Mycorrhiza (2007) 17:551–562
DOI 10.1007/s00572-007-0128-3

ORIGINAL PAPER

Deficit irrigation promotes arbuscular colonization of fine roots by mycorrhizal fungi in grapevines (*Vitis vinifera* L.) in an arid climate

R. Paul Schreiner · Julie M. Tarara · Russell P. Smithyman

Received: 10 January 2007 / Accepted: 21 March 2007 / Published online: 3 April 2007

Abstract Regulated deficit irrigation (RDI) is a common practice applied in irrigated vineyards to control canopy growth and improve fruit quality, but little is known of how imposed water deficits may alter root growth and colonization by beneficial arbuscular mycorrhizal fungi (AMF). Thus, root growth and mycorrhizal colonization were determined throughout the growing season for 3 years in own-rooted, field-grown, 'Cabernet Sauvignon' grapevines exposed to three RDI treatments. Vines under standard RDI were irrigated at 60 to 70% of full-vine evapotranspiration (FVET) from 2 weeks after fruit set until harvest, a standard commercial practice. Early deficit vines were exposed to a more extreme deficit (30% FVET) during the period from 2 weeks after fruit set until the commencement of ripening (veraison), and thereafter reverted to standard RDI. Late deficit vines were under standard RDI until veraison, then exposed to a more extreme deficit (30% FVET) between veraison and harvest. The production of fine roots was reduced in both the early and late deficit treatments, but the reduction was more consistent in the early deficit vines because the additional deficit was imposed when roots were more rapidly growing. The frequency of arbuscules in fine roots was greater in both of the additional deficit treatments than in the standard RDI, a response that appeared chronic, as the higher frequency of arbuscules was observed throughout the season despite the additional deficits being applied at discrete times. It appears that grapevines compensated for a lower density of fine roots by stimulating arbuscular colonization. Irrigation did not affect yield or quality of grapes, but reduced whole-vine photosynthesis during the additional deficit periods. It appears that high-quality grapes can be produced in this region with less water than that applied under the current RDI practice because the root system of the vine may be more efficient due to greater arbuscular colonization by AMF.

Keywords Cabernet Sauvignon · Drought stress · Mycorrhiza · Root growth

The use of trade, firm, or corporation names in this publication is for the information and convenience of the reader. Such use does not constitute an official endorsement or approval by the United States Department of Agriculture or the Agricultural Research Service of any product or service to the exclusion of others that may be suitable.

R. P. Schreiner (✉)
Horticultural Crops Research Laboratory, USDA-ARS,
3420 NW Orchard Avenue,
Corvallis, OR 97330, USA
e-mail: schreinr@science.oregonstate.edu

J. M. Tarara
Horticultural Crops Research Laboratory, USDA-ARS,
24106 N. Bunn Road,
Prosser, WA 99350, USA

R. P. Smithyman
Ste. Michelle Wine Estates,
660 Frontier Road,
Prosser, WA 99350, USA

Introduction

Regulated deficit irrigation (RDI) is used to manage irrigation in vineyards where seasonal rainfall and stored soil water are insufficient to meet evaporative demand during the growing season. The idea of RDI is to constrain vegetative growth in grapevines (*Vitis* spp.) by supplying less water than needed for maximum growth, but enough water to maintain a high rate of photosynthesis, which is less sensitive to water stress than is growth (Dry et al. 2001; Keller 2005; Williams and Matthews 1990). This practice ensures that ripening of fruit is not delayed by excessive

competition for photosynthates by vigorous shoots or compromised by excessive shading of clusters during the ripening period (Jackson and Lombard 1993; McCarthy 1997). Fruit quality often is improved in grapevines exposed to moderate water stress, which sometimes is attributed to a concentration effect from smaller berries (Bravdo et al. 1985; Hardie and Considine 1976; Matthews and Anderson 1988; Roby et al. 2004). Currently, most wine grape vineyards in arid eastern Washington state are subjected to RDI with irrigation supplied at 60 to 70% of full vine evapotranspiration (FVET) during the period between fruit set and harvest. Deficits are not applied before fruit set because water stress at this time can cause berry abortion or the loss of entire clusters (Hardie and Considine 1976). Further improvements in fruit quality or greater savings in water use, or both, may be possible by applying less water than under the current RDI practice. However, previous studies that have shown improvement in fruit quality in response to water deficits have compared irrigated vines to vines receiving no irrigation. Such comparisons are not meaningful in arid climates where the absence of irrigation for an extended time (~2 weeks) during the summer will induce partial defoliation.

The effects of water deficit on aboveground vine growth, leaf water potential, and leaf gas exchange have been well studied (de Souza et al. 2005; Esteban et al. 1999; Hardie and Considine 1976; Patakas et al. 2005), but little is known about RDI influences on root growth or colonization by arbuscular mycorrhizal fungi (AMF). A few studies have shown that root growth or the root/shoot ratio of grapevines can increase in response to reduced water inputs (Freeman and Smart 1976; Höfäcker 1977; Van Zyl 1988), which has led to a general misconception by many viticulturists that root growth is stimulated in dry soil. However, it is clear from a number of field and greenhouse studies that grapevine roots cannot grow in soil below a water potential of about −1.5 MPa, whether the soil was dry because of irrigation placement (i.e., drip versus overhead sprinkler) or because of competition from cover crops (Dry et al. 2000; Morlat and Jacquet 2003; Van Zyl 1988). The tolerance of grapevines to drought often has been attributed to their capacity to produce new roots selectively where soil water is available in the root zone (Dry et al. 2000; Morlat and Jacquet 1993; Richards 1983; Smart and Coombe 1983).

The fine roots of grapevines often are heavily colonized by AMF, and vine establishment and growth are highly dependent on the presence of AMF in some soils (Menge et al. 1983; Schreiner 2005a). AMF improve nutrient uptake by grapevines, particularly P (Biricolti et al. 1997; Karagiannidis et al. 1995; Petgen et al. 1998), and AMF have been shown to improve drought tolerance in 'Cabernet Sauvignon' grafted onto various rootstocks (Nikolaou et al. 2003a), attributed to increased P uptake. Further study using P-fertilizer, however, showed increased drought tolerance by mycorrhizal vines in the absence of increased P uptake (Nikolaou et al. 2003b). AMF can improve drought tolerance of their host plants independently of their impact on P nutrition by enhancing osmotic adjustment in roots, altering hormone synthesis or transport, enhancing protection from oxidative damage, and increasing plant access to soil water (Augé 2001; Ruiz-Lozano 2003). Augé (2001) concluded that in ~75% of published studies on AMF and drought stress, mycorrhizal plants depleted soil water to a greater degree than non-mycorrhizal plants before achieving the same level of water stress in shoots. The extent of AMF colonization in roots under drought is often, but not always, enhanced over that of well-watered plants. One limitation of the literature on AMF and plant water stress is that studies overwhelmingly were conducted in potted plants under glasshouse conditions. Recent findings of increased root colonization by AMF in response to lower soil water contents in two field studies with grapevines (Schreiner 2003; Schreiner and Linderman 2005) suggest that AMF may play a significant role in vine response to water stress.

