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ROOT DYNAMICS IN RESPONSE TO ABIOTIC AND BIOTIC STRESSORS IN *VITIS*

A Thesis in

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by

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ABSTRACT

Root system potential growth rate was examined in response to soil moisture heterogeneity. Root dynamics were studied using genetically identical grapevine shoot systems (*Vitis vinifera* cv Merlot) on genetically distinct root systems that promote high and low shoot vigor under three different levels of water stress severity. We utilized minirhizotrons to examine a more vigorous rootstock (1103P) and a less vigorous rootstock (101-14 Mgt) in an Oakville, CA vineyard with high soil clay content. Our results demonstrated that compared to a lower vigor root system, a grape root system of higher vigor exhibit greater morphological plasticity in response to heterogeneous moisture supply but similar tolerance as indicated by root survivorship in dry soil. The lower-vigor vines also developed more root biomass over several years, especially under conditions of no irrigation. Similar root lifespans in wet and dry soil suggested the possibility that hydraulic redistribution was a source of water transfer from areas of high soil moisture to those of low soil moisture through the plant during periods of low evaporative demand. We hypothesized that hydraulic redistribution prevented an appreciable reduction in root water potential and contributed to the prolonged root survivorship in dry soil. With thermocouple psychrometers, we measured water potentials of roots of the same plant with its roots system split into an irrigated pot and an unirrigated pot. We found reduced root survivorship was directly associated with plants in which hydraulic redistribution was reduced by 24 h light. Electrolyte leakage in dry roots was twice that compared to those in wet soil of the same plant. Our study demonstrated that besides a number of ecological advantages to protecting tissues against desiccation, internal hydraulic redistribution of water is a mechanism allowing extended root survivorship in dry soils. Plants with fast growth rates are hypothesized to be more tolerant of herbivory than slower growing plants. Our data on root systems that differ in potential growth rate supported the hypothesis proposed for leaves, that phylloxera insect infestation was proportional to number of growing tips. Lifespans of uninfested roots were similar for both root systems; however, the infested roots of the faster-growing root system were shorter than that of the slow-grower. The fast-growing rootstock had an

older age structure due to the mortality of young root tips. We did not find a trade-off between potential growth rate and relative rate of root infestation in these cultivars, but our study indicates that a fast-growing root system may more readily shed infested roots.

Keywords: minirhizotron, root survivorship, root production, hydraulic redistribution, herbivory

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To my brother, who taught me a lot about plant water relations, a little about getting your Ph.D., and nothing at all about real life.

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To all the members of the Eissenstat Lab where crazy is an everyday word.

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Preface

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Chapter 1

General Introduction

General overview

Water availability is a major determinant of plant distribution and abundance. A plants' survival often depends on its ability to withstand periods of little to no precipitation. In coping with soil moisture deficits, plants typically preferentially grow roots in regions of high soil moisture (Green and Clothier 1999; Fort et al. 1998; Coutts 1982). Portions of the root system near the soil surface often experience soil drying while deeper roots are still in relatively moist soil. When dry soils overlie relatively shallow water tables, an even more extreme condition results (e.g., Napa Valley, California). Soils, however, are not always wetter in deeper layers. For some soil types water percolation past the top layers of the soil may be restricted by a horizon with very low hydraulic conductivity, causing the soil after small rainfall or irrigation events to be wetter near the surface than in deeper layers. Root responses to heterogeneity in soil moisture, including root birth and death rates (North and Nobel 1998), anatomical changes (Stasovski and Peterson1991; Taleisnik et al. 1999) and physiological regulation through osmotic adjustment (Westgate and Boyer 1985; Sharp and Davies 1979), plant signaling to the leaves (Steudle 2000; Gowing, Davies and Jones 1990) and hydraulic redistribution (Emerman and Dawson 1996), are a few of the ways that roots can mitigate localized soil moisture deficits.

Several studies have been conducted with plants in containers examining root responses to soil moisture heterogeneity. Often studies examine whole-plant responses by utilizing split-root configurations where only one side of the root system is exposed to dry conditions (Stoll, Loveys and Dry 2000; Fort et al. 1998; Fort et al. 1997; Blum and Johnson 1992; Blackman and Davies 1985). Tan and coworkers went so far as to split tomato (Tan, Cornelisse and Buttery 1981) and peach (Tan and Buttery 1982) seedlings

roots into four separate quadrats to examine transpiration, stomatal conductance and photosynthesis in response to supplying different amounts of water to various portions of the root system. Ultimately, in peach, ¼ of the root system could be exposed to dry conditions and the plant still maintained the same transpiration, photosynthesis, stomatal conductance and xylem pressure potentials as those of the well watered (all four compartments) controls. When only one of the quadrants was watered (¾ dry), the peach seedlings reduced transpiration and photosynthesis by 40% and stomatal conductance and xylem pressure potential by 50% of the controls. Kosola and Eissenstat (1994) examined root growth, life span, and carbon allocation of fine roots exposed to vertical soil water heterogeneity. When water was supplied to the bottom portion of the root zone only, neither root dry weight nor total plant weight was affected in comparison to the well watered controls suggesting that plants with root access to deep soil water can maintain plant function. Few potted studies of this kind have been conducted in grape. Dry, Loveys and Düring (2000a, b) described changes in shoot growth, root development and leaf gas exchange under conditions of partial drying of the rootzone. When compared to well watered vines the authors found decreased shoot growth, stomatal conductance, and root survivorship in shallow depths when only part of the root system was watered. However, results of this study may be misleading because there were no controls used in several of the experiments and the number of roots on the glass walls of the containers may not reflect root numbers in the soil.

While studies have examined the effects of localized soil moisture deficits in potted seedlings, relatively few physiological studies have examined the effects of localized soil moisture deficits on the dynamics and physiology in mature plant root systems grown in unrestrictive soil volumes in the field. Container experiments often restrict root expansion and, thus, may not reflect the conditions in the field where roots are growing in largely unrestrictive soil volumes.

Wine grape biology

Grape vines in particular, with their expansive root systems (Jackson 2000) might be adversely affected by volume restrictions in experiments conducted with containers. Besides often expansive root systems, vines have other properties that make them a unique group. Most species of vines have large xylem vessels and have the capacity to transport water quickly (Lambers, Chapin and Pons 1998) and efficiently (Ewers, Fisher and Fichtner 1991). At least compared to other fruit crops, grapevines can grow well in regions of little summer precipitation, presumably because of deep expansive root systems and the ability to transport large quantities of water quickly (Champagnol 1984). Moreover, characteristics of the grapevine root system such as the ability to cope with water stress allow the vines to survive the harsh summers of Mediterranean climates in which they often grow (Mullins, Bouquet and Williams 1992).

During grapevine growth, water deficit plays an integral role in vine development and ultimately fruit yield and quality (Hardie and Considine 1976). In general, there has been only limited field-based research on grapevine root water status in response to soil water deficit. Previous determination of the effects of wet and dry soil conditions on spatial and temporal vine water status has been largely restricted to the shoot (Choné et al. 2001; Dry, Loveys and Düring 2000a; Greenspan, Shackel and Matthews 1994; Winkel and Rambel 1993; Hardie and Considine 1976).

General patterns of root dynamics

The easy access to plant canopies has resulted in the majority of theories concerning plant organ lifespan to be conducted on leaves (Wright et al. 2005; Reich, Walters and Ellsworth 1992). The inaccessibility of roots on the other hand makes measuring roots extremely laborious. However, fine roots lifespan has become increasingly recognized as an important contributor to plant function (Eissenstat et al. 2000). Even so, our current understanding of environmental or physiological factors that influence root lifespan remains minimal.

The most extreme response to reduced water availability is root death. Decreased root survivorship and reduced root birth of fine lateral roots potentially reduce levels of water and nutrient acquisition. There is a major gap in understanding patterns of root growth in response to decreased water in natural environments. Of interest in the field environment is how patterns of root growth and death respond to variations in soil moisture in different soil layers over the growing season. Anderson et al. (2003) showed longer survivorship of Concord grape roots in deeper soil layers compared to shallow layers. In a natural mixed hardwood forest, localized patches of water and N not only increased overall new root production, but also increased overall root longevity (Pregitzer, Hendrick and Fogel 1993). The authors suggested that root response to localized patches of water and nutrients may be plastic in the sense that root growth and distribution changed depending on the amount and location of resources.

Hydraulic redistribution

Grapevines are known to transport large quantities of water (Salleo, Lo Gullo and Oliveri 1985). In order to maintain water uptake required for plant growth and development, roots must physiologically regulate internal water potential to retain a soil-to-plant water potential gradient. It is quite possible that root systems mitigate localized soil moisture deficits by redistributing water to roots under the greatest moisture stress. Indeed, recent evidence suggests that roots in moist soil can redistribute water to those in dry soil, possibly preventing appreciable water stress (Smart et al. 2005; Green and Clothier 1999). While changes in water potential with soil drying may seem intuitive, this area is actually poorly understood. Water potential has been used to evaluate whole plant (Williams and Araujo 2002), stem (Choné et al. 2001), leaf (Winkel and Rambal 1993), and in a few instances root (Simonneau and Inra 1991; Nobel and Lee 1991) water status. However, there exists a good deal of controversy concerning hydraulic redistribution and its possible effects on both leaf and root water potential. The theory of hydraulic redistribution implies the nocturnal transfer of water from roots in wet zones to roots in dry zones (Richards and Caldwell 1987). Split-pot experiments with various plants

where one pot is watered to field capacity while the other pot is allowed to dry down have shown an increase in water potential in the dry pot when transpiration is suppressed (Fort et al. 1998; Fort et al. 1997; Blum and Johnson 1992; Coutts 1982). The increased water potential in the soil was located in the region where roots were present (Blum and Johnson 1992). This suggests that water moved through the plant and exuded from the roots in the dry soil. It is important to recognize the role that hydraulic redistribution might play in root response to water stress. Differentials in root water potential and physiological adjustment of individual roots should help to determine the presence or lack of water redistribution in the root system. Lowered water potential of roots in dry soil suggests increased axial resistance within the root system. An explanation for this resistance is xylem embolism. If a substantial drop in root water potential occurs, then this should be an indication of xylem embolisms in root orders less distal from the main framework roots (Jackson, Sperry and Dawson 2000). Roots provide an important place to examine embolisms since roots have the lowest threshold tensions within a plant (McCully, Huang, and Ling 1998). A relationship between plant water potential and root resistance to flow was examined in a small study on cotton seedlings (Byrne, Begg and Hansen 1976). Estimated root potential gradients suggested that root resistance to water flow did not follow a linear relationship with plant water potential because of the sharp increase in resistance when water stress surpassed -2 MPa. The authors propose root cavitation as the likely reason for the increase in flow resistance. In the context of this study, root water potential serves as a baseline measurement upon which initial examination of individual root response to water stress can be determined.

Herbivory Tolerance of fine roots

In addition to abiotic factors such as soil moisture deficits, roots can also be strongly influenced by biotic factors. While the study of insect herbivory on leaf tissue has been relatively well studied, the role of insect herbivory on below ground structures remains poorly understood. Like leaves, herbivores can cause severe damage to plant roots by disrupting resource flow and source: sink relationships (Brown and Gange 1990). Within

grapevines the root feeding louse phylloxera (*Daktulosphaira vitifoliae* FITCH) is a pest of great economic importance (Granett et al. 2001). The introduction of phylloxera resistant American rootstock species and inter-specific rootstock hybrids has currently curbed the danger of widespread vineyard damage for vinifera grapes. Research into the ecology of phylloxera and insect herbivory in general will contribute to our understanding of how plants cope with insect attacks on roots and the importance of insect herbivory on plant community dynamics.

How a plant reacts to root insect herbivores may be directly linked to the rate of growth of the plant where plants with fast growth rates are hypothesized to be more tolerant of herbivory than slower growing plants (Coley 1988). It has been suggested that it is not growth alone that forms the basis for this hypothesis but also plant phenological or morphological plasticity (van Schaik, Terborgh and Wright 1993). However, foliar research on the role of plant growth rate on plant responses to insect damage has triggered much controversy. As a resolution to controversy the use of actively growing meristematic tips has replaced the common usage of organ biomass as a method to determine insect infestation level (Price 1991). However, the use of the number of tips method supports a similar hypothesis to that of the plant growth rate method; faster plant growth obtains higher levels of plant herbivory (Fritz, Crab and Hochwender 2000; Woods et al.1996; Kimberling, Scott and Price 1990; Craig, Itami and Price 1989).

