Mycorrhiza (2005) 15: 345–356
DOI 10.1007/s00572-004-0336-z

ORIGINAL PAPER

Idoia Garmendia · Nieves Goicoechea · Jone Aguirreolea

Moderate drought influences the effect of arbuscular mycorrhizal fungi as biocontrol agents against *Verticillium*-induced wilt in pepper

Received: 17 May 2004 / Accepted: 15 October 2004 / Published online: 26 November 2004

Abstract Previous studies have shown that the arbuscular mycorrhizal fungus (AMF) *Glomus deserticola* (Trappe, Bloss and Menge) can diminish the negative effect of *Verticillium dahliae* Kleb. on pepper yield. On the other hand, it is known that AMF can be more beneficial for plant growth and physiology under dry conditions than when soil moisture is plentiful. Therefore, our objective was to assess if a moderate water deficit imposed on pepper plants before their inoculation with *V. dahliae* could improve the effectiveness of *G. deserticola* as biocontrol agent. In the present experiment, the delay in disease development in *Verticillium*-inoculated plants associated with AMF did not occur under well watered conditions. In addition, the establishment of mycorrhizal symbiosis and the development of structures by AMF were delayed when both symbiotic and pathogenic fungi infected the same root. Therefore, it is suggested that the equilibrium between pepper plant, *G. deserticola* and *V. dahliae* is so complex that small changes in competition between symbiotic and pathogenic fungi for host resources can modify the efficiency of AMF as a biocontrol agent. On the other hand, water deficit enhanced the deleterious effect of *V. dahliae* on fruit set and yield only when pepper plants were not associated with *G. deserticola*, which reinforces the idea that AMF may be more important for host plants subjected to stressful conditions. However, comparing well watered non-mycorrhizal and predroughted mycorrhizal plants, we found that moderate water deficit imposed before inoculation with *V. dahliae* did not improve the effectiveness of *G. deserticola* as a biocontrol agent.

Keywords Arbuscular mycorrhizal fungi · Biocontrol · Drought · Pepper · *Verticillium dahliae*

I. Garmendia · N. Goicoechea (✉) · J. Aguirreolea
Departamento de Fisiología Vegetal,
Universidad de Navarra,
c/Irunlarrea s/n,
31008 Pamplona, Spain
e-mail: niegoi@unav.es
Tel.: +34-948-425600
Fax: +34-948-425649

Introduction

Verticillium dahliae Kleb. alters the physiology of *Capsicum annuum* L. cv. Piquillo (Goicoechea et al. 2000, 2001) and drastically decreases pepper yield in the field (García-Mina et al. 1996). The use of methyl bromide to control this pathogen was phased out under the 1992 Montreal Protocol, thus finding alternative methods has become essential for agriculture in the future. Some of the most recent alternatives are crop rotation (Xiao et al. 1998), soil steaming (Van Loenen et al. 2003) and nitrogenous (Tenuta and Lazarovits 2002) or organic (Goicoechea et al. 2004) amendments. Another interesting alternative is the development of biological methods. In this sense, recent studies suggest that some fungal root endophytes can suppress *Verticillium* wilt in eggplant (Narisawa et al. 2002) and some arbuscular mycorrhizal fungi (AMF) can alleviate the negative effect of *Verticillium dahliae* on pepper yield (Garmendia et al. 2004a,b).

Goicoechea et al. (2000) found that *V. dahliae* modifies water status in pepper plants. This pathogen produces a progressive decrease in leaf water potential and a sharp reduction in leaf relative water content in the final stages of the disease. On the other hand, it is well known that AMF can affect the water balance of both watered and droughted host plants (see Augé 2001 for review). In addition, the beneficial effects of mycorrhizal symbiosis on plant growth and physiology can be even more relevant in dry conditions

than when soil moisture is plentiful (Peña et al. 1988; Sánchez-Díaz et al. 1990; Goicoechea et al. 1997a). In pepper, it has been found that AMF can improve drought resistance by enhancing uptake of soil water by hyphae (Davies et al. 1992) and/or increasing the root-to-shoot ratio (Davies et al. 2002).

Therefore, our objective was to study whether a moderate water deficit imposed on pepper plants before their inoculation with *V. dahliae* could improve the effectiveness of *G. deserticola* as a biocontrol agent.

Materials and methods

Biological material, growth conditions and experimental design

Seeds of *C. annuum* L. cv. Piquillo were germinated on washed sand. At 1 month old, 160 seedlings were transplanted to 1 l plastic containers filled with a mixture of vermiculite-sand-soil (2.5:2.5:1 v/v/v). Soil poor in available phosphorus (P) was chosen. It had a pH (H_2O) of 8.9, 0.3% organic matter, 0.08% nitrogen, 2.0 mg kg^{-1} P, 58.8 mg kg^{-1} potassium and 41.98% $CaCO_3$. Before preparing the mixture, the soil was sieved (2 mm) and steam sterilised at 100°C for 1 h on 3 consecutive days.

When transplanted to pots, seedlings were divided into two groups: (1) non-mycorrhizal plants (NM; 80 plants), and (2) plants inoculated with *Glomus deserticola* (Trappe, Bloss and Menge) (M; 80 plants). Mycorrhizal inoculum was supplied by the Estación Experimental del Zaidín (Granada, Spain). This isolate can improve plant tolerance to both abiotic (Ruiz Lozano and Azcón 1995) and biotic (Garmendia et al. 2004a,b) stresses. A soil-based inoculum (20 g per pot) including root fragments, spores and hyphae from a 3-month-old culture of leek and alfalfa was added to each pot at transplanting just below the pepper seedlings. Plants were fertilised and irrigated as described by Garmendia et al. (2004a).

At 6 weeks old, four NM and four M plants were harvested to determine biomass production and AMF presence or absence in the roots. Thereafter, 38 NM and 38 M seedlings were subjected to cyclic water deficit. Drought (D) consisted of withholding irrigation in a cyclic manner until the substrate water content in stressed treatments was equal to one-half of the water content in well watered pots [field capacity, FC—calculated as the maximum water retained by a pot after complete drainage of water excess (0.400 g water cm^{-3} substrate)]. When soil water content in stressed treatments was around 0.200 g cm^{-3} substrate, pots were watered with nutrient solution to FC (containing different P levels depending on mycorrhizal presence or absence). Irrigation with nutrient solution was performed to avoid a concomitant nutrient deficiency in water-stressed treatments. Afterwards, a new cycle of drought was imposed and, when the substrate water content in stressed treatments again reached one-half of FC, these pots were watered again. The soil water content during both drought periods was measured daily with an ML2 ThetaProbe (AT Delta-T Devices, Cambridge, UK). The duration of each cycle was approximately 1 week. In addition, 38 NM and 38 M seedlings were kept as well watered (WW) controls. Well watered peppers were irrigated alternately with water and nutrient solution in order that well watered and droughted plants were supplied the same amount of nutrients. Thus, at the end of the second cycle of drought there were four treatments (38 plants/treatment): well watered non-mycorrhizal (NMWW) and mycorrhizal (MWW) plants, and drought-stressed non-mycorrhizal (NMD) and mycorrhizal (MD) plants. To determine the effect of water deficit on the growth and physiology of the pepper plants as well as on mycorrhizal symbiosis, four NM and four M plants that had previously suffered water stress and their respective well watered controls were harvested at the end of the second drought cycle.

The day following the end of the second drought cycle, once the substrate water content had reached FC, 19 plants from each previously described treatment were inoculated with *V. dahliae* by adding a suspension of 3.6×10^7 conidia to the substrate of each pot (Hoyos et al. 1993). *V. dahliae* was isolated from diseased pepper grown in the field, and maintained on Messiaen culture medium prior to inoculation. The other 76 plants remained uninoculated. Finally, therefore, we had eight treatments with 19 plants each: NMWW inoculated with *V. dahliae* (NMWW+V) or not (NMWW−V); NMD+V and NMD−V; MWW+V and MWW−V, MD+V and MD−V. Pepper plants were grown in a greenhouse at 25/15°C day/night and received natural daylight for a photoperiod of 14 h. Six harvests were performed: the day before imposing the first cycle of drought, the day when *V. dahliae* was inoculated (day 0), on days 7, 14 and 43 after inoculation with pathogen, and at the end of the plant life cycle (3 months after pathogen inoculation).

