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The effect of high temperature and fallow period on infection of mung bean and cashew roots by the vesicular-arbuscular mycorrhizal fungus *Glomus intraradices*

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Abstract

The experiments described were designed to investigate the way in which high temperatures (30°C and above) affected the survival and infectivity of spores of *Glomus intraradices* formulated as the commercial inoculum NutriLink™. Infection of mung bean (*Vigna radiata*) occurred most rapidly at 30°C compared with either 22° or 38°C, although the final percentage of the root length infected (6 weeks) was similar at all three temperatures. Early rapid infection led to greater plant growth of this species at 30°. In cashew (*Anacardium occidentale*) no infection occurred at 38°C and this was associated with low plant growth, compared with the other temperatures at which infection reached 40–60% after 4 months. In both species differences in root temperature were associated with marked differences in the morphology and growth of the root systems, with poor root growth at 38°C.

Spores of *G. intraradices* retained infectivity with respect to mung bean for up to 6 weeks in moist fallow soil, although maximum infectivity was observed in soil without a fallow period. The effects of temperature on germination of spores buried in filter paper sandwiches in soil were consistent with the data for infection and growth. Germination was most rapid and reached the highest percentage at 3 weeks at 30°C. Lowest germination was attained at 38°C. We conclude that *G. intraradices* can retain its infectivity in moist soil at high temperatures, but that the extent to which the plants become infected and hence their response, depends not only on this but also on host factors such as root growth.

Introduction

In regions with high seasonal maximum temperatures, the soil in the rooting zone may reach and remain at high temperatures for several months of the year. These high temperatures may affect both root growth and the survival and infectivity of root-infecting microorganisms, including mycorrhizal fungi. One example of high soil temperatures is in northern Western Australia, near Kununurra, where temperature probes, 50 cm below the surface of bare black clay, registered mean temperatures of 36°C or above for 5 months of 1988 (Western Australia Department of Agriculture, personal communication). The success of mycorrhizal inoculation of crops under these conditions will depend on the ability of the fungus to survive as propagules, under both wet and dry conditions, and to form mycorrhizal associations at high temperatures.

The primary crop of interest in the current study is cashew (*Anacardium occidentale* L.), but

the results will be relevant to any crops that depend on mycorrhizal infection for maximum yield.

In assessing the suitability of a mycorrhizal isolate to high temperature, the effect of temperature on both survival and germination of propagules needs to be considered. Previous work has shown that the propagules of some isolates of VA mycorrhizal fungi can survive in dry soil at temperatures up to 70°C and subsequently infect roots of seedlings of ephemeral plants, following rain (McGee et al., 1987). In moist soil survival of propagules is likely to be lower unless susceptible plants are present, and marked reductions in the populations of infective propagules occur if moist soil remains fallow for long periods (Thompson, 1987).

Isolates and species of VAM differ in their germination response to temperature. This was demonstrated over the temperature range of 15° to 34°C by Schenck et al. (1975), who found that spores of an isolate of *Gigaspora heterogama* from Florida did not germinate at or below 20°C, but showed maximum germination at 34°C. In contrast, an isolate of *Glomus mosseae* from Washington showed maximum germination at 20°C and very low germination at 34°C. Other studies have also shown that isolates may differ in their optimum temperatures for spore germination, root colonization, and spore production (Graham et al., 1982; Tommerup, 1983). Furthermore, a single isolate may have a different optimum temperature for colonization than for spore production (Schenck and Smith, 1982).

Successful use of the commercially available isolate of *G. intraradices* (NutriLink™) at Kununurra will depend, in part, on its ability to survive high temperatures both in potting mixes under nursery conditions and in field soil after outplanting of inoculated plants. It appears that *G. intraradices* can survive relatively high temperatures both with respect to survival of spores and infection of roots. Nemec (1987) demonstrated that although a 1 hour treatment at 43°C and above (moisture conditions not stated) reduced the ability of spores of *G. intraradices* to subsequently infect plants, the spores did not become completely non-viable unless the temperature was raised to 60°C or above.

There have been a number of investigations of the effects of temperature on the infection of roots by mycorrhizal fungi (e.g. Furlan and Fortin, 1973; Hayman, 1974; Smith and Bowen, 1979), but only a few have considered temperatures above 30°C (e.g. Smith and Roncadori, 1986). They showed that although maximum root colonisation of cotton by *G. intraradices* occurred at 36°C, optimum growth response occurred at 30°C, indicating possible complex interactions between survival and germination of propagules and plant processes, such as root growth.

