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Arbuscular mycorrhiza colonization and development at suboptimal root zone temperature

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Abstract Temperature has a strong influence on the activity of living organisms. This study, involving two indoor experiments, evaluated the effects of root zone temperature (10, 15 and 23°C) on the formation and development of arbuscular mycorrhizae (AM). In the first trial, greenhouse-grown sorghum [*Sorghum bicolor* (L.) Moench] was either colonized by *Glomus intraradices* Schenck & Smith or left non-mycorrhizal. Root length, root and shoot weight and root colonization were measured after 5, 10 and 15 weeks of plant growth. Although suboptimal root zone temperatures reduced growth in both mycorrhizal and non-mycorrhizal plants, mycorrhizal plants were larger than non-mycorrhizal plants after 15 weeks at 15 and 23°C. At suboptimal root zone temperatures, mycorrhizal inoculation sometimes slightly reduced root development. AM colonization was more affected than root growth at suboptimal root zone temperatures. Colonization was markedly reduced at 15°C compared with 23°C, and almost completely inhibited at 10°C. The second experiment was conducted in vitro using transformed carrot (*Daucus carota* L.) roots supporting *G. intraradices*. Mycelium length and spore number were measured weekly for 15 weeks. Spore metabolic activity (iodonitrotetrazolium reduction), root length and percentage root colonization were measured after 15 weeks. *G. intraradices* sporulation was reduced at temperatures below 23°C, while spore metabolic activity was significantly reduced only at 10°C. Root length and in particular percentage colonization were decreased at suboptimal temperatures. A negative interaction between AM hyphal growth and root growth resulting in reduced probability of contact at suboptimal root zone temperatures is proposed to explain the greater reduction observed in root colonization than in root and hyphal growth.

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Introduction

Soil environmental conditions as well as plant nutrient level, light intensity and cropping systems affect the development of arbuscular mycorrhizal (AM) fungi and the formation of mycorrhizae (Evans and Miller 1990; Furlan and Fortin 1977; Jasper et al. 1989a, 1989b, 1991; Reinharts et al. 1994). Of the factors influencing mycorrhizal development and function, temperature is important (Bowen 1991; Fabig et al. 1989; Jakobsen and Andersen 1982). Mycorrhizal development is usually optimal, at least in plants of cool temperate climates, at 20–25°C (Matsubara et al. 2000; Zhang et al. 1995) and maximal spore germination occurs between 20°C and 28°C, depending on the species (Wang et al. 1997). These temperatures are much higher than those commonly encountered in the field in spring in cool climates.

AM fungi may benefit plants even at sub-optimal temperature. Charest et al. (1993) reported that mycorrhizae counteract chilling injury in maize (*Zea mays* L.). AM colonization increased nodule size and leaf N concentration in soybean grown at a root zone temperature of 15°C (Zhang et al. 1995). Mycorrhizal leek plants exposed to the same root zone temperature were better able to absorb ^{32}P from soil than non-mycorrhizal plants (Wang et al. 2002). In contrast, AM fungi did not improve the shoot or root growth of cotton (*Gossypium hirsutum* L.) at 18°C (Smith and Roncadori 1986).

In northern areas, the absence of plants in agricultural soils over winter as well as tillage operations can influence the formation of mycorrhizae in the next crop (Kabir et al. 1997, 1998). Any structures serving as propagules in agricultural soils under cool climates must be able to survive the winter months. Addy et al. (1994) reported that hyphae detached from roots of plants can survive winter temperatures and colonize plants the

following spring. AM fungi, or at least some of their structures, are quite resistant to cold temperatures. For example, AM spores and mycelium from 20 glomalean isolates from soil and root debris survived in storage at -80°C (Kuszala et al. 2001). To maximize benefit to plants in cool climates, AM fungi must remain viable for extended periods at low temperatures and retain infectiveness at cool soil temperatures in order to develop rapidly after seeding in spring. The stimulation of AM spore germination by a period of exposure to low temperature (Juge et al. 2002) suggests that some AM fungi possess mechanisms of dormancy that synchronize their activity to the seasonal cycle of cool climate ecosystems. Therefore, understanding the basic physiological responses of AM fungi to suboptimal root zone temperatures is of great importance.

Temperature strongly influences the physiology of living organisms and low temperatures are expected to markedly influence plant roots and mycorrhizal fungi development. The effects of suboptimal root zone temperature on the rate and extent of colonization are poorly understood; the responses observed so far have varied with the host plant and the fungus. A number of studies reported inhibition of mycorrhizal development at temperatures allowing plant growth. AM colonization of soybean was strongly repressed at a root zone temperature of 15°C (Zhang et al. 1995). Colonization of barley was similarly repressed at 15°C and inhibited at 10°C (Baon et al. 1994). Winter wheat did not form mycorrhizae after seeding in fall, when soil temperatures were low, although mycorrhizae developed in spring (Mohammad et al. 1998). In contrast, Daft et al. (1980) reported mycorrhizal development in dormant *Endymion non-scriptus* L. during winter months, when soil temperature was close to 5°C.