Disease assessment and estimation of AMF colonisation

In order to assess if *V. dahliae* had progressed from root to shoot, surface-disinfected cross-stem sections were cut and plated on Messiaen culture medium for fungus isolation and identification. Plates were incubated in the dark at 25°C for 10–15 days. Disease severity was non-destructively estimated by calculating a disease index as the sum of wilted, chlorotic and necrotic leaves related to total leaves per plant, expressed as a percentage (Goicoechea et al. 2000).

Root samples were cleared and stained (Phillips and Hayman 1970) and the percentage of root colonisation was assessed by examining a minimum of 80–90 1-cm root segments for each treatment (Hayman et al. 1976). In addition, mycorrhizal colonisation was characterised by assessing the presence or absence of arbuscules and vesicles.

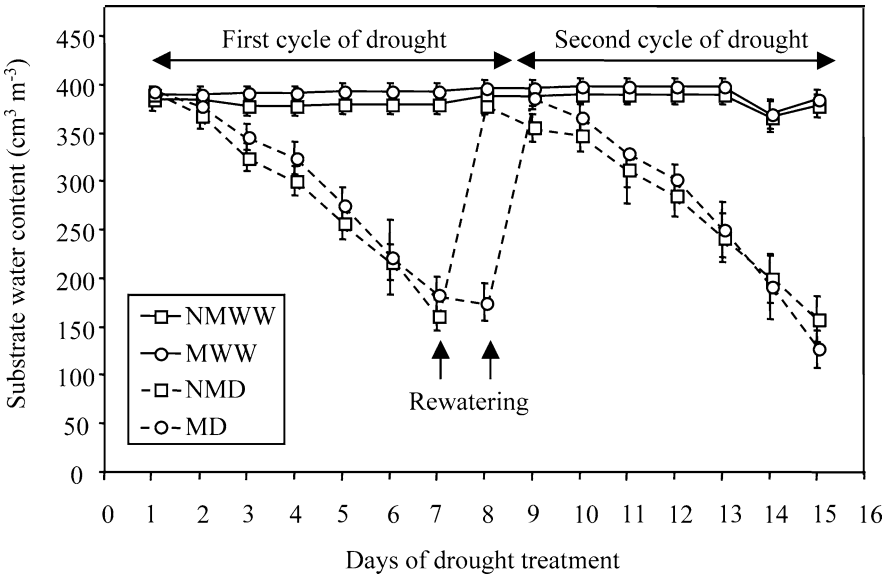

Fig. 1 Evolution of substrate water content ($cm^3\ m^{-3}$) in non-mycorrhizal (*NM*) and mycorrhizal (*M*) pots, well watered (*WW*) or subjected to drought (*D*) Values are means ± SD (n=38 pots)

Water status measurements, plant growth parameters and P in leaves

Water status measurements were made on the youngest fully mature leaves, collected at midday both at the beginning of the first drought cycle and at the end of the second drought cycle. Relative water content (RWC) was estimated by a modification of the method of Weatherley (1950). Water potential (Ψw) was determined on fresh leaves (disks of 0.95 cm^2) by using a thermocouple psychrometer (Tru Psi SC10X; Decagon, Pullman, Wash.) and after equilibrating the tissue for 2 h at 20°C. Osmotic potential (ψs) was measured with the same thermocouple psychrometer after equilibrating frozen leaf tissue (disks of 0.95 cm^2) for 2 h at 20°C. The leaf pressure potential (ψp) was estimated as ψp=ψw−ψs. Leaf osmotic potential at full turgor (ψs^{100}) was calculated according the expression $\psi s^{100}=\psi s \times RWC/100$ (Irigoyen et al. 1996).

Leaf, stem and root dry mass (DM) were determined after drying at 80°C for 2 days. Relative growth rates (RGR) of different organs were calculated between days 0 and 7 after inoculation with *V. dahliae*, as well as between days 7 and 14, 14 and 43 and, finally, between day 43 and final harvest (3 months after pathogen inoculation). Fruit DM was calculated after drying at 60°C for 45 days. Fruit set was calculated as the number of fruits to the sum of flowers (on plant and fallen flowers) and fruits. Activation of axillary buds and percentage of plants showing flowers and/or fruits on the main stem were recorded throughout disease development.

Phosphorus was determined spectrophotometrically (Allen et al. 1976) in leaf samples (0.5 g fresh weight, FW) previously digested with nitric-perchloric acid; P analyses were performed in samples harvested on days 0, 14 and 43 after inoculation with *V. dahliae*.

Statistics

Leaf, stem and root DM, water status parameters, percentages of mycorrhizal colonisation and incidence of arbuscules and/or vesicles the day the first drought cycle was imposed were evaluated by Student's *t*-test. Frequencies of *Verticillium*-inoculated plants showing activation of axillary buds, and the percentage of plants with flowers and/or fruits on the main stem were analysed by chi square (χ^2) test. Data were subjected to arc-sin transformation before applying the χ^2-test. Data on parameters measured

Table 1 Percentage of mycorrhizal colonisation and incidence of arbuscules (%) and vesicles (%) in well watered (*WW*) or droughted (*D*) mycorrhizal (*M*) plants at the beginning of the first drought cycle and at the end of the second drought cycle. Means ± SD (n=4 plants) were compared with the Student's *t*-test within each column. Values followed by a common letter are not significantly different ($P \leq 0.05$)

Treatment	Beginning of first drought cycle			End of second drought cycle		
	Mycorrhizal colonisation (%)	Arbuscules (%)	Vesicles (%)	Mycorrhizal colonisation (%)	Arbuscules (%)	Vesicles (%)
MWW	22.5	62.5	53.75	26.21 a	85.00 a	75.00 a
MD	ND[b]	ND	ND	24.59 a	86.67 a	83.33 a

[b]Not determined

Table 2 Leaves, stem and root dry matter (*DM*) (g plant^{-1}), root-to-shoot and leaves-to-stem DM ratios and shoot height (cm) in well watered (*WW*) or droughted (*D*) non-mycorrhizal (*NM*) and mycorrhizal (*M*) plants at the beginning of the first drought cycle (*BFD*) and at the end of the second drought cycle (*ESD*). *AMF* Arbuscular mycorrhizal fungi

Treatment	BFD[c]						ESD[d]					
	Leaves DM (g plant^{-1})	Stem DM (g plant^{-1})	Root DM (g plant^{-1})	Root/ shoot	Leaves/ stem	Shoot height (cm)	Leaves DM (g plant^{-1})	Stem DM (g plant^{-1})	Root DM (g plant^{-1})	Root/ shoot	Leaves/ stem	Shoot height (cm)
NMWW	0.187 a	0.032 a	0.149 a	0.70 a	5.91 a	6.87 a	0.741 a	0.256 a	0.470 a	0.55 b	3.07 b	13.83 a
MWW	0.157 a	0.026 a	0.130 a	0.74 a	5.87 a	7.00 a	0.680 a	0.223 a	0.431 a	0.48 b	3.14 b	13.00 a
NMD	ND[e]	ND	ND	ND	ND	ND	0.275 b	0.019 b	0.244 b	0.86 a	19.06 a	8.50 b
MD	ND	ND	ND	ND	ND	ND	0.514 ab	0.037 b	0.491 a	0.89 a	13.90 a	11.00 b
AMF	–	–	–	–	–	–	ns	ns	ns	ns	ns	ns
Drought	–	–	–	–	–	–	***	***	ns	***	***	*
Interaction	–	–	–	–	–	–	*	ns	*	ns	ns	ns

***P<0.001, **P<0.01, *P<0.05, *ns* non-significant

[c]Means ± SD (n=4 plants) were compared with Student's t-test within each column

[d]Data were analysed with a two-way ANOVA with AMF and drought as the main effects. Means ± SD (n=4 plants) were calculated and, when the F ratio was significant, least significant differences were evaluated by the Tukey-b test. Otherwise as for Table 1

[e]Not determined

after imposing drought and before inoculating with pathogen were subjected to a two-factor analysis of variance (ANOVA). The variance was related to the main treatments (AMF and drought) and to the interaction between them. Data on mycorrhizal colonisation on day 43 and at final harvest were analysed with two-way ANOVA with *V. dahliae* and drought as the main effects. After inoculating with *V. dahliae*, data were subjected to a three-factor ANOVA to partition the variance into the main effects and the interaction between AMF, drought and *V. dahliae*. Means ±SD were calculated and, when the F ratio was significant, least significant differences were evaluated by the Tukey-b test as available in the SPSS statistical package (version 9.0) (SPSS, Chicago, Ill.) for Windows 98 (Microsoft, Seattle, Wash.). Significance levels were always set at 5%.