The present study was undertaken to determine how the timing and extent of RDI applied to 'Cabernet Sauvignon' grapevines would influence the seasonal dynamics of fine root development and their colonization by AMF. Belowground responses to RDI practices were examined over 3 years to better understand how grapevines acclimate to added water deficit (greater water stress) during discrete periods. Aboveground vine responses in this study, including canopy development, cold-hardiness, yield, and fruit quality were examined by M. Keller (unpublished), and whole-vine gas exchange was examined by Perez Peña and Tarara (2004).

Materials and methods

The study was conducted in a commercial, drip-irrigated vineyard located ~10 km west of Paterson, WA, USA (45.88° N, 119.75°W). The vineyard is located on a 14% south-facing slope ~125 m above sea level and receives ~200 mm rainfall per year. The soil in the vineyard is a Burbank series loamy fine sand (sandy-skeletal, mixed, mesic Xeric Torriorthents) with an average depth of 1.2 m. The vineyard was planted in 1992 with own-rooted, 'Cabernet Sauvignon' vines on a spacing of 1.8 m between vines and 2.7 m between rows (2,000 vines per hectare). Rows are oriented north–south. Vines were trained to two trunks and a bilateral cordon at a height of ~1 m, and shoots were loosely trained vertically between two foliage wires spaced 25 cm apart, at a height of 20 cm above the cordons. Vines were spur-pruned during dormancy to leave 36 to 42 buds per vine. Shoots were not thinned during the AMF study (2001–2003). Fertilizer applications and pest and disease management practices were

applied uniformly across all treatments. Vines received a total of 44 kg N and 6 kg S ha^{-1} in 2001; 26 kg N and 4 kg S ha^{-1} in 2002; and 39 kg N and 5 kg S ha^{-1} in 2003 delivered through the drip irrigation system. Drip irrigation was applied using pressure-compensated emitters (1.8 l/h) spaced 1.2 m apart (three emitters for every two vines along a single drip line per row).

Three RDI treatments were imposed from 1999 to 2003 in a randomized complete block design replicated four times. Each block comprised 30 rows of vines with 56 to 70 vines per row. Each RDI treatment was applied to ten consecutive rows of vines per block. All plots were irrigated to field capacity just after budbreak, and thereafter, irrigation was withheld until shoots were 0.9 to 1.2 m long and the rate of shoot growth was minimal. Just after fruit set, the three RDI treatments were imposed. All were based on estimated FVET derived from a reference crop ET (ET_0; Doorenbos and Pruitt 1977) and a crop coefficient for fully-irrigated 'Cabernet Sauvignon' in eastern Washington (Evans et al. 1993) multiplied by 0.7 to account for the smaller canopy of RDI vines. The standard RDI (current industry practice) supplied 70% of FVET (1999 to 2002) or 60% of FVET (2003) from 2 weeks after fruit set until harvest with the goal of maintaining soil water content in the top 1 m of soil at 10% *v/v*. The 'early' and 'late' deficit treatments targeted the two stages of berry development when berry growth is most rapid (Mullins et al. 1992). The early deficit was irrigated at 35% FVET (1999 to 2002) and 30% FVET (2003) from shortly after fruit set until veraison (berry growth stage 1), and returned to the standard RDI (60 or 70% FVET) from veraison to harvest. The late deficit was under standard RDI until veraison (60 or 70% FVET), then was more severely stressed (30 or 35% FVET) between veraison and harvest (berry growth stage 3). After harvest (1999 to 2002), all plots received irrigation equivalent to 70% FVET for an additional 4 to 5 weeks followed by irrigation to field capacity in late October. In 2003, all plots were irrigated to field capacity immediately after harvest.

Meteorological data were obtained from Public Agriculture Weather System (PAWS) stations near Alderdale, WA (approximately 10 km west of the site) and near Paterson, WA (approximately 15 km east of the site). Only 5-year mean values were available from Alderdale, so Paterson data were used for long-term normals. Heat accumulation, expressed as growing degree days (GDD) on a daily basis was calculated as

$$GDD = \left(\frac{T_{\max} + T_{\min}}{2}\right) - T_{base}$$

where T_{max} and T_{min} are the daily maximum and minimum air temperatures (2 m height), and T_{base} is the base temperature for grapevine growth (10°C). No upper temperature threshold was applied. Accumulated GDD were computed from April 1 to October 31, the convention applied to vineyards in Washington state.

Volumetric soil water content (0 to 90 cm depth) was determined weekly throughout the growing season using the neutron scattering method (HydroProbe 503 DR, Pacific Nuclear, Martinez, CA, USA). Parallel polyvinyl chloride access tubes were installed equidistant between drip emitters in the vine row. Data were collected at 15-, 45-, and 75-cm depths from three access tubes per plot. The average soil water content in each access tube (three depths combined, representing 0 to 90 cm) was used for analysis (n=12 per RDI treatment). The desired soil water content at the end of each irrigation cycle was 10% (*v/v*) for standard RDI and 8.3% (*v/v*) for the additional deficits.

Fine root length density and colonization of roots by AMF were determined five times during each growing season as close as possible to the phenological stages of budbreak, bloom, veraison, harvest, and leaf fall (first frost). Root sampling at budbreak and bloom occurred before starting the RDI treatments. Sampling at veraison coincided with the end of the early deficit period, and sampling at harvest coincided with the end of the late deficit period. Samples collected at budbreak and bloom varied with respect to vine phenology by as many as 20 days (bloom 2001 and budbreak 2003); however, samples collected at veraison and harvest were obtained within five days of the observed phenological stage. Root samples were obtained by removing soil cores (3.1-cm diameter) comprising 0- to 50-cm depth within the drip irrigation zones (wetted area below emitters). Five intact soil cores were combined into a single sample for each plot (n=4), giving a total of 12 composite samples at each date. Samples were stored on ice and transported to Corvallis, OR. In each year, at all five phenological stages, each plot was sampled from a single set of 20 vines occupying two rows (ten contiguous vines in each row) at a randomly designated location. The relative sampling location (top, middle, or bottom of slope) within each block was assigned in 2001 and moved up or down slope each subsequent year to avoid excessive coring near the same vines. Soil cores initially were collected to a depth of 100 cm (budbreak and bloom, 2001), but less than 20% of the fine roots were retrieved from soil between 50 and 100 cm. In addition, the quantity of roots found between 50 and 100 cm did not differ significantly (p<0.05) among irrigation treatments. Thereafter, roots were collected only from 0 to 50 cm.