It is not clear how suboptimal root zone temperatures affect the growth of mycorrhizal plant roots and the formation of mycorrhizae. Such temperatures may negatively impact the plant, the fungus or mechanisms allowing symbiosis formation. To the best of our knowledge, no information has yet been published on the effect of suboptimal root zone temperature on AM mycelium growth and spore production.

The study of AM fungi is complicated by the obligate biotrophic nature of these microorganisms. Cultures of roots transformed with the *Agrobacterium rhizogenes* plasmid have been used to grow AM fungi in dual culture in vitro. In recent years, these have been used as a model to study AM fungi (Fontaine et al. 2001; Francisco and Ricardo 1999; Joner et al. 2000) or interactions between AM fungi and other soil organisms (Villegas and Fortin 2002). One of the advantages of this system is that both symbionts grow in a transparent medium, allowing non-destructive observations over long periods of time in easily maintained cultures. This simplified system has proven to be an easy, rapid and efficient way to study mycorrhizae.

The objectives of the present study were to assess the effect of suboptimal root zone temperatures on (1) *Glomus intraradices* (Schenck & Smith) growth, sporulation, spore metabolic activity and ability to colonize Ri T-DNA-transformed carrot (*Daucus carota* L.) roots in vitro, and (2) mycorrhiza formation using sorghum (*Sorghum bicolor* L.) as a model plant under greenhouse conditions.

Materials and methods

Experiment 1

Experimental design and sorghum growth conditions

The first experiment was conducted to compare the effect of suboptimal root zone temperatures (10 and 15°C) to the optimal temperature of 23°C on the formation of AM. A randomized complete block design was used with three factors including three levels of root zone temperature (10, 15 and 23°C), two levels of AM fungal inocula (–AM and +AM) and three sampling times (5, 10 and 15 weeks after seeding). Treatment combinations were replicated 5 times.

The soil used in this study was a St-Bernard clay loam with a pH of 6.7. The concentrations of Mehlich-3 (Sen Tran and Simard 1993) extractable elements in mg per kg soil were: P, 13.2; K, 115.5; Ca, 490.3; and Mg, 641.4. The soil was heat-pasteurized at 80°C for 4 h to eliminate indigenous mycorrhizal fungi, and amended with perlite at a soil:perlite ratio of 3:1. The soil was inoculated with a commercial inoculant, Mycorise (Les Entreprises Premier, Rivière-du-Loup, Québec) containing *G. intraradices* Schenck & Smith. A portion of inoculum was autoclaved at 121°C for 30 min and used to create a negative control treatment. The inocula were thoroughly mixed within the soil at a rate of 12 g per pot.

Soil temperature was adjusted to create three root zone temperatures (23, 15 and 10°C) using a thermostatically controlled water bath circulation system, set up in the greenhouse. A total of 10 2-l plastic pots were sealed to the bottom of each of nine water baths (68 × 42 cm) with a silicon sealant (GE Silicones Canada, Pickering, Ontario). A hole drilled in the base of each pot and through the bottom of the water bath allowed for drainage of extra water. A plastic bag containing 1,900 ml of inoculated soil was placed in each pot. Five sorghum seeds, surface sterilized twice with 0.5% NaOCl (2 min each time) and rinsed thoroughly with sterile, deionized water, were sown in each pot and thinned to three seedlings after emergence. The soil was maintained at field capacity by watering as needed with deionized water during the growth period. The experiment was conducted in a greenhouse for 100 days under controlled environment conditions with a 16/8 h day/night regime, 300 μmol m^{-2} s^{-1} photon flux density, 75% relative humidity and a day/night air temperature of 26/23°C. Sorghum shoots of all root zone temperature treatments were maintained at this 26–23°C air temperature regime. This situation, in the suboptimal temperature treatments mimicked spring conditions where young plant shoots are exposed to the sun and to warm daytime air temperatures, while their roots are in a still cool soil, in the suboptimal temperature treatments. Keeping all plant shoots under the same air temperature regime also allowed separation of the effect of temperature in the root zone from any confounding shoot temperature effects.

Sampling and data collection

Plants were harvested 5, 10 and 15 weeks after seeding. Roots were separated from shoots, washed with tap water and rinsed with deionized water. Fresh weights of shoots and roots were recorded. Shoot dry weight was measured after oven-drying at 65–70°C for 48 h. Total root length was estimated from the measurement of a 1.5-g subsample using a computerized image analysis system (WinRhizo, Regent Instruments, Québec). Remaining roots were

cleared 10 min in the autoclave (121°C) in 10% KOH and stained with 0.01% acid fuchsin (Kormanick and McGraw 1982) for 12 h at room temperature. The percentage of roots colonized by AM fungi was determined using the grid-line intersect method (Giovanetti and Mosse 1980).