Results

Soil water content in well watered pots was always maintained at FC (Fig. 1). Both the first and the second periods of drought ended when water content in non-irrigated pots reached one-half of FC. This value was achieved around 1 week after withholding irrigation.

The percentage of root cortex colonised by *G. deserticola* achieved 22.5% the day when the first cycle of drought was imposed (Table 1). On that date, 62.5% and 53.8% of mycorrhizal roots showed arbuscules and vesicles, respectively. Water deficit had no influence on mycorrhizal colonisation and development of fungal structures.

NM and M pepper plants were similar in size and DM partitioning on the first day of drought (Table 2). However, while in NM plants water deficit caused important decreases in leaf, stem and root DM, only stem DM declined

Table 3 Relative water content (*RWC*), water potential (Ψ_w), osmotic potential (Ψ_s), pressure potential (Ψ_p) and osmotic potential at full turgor (Ψ_s^{100}) in leaves from non-mycorrhizal (*NM*) and mycorrhizal (*M*) plants, well watered (*WW*) or subjected to drought (*D*), at the beginning of the first drought cycle and at the end of the second drought cycle

Treatment	RWC (%)		Ψ_w (MPa)		Ψ_s (MPa)		Ψ_p (MPa)		Ψ_s^{100} (MPa)	
	BFD[c]	ESD[d]	BFD[c]	ESD[d]	BFD[c]	ESD[d]	BFD[c]	ESD[d]	BFD[c]	ESD[d]
NMWW	96.94 a	97.15 a	−1.335 b	−1.340 a	−1.483 a	−1.695 a	0.128 b	0.326 b	−1.515 a	−1.740 a
MWW	97.96 a	94.74 a	−1.185 a	−1.193 a	−1.650 a	−1.680 a	0.462 a	0.489 a	−1.674 a	−1.784 a
NMD	ND	96.87 a	ND	−1.356 a	ND	−1.530 a	ND	0.273 b	ND	−1.582 a
MD	ND	95.49 a	ND	−1.141 a	ND	−1.620 a	ND	0.441 a	ND	−1.698 a
AMF	–	ns	–	ns	–	ns	–	***	–	ns
Drought	–	ns	–	ns	–	ns	–	ns	–	ns
Interaction	–	ns	–	ns	–	ns	–	ns	–	ns

***P<0.001, **P<0.01, *P<0.05, *ns* non-significant

[c]Means ± SD (n=4 plants) were compared with Student's t-test within each column

[d]Data were analysed with a two-way ANOVA with AMF and drought as the main effects. Means ± SD (n=4 plants) were calculated and, when the F ratio was significant, least significant differences were evaluated by the Tukey-b test. Otherwise as for Table 1

Table 4 Percentage of mycorrhizal colonisation and incidence of arbuscules (%) and vesicles (%) in well watered (*WW*) or predroughted (*D*) mycorrhizal (*M*) plants, inoculated (+*V*) or not (−*V*) with *Verticillium dahliae*

Treatment	Day of inoculation with *V. dahliae*[c]			43 days after pathogen inoculation[d]			Final harvest (90 days after pathogen inoculation)[d]		
	Mycorrhizal colonisation (%)	Arbuscules (%)	Vesicles (%)	Mycorrhizal colonisation (%)	Arbuscules (%)	Vesicles (%)	Mycorrhizal colonisation (%)	Arbuscules (%)	Vesicles (%)
MWW−V	26.21 a	85.00 a	75.00 a	54.72 a	98.75 a	95.00 a	53.75 a	96.25 a	98.67 a
MD−V	24.59 a	86.67 a	83.33 a	38.54 ab	91.25 a	93.75 a	53.82 a	100.00 a	100.00 a
MWW+V	ND[e]	ND	ND	44.02 ab	100.00 a	71.25 b	53.38 a	92.50 a	99.17 a
MD+V	ND	ND	ND	34.91 b	95.00 a	87.50 a	41.66 a	97.00 a	100.00 a
V. dahliae	–	–	–	ns	ns	***	ns	ns	ns
Drought	–	–	–	*	ns	ns	ns	ns	ns
Interaction	–	–	–	*	ns	*	ns	ns	ns

***$P<0.001$, **$P<0.01$, *$P<0.05$, *ns* non-significant

[c]Means ± SD (n=4 plants) were compared with Student's t-test within each column

[d]Data were analysed with a two-way ANOVA with *V. dahliae* and drought as the main effects. Means ± SD (n=4–6 plants) were calculated and, when the F ratio was significant, least significant differences were evaluated by the Tukey-b test. Otherwise as for Table 1

[e]Not determined

at the end of the second drought cycle in M plants. ANOVA results corroborated the significant interaction ($P<0.05$) between AMF and water deficit on leaves and root DM at the end of the second drought cycle. DM partitioning changed as a consequence of water stress with great increases in root-to-shoot and leaves-to-stem ratios in both NM and M plants.

RWC in leaves from NM and M pepper reached values greater than 95% regardless of substrate moisture (Table 3). In addition, we did not observe any significant effect of water deficit on Ψw and Ψs. Osmotic adjustment did not occur in any plant subjected to drought. The only parameter that differed between NM and M pepper was ψp. Peppers associated with *G. deserticola* always exhibited greater ψp

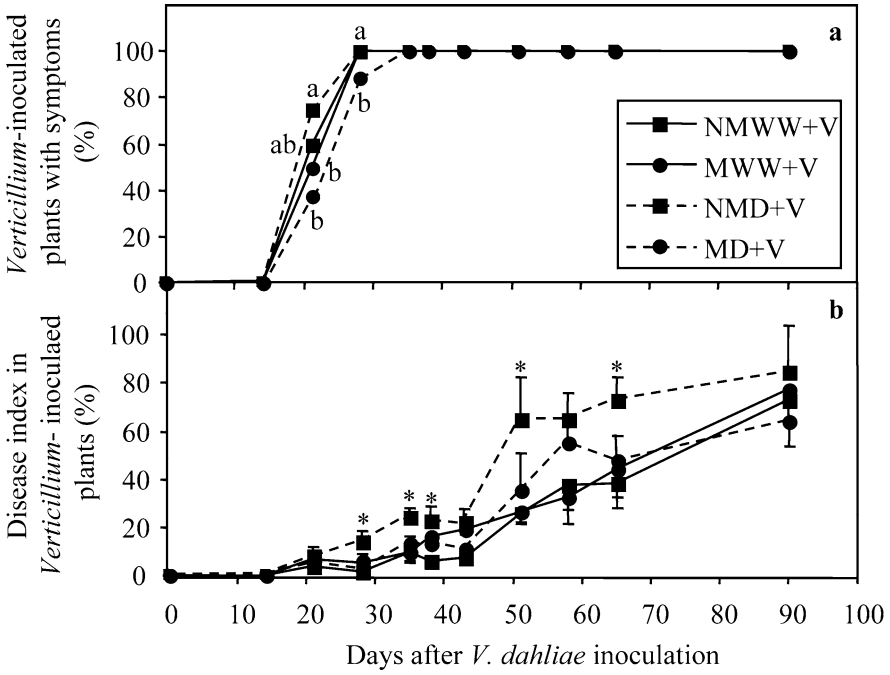

Fig. 2 Percentage of plants with visible symptoms of disease (**a**) and disease index (%) (**b**) in *Verticillium*-inoculated (+*V*) non-mycorrhizal (*NM*) and mycorrhizal (*M*) plants, well watered (*WW*) or predroughted (*D*). Data (n=5 plants) on percentage of plants showing disease symptoms were subjected to arc-sin transformation before applying χ^2-test. Different letters indicate significant difference. Data on disease index were analysed with a two-factor ANOVA. The variance was related to the main treatments [arbuscular mycorrhizal fungi (AMF) and drought] and to the interaction between them. Means ± SD (n=4–5 plants) were calculated and, when the F ratio was significant, least significant differences were evaluated by the Tukey-b test. *Asterisks* Treatments significantly different from the rest. Significance levels were always set at 5%

than NM plants under both well watered and stressful conditions.

