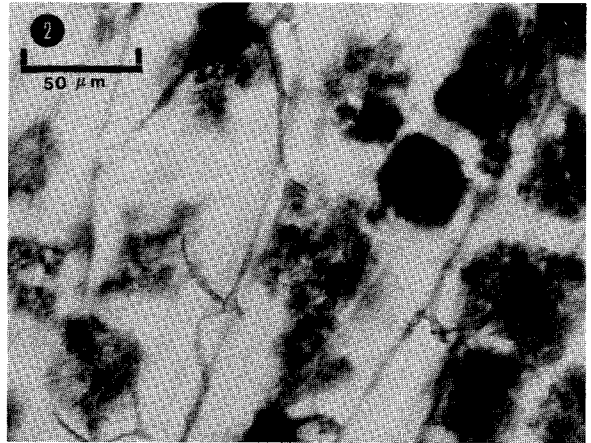
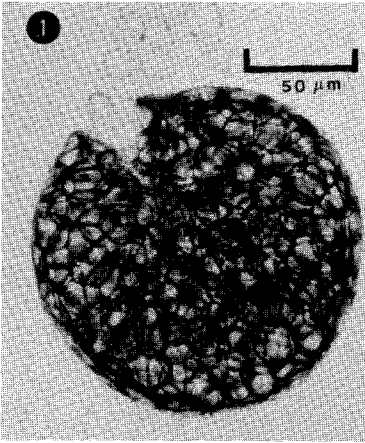
Values are means of three to five field samples  $\pm$  standard errors. LF, long fallow; SF, short fallow. — not determined. Some s.e.'s missing owing to compositing of field samples

Variable	Crop: Locality: Fallow length (months): Crop age (weeks):	Chickpea Macalister LF=14 SF=6 12	Sorghum Columboola LF=14 SF=6 4	Sorghum Columboola LF=14 SF=6 8	Sorghum Kilcummin LF=20 <sup>B</sup> SF=15 <sup>B</sup> 7	Sunflower Kilcummin LF=20 <sup>B</sup> SF=15 <sup>B</sup> 3	Sunflower Springsure LF=18 SF=3 —	Sunflower Springsure LF=14 SF=6 —	Wheat <sup>A</sup> Dirranbandi LF=7 SF=0.5 14
% root length with VAM	LF	17.5 $\pm$ 6.8	1.6 $\pm$ 1.0	2.5 $\pm$ 1.2	16.9 $\pm$ 6.1	2.6 $\pm$ 1.5	70.7 $\pm$ 4.6	63.9	39.0 $\pm$ 1.5
	SF	72.7 $\pm$ 5.9	25.0 $\pm$ 3.9	31.9 $\pm$ 1.7	69.7 $\pm$ 2.9	14.3 $\pm$ 2.2	71.8 $\pm$ 5.0	83.7	44.7 $\pm$ 5.3
Length of VAM-colonized root (cm/plant)	LF	—	6 $\pm$ 4.0	27 $\pm$ 19	783 $\pm$ 395	19 $\pm$ 12	4480 $\pm$ 85	2510	10369 $\pm$ 3892
	SF	—	129 $\pm$ 8.1	1015 $\pm$ 296	25829 $\pm$ 8152	94 $\pm$ 10	10200 $\pm$ 2811	4120	24270 $\pm$ 13466
Dry weight (g/plant)	LF	0.5 $\pm$ 0.1	0.2 $\pm$ 0.01	11.9 $\pm$ 2.1	6.6 $\pm$ 1.2	0.6 $\pm$ 0.2	31.1 $\pm$ 3.8	5.2	25.3 $\pm$ 6.9
	SF	2.9 $\pm$ 0.2	1.4 $\pm$ 0.2	57.8 $\pm$ 8.5	51.4 $\pm$ 10.3	6.9 $\pm$ 0.5	55.0 $\pm$ 7.1	14.5	28.0 $\pm$ 9.3
Plant phosphorus (%)	LF	—	0.09 $\pm$ 0.02	0.16 $\pm$ 0.02	0.12 $\pm$ 0.01	0.26	0.32 $\pm$ 0.02	0.20	—
	SF	—	0.25 $\pm$ 0.02	0.18 $\pm$ 0.02	0.18 $\pm$ 0.02	0.27	0.39 $\pm$ 0.02	0.24	—
Plant zinc (ppm)	LF	—	—	13.0 $\pm$ 2.0	69.2 $\pm$ 2.3	70.5	—	—	—
	SF	—	—	24.4 $\pm$ 2.3	62.9 $\pm$ 2.8	82.5	—	—	—
Plant nitrogen (%)	LF	—	2.2 $\pm$ 0.04	2.4 $\pm$ 0.14	2.5 $\pm$ 0.05	4.2	3.7 $\pm$ 0.15	3.0	—
	SF	—	4.2 $\pm$ 0.12	1.9 $\pm$ 0.09	2.4 $\pm$ 0.12	4.4	3.4 $\pm$ 0.06	3.4	—
P yield (mg/plant)	LF	—	0.2 $\pm$ 0.08	17.9 $\pm$ 2.6	8.1 $\pm$ 1.8	1.5	100 $\pm$ 14	10.4	—
	SF	—	3.4 $\pm$ 0.4	108.9 $\pm$ 27.2	96.7 $\pm$ 22.9	18.6	219 $\pm$ 35	34.8	—
Zn yield ( $\mu$ g/plant)	LF	—	—	160 $\pm$ 48.8	458 $\pm$ 89	41	—	—	—
	SF	—	—	1374 $\pm$ 67.2	3222 $\pm$ 663	569	—	—	—
N yield (mg/plant)	LF	—	4.6 $\pm$ 0.1	285 $\pm$ 38	166 $\pm$ 31	24.5	1128 $\pm$ 118	157.0	—
	SF	—	56.6 $\pm$ 7.1	1106 $\pm$ 215	1224 $\pm$ 222	306.4	1904 $\pm$ 259	487.0	—
Available soil P (ppm) bicarb.	LF	22.7 $\pm$ 2.2	12.8 $\pm$ 2.2	13.7 $\pm$ 6.5	4.0 $\pm$ 0.3	10.2 $\pm$ 0.6	19.2 $\pm$ 0.9	7.0	11.7 $\pm$ 3.2
	SF	21.3 $\pm$ 0.7	14.7 $\pm$ 1.5	17.7 $\pm$ 6.9	3.6 $\pm$ 0.2	9.0 $\pm$ 1.0	16.4 $\pm$ 1.0	5.0	9.7 $\pm$ 2.3
Available soil P (ppm) acid	LF	—	—	—	—	—	180.4 $\pm$ 6.9	16.0	33.0 $\pm$ 3.2
	SF	—	—	—	—	—	107.2 $\pm$ 4.0	12.0	29.3 $\pm$ 4.3
Available soil Zn (ppm) DTPA	LF	0.99 $\pm$ 0.4	0.28 $\pm$ 0.04	0.23 $\pm$ 0.05	0.24 $\pm$ 0.02	0.35 $\pm$ 0.03	0.32 $\pm$ 0.03	0.63	0.51 $\pm$ 0.01
	SF	0.4 $\pm$ 0.06	0.23 $\pm$ 0.03	0.31 $\pm$ 0.06	0.21 $\pm$ 0.02	0.35 $\pm$ 0.03	0.39 $\pm$ 0.11	0.25	0.52 $\pm$ 0.01
Soil pH	LF	6.9 $\pm$ 0.07	8.2 $\pm$ 0.09	—	—	—	—	—	8.2 $\pm$ 0.20
	SF	7.0 $\pm$ 0.03	8.1 $\pm$ 0.17	—	—	—	—	—	8.2 $\pm$ 0.13
Available soil NO <sub>3</sub> -N (ppm)	LF	53.1 $\pm$ 4.3	28.8 $\pm$ 2.3	28.1 $\pm$ 1.1	4.3 $\pm$ 0.4	4.6 $\pm$ 0.5	8.6 $\pm$ 1.4	13.7	0.0
	SF	10.6 $\pm$ 4.5	12.3 $\pm$ 0.2	20.6 $\pm$ 9.4	2.7 $\pm$ 0.1	2.7 $\pm$ 1.0	4.1 $\pm$ 0.9	6.4	0.0