Experiment 2

Experimental design and mycorrhizal culture

The second experiment was designed to compare the influence of suboptimal root zone temperature, 10 and 15°C, and an optimal temperature of 23°C on the growth of *G. intraradices* hyphae and on root colonization and sporulation by the fungus. Each treatment was replicated 9 times. Petri dishes (90 mm in diameter) were filled with 25 ml of minimal (M) medium. The medium used in this experiment contained in mg per liter distilled water: $MgSO_4.7H_2O$, 731; KNO_3, 80; KH_2PO_4, 4.8; $Ca(NO_3)_2$, 288; KCl, 65; NaFe.EDTA, 8; KI, 0.75; $MnSO_4.4H_2O$, 4.66; $ZnSO_4.7H_2O$, 2.65; H_3BO_3, 1.5; $CuSO_4.5H_2O$, 0.13; $Na_2MoO_4.2H_2O$, 0.0024; glycine, 3; thiamine, 0.1; pyridoxine, 0.1; nicotinic acid, 0.5; myo-inositol, 50; sucrose, 10,000 and phytagel, 4,000. The pH was adjusted to 5.5 before autoclaving at 121°C for 16 min. Plates were inoculated with a 1-cm^2 block of solid medium containing an Ri T-DNA-transformed root apex of carrot (*D. carota* L.) colonized with *G. intraradices*. The culture of *G. intraradices* grown on Ri T-DNA-transformed carrot roots was initially provided by Dr. J.A. Fortin (Institute de Recherche en Biologie Végétale, Montréal). Routine maintenance of the culture was by subculturing the mycorrhizal roots on M medium (Bécard and Fortin 1988) solidified with 0.4% (w/v) phytagel (ICN Biochemical Inc., Cleveland, Ohio) instead of 1% (w:v) Bacto-agar. Petri dishes were incubated upside-down in the dark at 23°C for 3 weeks until the hyphae had grown in the new medium. Twenty-seven plates with vigorous growth were selected and randomly placed in one of three incubators maintained at 10, 15 and 23°C.

Data collection

G. intraradices hyphal length was measured under a dissecting microscope at ×25 magnification using the modified grid-line intersect method previously described by Tennant (1975). Fourteen to sixteen 14.6 × 1.5-mm quadrats per Petri dish were randomly selected and counted once a week for 15 weeks. Spore numbers were also estimated from counts taken weekly from the same quadrats.

Total root length and percentage colonization were determined after 15 weeks. Roots were separated from the gellan gel by solubilization of the medium in 10 mM sodium citrate buffer (pH 6.0, 30°C) (Doner and Bécard 1991) and washed with distilled water. Root length was measured using the computerized image analysis. Percentage colonization was evaluated under a dissecting microscope using the grid-line intersect method (Giovanetti and Mosse 1980) after clearing in 10% (w:v) KOH and staining with 0.01% acid fuchsin (Kormanick and McGraw 1982) overnight at room temperature.

After spores were separated from the gellan gel as described above, washed and filtered (pore size 11 μm) under vacuum, the number of metabolically active spores was estimated by flooding the filters containing the spores with a solution made of equal parts of iodonitrotetrazolium (INT) (1 mg ml^{-1}) stock solution, nicotinamide adenine dinucleotide in the reduced form (NADH) (3 mg ml^{-1}) and 0.2 M Tris buffer at pH 7.4 (Sylvia 1988). The filters were incubated for 12 h at room temperature. This vital stain detects oxidase activity.

Statistical analysis

Statistical analysis for both experiments was carried out using the SAS software package (SAS Institute Inc. 1992). The Proc Univariate Procedure of SAS indicated that the experimental data was normally distributed. An analysis of variance was performed using the General Linear Model (GLM) procedure of SAS to test treatment effects, and protected Least Significant Difference (LSD) tests were used to compare treatment means when the analysis of variances indicated significance. Variation in spore numbers and hyphal length with time was also analyzed using the repeated measures analysis of variance procedure. In all procedures, $P<0.05$ were considered statistically significant.

Results

Experiment1

Sorghum total root length and growth rate were significantly reduced 5 weeks after seeding when root zone temperature was maintained at 15°C or 10°C (Tables 1, 2). There was no difference in root length between mycorrhizal and non-mycorrhizal plants (data not shown). Sorghum root fresh weight was much higher at 15°C than at 10°C in all mycorrhizal treatments and sampling times (Fig. 1). There was a temperature × mycorrhiza interaction on root fresh weight (Fig. 1). At optimal temperature, 5- and 15-week-old mycorrhizal plants had the highest root fresh weight but inoculation sometimes reduced root development at suboptimal temperatures. At 15°C, 5-week-old plants had lower root fresh weight when mycorrhizal, and AM inoculation also reduced the root fresh weight of 15-week-old plants grown at 10°C.