The percentage of mycorrhizal colonisation was 25% (±1%) when *V. dahliae* was inoculated (Table 4) and more than 75% of mycorrhizal roots showed arbuscules and vesicles. In healthy well watered plants, mycorrhizal symbiosis was completely established by day 43. In contrast, the development of mycorrhizal symbiosis was delayed by both drought and pathogen inoculation. The lowest percentage of mycorrhizal colonisation on day 43 was observed in predroughted plants inoculated with *V. dahliae*, and the lowest production of vesicles corresponded to well watered plants inoculated with the pathogen. ANOVA results showed a significant interaction ($P<0.05$) between *V. dahliae* and water deficit on mycorrhizal colonisation and incidence of vesicles on that date. At the end of the plant life cycle, the percentage of mycorrhizal colonisation was around 50% in all treatments, and almost all roots had arbuscules and vesicles.

While near 80% of predroughted NM plants showed visible symptoms of disease 20 days after inoculation with *V. dahliae*, only 40% of predroughted M pepper had developed symptoms (Fig. 2a). One week later almost all the plants showed wilted, chlorotic and/or necrotic leaves (Fig. 2a). The disease index did not exceed 20% until day 43 after *V. dahliae* inoculation in any pathogen-infected plant (Fig. 2b). However, the disease index in predroughted NM plants was greater than that exhibited by the rest of the *Verticillium*-inoculated plants between days 28 and 65. ANOVA results indicated a highly significant interaction ($P<0.001$) between AMF and drought from day 28 until day 43 after pathogen inoculation. Such interaction was less significant ($P<0.05$) between days 51 and 65 (data not shown). At the end of the plant life cycle there were no significant differences between any of the pathogen-treated plants.

The 1st week after inoculation with *V. dahliae*, all predroughted plants exhibited higher stem RGR than well watered ones (Table 5). NM plants inoculated with the pathogen and previously subjected to water deficit also showed extensive production of leaf tissue during this period of time. The most remarkable feature 1 week later was the significantly greater root growth in pathogen-infected plants as compared to healthy ones, independently of the prior water regime. From day 14 until day 43, *V. dahliae* caused defoliation and delayed stem development in both NM and M pepper. From day 43 until final harvest (day 90), healthy plants also suffered leaf abscission due to natural senescence. The low stem and root RGR during this period indicated that all plants had completed vegetative development.

Table 5 Leaves, stem and root relative growth rates (*RGR*) (mg mg^{-1} day^{-1}) in well-watered (*WW*) or predroughted (*D*) non-mycorrhizal (*NM*) and mycorrhizal (*M*) plants, inoculated (+*V*) or not (−*V*) with *Verticillium dahliae*. Data were analysed with a three-factor ANOVA to partition the variance into the main effects and the interaction between AMF, drought and *V. dahliae*. Means ± SD (n=4–5 plants) were calculated and, when the F ratio was significant, least significant differences were evaluated by the Tukey-*b* test. Otherwise as for Tables 1 and 2

Treatment	Days after *V. dahliae* inoculation											
	0–7			7–14			14–43			43–90		
	Leaves RGR	Stem RGR	Root RGR	Leaves RGR	Stem RGR	Root RGR	Leaves RGR	Stem RGR	Root RGR	Leaves RGR	Stem RGR	Root RGR
NMWW−V	0.075 b	0.030 d	0.100 a	0.019 bc	0.031 d	0.013 c	0.044 a	0.093 a	0.043 ab	−0.023 b	0.016 a	0.001 d
MWW−V	0.071 b	0.024 d	0.109 a	0.005 c	0.048 bcd	0.003 d	0.031 b	0.068 ab	0.023 c	−0.012 a	0.019 a	0.013 a
NMD−V	0.074 b	0.290 ab	0.113 a	0.053 a	0.055 bc	0.016 c	0.046 a	0.090 a	0.048 a	−0.016 b	0.009 bc	0.000 d
MD−V	0.051 b	0.219 bc	0.072 a	0.031 ab	0.041 cd	0.006 d	0.046 a	0.083 a	0.041 ab	−0.007 a	0.018 a	0.004 c
NMWW+V	0.041 b	0.030 d	0.053 a	0.063 a	0.086 a	0.069 a	−0.001 c	0.047 bc	0.029 bc	−0.015 b	0.004 c	0.001 d
MWW+V	0.065 b	0.041 d	0.062 a	0.033 ab	0.044 bcd	0.042 b	−0.010 c	0.038 c	0.017 c	−0.010 a	0.010 b	0.008 bc
NMD+V	0.126 a	0.320 a	0.092 a	0.062 a	0.071 ab	0.063 a	−0.011 c	0.034 c	0.018 c	−0.017 b	0.004 c	0.009 b
MD+V	0.069 b	0.205 c	0.052 a	0.034 ab	0.077 ab	0.058 b	−0.001 c	0.044 c	0.021 c	−0.011 a	0.008 bc	0.007 bc
AMF (M)	*	***	ns	***	*	***	ns	ns	*	***	*	***
Drought (D)	*	***	ns	***	*	ns	ns	ns	ns	ns	*	ns
V. dahliae (V)	ns	ns	ns	***	***	***	***	***	***	ns	***	ns
M×D	***	***	ns	ns	ns	ns	*	*	ns	ns	ns	***
M×V	ns	ns	ns	*	*	ns	*	ns	ns	ns	ns	ns
D×V	***	ns	ns	***	ns	ns	*	ns	*	ns	ns	***
M×D×V	ns	ns	ns	ns	***	ns	ns	ns	ns	ns	ns	ns