Table 1 (Continued)

Variable	Crop: Locality: Fallow length (months): Crop age (weeks):	Wheat <sup>C</sup> Dirranbandi LF=7 SF=0.5 14	Sudan Aubigny LF=12 SF=6 8	Sudan Aubigny LF=12 SF=6 15	Soybean Aubigny LF=12 SF=6 6	Maize Clermont LF=24 SF=10 5	Maize Clermont LF=14 SF=8 6	Sorghum Miles LF=23 SF=6 6	Sunflower Bowenville LF=11 SF=5 16
% root length with VAM	LF	21.5	3.4±1.5	31.4±8.4	38.5±2.9	5.0	16.3	25.1±4.9	8.1±1.2
	SF	40.4	34.7±0.3	62.9±7.1	44.7±3.3	43.5	57.4	77.2±3.1	49.8±6.1
Length of VAM-colonized root (cm/plant)	LF	2106	97±46	12040±4164	733±56	32	187	448±166	1280±438
	SF	11012	11496±1677	37100±5554	1448±301	622	1896	3893±753	17660±3450
Dry weight (g/plant)	LF	35.7	0.8±0.4	127.8±15.4	2.4±0.71	0.4±0.06	0.8±0.06	0.3±0.03	28.7±7.5
	SF	37.2	45.3±5.6	294.7±43.9	7.7±0.51	2.1±0.17	6.5±0.64	2.6±0.1	151.5±16.5
Plant phosphorus (%)	LF	—	0.13±0.01	—	—	—	—	0.20±0.01	0.22±0.01
	SF	—	0.16±0.02	—	—	—	—	0.31±0.01	0.27±0.01
Plant zinc (ppm)	LF	—	—	—	—	—	—	6.6±0.5	20.5±0.8
	SF	—	31.1±3.2	—	—	—	—	32.6±1.8	28.5±1.7
Plant nitrogen (%)	LF	—	2.1±0.10	—	—	—	—	3.5±0.11	2.2±0.05
	SF	—	1.7±0.18	—	—	—	—	3.9±0.08	2.1±0.08
P yield (mg/plant)	LF	—	1.5±0.9	—	—	—	—	0.6±0.05	65.1±18.3
	SF	—	72.8±12.5	—	—	—	—	8.0±0.5	412.3±53.2
Zn yield (µg/plant)	LF	—	—	—	—	—	—	2±0.4	603±172
	SF	—	1431±302	—	—	—	—	86±8.0	4278±431
N yield (mg/plant)	LF	—	22.9±14.1	—	—	—	—	10.3±1.0	633±168
	SF	—	783±154	—	—	—	—	101.4±3.8	3168±378
Available soil P (ppm) bicarb.	LF	14.0	43.0±12.6	33.7±1.5	29.0±5.9	22.0	22.0	7.8±0.5	50.5±3.8
	SF	16.0	53.0±10.7	39.0±2.1	34.7±2.7	26.0	22.0	9.4±0.4	62.5±1.8
Available soil P (ppm) acid	LF	26.0	797±52.0	965±7.6	875	670.0	38.0	5.6±0.7	182.8±5.9
	SF	31.0	923±7.3	875±2.9	802±16.9	810.0	24.0	7.4±0.8	217.8±9.5
Available soil Zn (ppm) DTPA	LF	0.98	2.06±0.33	1.43±0.07	1.33±0.18	0.49	0.83	0.17±0.01	0.51±0.06
	SF	0.75	3.13±0.09	3.37±0.52	1.60±0.15	0.45	1.43	0.23±0.01	2.80±0.60
Soil pH	LF	7.1	9.3±0.03	9.2±0.06	8.9±0.03	8.5	8.7	8.6±0.01	7.6±0.03
	SF	7.7	8.8±0.03	8.7±0.03	8.9±0.03	8.4	8.7	8.6±0.01	7.5±0.03
Available soil NO <sub>3</sub> -N (ppm)	LF	0.0	27.0±1.4	2.4±0.5	32.0±6.6	36.3	30.4	21.6±0.7	7.3±1.1
	SF	0.0	4.7±0.9	3.2±0.4	17.6±6.4	15.6	12.0	26.4±0.8	10.7±0.05

<sup>A</sup> Grey clay.<sup>B</sup> For these samples, LF had 40 months' fallow and SF had 30 months' fallow in the previous 45 months.<sup>C</sup> Red clay.



mycorrhizal colonization (compare treatments 11–15 with 1–5). The mycorrhizae were very arbuscular (Fig. 2). Gamma irradiation of cropped soil (treatments 16–20) or LF soil (treatments 23–24) virtually eliminated mycorrhizal colonization. Late colonization occurred in some pots of irradiated soil, possibly due to a very low percentage of VAM propagules surviving 10 kGy of gamma irradiation of this soil or to a low level of contamination in the course of the experiment.