Plant shoot height was significantly reduced at 10°C as compared with 15°C and the optimal temperature, at all sampling times (Table 1). Plants inoculated with *G. intraradices* had higher fresh weight than non-inoculated plants, but inoculation did not increase shoot height (data not shown). After 5 weeks, plants maintained at suboptimal root zone temperatures had lower shoot dry weight

Table 1 Effect of root zone temperature on total root length, shoot height and root colonization of sorghum, 5, 10 and 15 weeks after seeding. Values are treatment means (n=5). Standard errors are given in parentheses. Means within the same column (sampling time) and followed by the same letters are not significantly different at the P 0.05 according to LSD tests

Temperature (°C)	Root length (cm) Sampling time (week)			Shoot height (cm) Sampling time (week)			Root colonization (%) Sampling time (week)		
	5	10	15	5	10	15	5	10	15
10	100 (12) b	501 (47)b	519 (54)b	15.3 (0.8)b	34.8 (1.6)c	79.5 (3.8)b	0.2 (0.2)a	1.2 (0.4)c	10.0 (1.2)c
15	128 (16) b	609 (65)b	623 (66)b	57.5 (3.1)a	93.5 (4.2)b	133 (6.0)a	0.6 (0.3)a	9.8 (0.8)b	17.4 (2.0)b
23	215 (19) a	788 (71)a	816 (72)a	56.8 (2.8)a	108 (4.6)a	130 (6.2)a	0.8 (0.6)a	16.4 (1.5)a	59.2 (4.3)a

Table 2 Effect of root zone temperature on sorghum root elongation rate and root colonization rate 5, 10 and 15 weeks after seeding. Values are treatment means (n=5). Standard errors are given in parentheses. Means within the same column (sampling time) and followed by the same letters are not significantly different at the P 0.05 according to LSD tests

Temperature (°C)	Root elongation rate (cm day^{-1}) Sampling time (week)			Root colonization rate (cm day^{-1}) Sampling time (week)		
	5	10	15	5	10	15
10	2.8 (0.34)b	7.2 (0.67)b	4.9 (0.51)b	0.006 (0.006)a	0.09 (0.006)c	0.49 (0.01)c
15	3.7 (0.46)b	8.7 (0.93)b	5.9 (0.63)b	0.022 (0.004)a	0.85 (0.01)b	1.03 (0.02)b
23	6.1 (0.54)a	11.3 (1.01)a	7.8 (0.69)a	0.050 (0.003)a	1.85 (0.02)a	4.60 (0.04)a

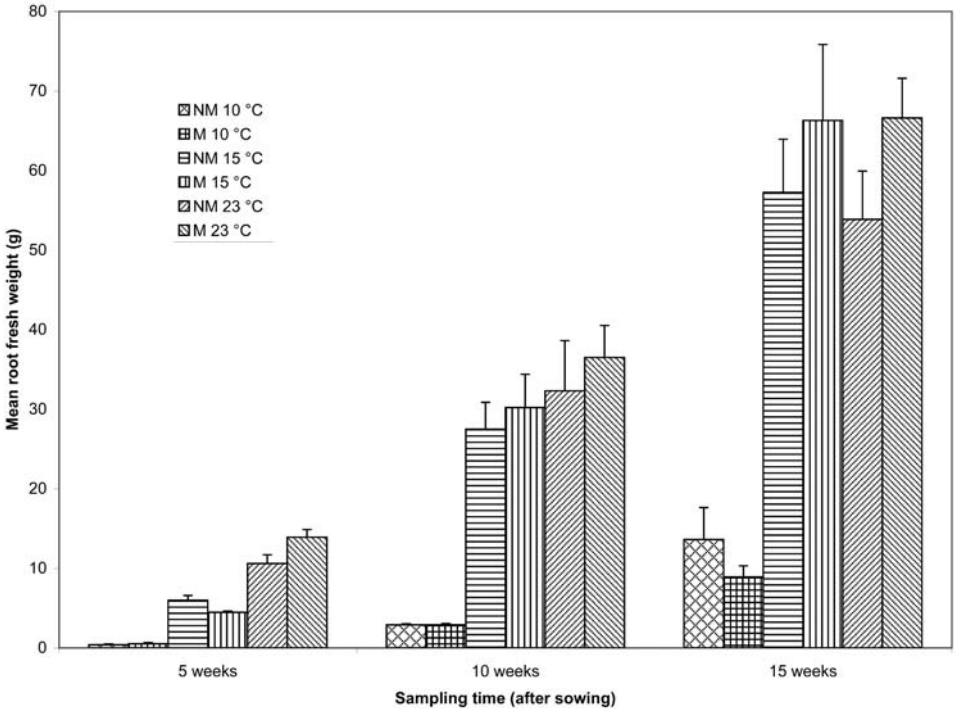

Fig. 1 Root fresh weight of sorghum plants grown at three root zone temperatures and either inoculated (*M*) or non-inoculated (*NM*) by a mycorrhizal fungus, *Glomus intraradices* Schenck & Smith. Each value is the mean of five experimental units. *Bars* above the columns represent standard errors