The chapters provide a closer look at both abiotic and biotic stressors on the grapevine fine root system. I have successfully examined fine root response to heterogeneous soil moisture in faster- and slower-growing grape root systems and the importance of internal hydraulic redistribution on prolonging fine root lifespan. In addition, my work on root production, lifespan and mortality of root systems that differ in potential growth rate and their influence of insect herbivory represents the first direct examination on the relationship between growth rate of root systems and root dynamics. Thus this work constitutes a substantial scientific contribution to the study of fine root dynamics and our collective understanding of the ecophysiology of root systems of different potential growth.

References

Anderson L.J., Comas L.H., Lakso A.N. and Eissenstat D.M. (2003) Multiple risk factors in root survivorship: a four-year study in Concord grape. *New Phytologist*, **158**, 489-501.

Byrne G.F., Begg J.E. and Hanssen G.K. (1976) Cavitation and resistance to water flow in plant roots. *Agricultural Meteorology*, **18**, 21-25.

Blackman P.G. and Davies W.J. (1985) Root to shoot communication in maize plants of the effects of soil drying. *Journal of Experimental Botany*, **36**, 39-48.

Blum A. and Johnson J.W. (1992) Transfer of water from roots into dry soil and the effect on wheat water relations and growth. *Plant and Soil*, **145**, 141-149.

Brown V.K. and Gange A.C. (1990) Insect herbivory below ground. *Advances in Ecological Research*, **20**, 1-58.

Champagnol, F. (1984) Elements de physiologie de la vigne et de viticulture generale. Francois Champagnol, Saint-Gely-du-Fesc (France).

Choné X., van Leeuwen C., Dubourdieu D. and Gaudillère J.P. (2001) Stem water potential is a sensative indicator of grapevine water status. *Annals of Botany*, **87**, 477-483.

Coley P.D. (1988) Effects if plant growth rate and leaf lifetime on the amount and type of anti-herbivore defense. *Oecologia*, **74**, 531-536.

Coutts M.P. (1982) Growth of sitka spruce seedlings with roots divided between soils of unequal matric potential. *New Phytologist*, **92**, 49-61.

Craig T.P., Itami J.K. and Price P.W. (1989) A strong relationship between oviposition preference and larval performance in a shoot-galling sawfly. *Ecology*, **70**, 1691-1699.

Dry P.R., Loveys B.R. and Düring H. (2000a) Partial drying of the rootzone of grape. I. Transient changes in shoot growth and gas exchange. *Vitis*, **39**, 3-7.

Dry P.R., Loveys B.R. and Düring H. (2000b) Partial drying of the rootzone of grape II. Changes in the pattern of root development. *Vitis*, **39**, 9-12.

Eissenstat D.M., Wells C.E., Yanai R.D. and Whitbeck J.L. (2000) Building roots in a changing environment: implication for root longevity. *New Phytologist.*, **147**, 33-42.

Emerman S.H. and Dawson T.E. (1996) Hydraulic lift and its influence on the water content of the rhizosphere: an example from sugar maple, *Acer saccharum*. *Oecologia*, **108**, 273-278.

Ewers, F.W., Fisher J.B. and Fichtner K. (1991). Water flux and xylem structure in vines. In: F.E. Putz and H.A. Mooney (eds.) *The biology of Vines*. Cambridge University Press, Cambridge, 127-160.

Fort C., Muller F., Label P., Granier A. and Dreyer E. (1998) Stomatal conductance, growth and root signaling in *Betula pendula* seedlings subjected to partial soil drying. *Tree Physiology*, **18**, 769-776.

Fort C., Fauveau M.L., Muller F., Label P., Granier A. and Dreyer E. (1997) Stomatal conductance, growth and root signaling in young oak seedlings subjected to partial soil drying. *Tree Physiology*, **17**, 281-289.

Fritz R.S., Crabb B.A. and Hochwender C.G. (2000) Preference and performance of a gall-inducing sawfly: a test of the plant vigor hypothesis. *Oikos*, **89**, 555-563.

Gowing D.J.G., Davies W.J. and Jones H.G. (1990) A positive root-sourced signal as an indicator of soil drying in apple, *Malus x domestica* Borkh. *Journal of Experimental Botany*, **41**, 1535-1540.

Granett J., Walker M.A., Kocsis L. and Omer A.D. (2001) Biology and management of grape phylloxera. *Annual Review of Entomology*, **46**, 387-412.

Green S. and Clothier B. (1999) The root zone dynamics of water uptake by mature apple trees. *Plant and Soil*, **206**, 61-77.

Greenspan M.D., Shackel K.A. and Matthews M.A. (1994) Developmental changes in the diurnal water budget of the grape berry exposed to water deficits. *Plant, Cell and Environment*, **17**, 811-820.

Hardie W.J. and Considine J.A. (1976) Response of grapes to water-deficit stress in particular stages of development. *American Journal of Enology and Viticulture*, **27**, 55-61.

Jackson R.B., Sperry J.S. and Dawson T.E. (2000) Root water uptake and transport: using physiological processes in global predictions. *Trends in Plant Science*, **5**, 482-488.

Jackson, R.S. (2000). Grapevine structure and function. In: S.L. Taylor (ed.) *Wine Science Priciples, Practice, Perception* Academic Press, San Diego, pp.45-95.

Kimberling D.N., Scott E.R. and Price P.W. (1990) Testing a new hypothesis: plant vigor and phylloxera distributions on wild grape in Arizona. *Oecologia*, **84**, 1-8.

Kosola K.R. and Eissenstat D.M. (1994) The fate of surface roots of citrus seedlings in dry soil. *Journal of Experimental Botany*, **45**, 1639-1645.

Lambers, H., F.S. Chapin III and Pons T.L. (1998). Plant Physiological Ecology. New York, Springer-Verlag p. 178.

McCully M.E., Huang C.X. and Ling L.E.C. (1998) Daily embolism and refilling of xylem vessels in the roots of field-grown maize. *New Phytologist*, **138**, 327-342.

Mullins M.G., Bouquet A. and Williams L.E. (1992) Biology of the grapevine. Cambridge, Cambridge University Press.

Nobel P.S. and Lee C.H. (1991) Variations in root water potentials: influence of environmental factors for two succulent species. *Annals of Botany*, **67**, 549-554.

North G.B. and Nobel P.S. (1998) Water uptake and structural plasticity along roots of a desert succulent during prolonged drought. *Plant, Cell and Environment*, **21**, 705-713.

Reich P.B., Walters M.B. and Ellsworth D.S. (1992) Leaf-lifespan in relation to leaf, plant and stand characteristics among diverse ecosystems. *Ecological Monographs* **62**, 365-392.

Richards J.H. and Caldwell M.M. (1987) Hydraulic lift: substantial nocturnal water transport between soil layers by *Artemisia tridentata* roots. *Oecologia*, **73**, 486-489.

Pregitzer K.S., Hendrick R.L. and Fogel R. (1993) The demography of fine roots in response to patches of water and nitrogen. *New Phytologist*, **125**, 575-580.

Price P.W. (1991) The plant vigor hypothesis and herbivore attack. *Oikos*, **62**, 244-251.

Salleo S., Lo Gullo M.A. and Oliveri F. (1985) Hydraulic parameters measured in 1-year-old twigs of some Mediterranean species with diffuse-porous wood: changes in hydraulic conductivity and their possible functional significance. *Journal of Experimental Botany***, 36**, 1-11.

Sharp R.E. & Davies W.J. (1979) Solute regulation and growth by roots and shoots of water-stressed maize plants. *Planta*, **147**, 43-49.

Simonneau T. and Inra R.H. (1991) The use of tree root suckers to estimate root water potential. *Plant, Cell and Environment*, **14**, 585-591.

Smart D.R., Carlisle E., Goebel M. and Núñez B.A. (2005) Transverse hydraulic redistribution by a grapevine (*Vitis rupestris* x *Vitis berlandieri* cv 420A). *Plant, Cell and Environment*, **28**, 157-166.

Stasovski E. and Peterson C.A. (1991) The effects of drought and subsequent rehydration on the structure and vitality of *Zea mays* seedling roots. *Canadian Journal of Botany*, **69**, 1170-1178.

Steudle E. (2000) Water uptake by roots: effects of water deficit. *Journal of Experimental Botany*, **51**, 1531-1542.

Stoll M., Loveys B. and Dry P. (2000) Hormonal changes induced by partial rootzone drying of irrigated grapevine. *Journal of Experimental Botany.*, **51**, 1627-1634.

Taleisnik E., Peyrano G., Córdoba A. and Arias C. (1999) Water retention capacity in root segments differing in the degree of exodermis development. *Annals of Botany*, **83**, 19-27.

Tan C.S. and Buttery B.R. (1982) The effect of soil moisture stress to various fractions of the root system on transpiration, photosynthesis, and internal water relations of peach seedlings. *Journal of the American Society of Horticulture Science*, **107**, 845-849.

Tan C.S., Cornelisse A. and Buttery B.R. (1981) Transpiration, stomatal conductance, and photosynthesis of tomato plants with various proportions of root system supplied with water. *Journal of the American Society of Horticulture Science.*, **106**, 147-151.

van Schaik C.P., Terborgh J.W. and Wright S.J. (1993) The phenology of tropical forests: adaptive significance and consequences for primary consumers. *Annual Review of Ecological Systematics*, **24**, 353-377.

Westgate M.E. & Boyer J.S. (1985) Osmotic adjustment and the inhibition of leaf, root, stem and silk growth at low water potentials in maize. *Planta*, **164**, 540-549.

Williams L.E. and Araujo F.J. (2002) Correlations among predawn leaf, midday leaf, and midday stem water potential and their correlations with other measures of soil and plant water status in *Vitis vinifera*. *Journal of the American Society of Horticulture Science*, **127**, 448-454.

Winkel T. and Rambal S. (1993) Influence of water stress on grapevines growing in the field: from leaf to whole-plant response. *Australian Journal of Plant Physiology*, **20**, 143-157.

Woods J.O., Carr T.G., Price P.W., Stevens L.E. and Cobb N.S. (1996) Growth of coyote willow and the attack and survival of a mid-rib galling sawfly, *Euura* sp. *Oecologia*, **108**, 714-722.

Wright I.J., Reich P.B., Cornelissen J.H.C., Falster D.S., Garnier E., Hikosaka K., Lamont B.B., Lee W., Oleksyn J., Osada N., Poorter H., Villar R. and Warton D.I. (2005) Assessing the generality of global leaf trait relationships. *New Phytologist* **166**, 485-496.

Chapter 2

Root foraging in response to heterogeneous soil moisture in two grapevines that differ in potential growth rate

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Running Head: root growth dynamics

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Abstract

We investigated the linkages between root system potential growth rate and root responses to soil moisture heterogeneity. Root dynamics were studied using genetically identical grapevine shoots (*Vitis vinifera* cv Merlot) on genetically distinct root systems that promote higher and lower shoot vigor. Three levels of drip irrigation were designed to replenish various percentages of evapotranspiration (0% Et_c, 40% Et_c, and 100% Et_c) in an Oakville, CA vineyard with high soil clay content. Growing-season pruning weights of shoots growing on the more vigorous rootstock (1103P) were double those on the less vigorous rootstock (101-14 Mgt), despite similar shoot water potentials and exposure to similar soil moisture deficits. Under irrigation, roots of higher-vigor vines exhibited more morphological plasticity in root production, as indicated by the growth of 75-95% of their total roots produced in irrigated zones of soil during the summer months compared to about 60-80% for the lower-vigor rootstock (May-August). The higher-vigor vine also exhibited a large shift in root diameter with a change in soil water content (coarser roots in irrigated soil) in contrast to the lower-vigor rootstock, which had similar root diameters in wet and dry soil. Compared to the rootstock of higher vigor, we did not observe higher tolerance of dry soil at the individual root level by the lower-vigor rootstock and, in fact, root survivorship in dry soil was similar for the two rootstocks. The lower vigor vine produced the majority of its roots during the relatively wet winter months suggesting avoidance to dry soil conditions. The higher vigor vine exhibited a high initial peak in root biomass in the first year with subsequent years exhibiting little change. In contrast, the lower vigor vine did not show a substantial initial root accumulation response to irrigation and instead slowly increased its root biomass over several years. Overall, our results demonstrated that a grape root system of higher vigor has greater morphological plasticity in response to heterogeneous moisture supply but similar tolerance to moisture stress as indicated by root survivorship in dry soil. The lower-vigor vines in contrast developed more root biomass over long term periods of several years, especially under conditions of no irrigation.