The majority of roots were obtained by quickly sieving soil (at ambient soil water content) through a 1-mm sieve using small aliquots at one time. After the entire sample had been passed through the sieve, soil subsamples were collected to determine soil water content (~75 g) gravimetrically (Gardner 1986) in all years, and to estimate

extraradical hyphal length (~50 g) of AMF in 2003. Fine roots retained on the 1-mm sieve were excised from larger woody roots when necessary and stored in cold tap water. Fine root fragments remaining in the soil after passing through the sieve were collected on a 500-μm sieve after vigorously washing and decanting the sample three times with cold tap water (Böhm 1979). Both root fractions were combined and transferred to a large Petri dish where fine roots were separated from other organic debris and dead roots under a stereoscope. Only primary roots that had an intact cortex varying in color from white to dark brown were retained for further analysis. These roots were blotted dry, weighed, and subsamples were stored in formaldehyde/acetic acid/ethanol (2:10:50%, *v/v*). Roots were cleared with KOH and stained using trypan blue to assess colonization by AMF (Schreiner 2003). Fine root length in each sample was determined by the grid line intercept method (Newman 1966), and fine root length density (millimeter per gram of soil) was calculated by dividing root length by the total soil dry mass per sample. Colonization by any AMF structures (hyphae, vesicles or arbuscules, henceforth referred to as total colonization) and colonization by arbuscules alone were determined at 50 to 100× magnification on randomly selected root fragments (~50) that were mounted between microscope slides (see Schreiner 2003). A minimum of 150 intersections was counted per sample. The length of extraradical AMF hyphae was determined in 2003 only using the filtration-gridline technique (Sylvia 1992) as modified by Bethlenfalvay et al. (1999). Hyphal lengths were assessed in duplicate subsamples (10 g) from each plot at each sampling date (n=8 per RDI treatment). Only non-septate hyphae greater than 3 μm in diameter with a morphology consistent with AMF (irregular branching pattern and irregular wall thickness) were counted.

Leaf blade nutrient concentrations were measured at veraison and harvest in 2003 only. Leaves opposite clusters (15–20) were collected from randomly selected shoots from the same vines where roots were collected. Leaf tissues were oven-dried at 70°C for 7 days and ground in a Wiley mill to pass through a 40-mesh screen. Leaf N was determined via combustion analysis (Tru Spec CN, Leco, St. Joseph, MI, USA). Leaf P, K, Ca, Mg, Fe, Mn, Cu, B, and Zn were determined by ICP-OES (Perkin Elmer Optima 4300 DV, Wellesley, MA, USA) after microwave digestion in HNO_3.

Data were analyzed by analysis of variance for each year independently because root sampling locations within each plot were moved annually. Sampling date, RDI treatment, and block were used as factors, including the interaction between sampling date and RDI treatment. Means were compared using Tukey's HSD test at 95% confidence. Fine root length density data were log-transformed before analysis to correct for violations in the assumption of homogeneity of variance, and the data presented represent back-transformed means. All analyses were carried out using Statistica (version 6.2, Statsoft, Tulsa, OK).

Results

Air temperatures recorded between April and October (PAWS, Alderdale) were similar across years, except that 2003 was warmer than 2001 and 2002 (Table 1). The sum of degree days (base 10°C; April 1 to Oct 31) was 1,736 in 2001, 1,669 in 2002, and 1,857 in 2003, whereas the long-term mean (1991–2003) is 1,624. Major grapevine phenological stages occurred at fairly consistent dates, particularly the times of veraison and harvest, which only varied by up to six calendar days among years. However, the dates of budbreak and bloom varied by as many as 10 (budbreak) to 12 (bloom) days among years. Rainfall during the growing season consistently was very low (<45 mm). Plant demand for water, indicated by ET_0, was highest in 2001. The maximum daily ET_0 during the study was 14 mm. Total irrigation applied to the standard RDI plots was approximately 298 mm in 2001, 257 mm in 2002, and 248 mm in 2003. Although 2003 was among the warmest on record, the amount of irrigation applied in that season primarily was due to the adoption of a smaller crop coefficient by the vineyard owner (60% FVET), and partly due to higher winter rainfall eliminating the need for supplemental irrigation at budbreak in 2003. Across the study, the early deficit vines received 79% (2001), 68% (2002), and 69% (2003) of the total irrigation applied to the standard RDI vines. The quantity of irrigation applied to late deficit vines was 78% (2001), 83% (2002), and 82% (2003) of that applied to the standard RDI vines.

Soil water content in all plots declined steadily during spring and early summer to about 9% *v/v* at the time of bloom across all RDI treatments (Fig. 1), reaching 6.9% in 2001, 7.8% in 2002, and 9.4% in 2003 before the onset of differential irrigation. After the RDI regimens commenced, differences in soil water content were apparent and predictable, with the lowest values in the early deficit treatment between fruit set and veraison and in the late deficit treatment between veraison and harvest. When early or late deficit plots were receiving the same amount of irrigation as the standard RDI, differences in soil water content generally were not significant. There was considerable temporal variability because of the practical limitations of weekly applications in a commercial operation, but both the early and late deficits clearly reduced the amount of soil water available to vines during the period that the deficit was applied as was intended.

Table 1 Grapevine phenological stages, meteorological variables, and irrigation applied to deficit-irrigated 'Cabernet Sauvignon' vines over 3 years

Year /growth stage	Dates	GDDs[a] (°C>10)	Precipitation[b] (mm)	ET_0^c (mm)	Irrigation (mm)		
					Standard RDI	Early deficit	Late deficit
2001							
Dormancy[d]	Nov 10–Apr 20		60				
Budbreak–bloom	Apr 20–May 31	271	10	287	37	34	37
Bloom–veraison	May 31–Aug 6	743	15	567	112	72	108
Veraison–harvest	Aug 6–Sep 14	477	3	262	103	97	52
Harvest–frost	Sep 14–Nov 8	228	16	177	46	33	34
Seasonal total		1719	44	1293	298	236	231
2002							
Dormancy[d]	Nov 8–Apr 25		114				
Budbreak–bloom	Apr 25–Jun 11	262	17	301	11	9	11
Bloom–veraison	Jun 11–Aug 9	771	13	499	140	78	134
Veraison–harvest	Aug 9–Sep 19	462	4	252	60	48	27
Harvest–frost	Sep 19–Oct 25	128	4	104	46	41	42
Seasonal total		1,623	38	1,156	257	176	214
2003							
Dormancy[d]	Oct 25–Apr 15		170				
Budbreak–bloom	Apr 15–Jun 3	232	12	269	0	0	0
Bloom–veraison	Jun 3–Aug 5	832	1	512	158	87	143
Veraison–harvest	Aug 5–Sep 13	486	19	233	42	40	13
Harvest–frost	Sep 13–Oct 21	284	5	148	48	45	48
Seasonal total		1834	37	1162	248	172	204

[a] *GDDs* Growing degree days >10°C
[b] Meteorological data from Washington State University's Public Agricultural Weather System (PAWS) station near Alderdale, WA ~10 km west of the experimental site
[c] ET_0=evapotranspiration for a grass reference crop calculated from the Penman Monteith equation (Doorenbos and Pruitt 1977)
[d] Dormant season rainfall was summed between the dates of the first frost of the preceding year and budbreak of the current year

That RDI treatments created differences in the soil water available to the vines was evident in measurements of whole-vine photosynthesis and transpiration from a companion study in the same vineyard (Perez Peña 2004; Perez Peña and Tarara 2004). Just after fruit set but before imposition of differential irrigation, there were no significant differences among RDI treatments in daily cumulative transpiration or net carbon fixation in whole vines. Before veraison, when the early deficit vines had been exposed to more severe water stress for about 4 weeks, daily cumulative transpiration was as much as 50% lower than in vines under standard RDI. Likewise, within 2 weeks of the imposition of the late deficit, those vines transpired up to 60% less than vines under standard RDI. Vines under the early deficit did not recover after returning to standard RDI between veraison and harvest; rates of transpiration were intermediate between those of vines under the late deficit and those under standard RDI. Seasonal maximum transpiration rates occurred around the time of veraison (mid-August) when canopies were at their maximum size and evaporative demand also was at its highest. By harvest, rates of transpiration and photosynthesis were low across all RDI treatments because of lower temperatures, shorter day length, and some canopy senescence. After harvest, differences among RDI treatments were not significant (Perez Peña 2004).