***$P<0.001$, **$P<0.01$, *$P<0.05$, *ns* non-significant

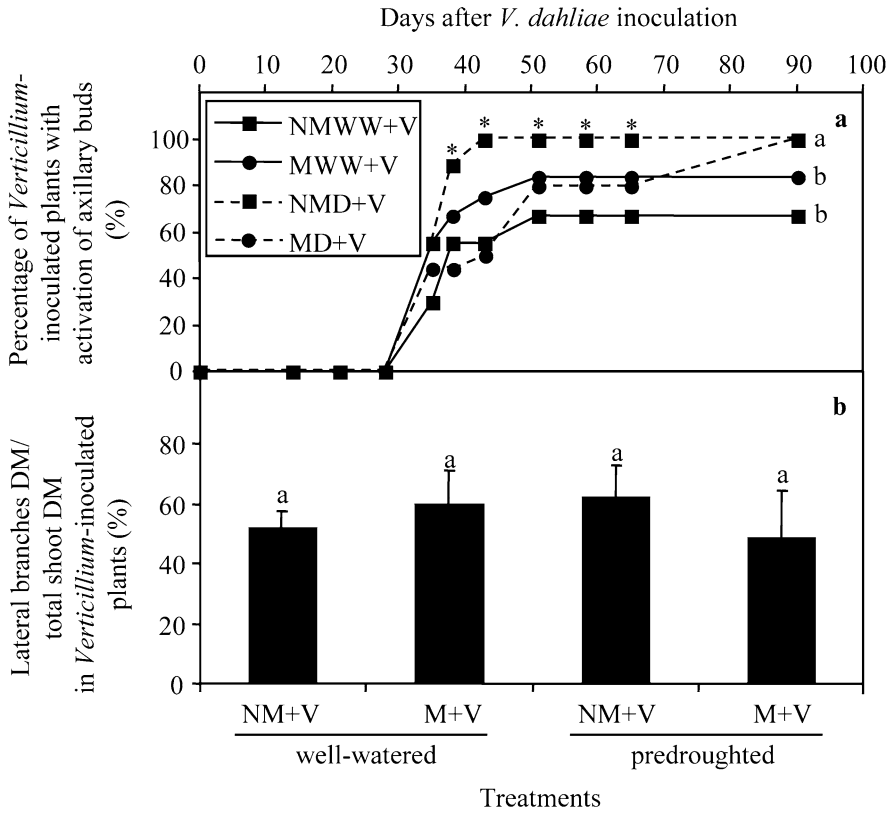

Fig. 3 Percentage of plants showing activation of axillary buds (**a**) and lateral branches to total shoot dry matter (DM) ratio (**b**) in *Verticillium*-inoculated (+*V*) non-mycorrhizal (*NM*) and mycorrhizal (*M*) plants, well watered (*WW*) or predroughted (*D*). Data on percentage of plants with activation of axillary buds were subjected to arc-sin transformation before applying χ^2-test. *Asterisks* Treatments significantly different from the rest. Values followed by the same letter do not differ significantly. Data on lateral branches to total shoot DM ratio were analysed with a two-factor ANOVA. The variance was related to the main treatments (AMF and drought) and to the interaction between them. Means ± SD (*n*=4–5 plants) were calculated and, when the *F* ratio was significant, least significant differences were evaluated by the Tukey-*b* test. Histograms with the same letter do not differ significantly. Significance levels were always set at 5%

V. dahliae caused activation of axillary buds in all treatments 1 month after inoculation (Fig. 3a). At final harvest, lateral branches were observed in all *Verticillium*-inoculated plants that had previously suffered water deficit. However, 100% of NM plants had developed lateral branches 43 days after pathogen inoculation. In contrast, the percentage of well watered pepper with secondary shoots reached around 70% and 80% in NM and M treatments, respectively, at the end of the experiment (Fig. 3a). The biomass of secondary branches at final harvest was similar among treatments and represented around 50–60% of the total shoot DM (Fig. 3b).

Total leaf P content is represented in Fig. 4. Despite the lower P nutrition received by plants associated with *G. deserticola*, P content was similar in leaves from NM and

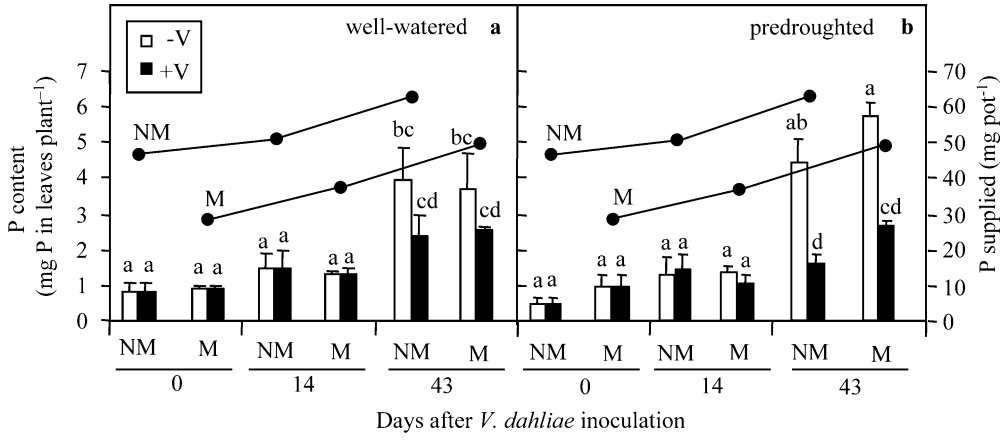

Fig. 4 Leaf phosphorus (P) content (mg plant^{-1}) and P supplied (mg pot^{-1}) to well watered or predroughted non-mycorrhizal (*NM*) and mycorrhizal (*M*) plants, inoculated (+*V*, *black bars*) or not (−*V*, *white bars*) with *Verticillium dahliae.* Values obtained before inoculating the pathogen were analysed with a two-factor ANOVA. The variance was related to the main treatments (AMF and drought) and to the interaction between them. Data after inoculating the pathogen were submitted to a three-factor ANOVA to partition the variance into the main effects and the interaction between AMF, drought and *V. dahliae*. Means ± SD (*n*=3 plants) were calculated and, when the *F* ratio was significant, least significant differences were evaluated by the Tukey-*b* test. Within each day after pathogen inoculation, values followed by the same letter are not significantly different ($P \leq 0.05$)

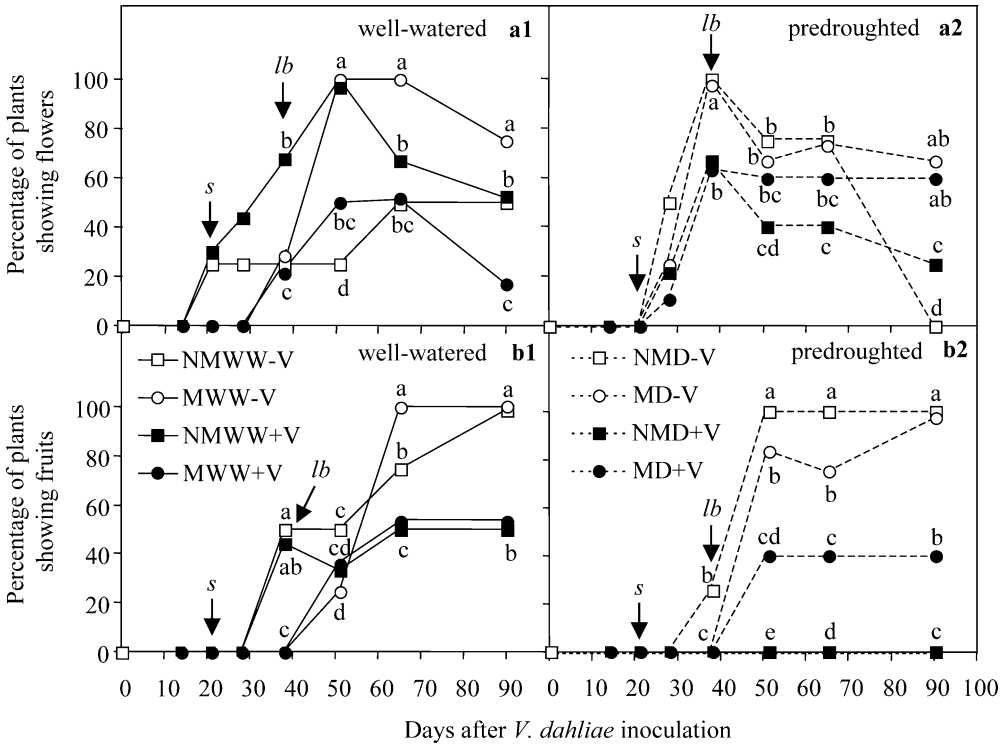

Fig. 5 Percentage of plants showing flowers (**a***1*, **a***2*) and fruits on main shoot (**b***1*, **b***2*) in well-watered (**a***1*, **b***1*) or pre-droughted (**a***2*, **b***2*) non-mycorrhizal (*NM*) and mycorrhizal (*M*) treatments, inoculated (+*V*) or not (−*V*) with *V. dahliae*. Data were subjected to arc-sin transformation before applying χ^2-test. Within each parameter and day after pathogen inoculation, values followed by the same letter are not significantly different. *s* First visible disease symptoms, *lb* appearance of first lateral branches. Significance levels were always set at 5%

M pepper the day on which *V. dahliae* was inoculated, independently of the previous water regime imposed on the plants. Likewise, no differences between treatments were observed 14 days later. However, on day 43, total P content in leaves from pathogen-infected plants was lower than that in healthy leaves, especially if plants had previously suffered drought. This decrease can be attributed to the defoliation observed in plants inoculated with *V. dahliae*. ANOVA results corroborated a significant interaction between drought and *V. dahliae* ($P<0.05$) on day 43 (data not shown).