than plants grown at optimal temperature (Fig. 2). After 10 and 15 weeks, the shoot dry weight of plants grown at root zone temperatures of 15 and 23°C were similar and higher than those of plants grown at a root zone temperature of 10°C (Fig. 2). Differences in shoot dry weight between mycorrhizal and non-mycorrhizal plants were not statistically significant.

Suboptimal root zone temperature had a large negative impact on sorghum root colonization by *G. intraradices* (Table 1). As expected, AM colonization of roots increased with sampling time. Colonization was slow to develop and there was no difference in the percentage of root colonization (Table 1) or colonization rate (Table 2) between the three root zone temperature treatments 5 weeks after seeding. The temperature effect developed with time and, 10 and 15 weeks after seeding, colonization rates were higher at higher root zone temperatures (Table 2). After 15 weeks growth, the colonization levels of sorghum plants grown at different root zone temperatures were significantly different, and were higher at higher temperatures (Table 1). At that time, the colonization level of sorghum grown at a root zone temperature of 10°C was only 17% of that of plants grown at optimal temperature. No colonization was observed in non-inoculated plants (data not shown).

Experiment 2

G. intraradices was significantly affected by suboptimal temperature, when grown in vitro (Fig. 3). Spore number was highest at 23°C and lowest at 10°C. Spores increased with time at all temperatures, but sporulation was most abundant at 23°C. The number of metabolically active spores was higher at 23°C than at 10°C (Table 3).

The length of hyphae increased over time at each temperature (Fig. 4), with the greatest hyphal length produced at optimal temperature and the lowest at 10°C. Hyphae growth was less negatively influenced by suboptimal root zone temperatures than sporulation. After 15 weeks at 10 and 15°C, hyphae length was reduced to

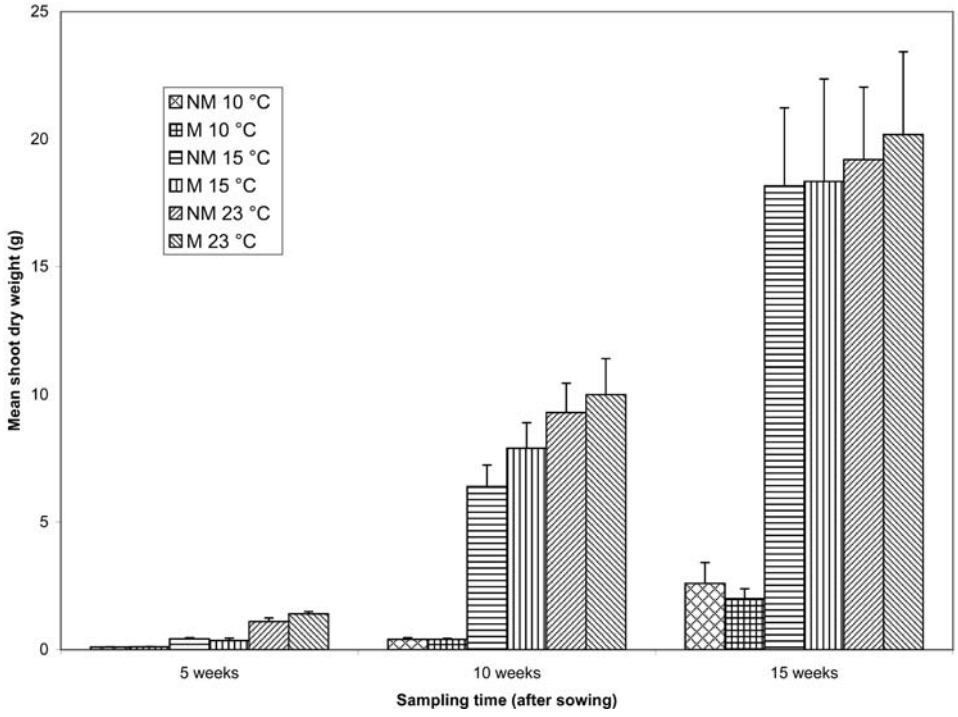

Fig. 2 Mean shoot dry weight of sorghum plants grown at three root zone temperatures and either inoculated (*M*) or non-inoculated (*NM*) by a mycorrhizal fungus, *G. intraradices* Schenck & Smith. Each value is the mean of five experimental units. *Bars* above the columns represent standard errors