**Table 2. Characterization of mycorrhizal propagules in long fallow and cropped soils used in the glasshouse experiment**

Values are means  $\pm$  standard errors

Soil history	No. of VAM spores/kg soil <sup>A</sup>		Dry wt of roots/kg soil <sup>B</sup>	Mycorrhizal root length (%)
	Yellow	Red-brown reticulate		
Long fallow	13 $\pm$ 6	487 $\pm$ 140	0.01	0
Cropped	340 $\pm$ 80	1953 $\pm$ 360	2.01	22.0

<sup>A</sup> Spore numbers are totals recovered on 250, 106 and 63  $\mu$ m mesh sieves. Cultures made from yellow spores were identified as *Glomus etunicatum* and *Glomus c. mosseae* by Dr I. R. Hall, Mosgiel, New Zealand. Red-brown reticulate spores were similar to *Acaulospora bireticulata* and *Gigaspora reticulata*, but no successful cultures were made and no spore attachments allowing generic identification were seen in sievings from the field soil.

<sup>B</sup> Recovered by wet sieving a single 1 kg soil composite through a 500  $\mu$ m mesh sieve.

This colonization in irradiated soil was low, mainly <1% and of little consequence, except in one replicate of treatment 16 with 18.4% root length colonized. Reinoculation with 20% w/w cropped soil into irradiated cropped soil (treatments 21–22) or LF soil (treatments 25–26) brought mycorrhizal colonization comparable to that in unirradiated cropped soil (treatments 6–7). These results indicate that differences between LF and cropped soil in rates of development of VAM colon-

**Fig. 1.** Red-brown reticulate spore recovered from cropped soil by wet-sieving. Spore ruptured to liberate lipid globules.

**Fig. 2.** Arbuscules in cortex of sunflower root from treatment 12 (long fallow soil inoculated with cropped soil and fertilized with basal nutrients). Interference contrast microscopy of unsectioned root stained for mycorrhizae with trypan blue.

**Fig. 3.** Phosphorus deficient sunflower plant with necrotic lower leaves from treatment 16 (gamma irradiated, cropped soil) at 54 days.

**Fig. 4.** Phosphorus deficiency symptoms in sunflower leaf illustrating dark brown necrosis of distal portion and green, non-chlorotic proximal portion.

**Fig. 5.** Phosphorus deficiency symptoms in sunflower leaf illustrating dark brown necrotic lesions and tip necrosis, the remaining areas being green with no chlorosis.

**Fig. 6.** Effects of gamma irradiation of cropped soil and reinoculation with unirradiated cropped soil on growth of sunflower at 62 days. Treatments, all with basal nutrients, left to right: left, cropped soil; centre, irradiated, cropped soil; right, irradiated, cropped soil (80% w/w) plus unirradiated cropped soil (20%).

**Fig. 7.** Effects of inoculation of long fallow soil with either cropped soil or irradiated, cropped soil on growth of sunflower at 62 days. Treatments, all with basal nutrients, left to right: left, long fallow soil; centre, long fallow soil (80% w/w) plus cropped soil (20%); right, long fallow soil (80%) plus irradiated cropped soil (20%).

ization are related to differences in inoculum level in the two soils rather than to any other factor that might influence per cent root colonization with VAM. Application of 50 mg P fertilizer/kg soil markedly reduced per cent root colonization with VAM (Table 4). Zinc fertilizer had no detrimental effect on VAM, and in fact appears to have increased per cent root colonization in cropped soil at 27 and 41 days (compare treatment 8 with 7). In general, basal nutrient containing N, K, S and Ca increased per cent root colonization in treatments with high VAM inoculum (compare treatment 7 with 6, 12 with 11, 22 with 21, and 26 with 25). This effect is probably due to the increased nitrogen supply (Thompson 1986*b*).

**Table 3. Chemical characterization of the long fallow and cropped soils used in the glasshouse experiment**  
Values are means  $\pm$  standard errors

Soil history	Available N		Bicarbonate-extractable P ( $\mu\text{g/g}$ )	DTPA-extractable Zn ( $\mu\text{g/g}$ )
	NH <sub>4</sub> -N ( $\mu\text{g/g}$ )	NO <sub>3</sub> -N ( $\mu\text{g/g}$ )		
Long fallow	0	12.0 $\pm$ 0.6	17.2 $\pm$ 0.7	0.8 $\pm$ 0.04
Cropped	0.2 $\pm$ 0.06	0.5 $\pm$ 0.06	6.0 $\pm$ 1.0	0.7 $\pm$ 0.09

Length of VAM colonized root (Table 4) was greater in cropped soil than in correspondingly treated LF soil (compare treatments 6–10 with 1–5). Inoculation of LF soil with cropped soil increased the length of VAM colonized root, particularly where basal nutrients were supplied (compare treatments 11–15 with 1–5). The length of root colonized with VAM was also influenced by P fertilizer (Table 4). At 27 and 41 days, P fertilizer tended to reduce length of VAM colonized root (compare treatments 9 with 7, and 14 with 12), but later at 72 days, P fertilizer resulted in greater lengths of VAM colonized roots. Irradiated soil had little or no VAM colonized root (except for one replicate of treatment 16 as noted above).

#### *Growth of sunflower*

Results at 68–72 days for plant height, stem diameter, total number of leaves, number of leaves exhibiting P deficiency symptoms, total area of green leaf, head diameter, time to flower and dry weights of plant components, are given in Table 5.