Keywords: minirhizotron, root survivorship, root production, *Vitis* rootstock, shoot vigor, localized water stress, plasticity, tolerance, avoidance, 1103 Paulsen, 101-14 Mgt

Introduction

Due to both spatial and temporal heterogeneity of resources in the soil, the efficient deployment of roots in resource-rich patches and reduced expenditures to roots in resource-poor patches is often an important element for success in a resource-limiting environment (Fitter 1994). Trade-offs between tissue construction and maintenance (Eissenstat and Yanai 1997) and between root and shoot biomass allocation (Sharp and Davies 1979) can substantially influence plant foraging for belowground resources (Fitter 1994).

Fast- and slow-growing species may respond differently to low resource availability (Lambers and Poorter 1992). For roots, studies that have examined the role of growth rate on root plasticity have focused on nutrient supply (Crick and Grime 1987; Eissenstat and Caldwell 1988; Doussan, Pagès and Pierret 2003; Hodge 2004 and references therein). Fast-growing species generally exhibit more morphological plasticity than slow-growing species, thus, allowing them to grow roots more quickly in areas rich in resources (Kembel and Cahill 2005, Crick and Grime 1987). It is not clear if these trends hold true for root systems exposed to localized patches of soil moisture. During periods of decreased water availability, some studies suggest increased root production or fine lateral initiation, as observed in field grown tomatoes (Reid and Renquist 1997) and in the perennial grass *Lolium perenne* (Jupp and Newman 1987). Other studies, in contrast, report that plants reduce total root growth (Steudle 2000, Richards and Cockroft 1975; Comas et al. 2005) and carbon allocation (Kosola and Eissenstat 1994) to roots in dry soil during periods of low soil moisture availability. In addition, several researchers observe that plants preferentially grow roots in regions of high soil moisture during periods of soil moisture deficits (Green and Clothier 1999, Fort et al. 1998, Coutts 1982). None of the studies reviewed compared responses of plants of different potential growth rate to soil moisture heterogeneity.

Highly plastic root systems can incur substantial risks if periods of favorable soil moisture are followed by long periods of dryness. Some plants, like cactus can produce very inexpensive “rain roots” that respond very quickly to ephemeral supplies of water

(Snyman 2006), whereas other plants like citrus may utilize a more conservative strategy of modest root growth in wet soil and maintenance of roots in dry soil (Eissenstat et al. 1999; Kosola and Eissenstat 1994). Allocation of carbon toward the root system to build "expensive" roots with a well developed exodermis and endodermis has also been shown to prevent root desiccation in dry soil (North and Nobel 1991).

Exploring how plants with different growth rates respond to heterogeneous water supply contributes to our understanding of how root foraging behavior may be linked to plant potential growth rate in perennial plants. Our study is unique in that we examined genetically identical shoot grafts (scions) on two genetically distinct root systems that differ in their effects on shoot growth rate (vigor). We quantified how root production, partitioning, and lifespan in grape vines of different potential growth rate respond to soil moisture heterogeneity along a gradient in whole-plant water stress severity. Compared to lower vigor vines, we hypothesized that vines with higher vigor would display greater plasticity in root growth and morphology as indicated by proportionally greater root production in moist soil and higher root mortality in dry soil. In addition, we predicted that these traits would be most accentuated in vines of greater water stress.

Materials and Methods

Experimental site

This experiment examined 1103 Paulsen (*V. berlandieri* x *V. rupestris*), a root system tending to provide high shoot vigor (HSV), and 101-14 Millardet de Gramanet (*Vitis riparia* x *V. rupestris*), a root system associated with lower shoot vigor (LSV). Horticultural rootstock trials and empirical evidence suggests 1103P is a vigorous rootstock with "good rooting ability" (Wolpert et al 2002) and the LSV root system was relatively drought tolerant when soils dried down slowly (Galet 1998). Vines were grown under different quantities of drip irrigation in an established Merlot (*Vitis vinifera* cv Merlot) experimental block in Oakville, CA (in cooperation with UC Davis). The vines

were 11 yrs old and planted in Bale (variant) gravelly loam (fine-loamy, mixed, superactive, thermic Cumulic Ultic Haploxeroll). The Oakville region averages 83 cm of precipitation annually and has a mean annual temperature of 14.3ºC (CIMIS 2003-2005). The vines were trained on a bilateral cordon with vertical shoot positioning (VSP) and was oriented SE to NW with rows with vines spaced 2.4 x 2.2 m apart. We utilized a completely randomized block design with irrigation amount (three levels) and two rootstock cultivars randomized in each block in a total of six blocks. The entire experimental vineyard comprised 1.05 hectares. In 2002 each experimental vine was reduced from two to one emitter, located 50 cm from the trunk. Irrigation amounts were applied bi-weekly and determined using crop evapotranspiration (Et_c) calculated from the evaporation of a Class A pan and corrected with crop coefficients (K_c) (Prichard 1992). Irrigation treatments consisted of 0% (no irrigation), 40% (deficit irrigation), & 100% Et_c (full irrigation) and were randomly assigned to each rootstock within the vineyard. Minirhizotron root observation tubes (1.3 m and 6 cm in outside diameter) of clear plastic (cellulose acetyl butyrate) were installed in April 2002 at an angle of 30˚ from the vertical to a depth of 1 meter. One minirhizotron tube was placed through the dripper zone about 60 cm from the trunk directly below the zone of soil receiving irrigation from the drip-emitter. The other minirhizotron for that vine was placed in the unirrigated zone on the opposite side of the vine, at a similar distance from the trunk. A total of 72 minirhizotrons were used with 2 tubes per vine x 3 irrigations x 2 rootstocks x 6 blocks. Surrounding vines served as buffers to provide environmental continuity for treatment vines and also to separate treatments. Plastic (PVC) plugs prevented water infiltration in the bottoms of the tubes and black electrical tape and rubber stoppers prevented light penetration in the portion of the tube above the soil surface. Radiant heating was prevented by covering the tops of the tubes with white metal radiation shields.

Root images were taken once every two weeks during the growing season and once a month during vine dormancy from January 2003 to December 2005. All images were analyzed using Win Rhizo Tron MF software (Regents Inc. Quebec, Canada) for root population counts, survivorship and production. Root births were estimated by calculating the date midway between the observation date when a root was first observed

and the previous observation date. Similarly, root death was estimated as being midway between the date the root was first observed dead and the previous observation date. Root death was identified by a black and shriveled appearance (Comas, Eissenstat and Lakso 2000) or if the root had disappeared from the window and did not re-appear. Roots that transected more than one minirhizotron observation window vertically within the same minirhizotron observation tube were only counted once. Root standing crop was determined by the difference in cumulative production and cumulative mortality of the fine roots.

Environmental

Environmental data were obtained from an on-site weather station (CIMIS 2003-2005). Volumetric soil water content was estimated using time domain reflectometry using the minirhizotrons as access tubes for a TRIME soil moisture probe at 20-50, 50-80 and 80-120 cm depths, intervals that corresponded with highest root densities (Mesa Systems Co., Medfield, MA). Volumetric soil moisture (θ_v; %) was converted to soil matric potential (MPa) by a soil water retention curve determined using a pressure plate extractor (Soil Moisture Equipment Co., Santa Barbara, CA, USA) under five levels of pressure (-0.01, -0.03, -0.1, -3, and -1.5 MPa). Bulk density measurements, acquired in a previous study at the same location (Carlisle et al. 2006), were 1.33 ± 0.10 g cm^{-3} at 6-12 cm depth and 1.45 ± 0.05 g cm^{-3} at 40-46 cm depth.

Predawn and solar noon stem water potentials were monitored throughout the growing season with a pressure chamber (Soil Moisture Inc., Santa Barbara, CA). Approximately every 10 days to 2 weeks, stem water potentials were determined in all 36 minirhizotron treatment vines by first placing a leaf in a plastic bag and then aluminum foil to prevent light penetration for 15 minutes before severing the leaf and placing in the pressure chamber.

Data Analysis

Root lifespan data were analyzed with Cox proportional hazards regression (PROC PHREG) (SAS Institute Inc., Cary, NC, USA). This type of analysis allows the influence of all other covariates to be held constant while the "hazard" of an individual covariate is determined (Cox 1972). The "hazard" of a covariate refers to the risk of mortality of a root at time *t*, where *t* is the product of a baseline hazard function of *k* covariates (Allison 1995).

Statistical Analysis System's PROC PHREG (SAS Institute Inc., Cary, NC, USA) uses the partial likelihood method (Cox 1972) to estimate a parameter coefficient of β for each tested covariate, and calculates a chi-square statistic to test the null hypothesis that each β equals zero. A parameter estimate can have either a negative or positive sign depending on the effect it has on the covariate. In this case, a negative sign indicates a decreased hazard of mortality with an increase in the covariate (Wells and Eissenstat 2001). Covariates tested included root diameter, number of daughter roots and the number of neighbor roots present in the window. Wilcoxon tests were performed to analyze for differences in survivorship of root systems and roots growing in wet versus dry soil. Further analyses on the effects of year of observation and depth of roots on root population size were completed using the GLM procedure in SPSS (SPSS Inc. v. 11.0, Chicago, IL). Soil moisture data were averaged over 2003-2005 and analyzed using ANCOVA with initial soil moisture values at all three depths as covariates (SAS Institute Inc., Cary, NC, USA).

Results

Environmental

Weather was characterized as regionally normal for 2003-2005 with wet, cool winters and warm, dry summers. Although a slightly longer, wet spring occurred in 2005, annual precipitation was similar to the long-term mean (Figure **2.1**).

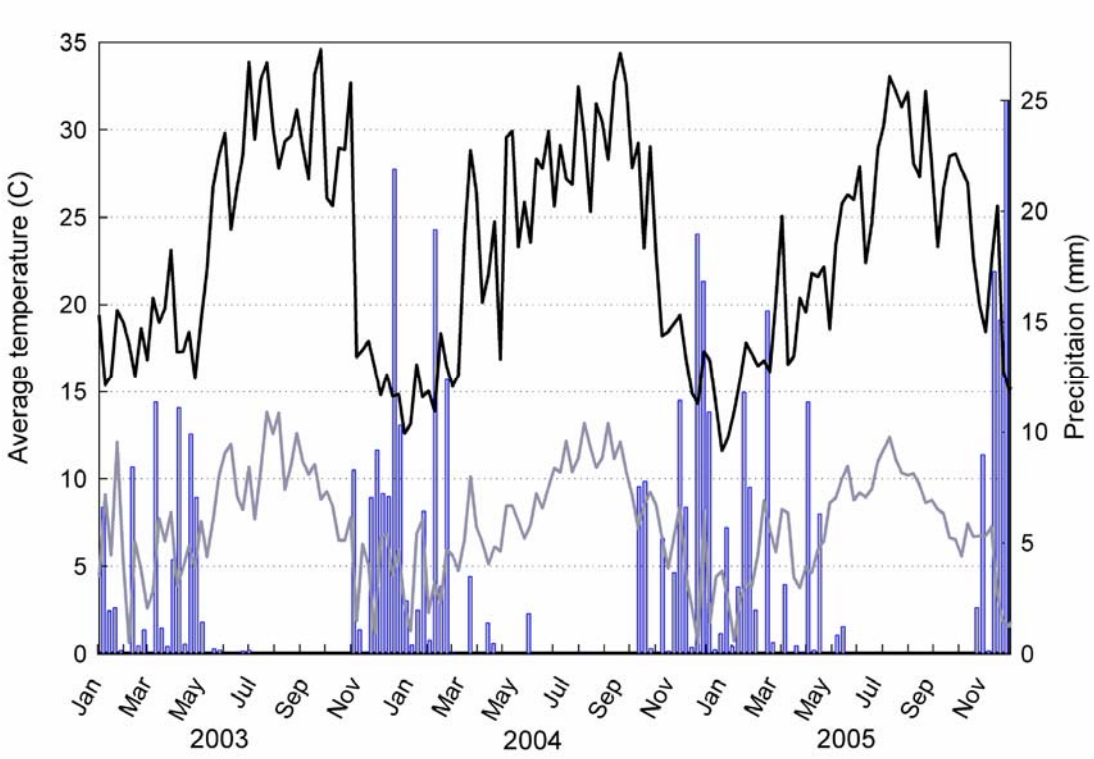

Figure **2.1**: Weekly maximum (– –) and minimum (– –) temperatures and precipitation (vertical bars) in Oakville, CA, for the years, 2003-2005.