Sampling date and irrigation significantly ($p<0.05$) influenced the density of 'Cabernet Sauvignon' fine roots in all years (Fig. 2; Table 2). Within seasons, the pattern of root length density among RDI treatments appeared temporally stable. Fine root length density increased during each season, reaching a peak at harvest regardless of RDI regimen (Fig. 2). In all years, the greatest increase in root length density occurred between bloom and veraison, while the early deficit RDI was being applied. Across RDI treatments, the length density of fine roots decreased after harvest in 2001 and 2002, but remained the same from pre- to post-harvest in 2003 in response to irrigation at FVET immediately after harvest. On an average basis, across the five sampling dates, fine root length density in standard RDI vines was significantly higher than that of late deficit vines in 2001 and significantly higher than that of early deficit vines in 2002 and 2003 (Table 2).

Total colonization of fine roots by AMF was not significantly affected by sampling date or RDI treatment in 2001 and 2002, although late deficit vines had significantly less total colonization in 2003 than did vines under either the

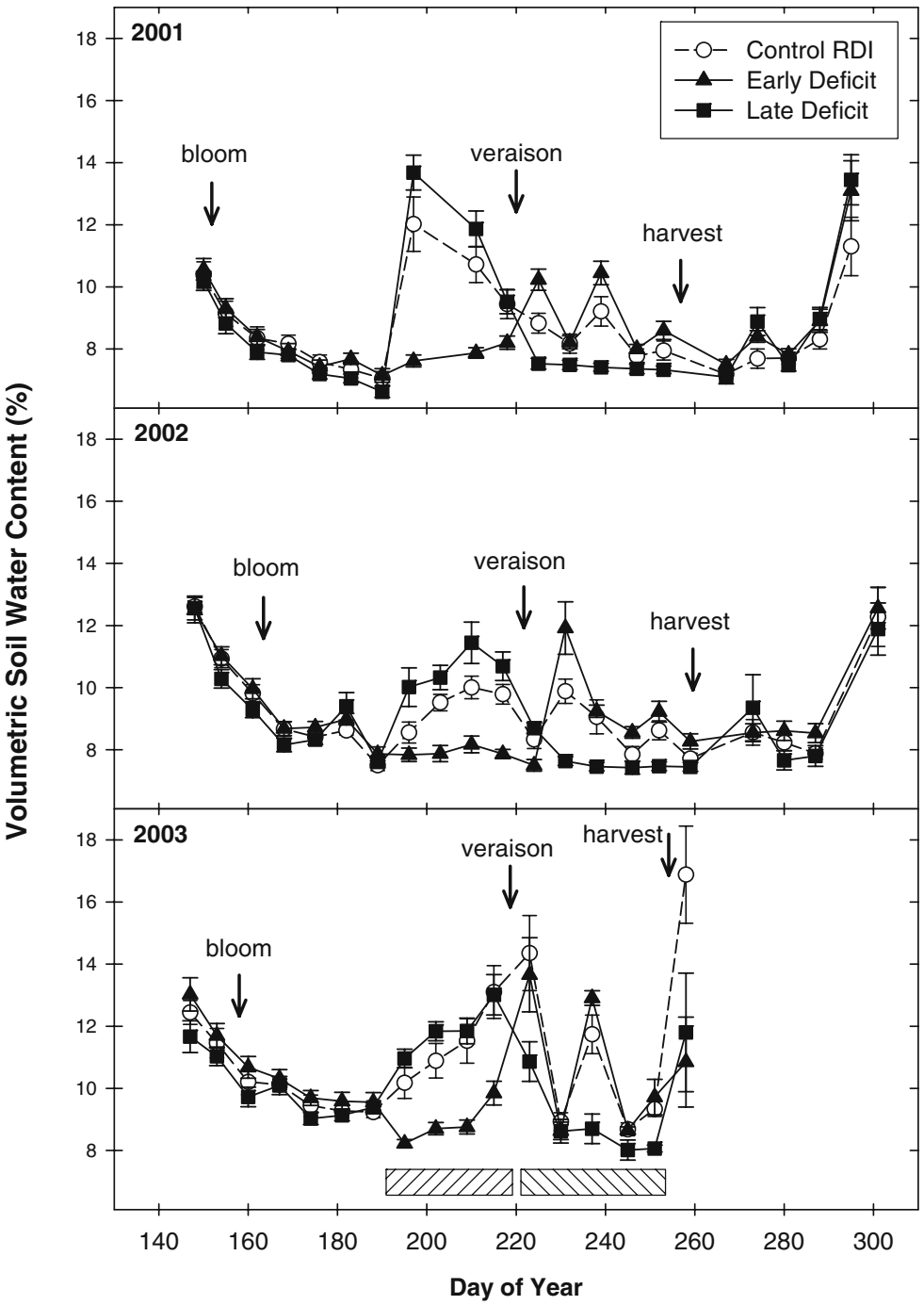

Fig. 1 Effect of RDI treatments on volumetric soil water content (0 to 90 cm) in a 'Cabernet Sauvignon' vineyard over 3 years. *Symbols* represent means (n=12), with *error bars* representing ±SEM. *Arrows* indicate the dates of bloom, veraison, and harvest in each year. The approximate periods of the early and late deficits are indicated by the *hatched blocks* at the *bottom* of the plot

early deficit or standard RDI (Table 2). However, the absolute difference in total AMF colonization (2003) between late deficit vines and those in other treatments was small (~2%) and probably not biologically meaningful. Arbuscular colonization consistently was affected by RDI treatment and sampling date (Fig. 3). Computed as a seasonal average, there was significantly higher percent arbuscular colonization in the roots under early deficit (2001, 2002, 2003) and the roots under late deficit (2001, 2002) than in the roots under standard RDI (Table 2). Across all RDI treatments, percent arbuscular colonization of fine roots remained relatively static after bloom in 2001, increased gradually from bloom through post-harvest in 2002, and followed a more parabolic pattern during the season in 2003, reaching maximum values just after veraison (Fig. 3).