Phosphorus fertilizer (treatments 4–5, 9–10, 14–15 and 19–20) resulted in marked increases in plant growth (Table 5), and irradiation without reinoculation resulted in marked decreases (treatments 16–18 and 23–24). Symptoms of phosphorus deficiency developed on lower leaves (Fig. 3) at about 50 days. The most common symptom was a marked necrosis developing rapidly at the distal end of the leaf with a definite boundary between the necrotic area and the unaffected, green, proximal portion of the leaf (Fig. 4). Less commonly, symptoms commenced as a number of chlorotic lesions which rapidly coalesced and became necrotic, unaffected portions of the leaves remaining quite green (Fig. 5). None of these symptoms developed in plants receiving P fertilizer and all were therefore considered to be P deficiency symptoms. In LF soil receiving basal nutrients the lower four to five leaves developed symptoms but later-produced leaves did not, in response to development of significant VAM colonization of the roots. The later leaves also

Table 4. Effects of soil and nutrient treatments on mycorrhizal colonization of sunflower

Code No.	Treatments		Nutrients <sup>B</sup>	% Root with vesicular-arbuscular mycorrhiza <sup>C</sup>					Length of mycorrhizal root (cm/plant)			
	Soil history <sup>A</sup>	Soil inoculum (20% w/w)		Root length (%)				Root cortex (%)	Root length (%)			
				Sampling time (days):					17	27	41	72
1	LF	0	0	0.0 (0.0)	0.0 (0.0)	10.3 (3.1)	34.9 (31.9)	29.8 (24.8)	0.0	0.0	15	2401
2	LF	0	B	0.0 (0.0)	1.4 (0.1)	11.2 (3.7)	36.8 (35.9)	34.6 (32.3)	0.0	0.7	25	2280
3	LF	0	B+Zn	0.0 (0.0)	1.4 (0.1)	9.6 (2.8)	33.6 (30.6)	35.0 (33.0)	0.0	0.5	17	3240
4	LF	0	B+P	0.0 (0.0)	1.3 (0.1)	0.0 (0.0)	16.5 (8.0)	11.8 (4.2)	0.0	0.5	0	4676
5	LF	0	B+P+Zn	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	21.2 (13.1)	14.3 (6.1)	0.0	0.0	0	6748
6	C	0	0	11.0 (3.7)	19.2 (10.9)	49.8 (58.3)	56.6 (69.7)	50.1 (58.9)	5.2	40.3	349	4489
7	C	0	B	10.9 (3.6)	25.6 (18.6)	55.9 (68.6)	62.5 (78.7)	53.0 (63.9)	4.9	62.7	481	5791
8	C	0	B+Zn	15.7 (7.3)	38.3 (38.5)	64.6 (81.6)	61.0 (76.5)	49.3 (57.5)	13.4	112.3	679	4759
9	C	0	B+P	15.8 (7.4)	16.1 (7.7)	33.3 (35.2)	43.6 (47.5)	37.2 (36.6)	12.9	48.0	290	13954
10	C	0	B+P+Zn	14.1 (5.9)	15.7 (7.3)	36.8 (35.8)	46.8 (53.2)	35.0 (33.0)	9.6	49.8	345	14049
11	LF	C	0	6.6 (1.3)	18.1 (9.6)	—	40.2 (41.7)	40.9 (42.9)	2.9	37.2	—	2433
12	LF	C	B	6.0 (1.1)	14.2 (6.1)	—	71.3 (89.7)	63.9 (80.7)	3.9	24.7	—	10381
13	LF	C	B+Zn	1.3 (0.1)	15.4 (7.1)	—	59.3 (74.1)	58.3 (72.4)	0.2	28.0	—	5347
14	LF	C	B+P	0.0 (0.0)	2.5 (0.2)	—	36.0 (34.7)	31.9 (28.0)	0.0	7.1	—	11878
15	LF	C	B+P+Zn	6.2 (1.2)	7.2 (1.6)	—	34.1 (31.5)	33.4 (30.3)	4.0	15.6	—	9154
16	IC	0	0	0.0 (0.0)	0.0 (0.0)	—	12.0 (4.3)	8.9 (2.4)	0.0	0.0	—	531
17	IC	0	B	0.0 (0.0)	1.3 (0.1)	—	5.7 (1.0)	1.3 (0.1)	0.0	0.6	—	76
18	IC	0	B+Zn	1.3 (0.1)	0.0 (0.0)	—	1.8 (0.1)	0.0 (0.0)	0.2	0.0	—	39
19	IC	0	B+P	0.0 (0.0)	0.0 (0.0)	—	0.0 (0.0)	0.0 (0.0)	0.0	0.0	—	0
20	IC	0	B+P+Zn	0.0 (0.0)	0.0 (0.0)	—	2.2 (0.2)	0.0 (0.0)	0.0	0.0	—	195
21	IC	C	0	8.7 (2.3)	16.0 (7.6)	—	62.7 (79.0)	53.0 (63.8)	2.7	28.4	—	8361
22	IC	C	B	10.2 (3.1)	20.1 (11.8)	—	68.9 (87.0)	58.8 (73.2)	3.9	61.0	—	7262
23	ILF	0	0	0.0 (0.0)	0.0 (0.0)	—	1.9 (0.1)	0.0 (0.0)	0.0	0.0	—	58
24	ILF	0	B	0.0 (0.0)	0.0 (0.0)	—	4.3 (0.6)	0.0 (0.0)	0.0	0.0	—	65
25	ILF	C	0	9.2 (2.6)	17.4 (9.0)	—	52.7 (63.3)	48.8 (56.7)	7.4	52.5	—	8856
26	ILF	C	B	10.9 (3.6)	18.0 (9.6)	—	66.6 (84.3)	61.2 (76.9)	6.4	62.3	—	23004
27	ILF	LF	B	0.0 (0.0)	1.4 (0.1)	—	37.0 (36.3)	38.2 (38.3)	0.0	1.1	—	4664
28	ILF	LF	B	9.7 (2.8)	27.4 (21.3)	—	63.9 (80.6)	62.9 (79.3)	4.1	122.8	—	8097
29	ILF	IC	B	0.0 (0.0)	1.4 (0.1)	—	39.6 (40.6)	36.6 (35.5)	0.1	0.8	—	3462
30	C	IC	B	13.4 (5.4)	30.9 (26.4)	—	60.3 (75.4)	55.8 (68.4)	10.1	97.4	—	5484
ANOVA: <i>F</i> test				**	**	**	**	**	**	**	*	**
l.s.d. <i>P</i> < 0.05				6.2	5.4	7.0	9.7	9.0	6.6	25.4	208	2992
l.s.d. <i>P</i> < 0.01				8.3	7.2	10.1	12.9	11.9	8.8	33.8	—	3980

\* Significant at *P* < 0.05. \*\* Significant at *P* < 0.01.<sup>A</sup> LF, long fallow; C, cropped; I, gamma irradiated.<sup>B</sup> B, basal (N, K, S, Ca); Zn, zinc (15 mg/kg soil); P, phosphorus (50 mg/kg soil).<sup>C</sup> Means of arcsin  $\sqrt{\%}$  transformations with equivalent means in parentheses.