No differences in soil moisture were found between LSV and HSV vines for either irrigation treatment (40% Et_c, P =0.582; 100% Et_c, P =0.727) and so data were combined (Figure **2.2**). Unirrigated soil had approximately 5% less soil moisture compared to the irrigated soil in the driest part of the year (June-Sept.) (28% irrigated, 23% un-irrigated; P<0.0001). Soil moisture exhibited slight increases with depth in dry soil (3-4% absolute increase) for the full irrigation treatment (100% Et_c) (P= 0.057) and no increase under deficit irrigation (40% Et_c) (P=0.793). Soil in the un-irrigated treatment (0% Et_c) had the largest increase in soil moisture with depth compared to other treatments (5-8% absolute increase)(P<0.0001). Soil water content on the irrigated side of the vine in the top 20-80 cm zone, the area where the majority of roots were located, averaged 30% (about -0.01 MPa) under irrigation designed to replace 100% Et_c and 27% (about -0.05 MPa) under 40% Et_c.

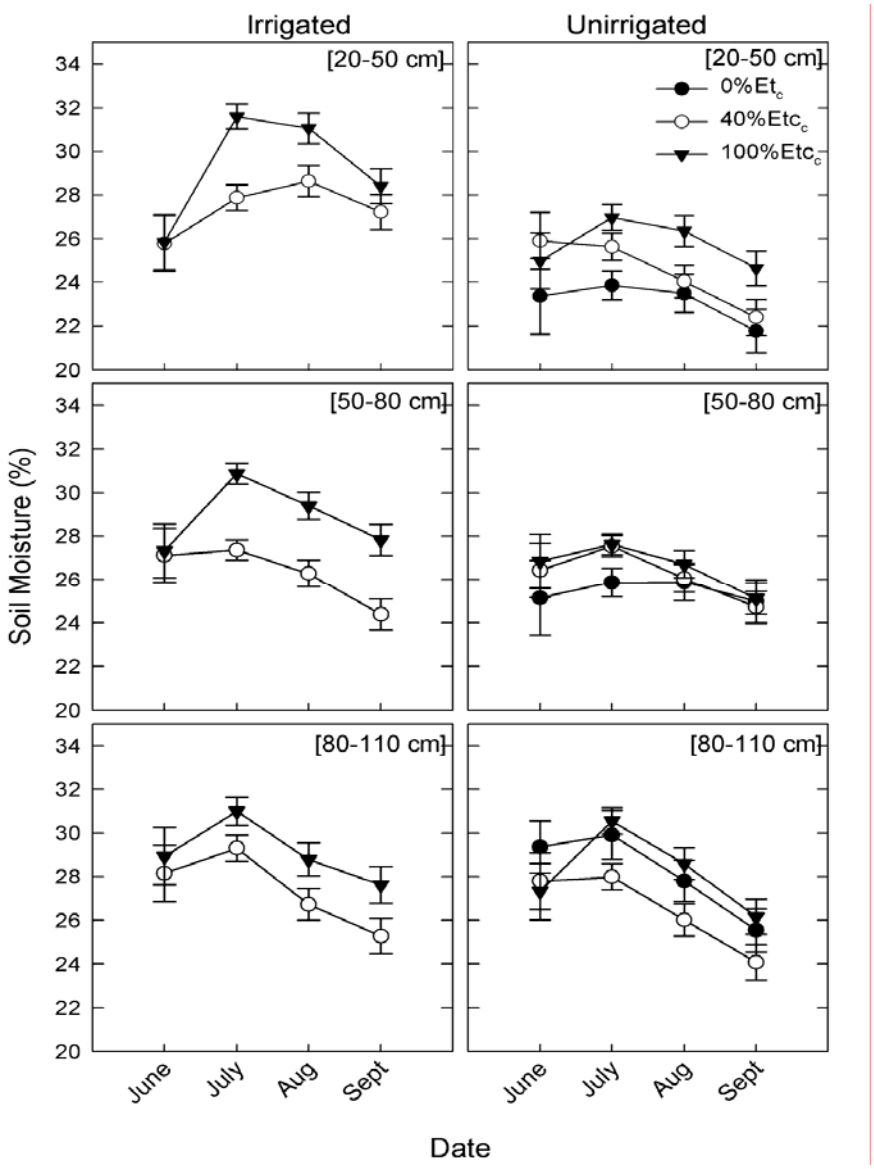

Figure **2.2**: Volumetric soil moisture (ls means; %) in a 'Merlot' wine grape vineyard under different levels of irrigation in Oakville, CA. Data were collected at 3 soil depths (20-50, 50-80 and 80-110 cm) in irrigated soil zones with irrigation designed for 40% and 100% replacement of vine evapotranspiration (Et_c) (left panels) and in unirrigated zones of soil of the same plant (right panels), averaged over 3 years (2003-2005) (± 1 SE). Significantly drier soil was maintained on the un-irrigated side of the vine in the deficit irrigation (40% Et_c) (P<0.0001) and full irrigation (100% Et_c) treatments (P<0.0001).

Soil water content on the irrigated side of the vine in the top 20-80 cm zone, the area where the majority of roots were located, averaged 30% (about -0.01 MPa) under irrigation designed to replace 100% Et_c and 27% (about -0.05 MPa) under 40% Et_c. Soil

water content on the unirrigated side of the vine was 26% (-0.15 MPa) for 100% Et_c irrigation, 24% (-0.4 MPa) for 40% Et_c, and 23% (-0.8 MPa) for no irrigation (40% Et_c) during August (a dry month) over the three years of the study (P<0.001). Deeper soil (>80 cm) retained more water with an average of 29% soil water content (-0.02 MPa) in the wet soil and 28% water content (-0.03 MPa) in the dry soil (P=0.150).

Shoot responses

Over the course of the study period, vine shoots produced almost double stem pruning weights on the HSV root system compared to that produced by vines on the LSV root system under all levels of water stress (P <0.001; Figure **2.3**).

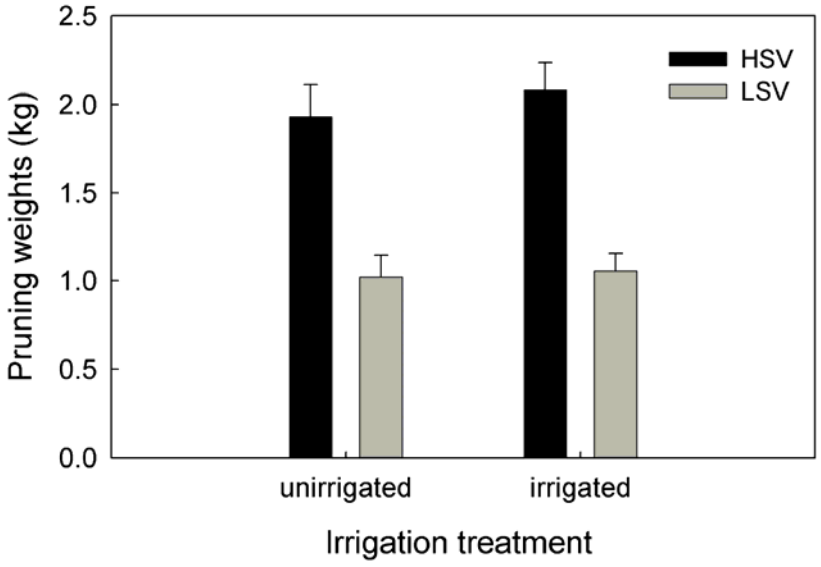

Figure **2.3**: Influence of root system on pruning weights (kg) of shoots of 'Merlot' grapevines under different levels of irrigation. Data were averaged over 3 years (2003-2005) (P=0.411) and represent vines on a root system associated with higher shoot vigor (HSV; 1103P; black bars) or on a root system associated with lower shoot vigor (LSV; 101-14 Mgt; gray bars) under all irrigation combined (P=0.308). The HSV root system produced more shoot biomass compared to the LSV root system (P<0.001). Irrigation did not influence pruning weights (P=0.160) (± 1 SE).

Despite differences in shoot vigor between the two root systems, there was no evidence that (shoot cane pruning) weights were affected by irrigation (data not shown; *P*=0.160). Absolute differences in mid-day stem water potentials through much of July and August, the period of lowest rainfall and highest temperatures of the growing season, were small with differences usually less than -0.1 MPa for the two rootstocks despite marginal significance (*P*=0.087) and so data were combined (Figure **2.4**).

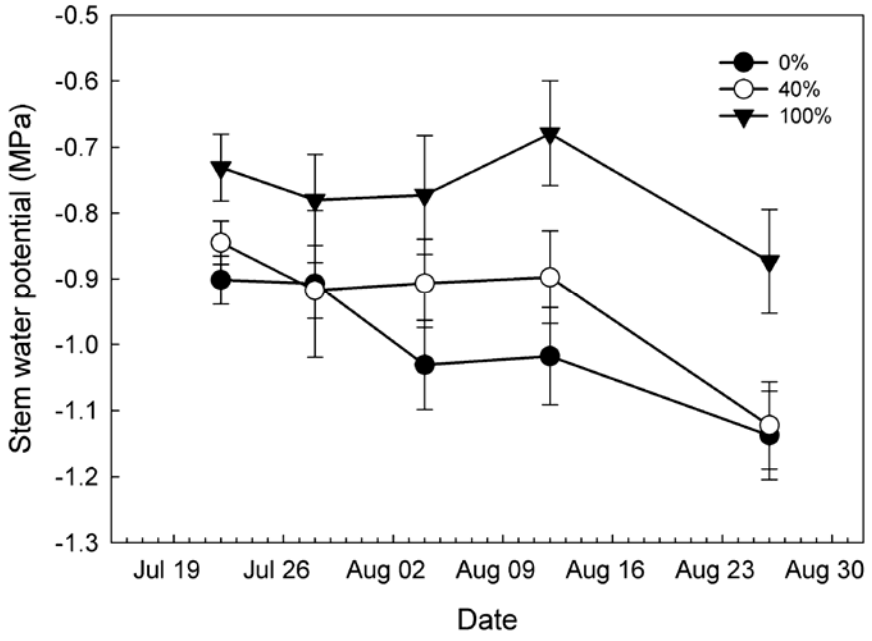

Figure **2.4**: Stem water potential (MPa) of 'Merlot' grape vines under different levels of irrigation in Oakville, CA, over the stressed portion of the growing season (July- August) in 2004. Irrigation treatments were 0% (●), 40% (○) and 100% (▼) replacement of evapotranspiration (Et_c) (± 1 SE) (*P*<0.0001). Data for vines on root systems associated with higher shoot vigor and lower shoot vigor were combined.

Irrigation level strongly influenced stem water potentials. Stem water potentials of vines in the 100% Et_c treatment were about 30% higher than those in the 40% Et_c treatment (*P*=0.005) and almost 50% higher than those in the 0% Et_c treatment

(P<0.001). The vines in the 0% Et_c and 40% Et_c treatments, however, exhibited similar mid-day stem water potentials (P=0.443).

Root Plasticity

Root growth plasticity was calculated by the growth of the root system in areas of high soil moisture as a proportion of total root growth observed with the two minirhizotrons, one in moist soil and one in dry soil during the growing season. During the dry summer months of July and August, the HSV root system exhibited higher relative root production sooner in the wet soil under the irrigation emitters compared to the LSV root system (Figure **2.5**A and 2.5B). During the early summer months of May and June, the HSV root system also responded up to 20% sooner to lateral heterogeneity in soil water created by 100% Et_c irrigation to one side of the vine (June, P<0.0001) and 10-20% sooner in heterogeneity caused by 40% Et_c in May (P<0.0001) and June (P=0.046). Increased plant moisture stress severity did not appear to accentuate the root growth response to lateral soil moisture heterogeneity.