The purpose of the experiments described below was to explore further the ability of the commercial isolate of *G. intraradices* to survive in potting mix and subsequently colonize plant roots at the high temperatures likely to be encountered on the Voyager Enterprises site at Kununurra. Experiments were carried out on mung bean (*Vigna radiata* L.) and cashew. Spore germination at different temperatures was also examined.

Materials and methods

Experimental design and treatments

Experiment 1. The effect of temperature on infectivity

To determine the effect of temperature on the ability of *G. intraradices* to infect plant roots, plants were grown in pots of Soil Mix 1 with or without inoculum (see below) placed in temperature tanks sited in a glasshouse (see below) to maintain the soil temperature at 22°, 30°, or 38°C. The pots were arranged in a randomized complete block design, with blocking on temperature. The assumption was made that the effect of temperature was the main cause of variation induced by block. There were 5 replications of each of the 6 treatments.

Three hundred grams of soil mix alone (control) or soil mix plus 10 g inoculum mixed throughout, were placed in 300 mL non-draining pots and watered with 90 mL H_2O. Mung bean seed was surface sterilized, soaked overnight, then pregerminated on water agar at 30°C for 2 days. Appropriate inoculum of *Rhizobium* was added to the seed prior to planting.

Germinated seed was planted into the pots, which were then placed in soil temperature tanks in an unshaded glasshouse (summer conditions) and allowed to grow for 2 or 6 weeks. Air temperatures reached as high as 48°C on very hot days. Night air temperatures were as low as 15°C. Mean daily maximum light intensity was approximately 900 μmol m^{-2} s^{-1}. Pots were watered to weight 3 times per week.

Experiment 2. The effect of temperature on infectivity

The design and methods were as in Experiment 1, except that plants were grown under winter conditions and to facilitate the separation of roots from soil mix, a modified soil mix (Soil Mix 2) was used. Air temperatures ranged from a night time minimum of 10°C to a daytime maximum of 25°C. The mean daily maximum light intensity was low (less than 450 μmol m^{-2} s^{-1}), as the glasshouse was coated with whitewash. Growth rate was low compared to Experiment 1 and the plants were harvested at 3 weeks.

Experiment 3. The effect of fallow temperature and time on infectivity

To determine the ability of *G. intraradices* to survive moist fallow in potting mix and subsequently to infect plant roots, pots with or without inoculum were placed at the 3 temperatures and and left unplanted (fallow) for 6, 3, 1, or 0 weeks prior to plant growth. The fallow periods were stagger-started so that all pots would be ready for planting with the test plant at the same time. After fallow, all temperature tanks were reset to 30°C and the pots were reassigned to the tanks in a randomized complete block design, with one or two replications per water bath. The block effect was confounded with the combined effects of starting date, batch of seed germination and tank to which the replication was assigned. Each treatment was replicated 5 times. After the fallow period mung bean seedlings were planted into the pots. Mycorrhizal infection and plant growth were measured after 3 weeks.

This experiment was conducted under similar glasshouse conditions to Experiment 2, except that light intensity was higher (mean daily maximum light intensity ranged from approximately 475 to 525 μmol m^{-2} s^{-1} over the 5 replications), as whitewash had been removed. Soil Mix 2 was used.

Experiment 4. The effect of soil temperature on cashew growth and mycorrhizal infection

To determine the effect of high temperature on cashew root growth and the formation of mycorrhizal associations with *G. intraradices*, cashew seedlings were grown with inoculum at root temperature of 22°, 30° or 38°C for 4 months. Each treatment was replicated 4 times.

Cashew seeds were surface sterilized for 20 minutes in 70% ethanol, soaked overnight in sterile water, then placed in moist sand at 30°C until germinated. Germinated seeds were planted into 1 L pots, which were then placed at the 3 temperatures. Each pot contained 1160 grams of Soil Mix 2, 40 g of inoculum mixed throughout, and 125 mL of water. Pots were watered to weight with reverse osmosis water 3 times per week.

Experiment 5. The effect of soil temperature on spore germination

Two experiments were carried out to determine the effect of temperature on spore germination. Spores were placed on filters buried in Soil Mix 1 at three soil temperatures for 6, 11, 16, or 21 days (see below). Each treatment was replicated 4 times.