Table 5. Effects of soil and nutrient treatments on components of sunflower growth at 68–72 days

Code No.	Treatments			Plant height (cm)	Stem diam. (mm)		Leaves		Head diam. (mm)	Time to flower (days)	Head	Dry wt of components (g)				Roots	
	Soil history <sup>A</sup>	Soil inoculum (20% w/w)	Nutrients <sup>B</sup>		Internode 1st	4th	No./plant Total	P deficit.				Total green area (cm <sup>2</sup> )	Leaves	Stem	Tops		
1	LF	0	0	82	3.8	3.4	16	1.3	145	20	65.3	0.6	0.6	1.6	2.8	0.3	
2	LF	0	B	86	5.9	6.6	19	4.3	460	31	65.7	1.7	1.7	2.8	6.1	0.6	
3	LF	0	B+Zn	93	6.3	7.3	20	4.7	565	35	64.7	2.0	2.0	3.6	7.5	0.4	
4	LF	0	B+P	117	8.3	8.0	24	0.0	912	56	65.0	5.5	3.7	9.9	19.1	4.2	
5	LF	0	B+P+Zn	121	8.7	8.6	25	0.0	903	51	64.0	4.7	4.0	11.1	19.8	4.0	
6	C	0	0	77	3.5	2.7	15	0.0	130	20	64.3	0.6	0.4	1.0	2.0	0.5	
7	C	0	B	79	4.4	4.5	16	1.3	361	33	60.7	1.9	1.2	1.9	5.1	0.9	
8	C	0	B+Zn	87	4.9	5.3	17	1.7	466	35	62.7	1.9	1.5	2.4	5.8	0.6	
9	C	0	B+P	108	7.9	7.9	21	0.0	947	65	57.3	7.0	3.2	7.6	17.8	2.4	
10	C	0	B+P+Zn	106	7.8	7.6	22	0.0	916	69	57.0	7.1	3.2	7.8	18.1	2.6	
11	LF	C	0	76	3.9	2.9	14	0.0	109	17	71.3	0.4	0.4	1.4	2.2	0.4	
12	LF	C	B	98	6.4	6.6	19	1.0	733	51	62.3	4.6	2.5	4.8	11.8	0.9	
13	LF	C	B+Zn	96	5.8	6.8	18	3.0	665	43	63.0	3.3	2.1	3.9	9.3	0.8	
14	LF	C	B+P	121	8.0	7.3	25	0.0	875	59	61.3	6.3	3.1	8.9	18.3	2.8	
15	LF	C	B+P+Zn	115	8.3	7.7	25	0.0	875	59	61.7	6.1	3.4	9.0	18.4	2.8	
16	IC	0	0	41	3.2	2.8	13	9.3	52	11	69.3	0.2	0.4	0.4	1.0	0.5	
17	IC	0	B	34	3.0	2.1	12	10.7	1	6	71.0	0.0	0.3	0.2	0.5	0.6	
18	IC	0	B+Zn	33	3.0	1.9	11	9.7	2	7	69.7	0.0	0.3	0.2	0.5	0.4	
19	IC	0	B+P	123	7.6	7.0	23	0.0	777	58	62.0	4.3	3.7	8.7	16.7	3.8	
20	IC	0	B+P+Zn	120	8.9	8.5	26	0.0	1005	66	60.7	7.4	4.4	10.8	22.6	4.0	
21	IC	C	0	75	4.2	4.3	16	4.0	215	28	63.3	1.0	0.8	1.7	3.5	0.5	
22	IC	C	B	74	4.7	5.2	17	4.0	417	31	64.7	1.6	1.3	1.9	4.8	0.8	
23	ILF	0	0	98	5.2	4.4	18	4.7	272	34	63.0	1.9	1.3	3.4	6.6	1.3	
24	ILF	0	B	66	4.0	3.0	14	14.0	3	13	66.3	0.2	0.6	0.8	1.6	0.4	
25	ILF	C	0	91	5.2	4.6	18	0.0	273	28	65.7	1.3	1.0	2.7	4.9	1.0	
26	ILF	C	B	102	7.6	8.0	21	0.0	1014	56	61.7	5.8	3.3	6.2	15.3	2.1	
27	ILF	LF	B	96	6.8	7.3	19	4.0	597	39	63.0	2.4	2.1	4.0	8.5	0.8	
28	C	LF	B	95	5.8	6.2	19	3.0	630	39	63.7	2.8	2.0	3.3	8.2	0.9	
29	LF	IC	B	86	5.7	6.6	19	4.3	599	28	67.3	1.5	1.7	3.0	6.2	0.8	
30	C	IC	B	80	4.7	4.9	17	2.7	397	35	61.7	2.0	1.5	2.2	5.6	0.6	
ANOVA: <i>F</i> test				**	**	**	**	**	**	**	**	**	**	**	**	**	**
l.s.d. <i>P</i> < 0.05				10	1.0	1.1	2	2.9	159	6	3.6	1.1	0.5	1.5	2.2	0.9	
l.s.d. <i>P</i> < 0.01				13	1.3	1.4	3	3.8	211	8	4.8	1.4	0.7	2.0	2.9	1.2	

\*Significant at *P* < 0.05. \*\*Significant at *P* < 0.01.

<sup>A</sup> LF, long fallow; C, cropped; I, gamma irradiated.

<sup>B</sup> B, basal (N, K, S, Ca); Zn, zinc (15 mg/kg); P, phosphorus (50 mg/kg).