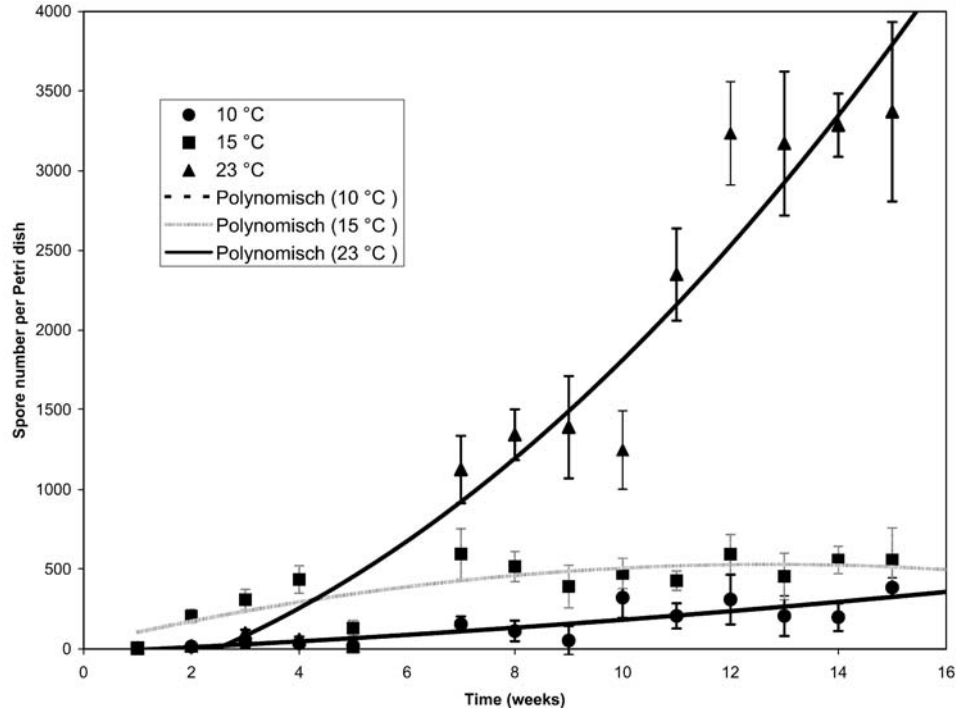

Fig. 3 Sporulation of *G. intraradices* grown on Ri-DNA-transformed carrot roots on M medium in vitro at three different temperatures. Data points represent the mean of nine replicates. *Vertical bars* represent standard errors

52% and 60% of the length of hyphae produced at optimal temperature.

The negative impact of suboptimal root zone temperatures on root elongation was relatively less with transformed carrot root than with sorghum. Root length was significantly decreased at 10°C (Table 3), but no difference in root length was observed at optimal temperature and 15°C. The percentage root colonization was relatively low at all three temperatures used, as is usually the case with in vitro cultures of *G. intraradices* (Chabot et al. 1992). Colonization of *G. intraradices* was higher at optimal temperature than at 15 or 10°C (Table 3). Colonization after 15 weeks at 10°C was decreased to 37% of the level at optimal temperature. There was no significant difference between colonization at 10 and 15°C.