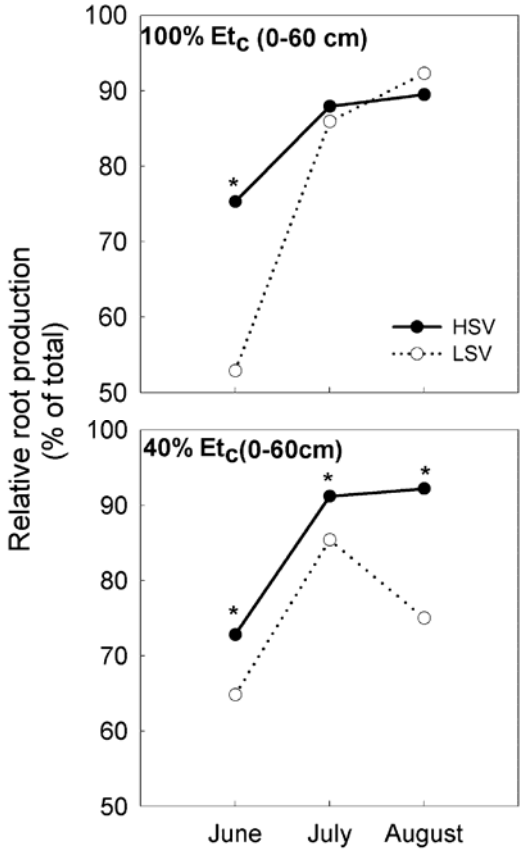

Figure **2.5**: Influence of grape vine potential growth rate on fine root production in irrigated soil zones for vines under different levels of drip irrigation. Vines were on a root system associated with higher shoot vigor (HSV; 1103P) (●) and on a root system associated with lower shoot vigor (LSV; 101-14 Mgt) (○) for two irrigation treatments: A. 100% replacement of evapotranspiration (Et_c) and B. 40% Et_c replacement. Data were calculated as the percentage of the number of roots visible with a minirhizotron in the top 0-60 cm of the irrigated region of the soil relative to the total roots produced in the top 60 cm of soil that were observed with two minirhizotrons, one in the irrigated soil region and a second in the unirrigated soil region. Data represent observations from June- August, averaged over 3 years (2003-2005) in a Merlot vineyard in Oakville, CA. Asterisks denote points of significance ($P < 0.05$).

In the unirrigated treatment, there was only limited evidence that the HSV root system exhibited greater preferential root production in the deeper soil (>60cm) as the growing season progressed, compared to the LSV root system (Figure **2.6**). Interestingly, during mid-summer the LSV root system allocated a greater percentage of its roots

deeper in the soil compared to the HSV root system (P<0.001 for both June and July). Only in August was there evidence that HSV exhibited more root growth in the deeper soil layers, but differences at this time between the root systems was not significant (P = 0.342).

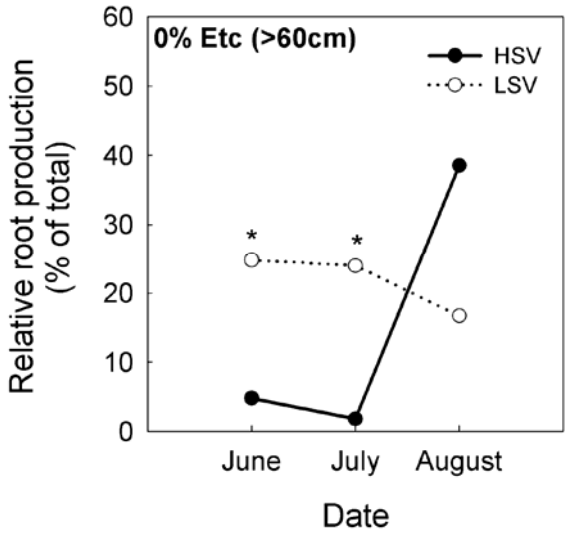

Figure **2.6**: Influence of potential growth rate of unirrigated grape vines on root growth plasticity in moist, deep soil layers. Data represent the percentage of roots produced in deep soil with little soil moisture depletion (soil depths >60 cm) relative to the total roots produced over the entire soil profile in Merlot vineyard in Oakville, CA. for a root system associated with higher shoot vigor (HSV; 1103P) (●) and a root system associated with lower shoot vigor (LSV; 101-14 Mgt) (○). Data represent observations from June-August, averaged over 3 years (2003-2005). Points of significance denoted by differences in letters (P < 0.05).

Roots of the HSV root system exhibited less tolerance to dry soil as evidenced by their root morphology. First-order roots (the finest laterals on the root system without daughter roots) of the HSV root system were approximately 30% thinner in diameter in dry soil during the dry season in the top 20 cm of soil compared to those in wet irrigated soil (Figure **2.7**) (P=0.039). In addition, in dry soil HSV roots near the surface were thinner than those in the moister, deeper soil layers (>60cm) (P<0.001). Root diameter varied with depth for the HSV root system, with roots in irrigated soil decreasing in root

diameter with soil depth, and in unirrigated soil, roots increased in diameter with soil depth (P= 0.029). In contrast to the HSV root system, the LSV root system exhibited greater tolerance to dry soil with similar root diameter in wet soil and dry soil (P=0.411) and similar diameter with depth (P=0.462).

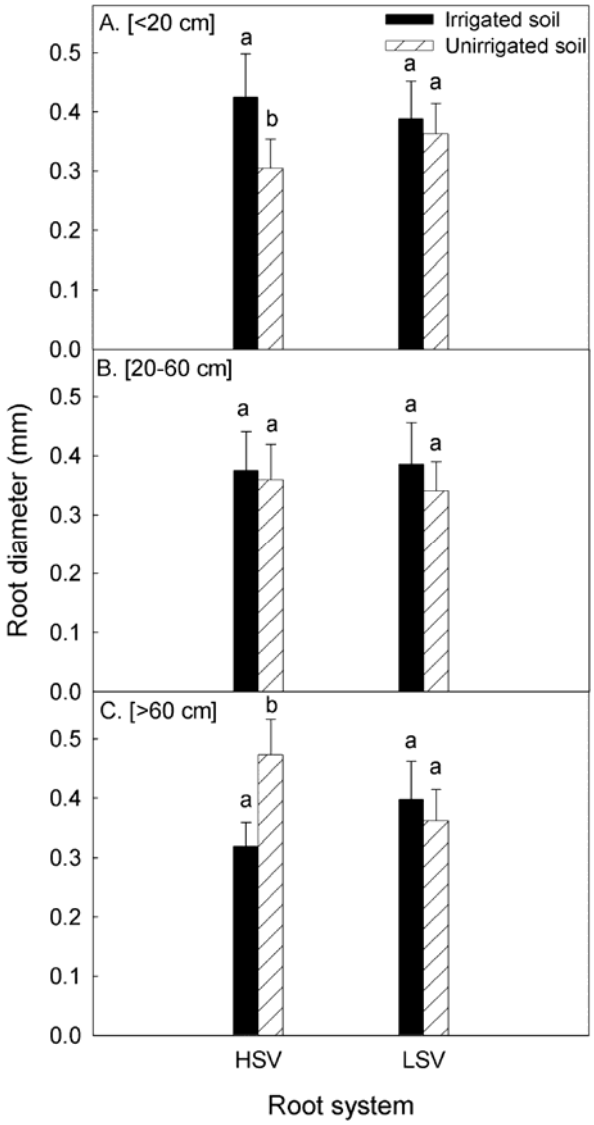

Figure **2.7**: Influence of root system potential growth rate on plasticity of root diameter (± 1 SE) in moist and dry soil. Data represent roots of grape vines in a Merlot vineyard in Oakville, CA over 3 depths (<20 cm, 20-60 cm and >60 cm) growing in irrigated soil (black bars) and unirrigated soil (hatched bars) of a root system associated with higher

shoot vigor (HSV; 1103P) (Significance of irrigated vs. unirrigated soil, P=0.029) and a root system associated with lower shoot vigor (LSV; 101-14 Mgt) (P=0.462) during the months of July-August for three consecutive years (2003-2005). Differences in lower case letters denote significance in root diameter between roots in irrigated soil and roots in un-irrigated soil ($P < 0.05$).

Tolerance/ Avoidance

Patterns of root survivorship indicated similar median lifespans of roots produced in un-irrigated soil zones during periods of low soil moisture when supplemental water was applied to the opposite side of the vine (P=0.081 for 40% Et_c; P=0.439 for 100% Et_c) (Figure **2.8**). We recognize that the lifespan of the roots shown extend beyond the period of high stress. However, during the most water stressed time period (June-September, see shaded area of Figure 2.8 and 2.9) root lifespan was very similar between the two rootstocks. Despite the difference in absolute numbers of roots produced during the winter, patterns of root survivorship of roots born in winter were the same for both root systems (P=0.627) (data averaged over all depths and irrigation sides; not shown).

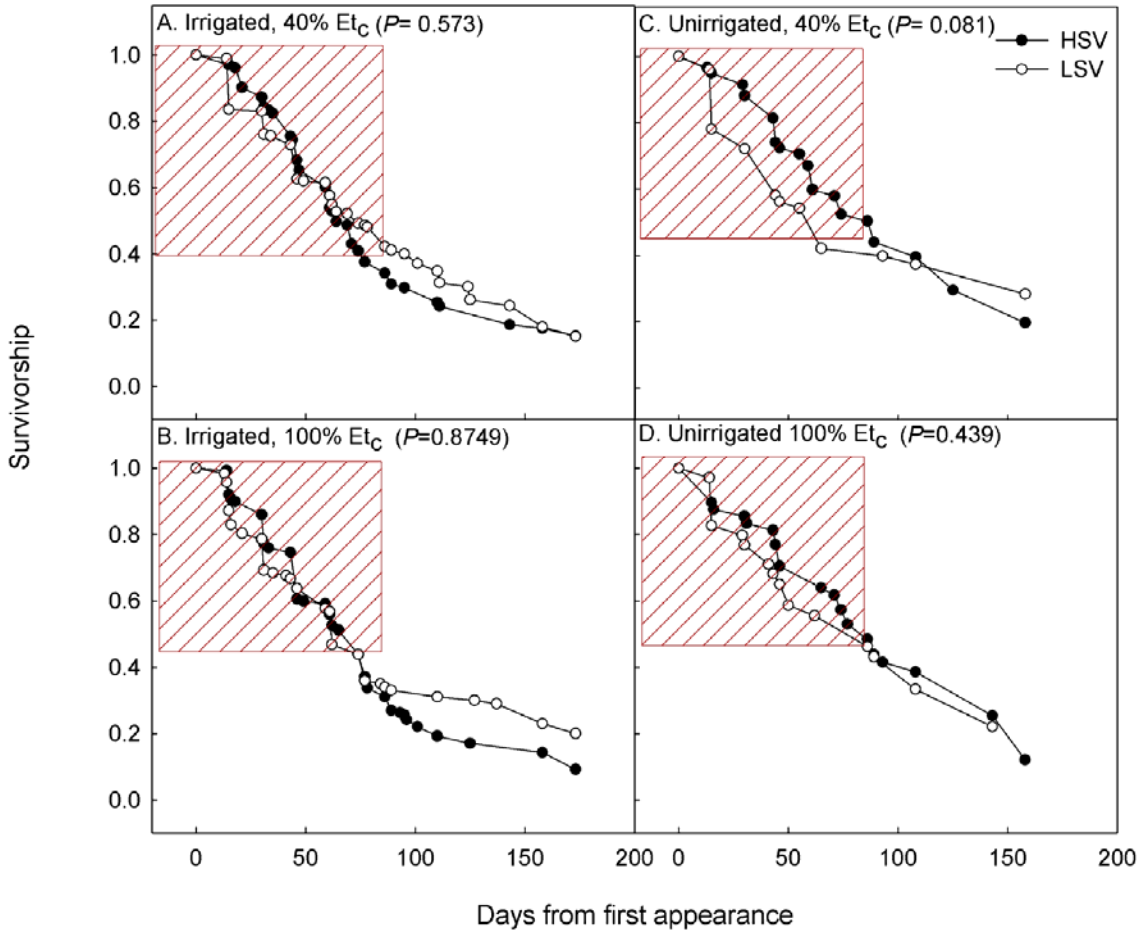

Figure **2.8**: Influence of root system potential growth rate on survivorship of roots in irrigated and unirrigated soil regions for grape vines at different levels of drip irrigation. Data represent roots born during the months of July-August in a Merlot vineyard in Oakville, CA for vines of high shoot vigor (HSV, rootstock 1103P) (+) and low shoot vigor (LSV, rootstock 101-14 Mgt)(○) over the years, 2003-2005. Roots were observed in the top 0-60 cm of irrigated (A and C) and unirrigated (B and D) zones of the soil where irrigation was added bi-weekly to replenish 40% of evapotranspiration (40% Et_c, A and B. Probability of significant difference between wet and dry sides, P = 0.573) or 100% Et_c (C and D, P =0.439). Shaded area indicates period of lowest soil moisture (July- September).

Likewise, roots produced in wet soil during the summer months also had similar lifespans for both root systems (P=0.573 for 40% Et_c; P=0.875 for 100% Et_c) (Figure 2.8). Roots born during dry mid-summer months (July-September) of vines in which no supplemental water was applied (0% Et_c), although not significant, displayed slightly

longer median lifespans for the LSV root system (Figure **2.9**); 95 days for roots of the LSV system and 67 days for roots of the HSV system; *P*=0.374).