Table 6. Effects of soil and nutrient treatments on nutrition of sunflower

Code No.	Treatments			P concn (%)			P uptake (mg/plant)			N concn (%)			N uptake (mg/plant)			Zn concn ( $\mu\text{g/g}$ )		Zn uptake ( $\mu\text{g/plant}$ )		
	Soil history <sup>A</sup>	Soil inoculum (20% w/w)	Nutrients <sup>B</sup>	Sampling time (days):			Tops			Tops			Tops			Tops		Tops		
				41	72	72	41	72	72	41	72	72	41	72	72	41	72	41	72	
1	LF	0	0	0.05	0.10	0.09	0.4	2.8	0.3	1.5	0.6	0.7	13	17	2	17	30	15	83	
2	LF	0	B	0.04	0.12	0.06	0.4	7.2	0.4	1.9	2.0	1.1	18	120	7	11	44	10	269	
3	LF	0	B+Zn	0.05	0.12	0.06	0.3	9.1	0.3	2.0	2.0	1.0	14	148	4	32	58	22	433	
4	LF	0	B+P	0.16	0.10	0.06	7.2	20.0	2.3	2.3	0.8	0.4	102	153	18	6	14	23	273	
5	LF	0	B+P+Zn	0.11	0.10	0.06	5.7	20.0	2.5	1.6	0.7	0.5	84	140	20	11	19	55	376	
6	C	0	0	0.15	0.13	0.09	1.1	2.6	0.5	1.5	0.6	0.7	12	12	3	34	58	26	99	
7	C	0	B	0.10	0.08	0.04	0.9	3.8	0.4	2.8	1.7	1.1	27	85	10	40	52	39	259	
8	C	0	B+Zn	0.12	0.08	0.05	1.1	4.3	0.3	3.2	1.7	1.1	30	100	7	111	90	102	518	
9	C	0	B+P	0.16	0.13	0.06	6.5	23.7	1.5	1.9	0.8	0.6	78	147	15	5	16	18	286	
10	C	0	B+P+Zn	0.16	0.13	0.06	6.8	23.5	1.5	1.5	0.9	0.6	65	159	16	23	26	9	468	
11	LF	C	0	—	0.11	0.08	—	2.4	0.3	—	0.5	0.8	—	10	4	—	38	—	83	
12	LF	C	B	—	0.13	0.06	—	15.0	0.4	—	1.3	0.7	—	147	6	—	37	—	426	
13	LF	C	B+Zn	—	0.11	0.04	—	10.4	0.3	—	1.6	0.7	—	145	6	—	61	—	559	
14	LF	C	B+P	—	0.10	0.06	—	18.5	1.8	—	0.7	0.5	—	132	16	—	15	—	266	
15	LF	C	B+P+Zn	—	0.10	0.06	—	18.4	1.6	—	0.8	0.5	—	137	15	—	25	—	448	
16	IC	0	0	—	0.15	0.04	—	1.4	0.2	—	1.8	1.3	—	17	5	—	29	—	77	
17	IC	0	B	—	0.04	0.04	—	0.2	0.2	—	2.0	1.3	—	10	8	—	—	—	—	
18	IC	0	B+Zn	—	0.04	0.06	—	0.4	0.3	—	2.1	1.5	—	11	6	—	—	—	—	
19	IC	0	B+P	—	0.07	0.05	—	10.9	2.0	—	0.9	0.8	—	153	30	—	12	—	201	
20	IC	0	B+P+Zn	—	0.07	0.05	—	16.2	2.0	—	0.8	0.7	—	180	25	—	22	—	504	
21	IC	C	0	—	0.11	0.08	—	3.9	0.3	—	0.7	1.0	—	25	3	—	41	—	144	
22	IC	C	B	—	0.07	0.06	—	3.6	0.5	—	1.6	1.1	—	80	8	—	46	—	222	
23	ILF	0	0	—	0.04	0.04	—	2.5	0.5	—	0.8	1.0	—	51	13	—	20	—	123	
24	ILF	0	B	—	0.03	0.05	—	0.5	0.2	—	1.6	1.2	—	26	4	—	31	—	69	
25	ILF	C	0	—	0.14	0.12	—	6.7	1.1	—	0.5	0.6	—	26	6	—	40	—	194	
26	ILF	C	B	—	0.10	0.05	—	14.7	0.9	—	1.1	0.7	—	169	14	—	33	—	504	
27	ILF	LF	B	—	0.15	0.06	—	12.7	0.4	—	1.9	1.0	—	158	8	—	42	—	348	
28	C	LF	B	—	0.08	0.04	—	6.1	0.4	—	1.6	0.9	—	133	9	—	49	—	396	
29	LF	IC	B	—	0.14	0.05	—	8.3	0.4	—	1.9	0.9	—	116	7	—	49	—	303	
30	C	IC	B	—	0.08	0.04	—	4.2	0.3	—	1.6	1.0	—	93	7	—	53	—	301	
ANOVA: F test				**	**	**	**	**	**	*	**	**	**	**	**	**	**	**	**	**
l.s.d. $P < 0.05$					0.02	0.05	0.03	1.0	3.5	0.5	0.6	0.2	0.3	15	28	6	10	8	22	99
l.s.d. $P < 0.01$					0.03	0.06	0.04	1.4	4.7	0.6	—	0.3	0.5	21	37	8	14	10	32	132

\*Significant at  $P < 0.05$ . \*\*Significant at  $P < 0.01$ .<sup>A</sup> LF, long fallow; C, cropped; I, gamma irradiated.<sup>B</sup> B, basal nutrients (N, K, S, Ca); Zn, zinc (15 mg/kg); P, phosphorus (50 mg/kg).

grew progressively larger. In irradiated soil with nil or extremely low levels of mycorrhiza, more leaves continued to develop P deficiency necrosis between days 56 (data not shown) and 70. Thus in irradiated cropped soil receiving basal nutrients an average 10.7 out of 12 leaves were necrotic at 70 days resulting in virtually no green leaf area (treatment 17 in Table 5, Fig. 6).

Measures of plant growth (Table 5) were quantitatively similar in unirradiated LF and cropped soils, but this appears to have resulted from a higher soil phosphorus status in LF soil (Table 3) and a higher VAM status in cropped soil (Table 2). Where better P status and better VAM status both occurred in LF soil inoculated with 20% cropped soil (treatments 12-13, Table 4; Fig. 7), plant growth (e.g. area of green leaf, Table 5) was considerably better than in either LF or cropped soil alone (e.g. treatments 2-3 and 7-8). Where the combination of lower P status and no VAM was achieved by irradiating cropped soil, growth was very poor indeed (Fig. 6). In the one replicate of irradiated cropped soil of treatment 16 that developed substantial late colonization with VAM (18.4% of root length), plant growth at 72 days was commensurately better (dry weight of tops four times heavier) than in the other two replicates with only *c.* 1% late colonization. Results with other treatment combinations can be interpreted in terms of their P and VAM statuses.

Zinc fertilizer had little effect on plant growth (Table 5). Generally basal nutrients markedly improved plant growth (Table 5) with some exceptions. Addition of basal nutrients to irradiated long fallow soil without VAM markedly increased the number of P-deficient leaves and reduced plant growth (compare treatment 24 with 23, Table 5). In contrast, basal nutrients improved growth of comparably treated mycorrhizal plants (compare treatment 26 with 25, Table 5).