Comparing MWW and NMWW healthy plants, flowering started 15 days earlier in NMWW plants than in those colonised by *G. deserticola* (Fig. 5a1). At final harvest, all NM and M plants had produced fruits (Fig. 5b1) and 80% of plants associated with *G. deserticola* still showed flowers on the main stem (Fig. 5a1). In well watered plants inoculated with *V. dahliae*, we observed that the percentage of NM plants with flowers was always higher than that of M plants (Fig. 5a1). However, the percentage of plants showing fruits at final harvest reached 60% in both treatments (Fig. 5b1), which indicates that many flowers in NM pepper were not transformed into fruits. Flowering in all predroughted plants began at the same time, regardless of the presence or absence of AMF or *V. dahliae* (Fig. 5a2). At final harvest, while healthy NM plants did not show flowers, 70% of healthy M plants still had flowers. Therefore, the symbiotic association prolonged the reproductive phase of healthy host plants, both well watered or under drought stress. The inoculation of predroughted plants with the pathogen had deleterious effects on the production of flow-

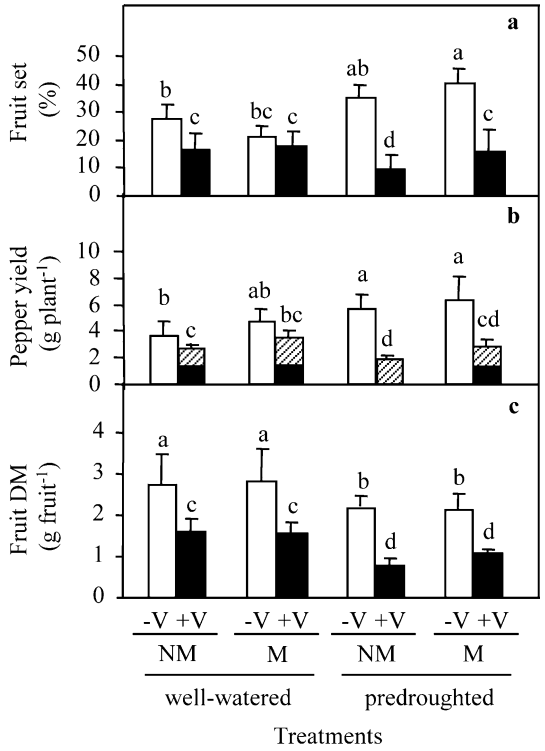

Fig. 6 Fruit set (%) (**a**), fruit yield (g plant^{-1}) (**b**) and fruit DM (g fruit^{-1}) (**c**) in well watered or pre-droughted non-mycorrhizal (*NM*) and mycorrhizal (*M*) plants, inoculated (+*V*) or not (−*V*) with *V. dahliae*. Data were submitted to a three-factor ANOVA to partition the variance into the main effects and the interaction between AMF, drought and *V. dahliae*. Means ± SD (n=4–5 plants) were calculated and, when the F ratio was significant, least significant differences were evaluated by the Tukey-*b* test. Within each graph, values followed by the same letter are not significantly different ($P\leq0.05$). Total yield in *Verticillium*-inoculated plants (**b**) includes peppers on main stem (*black bars*) and peppers on secondary shoots (*hatched bars*)

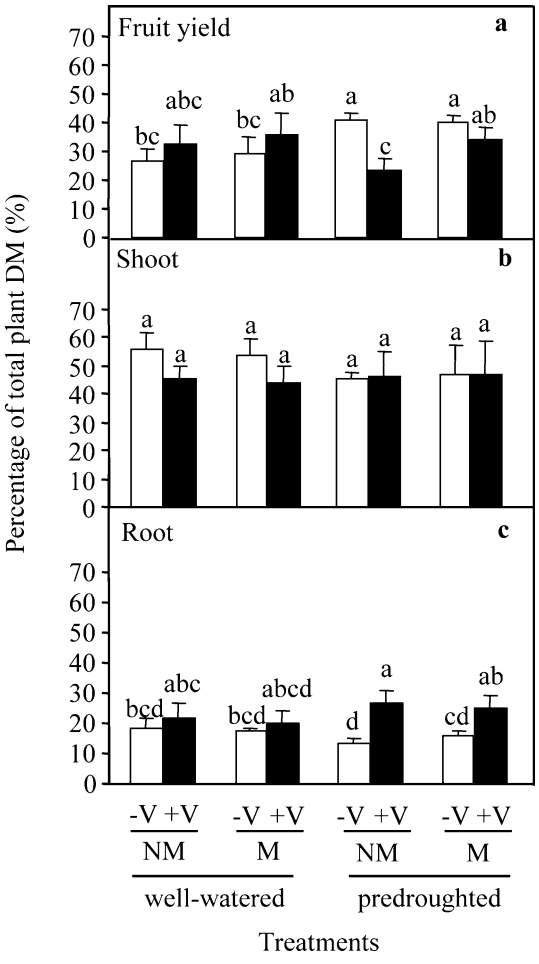

Fig. 7 Total plant DM partitioning into different organs expressed as a percentage in well watered or predroughted non-mycorrhizal (*NM*) and mycorrhizal (*M*) plants, inoculated (+*V*) or not (−*V*) with *V. dahliae*. Data were submitted to a three-factor ANOVA to partition the variance into the main effects and the interaction between AMF, drought and *V. dahliae*. Means ± SD (n=4–5 plants) were calculated and, when the F ratio was significant, least significant differences were evaluated by the Tukey-*b* test. Within each graph, values followed by the same letter are not significantly different ($P \leq 0.05$)

ers and, consequently, fruits (Fig. 5a2, b2). This negative effect was more evident in NM pepper; in this case, there were no fruits on main stems due to flower abortion and/or fall of incipient fruits. In contrast, flowering (Fig. 5a2) and fruit set (Fig. 5b2) in the M treatment occurred in 60% and 40% of plants, respectively.

Data on fruit set, yield per plant and individual fruit DM are shown in Fig. 6. Total yield in pathogen-infected plants (Fig. 6b) includes peppers produced on main shoot, and fruits produced on lateral branches. Individual fruit DM (Fig. 6c) in *Verticillium*-inoculated plants represents the medium weight of fruits harvested from main and secondary stems. Fruit set decreased in NMWW plants after pathogen inoculation (Fig. 6a). In contrast, MWW pepper showed similar fruit set regardless of the presence or absence of *V. dahliae* (Fig. 6a). The pathogen always decreased fruit set in plants previously subjected to water deficit; however, the symbiosis mitigated the effect of *V. dahliae* (Fig. 6a). The pathogen also caused decline in pepper yield (Fig. 6b) due to both lower fruit set (Fig. 6a) and smaller fruit size (Fig. 6c). In addition, a high percentage of fruits in diseased plants was harvested from secondary shoots (Fig. 6b). Peppers grown on lateral branches were smaller than those produced on main shoots and always showed green colour, indicating that their ripening was delayed (data not shown). In NMD pepper, *V. dahliae* caused total abscission of flowers and/or incipient fruits from the main stem.

In well watered healthy pepper, one-half of total plant DM was utilised to produce shoot biomass in both NM and M treatments (Fig. 7b). However, when healthy pepper had previously suffered drought, a large amount of energy and resources was employed to produce fruits, so that DM partitioning to fruits achieved 40% of total plant biomass (Fig. 7a). Inoculation with *V. dahliae* did not significantly influence DM partitioning if plants had not been previously subjectedpared to their healthy controls, NMD+V pepper showed an enhanced root production (Fig. 7c) to the detriment of yield (Fig. 7a). When plants were associated with *G. deserticola*, DM partitioning to fruits did not decrease as a consequence of the inoculation with the pathogen (Fig. 7a).