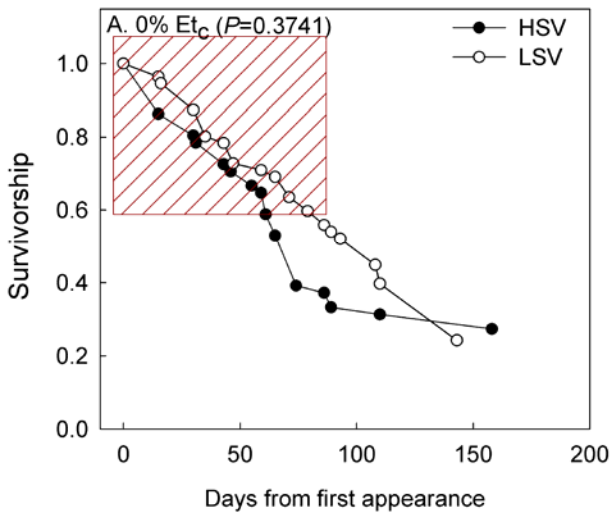

Figure **2.9**: Influence of potential growth rate on survivorship of roots in unirrigated soil. Data represent roots born during the months of July-August for vines of high shoot vigor (HSV, rootstock 1103P) (+) and low shoot vigor (LSV, rootstock 101-14 Mgt)(○) over the years, 2003-2005. (Roots were observed in the top 0-60 cm. of unirrigated soil (0% Et_c) in a Merlot vineyard in Oakville, CA. Probability of difference between rootstocks (*P*=0.374). Shaded area indicates period of lowest soil moisture (July- September).

While the HSV roots system exhibited a higher growth plasticity to soil moisture during periods of low soil moisture (Figure 2.5), the LSV root system exhibited greater avoidance of root growth during periods of water stress, producing a large portion of its roots during the cool, wet, winter months (Figure **2.10** D; *P*= 0.009). Both root systems displayed similar overall root production after 3 years of study (*P*=0.99). However, season played a large role in determining when roots of each root system were produced (season x root system interaction *P*=0.002). Averaged over all three years the LSV root system produced approximately three-fold more roots in the winter months (Dec-Feb.) than the HSV root system.

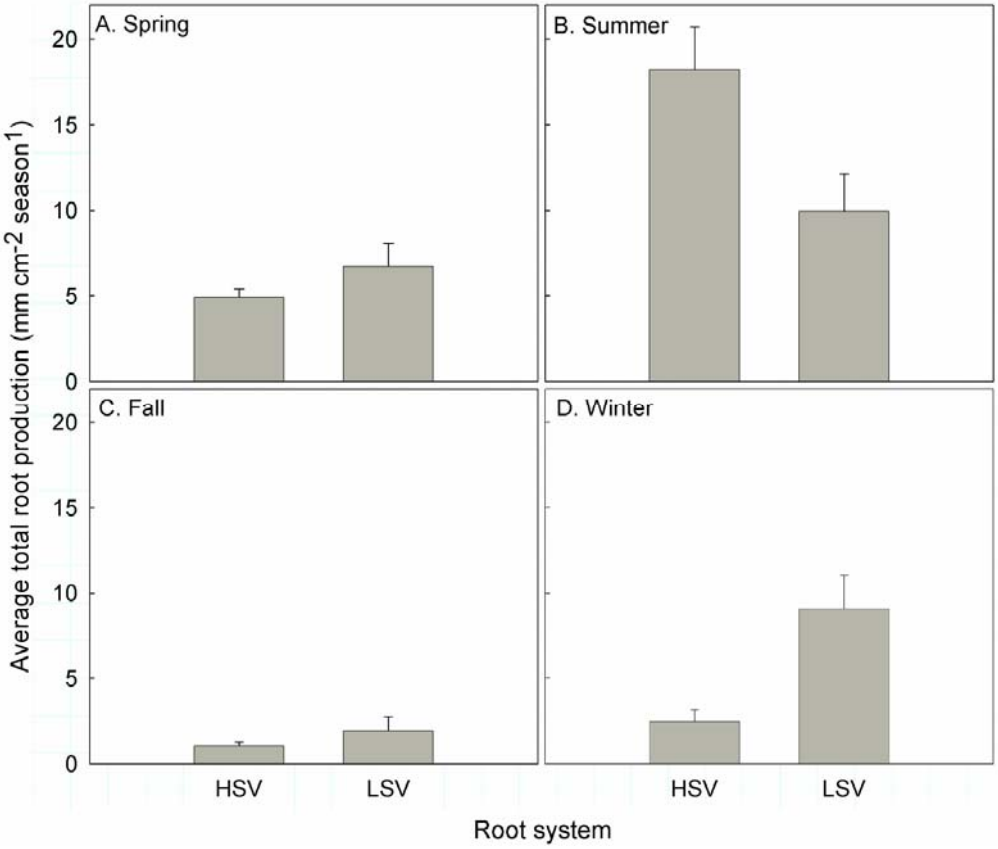

Figure **2.10**: Seasonal root production of two root systems that differ in potential growth rate (± 1 SE). Data represent total root length per observational window for three months for vines of higher shoot vigor (HSV, rootstock 1103P) (+) and lower shoot vigor (LSV, rootstock 101-14 Mgt)(○) over the years, 2003-2005 in a 'Merlot' wine grape vineyard in Oakville, CA (Season x root system interaction: *P*=0.002). Each season corresponded to the following months: A. Spring, March-May (Significance of difference between HSV and LSV: *P*=0.230); B. Summer, June-Aug (*P* =0.032); C. Fall, Sept-Nov (*P* =0.328); D. Winter, Dec-Feb (*P* =0.009).

Further indications of different root responses to soil moisture deficits are indicated by the root system standing crop (Figure **2.11**). The HSV root system demonstrated a high initial peak in standing crop root biomass in 2003. Subsequent years root standing crop appeared to reach a more steady-state, stable condition. In contrast, the LSV root system did not show as substantial a response to irrigation initially and instead increased its standing crop root biomass in the 0 to 90 cm zone over several years. Irrigation had opposite effects on the root systems, with the HSV root system having little

response to supplemental water during the growing season, with the exception of the first year, and the LSV root system producing the most roots in the absence of irrigation. Indeed, in the no-irrigation treatment, the LSV root system was characterized by a more extensive root system by the third year of the study than the HSV root system (*P*=0.001)(Figure 2.11).

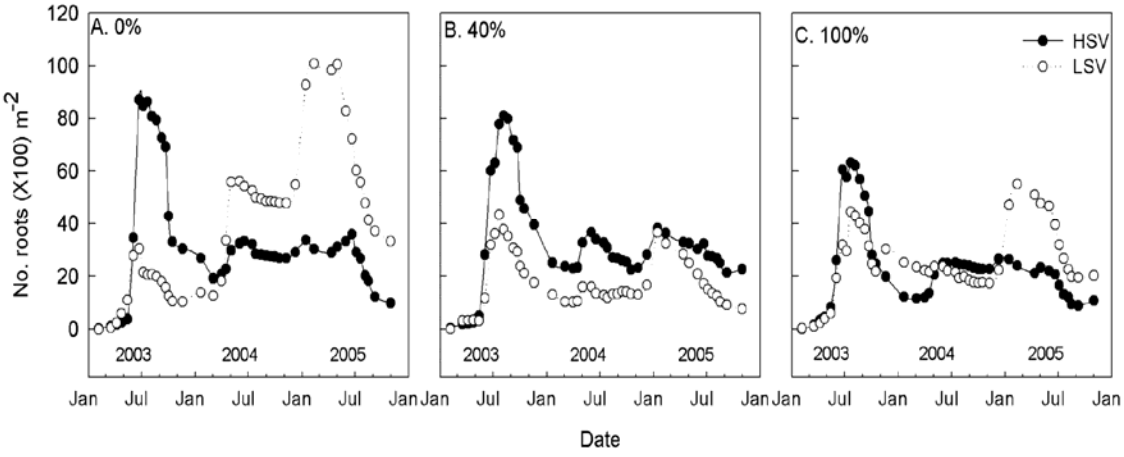

Figure **2.11**: Influence of grape vine potential growth rate on change in standing crop for vines under different levels of drip irrigation. Data represent root system standing crop from 2003-2005 in a 'Merlot' wine grape vineyard in Oakville, CA. Vines were on a root system associated with higher shoot vigor (HSV; 1103P) (●) and on a root system associated with lower shoot vigor (LSV; 101-14 Mgt) (○) for three irrigation treatments: A. no irrigation (0% Etc), B. irrigation added bi-weekly to replenish 40% of evapotranspiration (40% Etc) and C. irrigation added to replenish 100% of evapotranspiration (100% Etc). Roots from six vines were averaged for each treatment. Two tubes per vine were pooled.

Discussion

Various attempts have been made to describe root foraging responses to soil moisture heterogeneity (Steudle 2000: Green and Clothier 1999: Fort et al. 1998; Coutts 1982; Richards and Cockroft 1975); however, we are unaware of any studies that have evaluated the role of a plant's growth rate. Using a system where genetically identical

shoots were grafted to two genetically diverse root systems, we demonstrated that growth rate may influence root growth responses in both space and time across a gradient in plant water stress. We found the HSV root system exhibited proportionally more root growth in the irrigated soil zone sooner than the LSV root system during the period of most active growth (May – August; Fig. 2.5). Although the LSV root system also demonstrated preferential root growth in the irrigated zone, the response was slower (but not necessarily to a lower final extent) than that of the HSV root system, supporting the general theory that fast-growing species have higher plasticity and therefore a better chance of competing for resource patches than slow-growing species (Grime 1977).

Typically in Mediterranean climates, especially in heavy-textured soils of high water-holding capacity, later in the growing season the surface soil layers are relatively dry and only the deeper layers have adequate soil moisture to sustain root growth. Under conditions of no irrigation, more plastic plants may more readily grow roots in the deeper, moister soil regions later in the growing season compared to less plastic plants. As expected, the LSV root system did not respond as quickly as the HSV root system to localized soil moisture created by irrigation. We were unable to find clear patterns in preferential root growth to vertical soil moisture heterogeneity (Figure 2.6). Despite the HSV root system going into the summer stress period with 30% of its total root production in deep unirrigated soil layers (>60cm), this percentage dropped for June-July and did not recover until August. Interestingly the LSV root system produced about 25% of its root system in the deeper, moister soil layers for two out of the four months of high water stress. Therefore the HSV root system demonstrated higher plasticity in response to lateral heterogeneity in soil moisture than to vertical heterogeneity during periods of water stress. Root response to pulses of water such as that caused by irrigation demonstrates greater plasticity by the HSV root system due to the short temporal nature of the pulses compared to the longer duration of soil water in the >60 cm soil layers.

In addition to more rapid preferential root production in wet soil zones, we also demonstrated that the HSV root system exhibited more shrinkage in root diameter and therefore less tolerance to dry soil than the LSV root system (Figure 2.7). We suspect that the thicker diameter roots in wet soil in the HSV root system were a direct result of

the high water availability leading to larger, and possibly more numerous cortical cells (North and Nobel 1997; Mapfumo, Aspinall and Hancock 1994). The smaller diameter roots in the dry soil in the HSV root system may simply reflect greater susceptibility to root shrinkage compared to that of the LSV root system, an indication of less tolerance to dry soil (e.g., North and Nobel 1997). It is unclear if this has any functional significance to nutrient or water acquisition for the plant, but it clearly had little impact on root lifespan (Figure 2.8).

Despite previous arguments for extended root lifespan in slow-growing species (Grime 1977), both root systems demonstrated similar patterns of root survivorship in both wet and dry soil (Figure 2.8 and 2.9). We did see longer median lifespans of roots of the LSV than those of the HSV rootstock in unirrigated soil. This experiment did not support a hypothesized tolerance strategy of longer root survivorship in dry soil by the LSV root system. Soil resource heterogeneity can influence root longevity (Pärtel and Wilson 2001). One way in which roots may tolerate exposure to soil moisture deficit is through nocturnal hydraulic redistribution (see Chapter 3). Yet, water stress intensity may dictate the root systems ability to re-hydrate desiccated tissues (see Chapter 3). Many species have demonstrated the capability to redistribute water but perhaps the most severe condition can be found in desert species. Hydraulic redistribution has been shown to occur in desert species in soils as dry as (-5.0 MPa) (Williams et al. 1993) while in other desert species roots exposed to severe dry soil (<-5.0 MPa) have demonstrated large losses in root water permeability and root death (Nobel and Huang 1992). In our study, both root systems demonstrated tolerance of dry soil as demonstrated by similar median root lifespans and nocturnal hydraulic redistribution may have been the reason for the similarities in root lifespan observed in the wet and dry soil zones.