#### *Effects of VAM and fertilizer treatments on P, N and Zn nutrition*

The concentrations of phosphorus, nitrogen and zinc in plant tops and roots and total uptakes of these elements into tops and roots are given in Table 6. The highest concentrations and uptakes of P at 72 days were obtained with P fertilizer applied where VAM colonization was greatest, i.e. to cropped soil (treatments 9 and 10). Without VAM, plant concentrations and uptakes of P from fertilizer P were about halved (compare treatments 19-20 with 9-10). In general, results of P nutrition for various treatments parallel results for P deficiency symptoms (Table 5) and growth (Table 5). Irradiation of soil to kill VAM resulted in very poor uptake of P (treatments 16-18 and 23-24, Table 6). This effect could be largely overcome by addition of phosphorus fertilizer (treatments 19-20) or by inoculation with unirradiated cropped soil (treatments 21-22 and 25-26) to restore VAM colonization. Uptake of P in unirradiated LF soil was considerably increased by inoculation with cropped soil to increase rate of VAM colonization (compare treatment 12 with 2). If cropped soil inoculum was first irradiated to kill VAM propagules, it had little effect on P nutrition of LF soil (compare treatment 29 with 2), indicating the soil biological nature of the growth promoting factor in cropped soil. Addition of irradiated cropped soil to fallow soil would have increased P uptake if the growth promoting factor in cropped soil were chemical and decreased it if irradiation had induced phytotoxicity.

Plant concentrations and uptakes of zinc were generally greatest where zinc fertilizer had been applied and the plants were most strongly mycorrhizal (treatments

8 and 13 with greater uptake than treatment 3) (Table 6). Unfortunately there was insufficient plant material for zinc analysis at 72 days from treatment 18, i.e. irradiated soil with zinc fertilizer, to use as a mycorrhiza-free control to gauge the full effect of VAM on uptake of zinc fertilizer.

Nitrogen concentration was increased by basal nutrients. Nitrogen uptake was also increased by basal nutrients but only where phosphorus had been supplied or plants were mycorrhizal; e.g. compare treatments 7-8 with 17-18, or compare treatment 26 with 24. Where basal nutrients had been applied in the absence of VAM or P fertilizers, N/P ratios were very wide (57, 63 and 50 for treatments 17, 18 and 24 respectively at 72 days) and growth was poor. Growth of sunflower without VAM in irradiated long fallow soil was depressed by basal nutrients (compare treatment 24 with 23, Table 5), and this seemed related to depression of already low phosphorus concentrations in the plant tops (Table 6, treatment 24, 0.03%; treatment 23, 0.04%). Smith and Smith (1981) also noted depressions in growth of non-mycorrhizal plants caused by addition of nitrates. At the other extreme, mycorrhizal plants without basal nutrients, e.g. treatments 1, 6, 11, 21, and 25, had low nitrogen concentrations and uptakes (Table 6), and their growth (Table 5) probably was primarily limited by lack of nitrogen.

#### *Relative mycorrhizal dependency*

Relative mycorrhizal dependency was calculated at three levels of soil phosphorus, i.e. at 6  $\mu\text{g/g}$  in cropped soil, 17  $\mu\text{g/g}$  in LF soil and at 6  $\mu\text{g/g}$  plus another 50 mg soluble P fertilizer/kg soil. Relative mycorrhizal dependencies, ranging 85-90%, were similar in cropped soil with 6  $\mu\text{g P/g}$  soil (based on treatments 7 or 22 as mycorrhizal and 17 as non-mycorrhizal plants) and in LF soil with 17  $\mu\text{g P/g}$  soil (based on treatments 12 or 26 as mycorrhizal and 24 as non-mycorrhizal plants), even though absolute weights of mycorrhizal plants were greater in LF soil with greater available P. With a high rate of fertilizer phosphorus, RMD was only 6% (based on treatment 9 as mycorrhizal and 19 as non-mycorrhizal plants).

## **Discussion**

In this investigation, the most consistent difference between poor crops after long fallow and better crops after shorter fallow was the poorer colonization of the roots with VAM after long fallow. This difference in mycorrhizal colonization of crops growing in soils that are moderately low in available phosphorus and/or zinc can account for the phenomenon of long fallow disorder. Supporting evidence for a decline in VAM on fallowing has come from three studies in the United Kingdom. Salt (1977) found poorer VAM colonization of wheat grown after fallow than after faba beans in long-term field experiments at Rothamsted Experimental Station. Potatoes grown after long fallow at Leeds, U.K., yielded 20% more when inoculated with adjacent soil containing more VAM spores after a barley crop (Black and Tinker 1977). In a crop rotation experiment, Black and Tinker (1979) found the number of VAM spores and subsequent colonization of barley crops were greater after barley than after breaks of fallow or the non-mycorrhizal crop kale (*Brassica oleracea* L.). Owusu-Bennoah and Mosse (1979) obtained responses to inoculation with VAM from pot cultures, in growth of onions, lucerne and barley on a phosphorus-deficient, long fallow soil at Rothamsted Experimental Station.

Spores were not counted in soil samples taken from under farmers' crops in this investigation because their numbers might have changed from those existing before sowing, probably decreasing during early crop growth (Sutton and Barron 1972) and increasing as the crop matured after a period of maximum root growth (Hayman 1970). Counts done on the soils used in the pot experiment showed fewer spores and less colonized root that could act as propagules (Tommerup and Abbott 1981; Mosse and Thompson 1984) in long fallow soil than in cropped soil. Approximately 90% of all plant species can form VAM (Plenchette 1982) and all the crop species affected with long fallow disorder are known hosts, e.g. chickpea (Subba Rao *et al.* 1986), soybean (Ross 1971), sunflower (Ross and Harper 1973), sorghum and sudan grass (Ferguson and Menge 1982), maize (Gerdemann 1965; Khan 1972) and wheat (Hayman 1970; Khan 1975). There is relatively little host specificity with VAM fungi and almost any strain can colonize a host, e.g., Mosse and Thompson (1984). This is quite relevant to the phenomenon of long fallow disorder because most crop species can break the fallow and promote healthy growth of a subsequent crop.