The three-factor ANOVA (Table 6) showed a highly significant effect of the pathogen (P<0.001) on fruit set, pepper yield, and individual fruit DM, as well as on the percentage of total plant DM used to produce root biomass. In addition, the interaction between *V. dahliae* and water deficit was very significant (P<0.001) on fruit set, yield and plant DM partitioning into fruits and roots. The presence of *G. deserticola* significantly affected pepper

Table 6 Significance of three-factor ANOVA showing effects of AMF, drought (*D*) and *Verticillium dahliae* (*V*) on fruit set, pepper yield, individual fruit DM and total plant DM partitioning into different organs (fruits, shoot and root) in well watered (*WW*) or predroughted (*D*) non-mycorrhizal (*NM*) and mycorrhizal (*M*) plants, inoculated (+*V*) or not (−*V*) with *V. dahliae*

	AMF (M)	D	V	M×D	M×V	D×V	M×D×V
Fruit set	ns	*	***	*	ns	***	ns
Pepper yield	***	ns	***	ns	ns	***	ns
Fruit DM	ns	***	***	ns	ns	ns	ns
Fruit DM/ total plant DM	ns	*	ns	ns	ns	***	ns
Shoot DM/ total plant DM	ns	ns	ns	ns	ns	ns	ns
Root DM/ total plant DM	ns	ns	***	ns	ns	***	ns

***P<0.001, **P<0.01, *P<0.05, *ns* non-significant

yield ($P<0.001$), and the only significant interaction between AMF and drought was observed on fruit set ($P<0.05$). No significant interactions were found between AMF, drought and *V. dahliae*.

Discussion

In studies on water relations in plants confined to pots—with the consequent restriction of soil volume—it is essential to work with plants of comparable size, because unequal sizes lead to different rates of soil water depletion and, consequently, unequal environmental stress (Davies et al. 1993). The NM and M pepper plants in our study had similar biomass and DM partitioning the day on which the first cycle of drought began. In addition, drought was imposed before flowering because moisture stress at some critical stages of the reproductive phase may have long-term effects on fruit production (Katerji et al. 1993; Pellitero et al. 1993) and our aim was to study effects on yield due exclusively to infection with *V. dahliae*. On the other hand, as drought can sometimes reduce levels of root colonisation by AMF (Goicoechea et al. 1997a,b), pepper plants were subjected to moderate water deficit in order to mitigate possible detrimental effects on the establishment of *G. deserticola*. However, the resulting drought was strong enough to delay plant growth, especially in pepper not associated with AMF. Under field conditions, even small water deficits suffered by pepper plants at early stages in their development can restrict vegetative growth (Wien 1997). The association of pepper with AMF can enhance drought resistance (Davies et al. 2002) by facilitating soil water uptake by fungal hyphae (Davies et al. 1992). Leaf water status—RWC and ψw—at the end of the second drought cycle was similar in well watered and stressed plants, regardless the presence or absence of AMF. Other authors have also observed little difference in leaf ψw of pepper plants subjected to different soil moisture under field conditions (Horton et al. 1982) or to low water stress in the greenhouse (Davies et al. 1992). In addition, in agreement with Davies et al. (1993), we observed no osmotic adjustment in leaves from droughted pepper. The most remarkable observation concerning water parameters was the higher Ψp in M pepper compared to that measured in NM plants under both WW and D conditions, which suggests different cell wall elasticity in the two types of plants (Goicoechea et al. 1997b). Unequal cell wall elasticity could determine differences in growth pattern despite similar cell water content.

One of the mechanisms that could be involved in bioprotection against soil-borne pathogens by AMF is the improvement in plant nutrient, especially P, status (Azcón-Aguilar et al. 2002). In our experiment, NM and M pepper showed similar P content in leaf tissues on the day on which the pathogen was inoculated, as a consequence of the lower P supplied to plants associated with *G. deserticola* in order to achieve NM and M pepper comparable in size. On the other hand, the significant effect of *V. dahliae* on the incidence of vesicles in roots of M plants 43 days after pathogen inoculation suggests some degree of competition between pathogenic and symbiotic fungi for host resources when they infect the same plant (Larsen and Bodker 2001). Consequently, the efficiency of *G. deserticola* as a biocontrol agent could have been decreased. In fact, the disease index was similar in pathogen-treated NM and M plants where growth was maintained under conditions of plentiful soil moisture.

When comparing healthy peppers, we found that the beginning of the reproductive phase was accelerated in MD plants and there was increased fruit set compared to MWW plants. However, this enhanced fruit set in MD plants was not concomitant with higher pepper yield because fruits were slightly smaller in the stressed treatment. It is usual to observe that, as pepper number per plant increases, the size of individual fruits tends to be smaller (Wien 1997). We can presume that this tendency would be more evident if plants were growing in a restricted soil volume. In the NM treatment, drought decreased plant growth in the initial stages but accelerated fruit set and increased yield. Given sufficient time for recovery, pepper plants that had previously suffered water deficit can resume yield without detrimental effects (Alvino et al. 1990).

When comparing *Verticillium*-inoculated plants with the corresponding healthy controls, we found that water deficit reinforced the deleterious effect of the pathogen on fruit set and yield. The decline in fruit yield could be related to the heavier growth of lateral branches in pathogen-treated plants previously subjected to drought. While the percentage of plants with activation of axillary buds achieved values of 60–80% under well watered conditions, it reached 100% in predroughted plants in the final stages of their life cycle. DM partitioning is the result of the flow of assimilates from source organs to sink organs and is regulated mainly by the competitive ability of the later to attract assimilates (Marcelis 1996). The growth of secondary shoots in *Verticillium*-inoculated plants may have resulted in enhanced competition for assimilates to the detriment of fruit production. According to Clapham and Marsh (1987), the most obvious sign of assimilate competition among different organs on the pepper plant is the abscission of flowers and small fruits during the most active period of fruit growth, resulting in a cycling of flowering and fruit set. In our study, a large proportion of pepper yield in *Verticillium*-inoculated plants corresponded to fruits produced on lateral branches and, in the case of NMD plants, no fruits were harvested from the main shoot despite the fact that flowering also took place on the main stem.

Although the effects of the water deficit imposed on plants grown in pots with limited soil volume cannot be entirely extrapolated to field conditions, the results obtained in the present study reveal the importance of soil moisture during the vegetative stage of the pepper life cycle. According to our experience, even a moderate drought suffered by seedlings could enhance their susceptibility to *V. dahliae*, especially when plants are not associated with AMF. For intensively managed pepper it is usual to apply high rates of P-fertiliser and to fumigate with methyl bromide prior to

planting to reduce soil-borne pathogens (Chellemi 2000), which may severely limit mycorrhizal colonisation. Our study was conducted under low P supply to ensure adequate colonisation of pepper roots by AMF. This is a basic premiss in a research work focussed on the potential role of AMF as a biocontrol agent. However, we must be cautious with the hypothetical application of AMF in the field. The interactions of AMF with soil-borne pathogens under natural conditions have barely been investigated and, so far, biological control interactions appear relatively minor and to depend on the host-pathogen complex (Larsen and Bodker 2001). Moreover, the use of AMF in production agriculture is questionable when low yields result from the elimination of soluble P-fertilisers in order to achieve high levels of mycorrhizal colonisation. Most field-based studies have shown that AMF are generally not crucial for the nutrition, growth or health of plants in many agricultural systems (Ryan and Graham 2002). However, according to Azcón-Aguilar and Barea (1997), AM biotechnology can be both feasible and rewarding, mainly for crops that involve a transplant stage, as in horticultural systems where plants are produced by tissue culture or in nursery beds. Peppers are vegetable crops that can be grown in the greenhouse for commercial purposes (Wien 1997).

In conclusion, previous studies have shown that *G. deserticola* can alleviate the deleterious effect of *V. dahliae* on pepper (Garmendia et al. 2004a,b), especially if the pathogen attack takes place during the vegetative growth of the plants (Garmendia et al. 2004a). However, in the present experiment, the delay in the onset of disease development in *Verticillium*-inoculated plants associated with AMF did not occur clearly under well watered conditions. As experimental conditions in all studies were apparently identical, it can be presumed that the equilibrium between pepper plant, *G. deserticola* and *V. dahliae* is so complex that small changes in competition between symbiotic and pathogenic fungi for host resources could modify the efficiency of *G. deserticola* as a biocontrol agent.

Drought pretreatment can increase plant tolerance to abiotic stresses such as chilling (Irigoyen et al. 1996; Aroca et al. 2003). In contrast, in our study, a drought pretreatment enhanced the deleterious effect of *V. dahliae* on fruit set and yield, especially when pepper plants were not associated with *G. deserticola*. This reinforces the idea that mycorrhizal association may be more important for host plants subjected to stressful conditions (Peña et al. 1988; Sánchez-Díaz et al. 1990; Goicoechea et al. 1997a). However, the comparison between NMWW and MD plants showed that a moderate water deficit imposed before inoculation with *V. dahliae* did not improve the effectiveness of *G. deserticola* as a biocontrol agent.