Soil mixes

Soil mix 1 was 4:4:1 sand:peatmoss:perlite plus nutrients (0.20 kg $Ca(NO_3)_2$, 0.20 kg KCl, 0.10 kg $MgSO_4$ $7H_2O$, 0.06 kg $(NH_4)_2HPO_4$, 0.02 kg $MnSO_4$, 0.02 kg $FeSO_4$ $7H_2O$, 0.02 kg $CuSO_4$ $5H_2O$, 0.02 kg $ZnSO_4$ $7H_2O$, 0.018 kg $Na_2B_4O_7$ $10H_2O$, and 0.02 kg Na_2MoO_4 $2H_2O$) per 900 L of soil mix.

Soil mix 2 contained 2:1:1 sand:loam:perlite plus nutrients. Each pot contained 360 g of potting mix with or without inoculum plus 40 mL H_2O.

pH of the soil mixes was measured as follows: 100 mL of water was added to 40 g of soil mix, stirred, allowed to stand for 30 minutes, restirred and allowed to stand for a further 30 minutes. A pH reading was then taken using an

all-purpose calomel electrode. The pH of Soil mix 1 was approximately 6.4, that of Soil Mix 2 approximately 7.0 to 7.5.

Temperature tanks

The controlled temperature tanks were 300 L capacity stainless steel lined waterbaths. Each tank had a circulating pump, a refrigeration unit and a thermostat which held the water at a constant temperature (±1.5°C). Each tank had a styroform sheet over the water surface, with pots inserted through holes in the sheet. This shielded the above-ground portion of the plants from the influence of waterbath temperature.

The tanks were located adjacent to each other in the same glasshouse. Since tanks controlled only soil temperature, and the above-ground portion of all plants were subject to the same light, air and temperature conditions, it is biologically valid to assume that the majority of any effect on plant growth due to tank assignment was due to soil temperature.

Inoculum

The inoculum used was a commercial preparation of spores of *Glomus intraradices* in attapulgite clay, produced by NPI under the name NutriLink. The inoculum contained at least 1000 spores per gram.

Spore germination

Spores were separated from the clay carrier as follows: inoculum of *G. intraradices* (NutriLink) was suspended in water and poured over a 53 mm sieve. Spores and clay particles retained on the sieve were layered over a sucrose gradient (20% sucrose over 60% sucrose) and centrifuged for 3 minutes at approximately 1000 × g. The middle layer containing most of the spores was pipetted off and washed on a 53 mm sieve. The spores were transferred to a nematode counting dish.

Spores were placed, 3 per filter, on Millipore type RA filters (pore size 1.2 μm) (Tommerup, 1983). The filter sandwiches were buried (4 per pot) in pots of moistened soil mix as in Experiment 1. Data for presence or absence of germination on each filter and % spores germinated were collected. Two experiments were carried out with harvests at 6, 11, 16 and 21 d. The temperatures used were 22°, 30° and 38°C and 22°, 28° and 37°C in the two experiments respectively. Equipment failure resulted in data at 30°C only being available for the 6 and 11 d harvests. Each time and temperature treatment was in a separate pot. At each sampling time, the 4 filters from each of 4 replicate pots for each of the 3 temperature treatments were stained overnight with 0.063% trypan blue in lactic acid : glycerol : water. The filters were blotted dry and separated, and germination determined under a dissecting microscope.

Data collection and analysis

Measurements of shoot height and fresh weight were recorded at the time of sampling. The shoot portions of plants were dried overnight at 80°C, then dry weights were recorded. In the mung bean experiments, the fresh weight of the root system was recorded and the entire root system was preserved for determination of mycorrhizal infection and root length. In cashew experiments, observations were made of root morphology and a subsample of the roots was taken for evaluation of mycorrhizal infection.

Mung bean roots were cleared in 10% KOH and stained with 0.05% trypan blue in lactophenol (Philips and Hayman, 1971). Root length and mycorrhizal infection were determined using the grid intersect method under a dissecting microscope (Tennant, 1975).