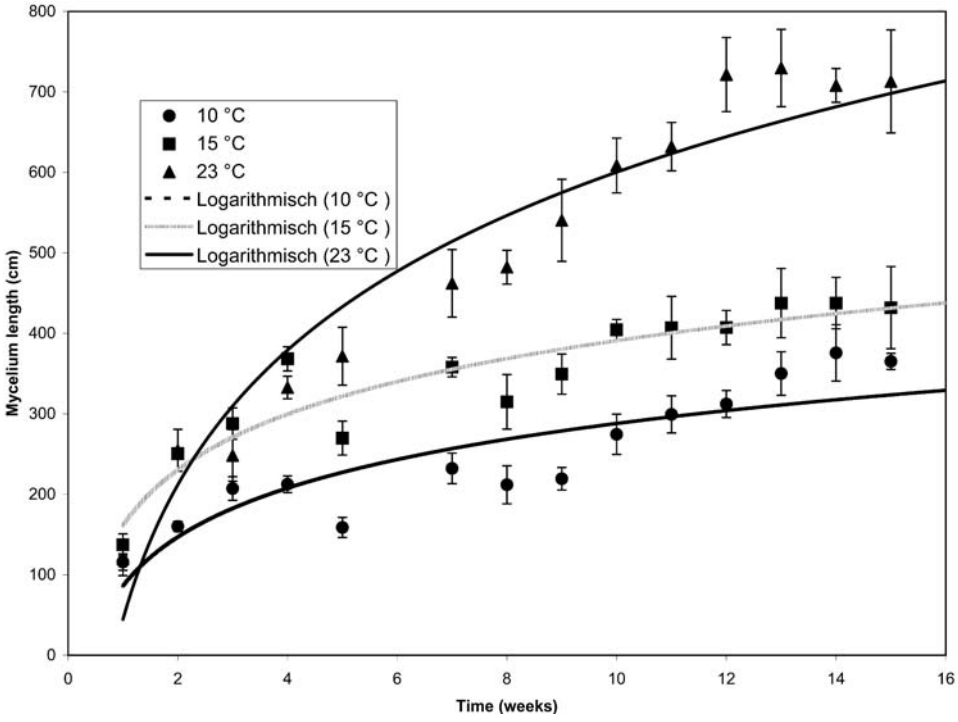

Fig. 4 Mycelium length of *G. intraradices* grown on Ri-DNA-transformed carrot roots on M medium in vitro at three different temperatures. Data points represent the mean of nine replicates. *Vertical bars* represent standard errors

Table 3 The effects of temperatures on root length, proportion of metabolically active spores and root colonization in in vitro dual cultures of Ri T-DNA-transformed carrot roots and *Glomus intraradices* Schenck & Smith. Values are treatment means (n=9). Standard errors are given in parentheses. Means within the same column and followed by the same letters are not significantly different at the P 0.05 according to LSD tests

Temperature (°C)	Root length (cm)	Active spore (%)	Colonization (%)
10	547.6 (25)b	89.7 (2.9)b	2.2 (0.3)b
15	676.1 (31)a	94.4 (3.4)ab	1.6 (0.3)b
23	658.4 (28)a	96.1 (3.3)a	5.9 (0.6)a

Discussion

Suboptimal root zone temperatures decreased root growth and AM colonization in both the in vitro and greenhouse systems. The impacts of suboptimal temperatures were less severe in vitro than in the greenhouse. The better growth of carrot at suboptimal temperatures might be explained by the effect of the RI T-DNA plasmid, but the relatively low impact of suboptimal root zone temperature on carrot root colonization by *G. intraradices* cannot have the same cause, since mycorrhizal transformed carrot roots usually have very low levels of colonization. Conversely, the higher susceptibility to suboptimal root zone temperature of sorghum root growth and colonization by *G. intraradices* may be due to the tropical origin of the plant.

The origin of the host plant species does not explain the impact of suboptimal root zone temperatures in all cases reported in the literature. Previous studies reported a strong repression of colonization at 15°C in soybean (Zhang et al. 1995), another plant of tropical origin, and in barley (Baon et al. 1994), a temperate climate crop plant. Smith and Bowen (1979) reported similar repression in *Medicago trunculata* and *Trifolium subterraneum*, further temperate climate species, at 16°C. Volkmar and Woodbury (1989), in contrast to Baon et al. (1994), found no effect of root zone temperature on the colonization of barley by AM species indigenous to the Canadian prairies when the inoculum was dispersed through the soil, but found higher colonization rates at a root zone temperature of 12°C than 16 or 20°C when the inoculum was placed 5 cm below the soil surface. Reduced root elongation under suboptimal root zone temperature can lead to higher percentages of root colonization if AM fungi growth and infectivity is not reduced to the same extent as root growth. Volkmar and Woodbury (1989) found reduced root growth at suboptimal root zone temperature. It is unclear in their study whether higher AM colonization percentage at 12°C was due to a more active colonization process at this temperature, or to a dilution of colonized root areas in larger barley root systems at 16 and 20°C.

Contrasting results sometimes obtained using the same plant species (e.g. for barley, Baon et al. 1994; Volkmar and Woodbury 1989) suggest that colonization depends at least in part on the metabolic activity of the fungal

isolates at suboptimal temperature and on the maintenance of their "symbiotic" properties. Several studies report a detrimental effect of suboptimal root zone temperatures around 15°C on different agricultural plants. In contrast, Daft et al. (1980) reported mycorrhizal formation at a root zone temperature around 5°C in English bluebells, a bulbous spring-flowering forest floor species. Clearly, information is needed on the impact of host plant species with different growth habits on AM formation at suboptimal root zone temperature and, conversely, on the response of AM fungal species from different geographical origins to suboptimal root zone temperature.

In this study, AM inoculation increased sorghum growth at optimal temperature, but decreased it sometimes at suboptimal root zone temperatures. Decrease in plant development due to AM colonization under suboptimal temperature was also found by Hayman (1974). Negative effects of mycorrhizae on plant growth at suboptimal temperatures have been attributed to the inability of an established fungus to take up (Bowen et al. 1975) or transport (Hayman 1977) phosphorus while still utilizing host carbon (Daniels-Hetrick and Bloom 1984). In contrast to these previous experiments, sorghum shoots here were kept at 23–26°C in all root zone temperature treatments. This reduced the likelihood of reduced C fixation and, hence, the likelihood of C export to roots and AM fungi exceeding shoot photosynthetic capacity. In spite of this, sorghum growth was slightly reduced by mycorrhizal inoculation. In the present case, the tendency of mycorrhizal plants to be smaller may also be related to a C drain effect, as previously proposed, or to another dysfunction of sorghum metabolism at suboptimal root zone temperature.