In the glasshouse experiment, the more rapid mycorrhizal colonization of sunflower roots in cropped soil than in long fallow soil was no doubt a result of the difference between the two soils in propagule density. Sanders and Reed (1978) showed that differences in VAM inoculum density resulted in large differences in development of root colonization of annual crops. A complicating factor in the pot experiment was that the long fallow soil had 11  $\mu\text{g}$  more available P/g soil than the cropped soil. This was not apparently due to the different fallow lengths, for similar differences were not recorded in the samples taken from farmers' fields. Black and Tinker (1977) encountered an opposite difference in their field experiment with fallow soil having about 10  $\mu\text{g}$  less available P/g soil than cropped soil. Despite more available P in LF than in cropped soil, sunflower plants in LF soil developed more P deficiency symptoms of the leaves, until the later developing VAM colonization took effect. Sometimes, crops in the field grow out of long fallow disorder, and this is most likely a response to late development of substantial VAM colonization. When cropped soil was inoculated into LF soil the plants grew vigorously as the better VAM colonization took advantage of the better P supply. This effect could not be reproduced by inoculating with irradiated cropped soil, indicating the soil biological, rather than chemical, nature of the transferable growth-stimulating agent. Hayman and Mosse (1979) also found that both mycorrhizal inoculation and phosphorus fertilizer were required for white clover to grow strongly in hill pastures, and Abbott and Robson (1977) demonstrated that maximum growth difference between mycorrhizal and non-mycorrhizal subterranean clover occurred at moderate rather than very low soil P levels.

The importance of VAM for growth of sunflower in both LF and cropped soil was shown by gamma irradiation of the soil to kill VAM propagules. Virtually all leaves on plants with nil or extremely low levels of VAM and without P fertilizer eventually became necrotic, particularly in cropped soil. Depressions of plant growth from soil sterilization by extreme heating have been ascribed to the generation of toxic levels of various inorganic and organic components; e.g. Rovira and Bowen (1966); Thompson (1974); Smith and Smith (1981). As gamma irradiation of soil at a dose 2.5 times that used here, does not generate these phytotoxins to any marked degree (Bowen and Rovira 1961; Rovira and Bowen 1966), they are unlikely

to have been a factor in the present investigation. Also, addition of irradiated soil to untreated soil did not cause growth depressions indicative of phytotoxins. Smith and Smith (1981), in their experiment on mycorrhizae of subterranean clover grown in mixtures of autoclaved and untreated soil, showed that growth depressions of plants in the autoclaved soil were related to poor phosphorus nutrition in the absence of mycorrhizal colonization. The mycorrhizal and plant growth data from the present experiment demonstrate this again.

In this pot experiment, sunflower was clearly responsive to phosphorus and the beneficial effects of VAM were mediated through improved P nutrition. There was some evidence that VAM improved zinc nutrition of sunflower, but this did not result in growth responses in this soil with 0.7–0.8  $\mu\text{g}$  DTPA-extractable Zn/g soil. In more zinc-deficient soils from the Darling Downs, long fallow disorder of linseed (*Linum usitatissimum* L.) is expressed as both P and Zn deficiencies. In these soils, VAM have been shown to overcome both deficiencies and considerably improve plant growth (Thompson 1986a).

Plant response to VAM was greatest in the presence of the basal nutrient treatment. Similar effects were noted by Verkade and Hamilton (1981), who found that seedlings of the tree species *Liriodendron tulipifera* responded more to mycorrhizal inoculation when given nitrogen fertilizer. No doubt this effect is one of limiting factors, i.e. plants can respond to better phosphorus nutrition resulting from mycorrhizal colonization only if nitrogen is not primarily limiting.

There have been a small number of studies involving sunflower and VA mycorrhizae. Although Ross and Harper (1973) showed sunflower to be heavily mycorrhizal with a strain of *Glomus geosporum*, in subsequent studies differing growth responses to VAM have been obtained. In Pakistan, mycorrhizal sunflower transplanted into the field grew 85% taller than non-mycorrhizal controls (Iqbal and Qureshi 1977), although transplanting caused temporary reductions in per cent colonization with VAM and plant growth (Iqbal and Malik 1978). Graw *et al.* (1979) found sunflower growth was little improved by strong development of mycorrhizae that increased P uptake, and they considered sunflower to be a species that readily obtained phosphorus without mycorrhizae. Furthermore, Koide (1985) found mycorrhizal colonization of sunflower, cv. S304, by *Glomus fasciculatum* depressed leaf area for 7–8 weeks and for the duration of the experiment in soils with respectively 5 and 15  $\mu\text{g}$  bicarbonate-extractable P/g soil by the Olsen method. In contrast, the present study showed sunflower in soil without P fertilizer grew much better with VAM, the RMD ranging 85–90%. Differing results could arise from differences in effectivity of endophyte species (Mosse 1972; Powell 1982), plant cultivar (Hall 1978; Azcon and Ocampo 1981), light intensity (Diederichs 1983) or soil phosphorus level (Mosse 1973b). As the Olsen method extracts less phosphate from vertisols than the Colwell method (J. Burford, personal communication) the present soils are probably more deficient than Koide's (1985) soils. However, even with 50 mg soluble P fertilizer/kg soil in the present study there was no indication of depressions in plant growth caused by VAM.

The lack of evidence for inhibition of VAM by zinc fertilizer (15 mg Zn/kg soil) is a significant finding, as soluble zinc can be fungitoxic and zinc fertilizers are applied at high rates to vertisols. McIlveen and Cole (1979) also found that development of VAM between *G. mosseae* and soybean roots in a silty clay loam soil was enhanced by zinc fertilizer at 18 mg Zn/kg soil but decreased by 45 and 135 mg Zn/kg soil.

The results of this paper indicate that long fallow disorder arises from a decline in number of propagules of natural populations of VAM fungi in the soil, and explain the dynamic nature of the disorder as VAM populations fluctuate in response to agronomic practices. Extreme problems can be reduced by avoiding overlong fallows whenever possible, but even average crops on normal fallows might respond to better colonization with VAM. This new understanding draws attention to the important role that VAM must be playing in the nutrition of a whole range of crop species grown on vertisols in this northern cereal belt, where use of phosphorus and zinc fertilizers is limited. Furthermore, it also suggests that more efficient use of available supplies of phosphorus and zinc from soil and fertilizer sources could be achieved through proper scientific management of natural VAM populations in crop production systems. More comprehensive research on managing natural populations is warranted. This seems the best strategy at present, but artificial inoculation with selected VAM cultures might also be a valuable practice in these soils if future technology makes inoculum production feasible.

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