The length density of fine roots with arbuscules (millimeter of arbuscular root per gram of soil) was significantly greater (p=0.003) in both early and late deficit vines than standard RDI vines in 2002, but was not affected by RDI treatment in 2001 or 2003 (data not shown). The length density of

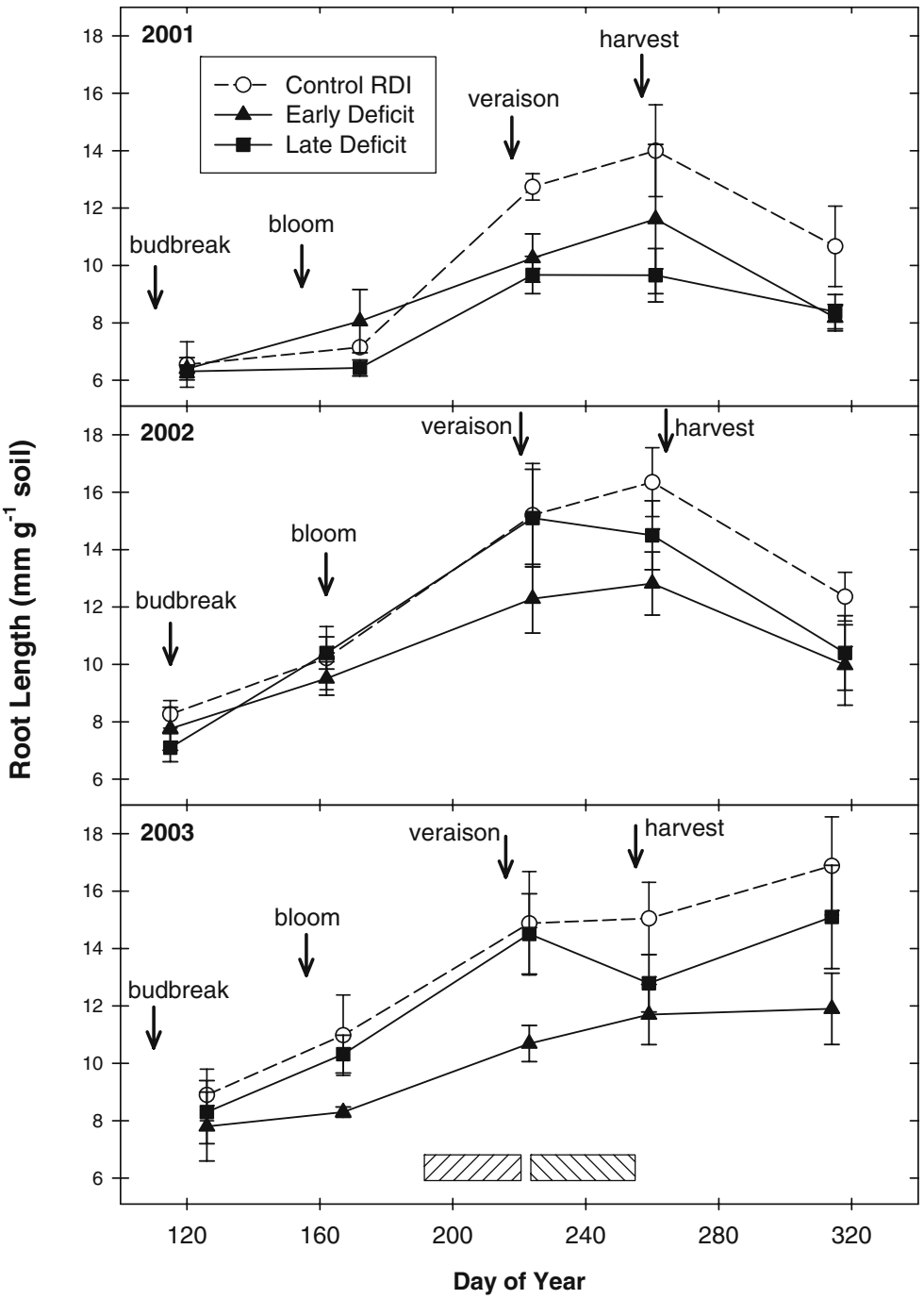

Fig. 2 Seasonal changes in fine root length density (0–50 cm) of 'Cabernet Sauvignon' grapevines in three deficit irrigation treatments over 3 years. *Symbols* represent back-transformed means (n=4), with *error bars* representing ±SEM. Main effects of sample date and irrigation treatment were significant (p<0.05) in each year, but the interaction between date and irrigation treatment was not. *Arrows* indicate the dates of budbreak, bloom, veraison, and harvest in each year. The approximate periods of the early and late deficits are indicated by the *hatched blocks* at the *bottom* of the plot

extraradical hyphae of AMF (measured in 2003 only) was not significantly affected by RDI treatment, although hyphal length density declined (p<0.001) over the course of the growing season from a high of 51.4 (±1.2 SEM) m/g soil at budbreak to a low of 35.0 (±2.1 SEM) m/g soil at leaf fall.

Nutrient concentrations measured in leaves in 2003 were not affected by irrigation treatment, but were affected by sampling date. For example, leaf P was 1.08 (±0.002 SEM) g/kg dry mass at veraison and 0.96 (±0.003 SEM) g/kg dry mass at harvest, and leaf N was 22.38 (±0.13 SEM) g/kg dry mass at veraison and 19.28 (±0.19 SEM) g/kg dry mass at harvest. Likewise, nutrient concentrations in petioles collected from bloom to harvest (six sampling dates) in 2001 were not affected by RDI treatment (data not shown). With the exception of P, all macro- and micro-nutrient concentrations in leaf blades (2003) or petioles (2001) fell within a range considered to be healthy for wine grapes (Cook 1966; Gärtel 1996; Robinson 1992). Phosphorus concentrations of 'Cabernet Sauvignon' leaf blades or petioles were close to the limit considered deficient for wine grapes, although above the concentration where a positive vine response to added P fertilizer has been observed (Cook et al. 1983; Skinner et al. 1988).

Table 2 Effect of RDI treatments on fine root length density and colonization by AMF per season

Treatment	Fine root length density (mm/g soil)			Total AMF colonization (% root length)			Arbuscular colonization (% root length)		
	2001	2002	2003	2001	2002	2003	2001	2002	2003
Control	9.6a	12.0a	12.6a	90.2	91.1	96.1a	20.8b	24.4b	29.1b
Early deficit	8.5ab	10.1b	9.7b	91.0	93.2	96.2a	27.8a	39.5a	40.0a
Late deficit	7.9b	11.0ab	11.7ab	91.6	93.7	94.2b	31.0a	36.2a	34.6ab
p value	0.020	0.042	0.005	0.511	0.106	0.005	<0.001	<0.001	<0.001

Values are means of an irrigation treatment across five sampling dates (n=20). Means within a column followed by the same letter are not significantly different (Tukey's HSD, 95% confidence).

Discussion

Results indicate that reducing the quantity of irrigation water applied to 'Cabernet Sauvignon' grapevines to 30 to 35% FVET for discrete periods during the growing season reduced the growth of fine roots but enhanced arbuscular colonization of roots over that of irrigation at 60 to 70% FVET. These findings support previous field observations where mycorrhizal colonization was negatively correlated with soil water content in grafted (Schreiner 2003) and in own-rooted 'Pinot noir' grapevines (Schreiner and Linderman 2005): Both total colonization and arbuscular colonization increased as soil water content decreased. By contrast, only arbuscular colonization was affected by reduced irrigation in the present study, which could be a cultivar-specific response. In other plants, AMF colonization of roots has been found to increase or decrease with roughly equal frequency in response to drought (Augé 2001), but arbuscule frequency was rarely examined. Elsewhere, root colonization by AMF was unaffected by drought in 'Cabernet Sauvignon' grapevines grown in pots, but arbuscule frequency was not assessed (Nikolaou et al. 2003a, b). Had we only examined total AMF colonization in the present study, we would have concluded that water deficits more extreme than those imposed under standard RDI had no effect. Our data are the first to show a specific increase in arbuscule frequency in grapevine roots resulting from controlled water deficit.