A subsample of 40 root pieces 1 cm long was used to evaluate cashew mycorrhizas. Cashew roots are very dark in colour and difficult to clear. The methods previously reported for cashew (Bepaiah et al., 1989; Krishna et al., 1983) were based on modifications of the method of Phillips and Hayman (1970). However, modified methods of Brundrett et al. (1984) were found to be more appropriate in the current experiment. Root pieces were cleared in 10% KOH at 80°C for 3 to 5 hours, then bleached with 1 *N* HCl for 3 minutes. The pieces were then stained for 0.5 to 1 hour at 80°C with 0.1% chlorazol Black E in 1:1:1 water:lactic acid : glycerol. The pieces were rinsed and allowed to

stand in 50:50 lactic acid:glycerol at least overnight. The pieces were bleached with 3% NaOCl at room temperature until roots were creamy white (<5 minutes) then rinsed thoroughly. The root pieces were then re-stained with 0.1% Chlorazole Black E at 80°C for up to 1 hour. The roots were stored in 50% glycerol until evaluation.

The NaOCl step was necessary to bleach or remove the dark pigments in the cashew roots. However, it also affected the subsequent staining. The addition of a staining step prior to bleaching, followed by restaining, overcame this problem. Infection was evaluated using a modification of the method of Trouvelot et al. (1986).

All data except spore germination were analyzed by analysis of variance within the general linear models procedure of SAS. Significant differences were determined at $\alpha = .05$. The spore germination data was binomially distributed, and Genstat was used in the analysis of this data.

Results

Experiment 1. The effect of temperature on infectivity (summer conditions)

Table 1 shows that after 2 weeks of growth, inoculated mung bean plants had significantly ($p = 0.001$) greater shoot dry weight than noninoculated plants at 30°C, but not at 22°C and 38°C. This growth response was associated with a significantly ($p = 0.05$) greater fraction of the root length infected by mycorrhizas at 30° than at the other temperatures.

After 6 weeks growth, shoots of inoculated plants were significantly ($p = 0.001$) larger than noninoculated plants at 30° and 38°, but not at 22°C (Fig. 1b). The roots of plants at all 3 temperatures were approximately 60 to 65% infected, with no significant differences (Table 1).

In the absence of mycorrhizal inoculum, soil temperature had no significant effect on shoot dry weight after either 2 weeks or 6 weeks (Table 1).

Table 1 also shows that in noninoculated plants, root growth decreased significantly with

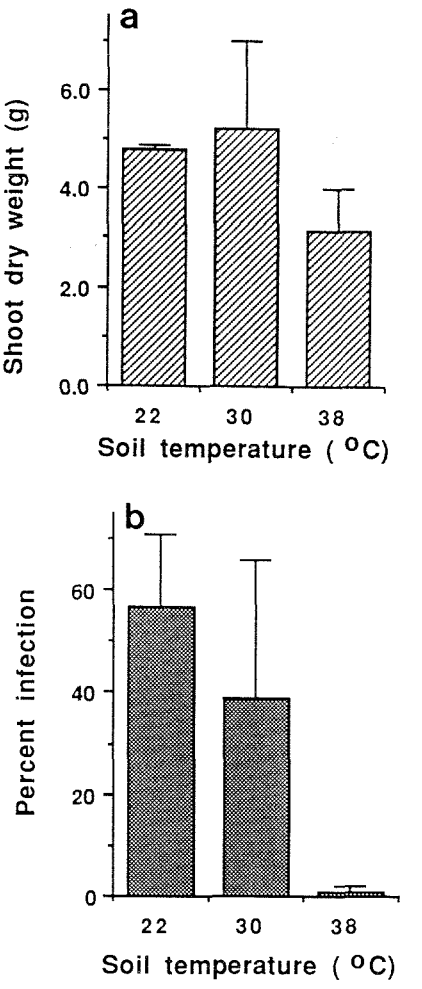

Fig. 1. Experiment 4. The effect of soil temperature on **(a)** shoot dry weight and **(b)** mycorrhizal infection of inoculated cashew seedlings. Means and standard deviations of means.

increasing temperature. Inoculation significantly increased root growth (fresh weight) within 2 weeks. Root fresh weight was not accurately determined after 6 weeks growth, since the extensive root systems could not be separated adequately from the potting mix.

Root morphology was affected by temperature. At 22°C, roots were coarse and extended to the bottom of the pot. At 38°, roots were fine and concentrated in the upper 1/3 of the pot. At 30°C, root development was intermediate, with both fine and coarse roots and with fairly extensive root development throughout the pot.