Suboptimal root zone temperature delayed the formation of AM. Although both plant root length and percentage colonization decreased with decreasing root zone temperature, a proportionately greater decrease occurred in root colonization than in root growth. Since mycorrhizal development was not proportional to root growth reduction in this study, the mechanisms responsible for the repression of AM development at suboptimal root zone temperatures should be located at the level of AM initiation or at the level of the fungus itself. At 10°C, AM hyphal in vitro growth was half that of the 23°C control. The extent of this reduction in hyphal growth is similar to the reduction in root growth observed in sorghum (63%), suggesting that the suboptimal root zone temperature impacts on AM initiation.

The speed of AM colonization in a given rooting volume depends on the frequency of hyphae and root interceptions. These events are functions of hyphal growth rate and root growth rate. The reduction of both root and AM hyphal growth rates were observed at suboptimal root zone temperatures. After 15 weeks at 10°C, for example, root growth rate was reduced to 63% and 83% of that of the 23°C controls in the greenhouse and the experiment in vitro, respectively, and AM hyphal growth, which could only be measured in vitro, was reduced to 52% of the 23°C control. Hence, the reduction in the extent of root colonization under suboptimal root zone temperature after 15 weeks of growth should be the result of the negative interaction created by reduced root and mycelium growth. Since the frequency of hyphae-root interception depends on hyphal and root growth over a period of time, a greater reduction in colonization rate must be expected from the negative interaction effect of reduced root and hyphae growth rate. Accordingly, at 10°C, sorghum and carrot root colonization levels were reduced to 17% and 37% of the 23°C controls, respectively. The reduced probability of contact between plant roots and AM hyphae in the growth containers may explain the greater reduction in root colonization than in root and hyphal development in this study.

Root zone temperature-induced reduction in hyphae-root interception frequency, following reduced root and hyphae elongation rates, may be more important under conditions of low inoculum density level. After 5 weeks, sorghum colonization was low at all the root zone temperatures, but after 10 and 15 weeks, root colonization had developed along with a strong effect of root zone temperature on mycorrhizal development. High density of infective inoculum may explain the discrepancy observed in the impact of suboptimal root zone temperatures with different inoculum placement strategies. For example, Volkmar and Woodbury (1989) found no effect of root zone temperature when the inoculum was dispersed throughout the soil but did find an effect of root zone temperature on the colonization of roots beyond the source of inoculum when it was placed 5 cm below the soil surface. The inoculum dispersed throughout the soil might have been so abundant that primary infection was sufficient to saturate the roots.

The conditions of the experiment in vitro differed dramatically from natural soil conditions. However, the sudden application of temperature treatments should have had very little impact beyond that of temperature. Diurnal variation in root zone soil temperature in the range of 10°C is not uncommon (Huang et al. 1994; Uvarov 1995), particularly in bare agricultural soils (Brady and Weil 2002) as found in spring. The in vitro system, on the other hand, is extremely artificial. The physico-chemical environment of mycorrhizae grown in vitro is a sterile gellified nutrient solution with no adsorption site to buffer root and AM hyphal effluents or nutrient uptake, and the host plant has no shoot. Nonetheless, mycorrhizal cultures on Ri T-DNA-transformed roots have proven very useful to study mycorrhizal fungi, (Fontaine et al. 2001; Francisco and Ricardo 1999; Joner et al. 2000). Also in this experiment the data must be interpreted with caution. It is clear that reduced mycelial growth rates were sustained for 15 weeks after application of the 10 and 15°C treatments, but whether or not suboptimal root zone temperature-induced reduction in hyphal growth rate and sporulation experienced by *G. intraradices* would be the same in soil as in vitro remains to be determined.

Ways to measure the impact of suboptimal root zone temperature on AM hyphal and spore production in a

more natural situation should be developed. Temperature-controlled mini-rhizotrons used in connection with techniques of immunofluorescence, e.g. the Mab32B11 antibody (Wright 2000), to reveal mycorrhizal hyphae may be an avenue worth exploring.

In conclusion, suboptimal root zone temperature has the potential to reduce the growth of both host plant roots and AM hyphae. Reduced root and hyphal growth leads to a reduced frequency of root and infective hyphae encounters within the rooting volume. This negative interaction could explain the greater negative impact of suboptimal root zone temperature on AM colonization of root than on root or hyphal growth.

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