Root growth during predictable periods of high soil moisture is beneficial to plants in drought-prone environments (Lyr and Hoffman 1967; Hayes and Seastedt 1987). The LSV root system exhibited an entirely different root growth strategy than the HSV root system to seasonal patterns of water and overall root development in response to supplemental water. The LSV root system produced a large number of its roots during the winter months suggesting the avoidance of periods when soils were dry (Figure 2.10).

Moreover in contrast to the HSV root system, the slow accumulation of root density, especially under no-irrigation in the LSV root system (Figure 2.11) indicates a more conservative strategy by the LSV root system. Rather than rapid root proliferation in wet soil zones during the growing season, the LSV root system seems more adjusted to growing roots during periods of soil moisture that are somewhat more predictable in Mediterranean climates. Similar observations of fine root growth during periods of ample soil water were recently reported in three Great Basin species *A. tridentata, B. tectorum* and *A. desertorum* under drought stress conditions (Peek 2005). This strategy, coupled with continued root system development and lower shoot vigor, presumably allows a plant in climatic regions with seasons of predictable high soil moisture to cope with severe drought stress in summer seasons.

The patterns documented here provide indications regarding the evolution of inherent grape root-system growth patterns in response to water stress. These results contribute to our understanding of the diverse root foraging responses of fast- and slow-growing plants to heterogeneous soil moisture that may occur in ecological communities and further emphasizes the important role of root system plasticity and seasonality of growth for plant success under different environmental conditions.

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References

Allison P.D. (1995) Survival analysis using the SAS system: a practical guide. SAS Institute, Cary, North Carolina, USA.

Carlisle E.A., Steenwerth K.L. and Smart D.R. (2006) Effects of land use on soil respiration: conversion of oak woodlands to vineyards. *Journal of Environmental Quality* **35**, 1396-1404.

CIMIS (2003-2005) California Irrigation Management and Information System http://www.cimis.water.ca.gov/.

Comas L.H., Andersen L.J., Dunst R.M., Lakso A., and Eissenstat D.M. (2005) Canopy and environmental control of root dynamics in a long-term study of Concord grape. *New Phytologist*, **167**, 829-840.

Comas L.H., Eissenstat D.M. and Lakso A.N. (2000) Assessing root death and root system dynamics in a study of grape canopy pruning. *New Phytologist*, **147**, 171-178.

Coutts M.P. (1982) Growth of Sitka spruce seedlings with roots divided between soils of unequal matric potential. New Phytologist, **92**, 49-61.

Cox D. (1972) Regression models and life tables. *Journal of the Royal Statistical Society* **34**,187-220.

Crick J.C. and Grime J.P. (1987) Morphological plasticity and mineral nutrient capture in two herbaceous species of contrasted ecology. *New Phytologist* **107**, 403-414.

Doussan C., Pagès L. and Pierret A. (2003) Soil exploration and resource acquisition by plant roots: an architectural and modeling point of view. *Agronomie* **23**, 419-431.

Eissenstat D.M., Whaley E.L., Volder A. and Wells C.E. (1999) Recovery of citrus surface roots following prolonged exposure to dry soil. *Journal of Experimental Botany*, **50**, 1845-1854.

Eissenstat D.M. and Yanai R.D. (1997) The ecology of root lifespan. *Advances in Ecological Research* **27**,1-60.

Eissenstat D.M. and Caldwell M.M. (1988) Competitive ability is linked to rates of water extraction. A field study of two aridland tussock grasses. *Oecologia* **75**, 1-7.

Fitter A.H. (1994) Architecture and biomass allocation as components of the plastic response of root systems to soil heterogeneity. In *Exploitation of environmental heterogeneity by plants*: *Ecophysiological processes above- and belowground*, eds. Martyn M. Caldwell and Robert W. Pearcy, 305-323. San Diego: Academic Press.

Fort C., Muller F., Label P., Granier A. and Dreyer E. (1998) Stomatal conductance, growth and root signaling in *Betula pendula* seedlings subjected to partial soil drying. *Tree Physiol*ogy **18**,769-776.

Galet P. (1998). Grape varieties and rootstock varieties**.** Oenoplurimedia Chaintre, France.

Green S. and Clothier B. (1999) The root zone dynamics of water uptake by mature apple trees. *Plant and Soil* **206**, 61-77.

Grime J.P. (1977) Evidence for the existence of three primary strategies implants and its relevance to ecological and evolutionary theory. *The American Naturalist* **111**, 1169-1194.

Hayes D. and Seastedt T. (1987) Root dynamics of tallgrass prairie in wet and dry years. *Canadian Journal of Botany*, **65**, 787-791.

Hodge A. (2004) The plastic plant: root responses to heterogeneous supplies of nutrients. *New Phytologist* **162**, 9-24.

Jupp A.P. and Newman E.I. (1987) Morphological and anatomical effects of severe drought on the roots of *Lolium perenne* L. *New Phytologist* **105**, 393-402.

Kembel S.T. and Cahill J.F. Jr. (2005) Plant phenotypic plasticity belowground: a phylogenetic perspective on root foraging trade-offs. *American Naturalist* **166**, 216-230.

Kosola K.R. and Eissenstat D.M. (1994) The fate of surface roots of citrus seedlings in dry soil. *Journal of Experimental Botany* **45**,1639-1645.

Lambers H. and Poorter H. (1992) Inherent variation in growth rate between higher plants: a search for physiological causes and ecological consequences. *Advances in Ecolgical Research* **23**,187-261.

Lyr H. &and Hoffman G. (1967) Growth rates and growth periodicity of tree roots. *International Review of Forest Research*, **2**, 181-206.

Mapfumo E., Aspinall D. and Hancock T. (1994) Growth and development of roots of grapevine (*Vitis vinifera* L.) in relation to water uptake from the soil. *Annals of Botany* **74**, 75-85.

Nobel P.S. and Huang B. (1992) Hydraulic and structural changes for lateral roots of two desert succulents in response to soil drying and rewetting. *International Journal of Plant Science* **153**, S163-S170.

North G.B. and Nobel P.S. (1997) Root-soil contact for the desert succulent *Agave deserti* in wet and drying soil. *New Phytol.ogist* **135**, 21-29.

North G.B. and Nobel P.S. (1991) Changes in hydraulic conductivity and anatomy caused by drying and rewetting roots of Agave deserti (Agavaceae). *American Journal of Botany* **78**, 906-915.

Pärtel M. and Wilson D. (2001) Root and leaf production, mortality and longevity in response to soil heterogeneity. *Functional Ecology* **15**, 748-753.

Peek M.S., Leffler A.J., Ivans C.Y., Ryel R.J. and Caldwell M.M. (2005) Fine root distribution and persistence under field conditions of three co-occurring Great Basin species of different life form. *New Phytologist*, **165**, 171-180.

Pritchard T.L. (1992) A volume balance approach to quality wine grape irrigation. In: *Viticultural practices* (eds W. M.A. & K. W.M), pp. 12-23. University of California, Davis.

Reid J.B. and Renquist A.R. (1997) Enhanced root production as a feed-forward response to soil water deficit in field-grown tomatoes. *Australian Journal of Plant Physiology* **24**, 685-692.

Richards D. and Cockroft B. (1975) The effect of soil water on root production of peach trees in summer. *Australian Journal of Agricultural Research*, **26**, 173-180.

Sharp R.E. and Davies W.J. (1979) Solute regulation and growth by roots and shoots of water-stressed maize plants. *Planta* **14**,:43-49.

Snyman H.A. (2006) A greenhouse study on root dynamics of cactus pears, *Opunita ficus-indica* and *O. rubusta*. *Journal of Arid Environments* **65**, 529-542.

Steudle E. (2000) Water uptake by roots: effects of water deficit. *Journal of Experimental Botany* **51**, 1531-1542.

Wells C.E. and Eissenstat D.M. (2001) Marked differences in survivorship among apple roots of different diameter. *Ecology* **82**, 882-892.

Williams K., Caldwell M.M. and Richards J.H. (1993) The influence of shade and clouds on soil water potential: the buffered behavior of hydraulic lift. *Plant and Soil* **157**, 83-95.

Wolpert J., Walker M.A., Bettiga L., Bianchi M., Hirschfelt D., McGourty G., Smith R. and Weber E. (2002) *Field evaluation of winegrape rootstocks.* American vineyard foundation California rootstock commission viticulture consortium pgs 1-112.

Chapter 3

Importance of internal hydraulic redistribution for prolonging lifespan of roots in dry soil

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Abstract

Hydraulic redistribution could mitigate drought stress of roots in dry soil layers by transferring water from areas of high soil moisture to roots in areas of low soil moisture through the plant during periods of low evaporative demand. Using minirhizotrons, we observed similar lifespans of roots in wet and dry soil for the grapevine, 'Merlot' (*Vitis vinifera*) on the rootstock 101-14 Millardet de Gramanet (*Vitis riparia* x *Vitis rupestris*) in a Napa County, California vineyard. We hypothesized that hydraulic redistribution would prevent an appreciable reduction in root water potential and contribute to prolonged root survivorship in dry soil. In a greenhouse study, grapevine root systems were divided using split pots and grown for six months. With thermocouple psychrometers, we measured water potentials of roots of the same plant in both wet and dry soil under three treatments: control (*C*); 24 h light + supplemental water (*LW*); and 24 h light only (*L*). Roots in the dry side of split-pots had similar survivorship as roots in the wet side of the split pots (P=0.136) in the *C* treatment. In contrast, reduced root survivorship was directly associated with plants in which hydraulic redistribution was experimentally reduced by 24 h light. Dry-side roots of plants in the *LW* treatment lived half as long as the roots in the wet soil despite being provided with supplemental water (P<0.0004). Additionally, predawn water potentials of roots in dry soil under 24 h of illumination (*L* & *LW*) exhibited values nearly twice as negative as that of *C* plants (P=0.034). Estimates of root membrane integrity using electrolyte leakage were consistent with patterns of root survivorship. Plants in which hydraulic redistribution was reduced exhibited more than twice the amount of electrolyte leakage in dry roots compared to those in wet soil of the same plant. Our study demonstrates that besides a number of ecological advantages to protecting tissues against desiccation, internal hydraulic redistribution of water is a mechanism consistent with extended root survivorship in dry soils.

Keywords: root survivorship, hydraulic redistribution, root water potential, electrolyte leakage, split-pot

Introduction

Roots take up water and serve as a major water conduit in the soil-plant-atmosphere continuum. While transpiration is the major driving force of water movement, water can redistribute within the plant at night or during periods of minimal transpiration, and may exit the roots and hydrate the rhizosphere (Richards and Caldwell 1987). The degree and rate that water redistributes within the plant tissues is important, and species vary in their resistance along this pathway. During extended periods when transpiration is negligible such as nighttime, hydraulic redistribution removes gradients in water potential among leaves and roots of different orders of branching (Hinckley et al. 1978; Boyer 1995). However, hydrologic factors that would increase transport resistance, including breaks in direct hydraulic contact (Nobel and Cui 1992), soil moisture heterogeneity (Ourcival and Berger 1995), and periods of nocturnal transpiration (Donovan, Richards and Linton 2003; Donovan et al. 1999) can disrupt internal water transport. Internal hydraulic redistribution may alleviate plant water stress by maintaining cell turgor for plant growth (Hsiao and Xu 2000), supplying water for night-time increases in leaf tugor (Blum and Johnson 1992) and presumably maintaining leaf water content in plants exposed to drought (Nardini and Pitt 1999). Internal hydraulic redistribution has also been hypothesized to mitigate drought conditions by refilling root xylem embolisms (McCully 1999), and preserving plant root viability (Huang 1999). Even under severe drought conditions if a portion of the root system is maintained in wet soil, internal hydraulic redistribution can continue to occur and fine roots in the dry soil layers can retain their function (Williams et al. 1993).

We are not aware of any studies that have examined the influence of internal hydraulic redistribution on root water potential status and root tolerance of desiccation, although a number of studies have now investigated water transfer from wet to dry soil through the root system (Baker and van Bavel 1986; Richards and Caldwell 1987; Dawson 1993). Espeleta et al. (2004) demonstrated indirectly that hydraulic lift influences root lifespan by enclosing roots in “root chambers” and measuring soil water potential. Roots used in the study were relatively large in diameter (0.5 to 1.0 cm) and

although 2 roots were placed in the chamber at the start of the experiment there did not appear to be any control over the number of roots that resulted in the final soil water potential. We are not aware of any past examination of the influence of internal water redistribution on root water potential and lifespan of individual roots of the finest laterals of a root system.