Table 1. Experiment 1. The effect of soil temperature and the presence (+) or absence (−) of mycorrhizal inoculum on mung bean growth and mycorrhizal infection after 2 and 6 weeks. Means and standard deviations of means

Plant age	2 weeks						6 weeks					
Soil temperature (°C)	22		30		38		22		30		38	
Inoculum	+	−	+	−	+	−	+	−	+	−	+	−
Infection (%)	16.4	−	55.9	−	23.2	−	62.4	−	65.2	−	60.5	−
SD	3.1		4.3		3.8		3.7		5.6		5.5	
Shoot dry wt (g)	0.14	0.14	0.21	0.14	0.18	0.15	0.79	0.63	1.17	0.45	0.83	0.52
SD	0.02	0.01	0.02	0.04	0.02	0.03	0.12	0.21	0.34	0.11	0.17	0.11
Root fresh wt (g)	1.74	1.56	1.87	1.41	1.43	0.93	–	–	–	–	–	–
SD	0.24	0.11	0.24	0.26	0.29	0.31						

The differences in root morphology were quantified using the ratio between root length and root fresh weight. The length per g of root increased with increasing temperature, with significantly lower values at 22° (450 cm g^{-1}) than at 30° or 38°C (826 and 751 cm g^{-1}, respectively). This corresponds to the observed coarser morphology at 22°C. The ratio between root length and weight was not significantly different between 30° and 38°C.

Experiment 2. The effect of temperature on infectivity (winter conditions)

Under winter growing conditions, the percentage of root length infected in inoculated plants was much lower than in Experiment 1. The percentages of root length which were infected at 30° and 38° were not different from each other, but were significantly ($p = 0.01$) greater than at 22°C (Table 2).

Table 2. Experiment 2. The effect of soil temperature and presence (+) or absence (−) of mycorrhizal inoculum on mung bean growth and mycorrhizal infection after 3 weeks under winter conditions. Means and standard deviations of means

Soil temperature (°C)	22		30		38	
Inoculum	+	−	+	−	+	−
Infection (%)	11.3	−	27.6	−	29.1	−
SD	5.2		7.9		7.3	
Shoot dry wt (g)	0.06	0.05	0.08	0.08	0.08	0.08
SD	0.02	0.01	0.03	0.01	0.02	0.02

Shoot growth was not significantly affected by inoculation, but was increased by increasing soil temperature (Table 2). Root fresh weight was not significantly affected by any treatment.

Root morphology and the ratio between root length and weight followed similar patterns to Experiment 1 (372, 784 and 787 cm g^{-1} at 22°, 30° and 38°C, respectively).

Experiment 3. The effect of fallow temperature and time on infectivity

Results are presented in Table 3. The maximum mean level of infection (33.8%) was observed after a fallow period of one week at 38°C, but was not significantly different from the level observed without fallow pretreatment. The latter, therefore, can be taken as the maximum level of infection for the plant growth conditions.

At a soil temperature of 30°C, increasing fallow time caused a significant ($p = 0.01$) decrease in the subsequent percentage infection. The percentage infection which occurred following fallow at 22°C was not significantly different from the corresponding levels of infection following fallow at 30°C at all fallow times. In contrast, the levels of infection which occurred following fallow at 38°C were not significantly decreased by fallow of up to six weeks, and were not significantly different from the maximum level of infection.

Inoculation did not have a significant effect on plant growth following any fallow treatment, so growth data are presented for only the inoculated plants. There was no increase in growth corresponding to increased levels of infection.

Table 3. Experiment 3. The effect of fallow temperature and length of fallow time on subsequent mycorrhizal infection and growth of mung bean plants grown for 3 weeks in inoculated soil mix under winter conditions

Fallow time (weeks)	0			1			3			6		
Fallow temp. (°C)	22	30	38	22	30	38	22	30	38	22	30	38
Infection (%)	–	31.8	–	30.1	28.9	33.8	24.4	19.1	30.2	11.9	10.0	29.4
SD		11.6		5.8	6.4	13.1	8.3	4.2	13.1	8.4	6.4	8.7
Shoot dry wt (g)	–	67.4	–	84.0	80.0	81.4	72.0	87.8	91.4	82.6	84.8	93.8
SD		22.1		22.5	19.5	28.1	14.4	18.6	19.7	19.2	21.3	40.9

Experiment 4. The effect of soil temperature on cashew growth and mycorrhizal infection

Figure 1 shows that soil temperature affected both the growth of cashew plants (Fig. 1a) and the level of mycorrhizal infection which occurred (Fig. 1b). Plants grown at 38°C were significantly smaller (Duncan's multiple range test) than plants grown at 30°, though plants grown at 22° were not significantly different from plants grown at either 30° or 38°C.