What is not known from this study is the benefit to the plant, if any, of greater arbuscular colonization in the roots of the more severely stressed vines. AMF were shown to facilitate increased stomatal conductance and carbon assimilation of drought-stressed 'Cabernet Sauvignon' grapevines grown in pots, and this benefit occurred whether or not AMF had facilitated increased P concentrations in leaves (Nikolaou et al. 2003a, b). The mycorrhizal vines in these studies may have had greater access to soil water than non-mycorrhizal vines, which allowed for higher rates of transpiration to occur under drought. The extent to which higher arbuscular colonization may have facilitated plant access to limited soil water in the more severe deficit treatments in our field study is not known. Early and late deficit vines transpired less water and fixed less carbon than standard RDI vines when the more extreme deficit was applied. The higher frequency of arbuscules that we observed in early and late deficit vines apparently did not enhance vine access to water to a level that was equal to that of standard RDI vines. However, had arbuscular colonization not been affected by the severity of RDI, vines exposed to the more severe water deficits may have suffered even greater damage from drought (such as oxidative damage to photosystems, loss of enzyme activity, and leaf senescence) than just the stomatal limitation of gas exchange that was observed (Perez Peña 2004).

We hoped to test the hypothesis (2003) that greater arbuscular colonization of roots would lead to more extraradical hyphae in soil in the early and late deficit vines, thus, providing greater hydraulic contact between roots and soil. Greater extraradical hyphal lengths, which appeared to facilitate subsequent soil water uptake, were observed in mycorrhizal pepper plants exposed to repeated drought cycles (Davies et al. 1992). However, although percent arbuscular colonization in roots in our study was greater in the early deficit vines in 2003, both the length of fine roots with arbuscules per unit mass of soil and the length of extraradical hyphae per unit mass of soil were the same among RDI treatments in 2003. It is unfortunate that we did not measure hyphae in 2002 when the length of fine roots with arbuscules per unit mass of soil was significantly higher in the early and late deficit treatments than in the standard RDI. The length density of extraradical hyphae in samples from this 'Cabernet Sauvignon' vineyard was about threefold higher than found previously in a rain-fed 'Pinot noir' vineyard sampled in the same manner (Schreiner 2005b), suggesting that AMF are more abundant in the irrigated 'Cabernet Sauvignon' vineyard. However, this is probably not the case because the vine roots (and hence, extraradical hyphae) were heavily concentrated under drip emitters in the 'Cabernet Sauvignon' vineyard, while roots in the rain-fed 'Pinot noir' vineyard were distributed over a larger area.

We found no effect of RDI treatment on nutrient concentrations in leaf blades (2003), nor did RDI treatment affect nutrient concentrations in petioles collected throughout

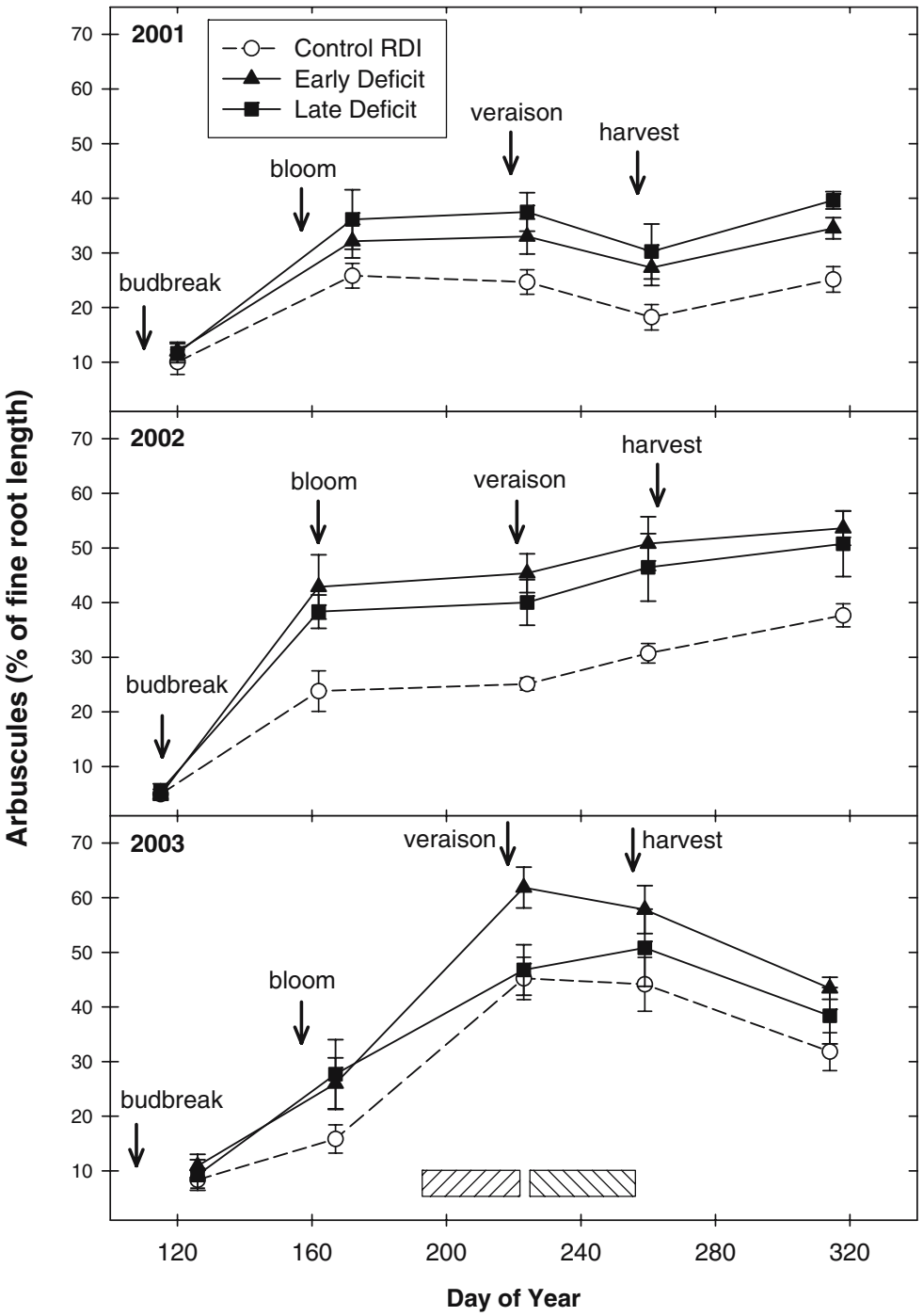

Fig. 3 Seasonal changes in arbuscular colonization of fine roots (0–50 cm) of 'Cabernet Sauvignon' grapevines in three deficit irrigation treatments over 3 years. *Symbols* represent means (n=4), with *error bars* representing ±SEM. Main effects of sample date and irrigation treatment were significant (p<0.05) in each year, but the interaction between date and irrigation treatment was not. *Arrows* indicate the time of budbreak, bloom, veraison, and harvest in each year. The approximate periods of the early and late deficits are indicated by the *hatched blocks* at the *bottom* of the plot

2001. However, evidence from field studies with other plant species (Sylvia et al. 1993; Kaya et al. 2003) suggests that vines in the early and late deficit treatments in our 'Cabernet Sauvignon' vineyard may have relied more heavily upon AMF to maintain nutrient uptake, particularly P. No differences were found in leaf nutrient concentrations among RDI treatments, but fine root length was reduced, while arbuscular colonization was increased in early and late deficit vines; thus, these vines probably relied more on AMF to supply nutrients than did the vines under standard RDI.