Although the present study constitutes a laboratory exercise, the results obtained may contribute to clarifying some basic aspects of the complex interactions between *V. dahliae*, *G. deserticola* and drought in pepper plants under controlled conditions. However, more research is required to determine whether AMF can be used as biocontrol agents against *Verticillium*-induced wilt in industrialised agricultural systems.

Acknowledgements The Plan de Investigación Universidad de Navarra (PIUNA) and Gobierno Vasco (AE-2000) supported this work.

References

Allen SE, Grimmshaw HM, Parkinson JA, Quarmby C, Roberts JP (1976) Methods in plant ecology. In: Chapman SB (ed) Chemical Analysis. Blackwell, Oxford, pp 411–466

Alvino A, Centritto M, Di Lorenzi F, Tedeschi P (1990) Gas exchange of pepper grown in lysimeter under different irrigation regimes. Acta Hortic 278:137–147

Aroca R, Irigoyen JJ, Sánchez-Díaz M (2003) Drought enhances maize chilling tolerance. II. Photosynthetic traits and protective mechanisms against oxidative stress. Physiol Plant 117:540–549

Augé RM (2001) Water relations, drought and vesicular-arbuscular mycorrhizal symbiosis. Mycorrhiza 11:3–42

Azcón-Aguilar C, Barea JM (1997) Applying mycorrhiza biotechnology to horticulture: significance and potentials. Sci Hortic 68:1–24

Azcón-Aguilar C, Jaizme-Vega MC, Calvet C (2002) The contribution of arbuscular mycorrhizal fungi for bioremediation. In: Gianinazzi S, Schüepp H, Barea JM, Haselwandter K (eds) Mycorrhizal technology in agriculture; from genes to bioproducts. Birkhäuser, Berlin, pp 187–197

Chellemi DO (2000) Adaptation of approaches to pest control in low input agriculture. Crop Prot 19:855–858

Clapham WM, Marsh HV (1987) Relationship of vegetative growth and pepper yield. Can J Plant Sci 67:521–530

Davies FT Jr, Potter JR, Linderman RG (1992) Mycorrhiza and repeated drought exposure affect drought resistance and extraradical hyphae development of pepper plants independent of plant size and nutrient content. J Plant Physiol 139:289–294

Davies FT Jr, Potter JR, Linderman RG (1993) Drought resistance of mycorrhizal pepper plants independent of leaf P concentration -response in gas exchange and water relations. Physiol Plant 87:45–53

Davies FT Jr, Olalde-Portugal V, Aguilera-Gomez L, Alvarado MJ, Ferrera-Cerrato RC, Boutton TW (2002) Alleviation of drought stress of Chile ancho pepper (*Capsicum annuum* L. cv. San Luis) with arbuscular mycorrhiza indigenous to Mexico. Sci Hortic 92:347–359

García-Mina JM, Jordana R, Aguirreolea J, Hernández MA (1996) The effect of a special organic amendment on the development of pepper plants cultivated in a soil infested with *Verticillium dahliae*. In: Rodríguez-Barrueco C (ed) Fertilizers and environment. Kluwer, Dordrecht, pp 301–303

Garmendia I, Goicoechea N, Aguirreolea J (2004a) Plant phenology influences the effect of mycorrhizal fungi on the development of *Verticillium*-induced wilt in pepper. Eur J Plant Pathol 110: 227–238

Garmendia I, Goicoechea N, Aguirreolea J (2004b) Effectiveness of three *Glomus* species in protecting pepper (*Capsicum annuum* L.) against verticillium wilt. Biol Control 31:296–305

Goicoechea N, Antolín MC, Sánchez-Díaz M (1997a) Gas exchange is related to the hormone balance in mycorrhizal or nitrogen-fixing alfalfa subjected to drought. Physiol Plant 100:989–997

Goicoechea N, Antolín MC, Sánchez-Díaz M (1997b) Influence of arbuscular mycorrhizae and *Rhizobium* on nutrient content and water relations in drought stressed alfalfa. Plant Soil 192:261–268

Goicoechea N, Aguirreolea J, Cenoz S, García-Mina JM (2000) *Verticillium dahliae* modifies the concentrations of proline, soluble sugars, starch, soluble protein and abscisic acid in pepper plants. Eur J Plant Pathol 106:19–25

Goicoechea N, Aguirreolea J, Cenoz S, García-Mina JM (2001) Gas exchange and flowering in *Verticillium*-wilted plants. J Phytopathol 149:281–286

Goicoechea N, Aguirreolea J, García Mina JM (2004) Alleviation of verticillium wilt in pepper (*Capsicum annuum* L.) by using the organic amendment COA H of natural origin. Sci Hortic 101: 23–37

Hayman DS, Barea JM, Azcón R (1976) Vesicular-arbuscular mycorrhiza in southern Spain: its distribution in crops growing in soil of different fertility. Phytopathol Mediterr 15:1–6

Horton R, Beese F, Wierenga PJ (1982) Physiological response of chilli pepper to trickle irrigation. Agron J 74:551–555

Hoyos GP, Laurer FI, Anderson NA (1993) Early detection of *Verticillium* wilt resistance in a potato breeding program. Am Potato J 70:535–541

Irigoyen JJ, Pérez de Juan J, Sánchez-Díaz M (1996) Drought enhances chilling tolerance in a chilling-sensitive maize (*Zea mays*) variety. New Phytol 134:53–59

Katerji N, Mastrorilli M, Hamdy A (1993) Effects of water stress at different growth stages on pepper yield. Acta Hortic 335:165–171

Larsen J, Bodker L (2001) Interactions between pea root-inhabiting fungi examined using signature fatty acids. New Phytol 149: 487–493

Marcelis LFM (1996) Sink strength as a determinant of dry matter partitioning in the whole plant. J Exp Bot 47:1281–1291

Narisawa K, Kawamata H, Currah RS, Hashiba T (2002) Suppression of *Verticillium* wilt in eggplant by some fungal root endophytes. Eur J Plant Pathol 108:103–109

Pellitero M, Pardo A, Simon A, Suso ML, Cerrolaza A (1993) Effect of irrigation regimes on yield and fruit composition of processing pepper (*Capsicum annuum* L.). Acta Hortic 335: 257–263

Peña JI, Sánchez-Díaz M, Aguirreolea J, Becana M (1988) Increased stress tolerance of nodule activity in the *Medicago-Rhizobium-Glomus* symbiosis under drought. J Plant Physiol 133:79–83

Phillips JM, Hayman DS (1970) Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. Trans Br Mycol Soc 55:158–161

Ruiz-Lozano JM, Azcón R (1995) Hyphal contribution to water uptake in mycorrhizal plants as affected by the fungal species and water status. Physiol Plant 95:472–478

Ryan MH, Graham JH (2002) Is there a role for arbuscular mycorrhizal fungi in production agriculture? Plant Soil 244: 263–271

Sánchez-Díaz M, Pardo M, Antolín MC, Peña JI, Aguirreolea J (1990) Effect of water stress on photosynthetic activity in the *Medicago-Rhizobium-Glomus* symbiosis. Plant Sci 71:215–221

Tenuta M, Lazarovits G (2002) Ammonia and nitrous acid from nitrogenous amendments kill the microsclerotia of *Verticillium dahliae*. Phytopathology 92:255–264

Van Loenen MCA, Turbett Y, Mullins CE, Feilden NEH (2003) Low temperature-short duration steaming of soil kills soil-borne pathogens, nematode pests and weeds. Eur J Plant Pathol 109: 993–1002

Weatherley PE (1950) Studies in the water relations of the cotton plant. I. The field measurements of water deficits in leaves. New Phytol 49:81–87

Wien HC (1997) Peppers. In: Wien HC (ed) The physiology of vegetable crops. CAB International, Wallingford, pp 259–293

Xiao CL, Subbarao KV, Schulbach KF, Koike ST (1998) Effects of crop rotation and irrigation on *Verticillium dahliae* microsclerotia in soil and wilt in cauliflower. Phytopathology 88:1046–1055