The level of infection was much lower in plants grown at a soil temperature of 38° (mean infection $< 1\%$) than at either 22° or 30° (mean infection 57% and 39%, respectively). The levels of infection which occurred at 30° and 38° were not significantly different from each other (Fig. 1b).

Morphology of cashew root was significantly affected by temperature. At 38°, the lateral root system was very finely branched, with short interbranch distances (a few mm to 1 cm). The roots were very brittle and the root system appeared poorly developed. At 22°, the interbranch distances were comparatively long (>1.5 cm). The roots were stringy and formed a fairly open network through the pot. The lateral branching structure at 30° was intermediate between the patterns at 22° and 38°C. Generally, the roots at 30°C were more extensive than at 22° or 38°C, forming a dense mat throughout the pot. One plant at 30° had very poor root development compared to the other plants; this plant had no mycorrhizal infection.

Experiment 5. The effect of temperature on spore germination

Inspection of data from both experiments as % filters with at least one spore germinated, compared with % germination of the whole spore population gave no indication that there was a synergistic effect caused by the presence of 3 spores per filter. This was not examined statistically. The maximum percentage germination in the two experiments differed, being 90 and 75% in the first and second respectively, presumably reflecting loss of viability during the 4 month storage period between the experiments. The same overall trends were apparent in the two experiments, with the most rapid germination occurring at the intermediate temperature and the lowest % germination at the final sampling time at the highest temperature. Figure 2 shows the results for the second experiment, in which there were highly significant ($p < 0.001$) effects of both time and temperature on the % germination.

Auxilliary cells or secondary spores, similar to the vesicle-like structures described on hyphae of germinated *Endogone* spores by Mosse (1959),

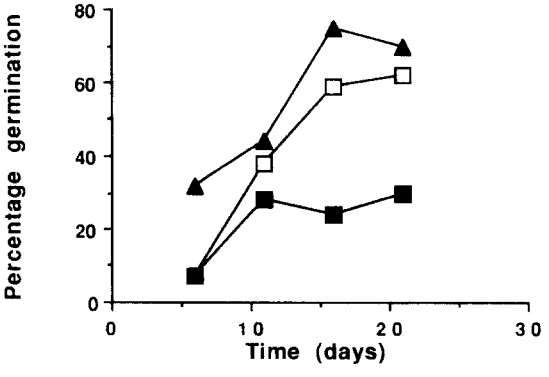

Fig. 2. The effect of soil temperature on the percentage of spores germinated after different times. □, 22°C; ▲, 28°C; ■, 37°C. The main effects of time and temperature were significant at $p < 0.001$.

were observed within 6 days at 38°, and within 11 days at all temperatures.

Discussion

Extended periods of fallow in moist soil were expected to lead to a decrease in ability of mycorrhizal spores to infect plants subsequently grown in the soil. The maximum extent of infection was observed in plants grown in soil which had not had a fallow treatment, but the results from Experiments 1 through 3 clearly show that *G. intraradices* maintains its infectivity with respect to mung bean at high temperatures, and that fallow for up to 6 weeks does not significantly diminish this. Considering these results, it is quite surprising that mycorrhizal associations were almost eliminated at 38°C in cashew. This could reflect either the effect of extended high temperatures on the fungus or the effect of high temperature on the susceptibility of cashew roots to mycorrhizal infection (see below).

Comparison of infection data at 2 weeks and 6 weeks in Experiment 1 shows that temperature did not affect the final level of mycorrhizal infection, but did affect the time taken to reach that level. The corresponding data for shoot dry weight make it clear that although levels of infection were equivalent at 6 weeks, the earlier infection at 30°C resulted in a much greater growth response than had occurred at 22° or 38°C. This supports previous observations that early infection is an important determinant of the magnitude of response to mycorrhizal infection (e.g. Abbott and Robson, 1981; Menge, 1983; Smith et al., 1979).