The dependence of fine root growth on soil water content was evident across RDI treatments. Standard RDI (highest amount of irrigation) consistently had the highest length density of fine roots over the growing season, while the early and late deficit treatments often had lower root length density. These data contrast with some studies (Freeman and Smart 1976; Höfäcker 1977) where a higher number of fine roots were produced by grapevines receiving less irrigation. However, the wet treatments in those studies were irrigated excessively (e.g., 300% FVET; Freeman and Smart 1976).

Van Zyl (1988) showed that the maximum number of root tips was produced by vines that were irrigated at 50% plant available water (PAW), with fewer roots produced when either more (irrigated at 90% PAW) or less (irrigated at 25% PAW) water was applied to 'Colombar' on 99 Richter rootstock. Our results agree with those of Van Zyl (1988).

We confined our root sampling to the soil zones that were wetted by irrigation, i.e., just beneath drip emitters in the vine rows. Hand-trenching (2001) revealed that no vine roots were growing in the alleyway (between rows of vines), and only large woody roots were growing horizontally between drip emitters in the vine row. Exceptionally few fine roots were attached to these woody roots between drip emitters. Because most fine roots in this vineyard were under the drip emitters, it is not surprising that the fine root density was much higher than that reported previously in grapevines (Mohr 1996; Schreiner 2005b).

A clear seasonal growth pattern of fine roots was apparent in this vineyard independent of irrigation treatment. Fine root length density increased nearly linearly from budbreak to veraison or harvest, in agreement with previous reports (Freeman and Smart 1976; Mohr 1996; Van Zyl 1988), but in contrast with results from a rain-fed vineyard where maximum root growth occurred after harvest (Schreiner 2005b). The seasonal pattern of arbuscule frequency in fine roots in the present study was similar to that of the rain-fed vineyard (Schreiner 2005b); arbuscular colonization increased after budbreak and generally remained high until the end of the growing season. The effect of irrigation on arbuscular colonization in the current study was more chronic than expected, with consistently higher arbuscular colonization in both early and late deficit vines. One would have expected a specific increase to be constrained to the period of the more extreme water deficit. However, increased arbuscular colonization was obvious by bloom, before the onset of differential RDI, suggesting that vines responded to the degree of water deficit from the previous growing season. Our analysis began after vines had been exposed to differential RDI for 2 years. It is unknown what stimulated greater arbuscular colonization before each season's deficits (2001 to 2003), but the response was correlated with the length of fine roots in the early and late deficit vines. For example, late deficit vines had the highest overall arbuscular colonization in 2001 when that treatment also had the lowest density of fine roots. Likewise, early deficit vines had the highest arbuscular colonization in 2002 and 2003 when that treatment had the lowest overall root length. Based on these observations, we believe that increased arbuscular colonization under more severe water deficit was largely a response to reductions in fine root growth.

The opposing response of root length density and arbuscular colonization was also evident during the post-harvest period, independent of irrigation treatment. Fine root length density decreased between harvest and leaf fall in 2001 and 2002 when irrigation was maintained at 60% of FVET, but remained the same after harvest in 2003 when vines were irrigated to field capacity right after harvest. Arbuscular colonization responded in the opposite fashion, increasing after harvest in 2001 and 2002 and decreasing after harvest in 2003. Thus, when soil water is more readily available, growth of fine roots increases, and arbuscular colonization decreases. This effect was not simply due to a dilution effect of root growth outpacing the spread of AMF within roots because total AMF colonization did not change. This is true also for the effect of irrigation deficit on root colonization. The increase in AMF colonization was specific to arbuscules, suggesting that the plant either produced some sort of specific signal(s) perceived by the fungi in roots that stimulated a greater number of arbuscules to form or that the life span of existing arbuscules increased in response to drought.

The RDI treatments in this study did not have a consistent effect on aboveground vine growth, yield, or fruit quality over 5 years (1999–2003) that different RDI treatments were imposed in this vineyard (M. Keller, unpublished data). For example, average shoot dry mass was lower in the early deficit vines in 2 years, and dormant season pruning weights were lower in 1 year, but these variables were not affected in any other year. Yield was not reduced, nor was fruit quality altered in the early or late deficit treatments in any year, although yields in this vineyard were considered low by commercial production standards. Therefore, production of high-quality wine grapes appears to be possible with less irrigation than is applied under the current RDI practice partly because increased arbuscular colonization by AMF may have compensated for reduced root length that was brought about by more severe water deficits. The additional water deficits applied in this study apparently allowed for greater expression of AMF activity in the roots of grapevines.

Acknowledgments We thank Matthew Scott, John Carter, and Stepfanie Lair for help in collecting and processing root samples. We also thank Mimi Nye for vineyard management and Markus Keller for sharing his results.

References

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

Bethlenfalvay GJ, Cantrell IC, Mihara KL, Schreiner RP (1999) Relationships between soil aggregation and mycorrhizae as influenced by soil biota and nitrogen nutrition. Biol Fertil Soils 28:356–363

Biricolti S, Ferrini F, Rinaldelli E, Tamantini I, Vignozzi N (1997) VAM fungi and soil lime content influence rootstock growth and nutrient content. Am J Enol Vitic 48:93–99

Böhm W (1979) Methods of studying root systems. Springer, Berlin Heidelberg New York

Bravdo B, Hepner Y, Loinger C, Cohen S, Tabacman H (1985) Effect of irrigation and crop level on growth, yield and wine quality of Cabernet Sauvignon. Am J Enol Vitic 36:132–139

Cook JA (1966) Grape nutrition. In: Childers NF (ed) Nutrition of fruit crops. Somerset, Somerville, MA, pp 777–812

Cook JA, Ward WR, Wicks AS (1983) Phosphorus deficiency in California vineyards. Calif Agric 37:16–18

Davies FT, 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

de Souza CR, Maroco JP, dos Santos TP, Rodrigues ML, Lopes C, Pereira JS, Chaves MM (2005) Control of stomatal aperture and carbon uptake by deficit irrigation in two grapevine cultivars. Agric Ecosyst Environ 106:261–274

Doorenbos J, Pruitt WO (1977) Guidelines for predicting crop water requirements. Irrigation and Drainage Paper 24. Food and Agriculture Organization of the United Nations, Rome

Dry PR, Loveys BR, Düring H (2000) Partial drying of the rootzone of grape. II. Changes in the pattern of root development. Vitis 39:9–12

Dry PR, Loveys BR, McCarthy MG, Stoll M (2001) Strategic irrigation management in Australian vineyards. J Int Sci Vigne Vin 35:45–61