The levels of infection attained in mung bean were markedly higher under summer conditions of high light intensity and high air temperatures than under winter conditions of low light intensity and cool air temperatures as shown previously (Hayman, 1974). Growth response to mycorrhizal infection was also reduced under winter conditions. This is consistent with the frequent observation that under low light intensity mycorrhizal infection may be supressed and growth responses reduced or eliminated (Graham et al., 1982; Hayman, 1974; Smith and Gianinazzi-Pearson, 1990; Son and Smith, 1988). In contrast, Furlan and Fortin (1977) found that a low light regime was associated with earlier infection and earlier onset of a growth response; our results do not confirm their observations. The interactions of light intensity with plant growth and mycorrhizal infection are complex (see Tester et al., 1986) and are further affected by phosphate supply (Graham et al., 1982; Smith and Gianinazzi-Pearson, 1990; Son and Smith, 1988). It is clear that light intensity, as well as other conditions, must be carefully controlled to obtain consistent results in mycorrhizal experiments. This has implications for the extrapolation of results from experiments done under controlled conditions to field situations, where conditions may fluctuate on both daily and seasonal bases.

It is difficult to compare the results of Experiments 1 and 2 directly, because after 3 weeks growth the plants in Experiment 2 had not attained the size or stage of development which had occurred within 2 weeks in Experiment 1. However, the lack of growth response to inoculation in Experiment 2 is consistent with the lower levels of infection which occurred in that experiment, compared with Experiment 1 in which marked growth differences were observed. Lower levels of infection in Experiments 2 and 3 compared with Experiment 1 may have been due to the higher pH (7.0 to 7.5 in Experiments 2 and 3, 6.4 in Experiment 1). Nutrilink is recommended for use in soils of pH 6.0 to 7.0.

Tommerup (1983) demonstrated that spores of *Glomus caledonium* could maintain the capacity to infect plants for up to 8 weeks under moist fallow conditions, though the capacity was greatly reduced after 4 weeks. Despite the fact that plants grew relatively slowly in Experiment 3, the data for percentage infection of the roots clearly demonstrate that time and temperature of fallow had an effect on the survival and infectivity of *G. intraradices*. A possible explanation for the higher infection following fallow at 38°C than following fallow at other temperatures is that spore germination may have been delayed at this temperature, so that a larger number of spores remained infective at the time of planting. Data from the second spore germination experiment would support this. Alternatively, the spores may have germinated early in the fallow period

and yet maintained their infective capacity better at 38° than at other temperatures. This infective capacity may be retained by the original spores; spores of the mycorrhizal fungus *Gigaspora gigantea* were able to germinate up to 10 times after germ tubes were severed (Koske, 1981). The role of the secondary spore-like structures in survival and infection is unknown. The undisturbed network of hyphae in the fallowed potting mix may also be a considerable source of inoculum (see Jasper et al., 1989) and may have added to the infective capacity of the inoculum. Soil microorganisms which reduce the longevity of mycorrhizal hyphae in the soil may have been inhibited by the high temperatures. Menge (personal communication, 1991) has observed that mycorrhizal fungi are generally more tolerant of high temperatures than competing microorganisms in the soil.

This investigation highlights the need to consider root physiology and growth as well as fungal survival. Temperature affected root growth, morphology and distribution in the pots in both mung bean and cashew. For cashew it seems highly probable that failure of infection at 38°C was linked to the very poor root growth at that temperature, as it is clear that fungal infectivity was not adversely affected. Further investigation of this problem would be worthwhile as it has practical significance for the establishment of cashew in soils at high temperature. This work would require the plants to be grown in very large pots, so as to avoid physical restriction of the roots.

In summary, *Glomus intraradices* (as spores in Nutrilink) retains infective capacity with respect to mung bean after 6 weeks incubation at 38°C in moist fallow soil. It would therefore be suitable for inoculation of crops in soils of high temperature, as long as the root growth of the crops was not adversely affected by those temperatures. However, persistence of inoculum for longer periods in field soils under bare fallow has not been established. It seems likely that, as with indigenous mycorrhizal fungi in other habitats, long, bare fallow would result in severe decline in the population of propagules and consequent failure of infection in field crops (see Thompson, 1987). Survival in the field involves not only spores, but also infected root fragments and hyphae and we have not examined their response to fallow or high temperatures in the current work.

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References

Abbott L K and Robson A D 1981 Infectivity and effectiveness of vesicular-arbuscular mycorrhizal fungi: Effect of inoculum type. Aust. J. Agric. Res. 32, 631–639.

Brundrett M C, Piché Y and Peterson R L 1984 A new method for observing the morphology of vesicular-arbuscular mycorrhizae. Can. J. Bot. 62, 2128–2134.

Furlan V and Fortin J A 1973 Formation of endomycorrhizae by *Endogone calospora* on *Allium cepa* under three temperature regimes. Nat. Canad. 100, 467–477.

Furlan V and Fortin J A 1977 Effects of light intensity on the formation of vesicular-arbuscular mycorrhizas on *Allium cepa* by *Gigaspora calospora*. New Phytol. 79, 335–340.

Graham J H, Leonard R T and Menge J A 1982 Interation of light intensity and soil temperature with phosphorus inhibition of vesicular-arbuscular mycorrhizal formation. New Phytol. 91, 683–690.

Hayman D S 1974 Plant growth responses to vesicular-arbuscular mycorrhiza. IV. Effect of light and temperature. New Phytol. 73, 71–80.

Jasper D A, Abbott L K and Robson A D 1989 Soil disturbance reduces the infectivity of external hyphae of vesicular-arbuscular mycorrhizal fungi. New Phytol. 112, 93–99.

Koske R E 1981 Multiple germination by spores of *Gigaspora gigantea*. Trans. Br. Mycol. Soc. 76, 328–330.

McGee P A, Smith S E and Smith F A 1987 The survival of VA mycorrhizal propagules in arid environments. *In* Mycorrhizae in the Next Decade. p 152. Eds. D M Sylvia, L L Hung and J H Graham. Institute of Food and Agricultural Sciences, University of Florida, Gainsville, FL.

Menge J A 1983 Utilisation of vesicular-arbuscular mycorrhizal fungi in agriculture. Can. J. Bot. 61, 1015–1024.

Mosse B 1959 Observations on the extramatrical mycelium of

a vesicular-arbuscular endophyte. Trans. Br. Mycol. Soc. 42, 439–448.

Nemec S 1987 Effect of storage temperature and moisture on *Glomus* species and their subsequent effect on citrus rootstock seedling growth and mycorrhiza development. Trans. Br. Mycol. Soc. 89, 205–212.

Phillips J M and Hayman D S 1970 Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. Trans. Br. Mycol. Soc. 55, 158–160.

Schenck N C and Smith G S 1982 Responses of six species of vesicular-arbuscular mycorrhizal fungi and their effects on soybean at four soil temperatures. New Phytol. 92, 193–201.

Schenck N C, Graham S O and Green N E 1975 Temperature and light effects on contamination and spore germination of vesicular-arbuscular mycorrhizal fungi. Mycologia 67, 1189–1192.

Smith G S and Roncadori R W 1986 Responses of three vesicular-arbuscular mycorrhizal fungi at four soil temperatures and their effects on cotton growth. New Phytol. 104, 89–95.

Smith S E and Bowen G D 1979 Soil temperature, mycorrhizal infection and nodulation of *Medicago truncatula* and *Trifolium subterraneum*. Soil Biol. Biochem. 11, 469–473.

Smith S E and Gianinazzi-Pearson V 1990 Phosphate uptake and arbuscular activity in mycorrhizal *Allium cepa* L.: Effects of photon irradiance and phosphate nutrition. Aust. J. Pl. Physiol. 17, 177–188.

Smith S E, Nicholas D J D and Smith F A 1979 The effect of early mycorrhizal infection on nodulation and nitrogen fixation in *Trifolium subterraneum*. Aust. J. Pl. Physiol. 6, 305–311.

Son C L and Smith S E 1988 Mycorrhizal growth responses: Interactions between photon irradiance and phosphorus nutrition. New Phytol. 108, 305–314.

Tennant D 1975 A test of a modified line intersect method of estimating root length. J. Ecol. 63, 995–1001.

Tester M., Smith S E, Smith F A and Walker N A 1986 Effects of photon irradiance on the growth of shoots and roots, on the rate of initiation of mycorrhizal infection and on the growth of infection units in *Trifolium subterraneum* L. New Phytol. 103, 375–390.

Thompson J P 1987 Decline of vesicular-arbuscular mycorrhizae in Long Fallow disorder of field crops and its expression in phosphorus deficiency of sunflower. Aust. J. Agric. Res. 38, 847–867.

Tommerup I C 1983 Temperature relations of spore germination and hyphal growth of vesicular-arbuscular mycorrhizas in soil. Trans. Br. Mycol. Soc. 81, 381–387.

Trouvelot A, Kough J L and Gianinazzi-Pearson V 1986 Mesure du taux de mycorhization VA d'un systeme radiculaire. Recherche de methodes d'estimation ayant une signification foncitionelle. *In* Physiological and Genetical Aspects of Mycorrhizae. Eds. V Gianinazzi-Pearson and S Gianinazzi. pp 217–221. INRA, Paris.