Esteban MA, Villanueva MJ, Lissarrague JR (1999) Effect of irrigation on changes in berry composition of Tempranillo during maturation. Sugars, organic acids, and mineral elements. Am J Enol Vitic 50:418–434

Evans, RG, Spayd SE, Wample RL, Kroeger MW, Mahan MO (1993) Water use of *Vitis vinifera* grapes in Washington. Agric Water Manag 23:109–124

Freeman BM, Smart RE (1976) A root observation laboratory for studies with grapevines. Am J Enol Vitic 27:36–39

Gardner WH (1986) Water content. In: Klute A (ed) Methods of soil analysis. Part I. Physical and mineralogical methods. American Society of Agronomy, Madison, WI, pp 493–544

Gärtel W (1996) Grapes. In: Bennett WF (ed) Nutrient deficiencies and toxicities in crop plants. APS, St. Paul, MN, pp 177–183

Hardie WJ, Considine JA (1976) Response of grapes to water-deficit stress in particular stages of development. Am J Enol Vitic 27:55–61

Höfäcker W (1977) Untersuchungen zur stoffproduktion der Rebe under dem einfluss wechselnder bodenwasserversorgung. Vitis 16:162–173

Jackson DI, Lombard PB (1993) Environmental and management practices affecting grape composition and wine quality—a review. Am J Enol Vitic 44:409–430

Karagiannidis N, Nikolaou N, Mattheou A (1995) Influence of three VA-mycorrhiza species on the growth and nutrient uptake of three grapevine rootstocks and one table grape cultivar. Vitis 34:85–89

Kaya C, Higgs D, Kirnak H, Tas I (2003) Mycorrhizal colonization improves fruit yield and water use efficiency in watermelon (*Citrullus lanatus* Thunb). grown under well-watered and water-stressed conditions. Plant Soil 253:287–292

Keller M (2005) Deficit irrigation and vine mineral nutrition. Am J Enol Vitic 56:267–283

Matthews MA, Anderson MM (1988) Fruit ripening in *Vitis vinifera* L.: response to seasonal water deficits. Am J Enol Vitic 39:313–320

McCarthy MG (1997) The effect of transient water deficit on berry development of cv. Shiraz (*Vitis vinifera* L.). Australian Journal of Grape and Wine Research 3:102–108

Menge JA, Raski DJ, Lider LA, Johnson ELV, Jones NO, Kissler JJ, Hemstreet CL (1983) Interactions between mycorrhizal fungi, soil fumigation and growth of grapes in California. Am J Enol Vitic 34:117–121

Mohr HD (1996) Periodicity of root tip growth of vines in the Moselle valley. Vitic Enol Sci 51:83–90

Morlat R, Jacquet A (1993) The soil effects on the grapevine root system in several vineyards of the Loire Valley (France). Vitis 32:35–42

Morlat R, Jacquet A (2003) Grapevine root system and soil characteristics in a vineyard maintained long-term with or without interrow sward. Am J Enol Vitic 54:1–7

Mullins MG, Bouquet A, Williams LE (1992) Biology of the grapevine. Cambridge University Press, Cambridge

Newman EI (1966) A method of estimating the total length of root in a sample. J Appl Ecol 3:139–145

Nikolaou N, Angelopoulos K, Karagiannidis N (2003a) Effects of drought stress on mycorrhizal and non-mycorrhizal Cabernet Sauvignon grapevine, grafted onto various rootstocks. Exp Agric 39:241–252

Nikolaou N, Koukourikou M, Angelopoulos K, Karagiannidis N (2003b) Cytokinin content and water relations of 'Cabernet Sauvignon' grapevine exposed to drought stress. J Hortic Sci Biotechnol 78:113–118

Patakas A, Noitsakis B, Chouzouri A (2005) Optimization of irrigation water use in grapevines using the relationship between transpiration and plant water status. Agric Ecosyst Environ 106:253–259

Perez Peña JE (2004) Whole-canopy photosynthesis and transpiration under regulated deficit irrigation in *Vitis vinifera* L. cv. Cabernet Sauvignon. PhD dissertation. Washington State University, Pullman, WA

Perez Peña JE, Tarara JM (2004) A portable whole canopy gas exchange system for multiple mature field-grown grapevines. Vitis 43:7–14

Petgen M, Schropp A, George E, Römheld V (1998) Einfluss unterschiedlicher inokulationstiefen mit dem arbuskulären mykorrhizapilz *Glomus mosseae* auf die mykorrhizierung bei reben (*Vitis* sp.) in wurzelbeobachtungskästen. Vitis 37:99–105

Richards D (1983) The grape root system. Hortic Rev 5:127–168

Robinson JB (1992) Grapevine nutrition. In: Coombe BG, Dry PR (eds) Viticulture. Vol 2: practices. Winetitles, Adelaide, pp 178–208

Roby G, Harbertson JF, Adams DA, Matthews MA (2004) Berry size and vine water deficits as factors in winegrape composition: anthocyanins and tannins. Australian Journal of Grape Wine Research 10:100–107

Ruiz-Lozano JM (2003) Arbuscular mycorrhizal symbiosis and alleviation of osmotic stress. New perspectives for molecular studies. Mycorrhiza 13:309–317

Schreiner RP (2003) Mycorrhizal colonization of grapevine rootstocks under field conditions. Am J Enol Vitic 54:143–149

Schreiner RP (2005a) Mycorrhizas and mineral acquisition in grapevines. In: Christensen LP, Smart DR (eds) Proceedings of the soil environment and vine mineral nutrition symposium. American Society for Enology and Viticulture, Davis, CA, pp 49–60

Schreiner RP (2005b) Spatial and temporal variation of roots, arbuscular mycorrhizal fungi, and plant and soil nutrients in a mature Pinot noir (*Vitis vinifera* L.) vineyard in Oregon, USA. Plant Soil 276:219–234

Schreiner RP, Linderman RG (2005) Mycorrhizal colonization in dryland vineyards of the Willamette Valley, Oregon. Small Fruits Review 4:41–55

Skinner PW, Cook JA, Matthews MA (1988) Responses of grapevine cvs Chenin blanc and Chardonnay to phosphorus fertilizer applications under phosphorus-limited soil conditions. Vitis 27:95–109

Smart RE, Coombe BG (1983) Water relations of grapevines. In: Kozlowski TT (ed) Water deficits and plant growth. Academic, New York, pp 137–196

Sylvia DM (1992) Quantification of external hyphae of vesicular–arbuscular mycorrhizal fungi. Methods Microbiol 24:53–65

Sylvia DM, Hammond LC, Bennett JM, Haas JH, Linda SB (1993) Field response of maize to a VAM fungus and water management. Agron J 85:193–198

Van Zyl JL (1988) Response of grapevine roots to soil water regimes and irrigation systems. In: Van Zyl JL (ed) The grapevine root and its environment. Department of Agriculture and Water Supply, Pretoria, South Africa, pp 35–43

Williams LE, Matthews MA (1990) Grapevine. In: Stewart BA, Nielsen DR (eds) Irrigation of agricultural crops. American Society of Agronomy, Madison, pp 1019–1055