Prolonged fine root lifespan can have beneficial implications for the plant as a whole including maintained nutrient uptake capacity (Matzner and Richards 1996; Eissenstat et al. 1999), and mineral nutrient retention (Aerts et al. 1989). Some reports have indicated that during periods of decreased water availability the very small movement of water associated with hydraulic redistribution has been adequate enough to prevent root mortality in some species, like oak seedlings (Querejeta et al. 2003), citrus seedlings (Kosola and Eissenstat 1994) and mature grape vines (Anderson et al. 2003), while others have reported it does not in species such as some bunch grasses (*S. scoparium*; Espeleta et al. 2004), mesic oaks (*Q. margaretta*; Espeleta et al. 2004) and tomato (Reid et al. 1996).

Very fine roots of the ultimate laterals, which may readily lose water as a consequence of higher surface area to volume and/or lack of suberization, may be more susceptible to drought (Huang et al. 1997). Hydraulic constraints of fine roots such as small xylem vessel diameter and a high incidence of xylem cavitation are believed to contribute to shortened root lifespan (Sperry, Stiller and Hacke, 2002). Nonetheless, roots may exhibit plasticity in as much as roots exposed to water stress have been shown to invest in apoplastic barriers such as increased suberization of the exodermis and endodermis, presumably reducing hydraulic flow back into the soil during nocturnal hours where the water potential gradient is in the direction of the soil (North and Nobel 1991). Exodermal secondary cell wall development is hypothesized to provide structural support against radial weakening of the root, thus preventing root collapse and the formation of air gaps between the root and the surrounding soil (Taleisnik et al. 1999). Finally, aquaporins within root cells may close and decrease water loss (Steudle 2000). All of the above factors may serve to increase root lifespan during episodes of drought stress.

We studied the links between internal hydraulic redistribution and root lifespan in grape vines. Researchers have demonstrated previously both the existence of hydraulic redistribution (Smart et al. 2005) and similarity in fine root lifespan of grape roots grown in wet and dry soil (Anderson et al. 2003). The objectives of this study were to examine consequences of internal hydraulic redistribution on root water potential and fine root longevity. We hypothesized that roots in wet soil and dry soil have similar lifespans when internal hydraulic redistribution occurs. Restricting water movement in the roots by continuous illumination at "night" should lead to a sustained decline in water potential and more rapid death of branch roots in dry soil as a consequence of restricting redistribution of water from branch roots in moist soils.

Methods

Field experiment

The *Vitis vinifera* cv. Merlot vineyard was about 8 to 11 yrs old during the period of study and planted in Bale (variant) gravelly loam (fine-loamy, mixed, superactive, thermic Cumulic Ultic Haploxeroll). The Mediterranean climate of the Oakville region, Napa County, California, USA averages 83 cm of annual precipitation and has a mean annual temperature of 14.3ºC (CIMIS, 2004). The vineyard had a NE to SW row orientation with vines spaced 2.4 m between rows and 2.2 m within the row and trained on a bilateral cordon with vertical shoot positioning (VSP) (Winkler et al. 1974).

The entire experimental vineyard covered 1 ha and had three irrigation treatments and three rootstock cultivars laid out in a completely randomized block design. Surrounding vine rows served as buffers to separate treatments. Each vine had one emitter, located 50 cm from the trunk on one side of the vine. The irrigation treatments (no irrigation, 40% (deficit irrigation), and 100% Et_c) were determined using crop evapotranspiration (Et_c) calculated from the evaporation of a Class A pan and the Penman

Montieth equation(Et_0) and corrected with crop coefficients (K_c) put forward by Prichard (1992). In this study, only vines in the 100% Et_c treatment on 101-14 Millardet de Gramanet (*Vitis riparia* x *Vitis rupestris*) rootstocks (101-14 Mgt.) are reported.

In April 2002, clear plastic (cellulose acetyl butyrate) root observation tubes (minirhizotrons) were installed. Tubes were 1.3 m long and 6 cm outside diameter. The tubes were sealed with PVC plugs and the tops were wrapped with black electrical tape and sealed with rubber stoppers to prevent light penetration. When not in use, the tops of the tubes were covered with white metal radiation shields to prevent radiant heating. Each minirhizotron tube was inserted parallel to the vine row at a 30° angle from vertical, and about 60 cm from the trunk. One minirhizotron tube placed through the dripper zone and the other in the unirrigated zone on the opposite side of the vine. Thus, there were 2 tubes per vine representing irrigated and non-irrigated treatments x 1 irrigation level x 1 rootstock x 6 blocks, for a total of 12 tubes in this study.

Root images were captured using a BTX-100x camera equipped with BTC I-Cap version 4.01 imaging software (Bartz Technology, Santa Barbara, CA, USA). Images were captured approximately every two weeks during the growing season (April-October) and every four weeks during the dormant period (November- March). Root diameters were measured with WinRhizo Tron MF software (Regents Inc. Quebec, Canada). Root births were estimated by calculating the date midway between the observation date when a root was first observed and the previous observation date. Similarly, root death was estimated as being midway between the date the root was first observed dead and the previous observation date. Root death was identified by a black and shriveled appearance (Comas, Eissenstat and Lakso 2000) or if the root had disappeared from the window and did not re-appear. Roots that transected more than one minirhizotron observation window vertically within the same minirhizotron observation tube were only counted once. Volumetric soil water content was estimated using time domain reflectrometry.

The minirhizotrons were used as access tubes for soil moisture determination using a TRIME soil moisture probe (Mesa Systems Co., Medfield, MA, USA) at 20-50, 50-80 and 80-120 cm depths These intervals corresponded with the highest root densities.

A soil water retention curve was determined using a pressure plate extractor (Soil Moisture Equipment Co., Santa Barbara, CA, USA) under five levels of pressure to relate volumetric soil moisture (%) estimated by TRIME to soil matric potential (MPa). Bulk density measurements come from a parallel study in the same vineyard and were determined to be 1.33 ± 0.10 Mg m^{-3} at 6-12 cm depth and 1.45 ± 0.05 Mg m^{-3} at 40-46 cm depth (Carlisle et al. 2006)

Greenhouse experiment

The study took place at The Pennsylvania State University greenhouses, University Park, PA, USA. Green cuttings from rootstock 101-14 Mgt. were collected from the Oakville vineyards in August, 2005, rooted on a misting bench, and then shipped to University Park, PA. Plants were transplanted into a mixture of 50% sand and 50% Hagerstown series soil, which is characterized by a dark brown silt loam layer (20 cm) in 5L pots. Greenhouse temperatures during the daytime were 25°± 3°C and during the nighttime were 15.5°± 3°C.

In November 2005 6-month-old grape vine root systems were split into 2, 5-L containers with a 90°, 5-cm PVC elbow with the corner portion removed to bridge the two containers (Eissenstat 1990). The root system was evenly divided between the two outlets of the elbow bridge while the shoot protruded from the central hole. Pots were arranged in a completely randomized design with three replicates per treatment. Treatments were designed to control transpiration at night and limit hydraulic redistribution by the root system (Caldwell and Richards 1989). Treatments were: control (*C*), supplemental nighttime light + supplemental water (*LW*), and nighttime light only (*L*). Control plants were kept under natural light conditions (12 h of illumination) while treatments that included supplemental light were kept under 12 h of natural illumination and 12 h of supplemental illumination during the night (minimum 300 μmol m^{-2} s^{-1} PPFD). Wet and dry sides of the plant were randomly chosen; wet-side pots of the *C* and *L* treatments were watered 400 ml once daily with drip irrigation emitters. The *LW* treatment was watered once daily with 600 ml daily to maintain leaf water potentials

similar to that of the *C* treatment. Leaf water potentials of *C* and *LW* treatments were maintained within 0.1 MPa (measured with a Scholander pressure chamber, Soil Moisture Equipment Co., Santa Barbara, CA, USA). For each pot, roots were tracked weekly on 3, 15 cm tall x 8 cm wide acetate windows located 120 degrees apart on each pot. Root births and deaths were estimated in the same way as in the field study (see above). If the roots did not die or disappear by the end of the experiment, then the data were treated as censored.

Time-domain reflectometry was used to measure volumetric soil water daily (TDR 100, Campbell Scientific, Logan, UT, USA). Probes were constructed of three parallel stainless steel rods, 20 cm in length and 3 mm in diameter. Probes were inserted perpendicular to the soil surface and remained in place for the duration of the experiment. A soil water retention curve was determined in the same way as in the field study (see above).

Root water potential was determined using thermocouple psychrometry. Control plants were covered with 100% shade cloth on the evening of measurement to prevent nighttime transpiration (Caird, Richards and Donovan 2007). Roots less than 1 week old were collected at predawn (5.00 h) from both the wet and dry pots every other day for two weeks. Using a razor blade, the acetate window was carefully cut and peeled away, and a 1st-order root (root with no laterals) about 2 cm in length was severed with the razor blade and removed to a humidified box to prevent water loss. Roots segments were tapped to remove adhering soil particles, loaded into the thermocouple psychrometer chambers (series 74, J.R.D. Merrill Specialty Equipment, Logan, UT, USA) and placed in a cooler until they were brought back to the lab and connected to a computerized data acquisition system (CR-7 data logger, Campbell Scientific Incorporated, Logan, UT, USA). Individual root sampling averaged 10 s per root and total sampling time did not exceed 30 minutes on any given day. Thermocouple psychrometers were placed in an insulated water bath at 25 °C and measured every 30 minutes for at least six hours to allow for temperature and vapor equilibrium. Once equilibrium was reached three measurements were averaged to estimate water potential. Diurnal patterns of root rehydration were determined by simultaneous sampling of root and leaf water potentials

at predawn (5.00 h) and mid-day (13.00 h) on two randomly chosen days. Osmotic potential (Ψ_s) was determined on the same samples by freezing the sample tissue in liquid N and equilibrating the psychrometer in the water bath for another six hours. Thermocouple psychrometers were calibrated with three salt solutions of known osmolality every ten measurements. Due to the limited number of available psychrometers and results of a preliminary study that found a reasonably close relationship of thermocouple psychrometers with the pressure chamber technique (R^2= 0.744; *P*=0.003), leaf water potentials were measured with a Scholander-type pressure chamber (Soil Moisture Equipment Co., Santa Barbara, CA, USA) immediately following root water potential measurements. Leaf osmotic potentials were measured on expressed sap by vapor pressure osmometry (Boyer 1995). Leaves were severed from the plant, placed in plastic syringes and frozen until measurement. Leaves were allowed to thaw for 12 h and a sample of sap was placed on filter paper for measurement (Wescor 5500 vapor pressure osmometer, Wescor Inc., Logan, UT, USA).

Electrolyte leakage was determined on a separate set of new 1st-order laterals of similar length and weight (Huang, Lakso and Eissenstat 2005) (Eq. **3.1)**. Roots were thoroughly rinsed of all soil particles and immersed in 40ml of deionized water. Percent electrolyte leakage of the sample was estimated by measuring the electrical conductivity (EC) of the water at immersion ($EC_{initial}$), after 30 min. (EC_{30}) and after disrupting root cell membranes by boiling the sample for 5 min (EC_{boil}). Membrane leakage was estimated as a percent of total electrolytes in the roots:

$$\text{Electrolyte leakage (\%)} = 100 * (EC_{30} - EC_{initial})/(EC_{boil} - EC_{initial}) \qquad \textbf{3.1}$$

Statistical methods

Root lifespan data were analyzed by Cox proportional hazards regression (PROC PHREG; SAS Institute Inc., Cary, NC, USA). This type of analysis allows the influence

of all other covariates to be held constant (Cox 1972). Wilcoxon tests were used to determine significance in root lifespan for field data (SAS Institute Inc., Cary, NC, USA). Components of water potential data were transformed using (log + 1) to correct for heteroscedasticity. Soil volumetric moisture and root water potentials over time were analyzed using GLM repeated measures (SPSS Inc. v. 11.0, Chicago, IL, USA). Root predawn and midday water potential were analyzed using T-tests and root electrolyte leakage was analyzed using ANOVA (SPSS Inc. v. 11.0, Chicago, IL, USA).

Results

Field Experiment

The years 2003 through 2005 were typical for weather patterns of Napa Valley, California with cool wet winters and warm summers with no rainfall. Soil moisture in the top 80 cm, the area where the majority of roots are located, averaged 27% (about -0.03 MPa) on the irrigated side of the vine and 22% (-0.9 MPa) on the unirrigated side of the vine during August (a dry month) of all three years combined (P=0.0001). Deeper soil (> 80 cm) retained more water with an average of 29% water (>-0.0 MPa) in the wet soil and 28% water (-0.02 MPa) in the dry soil (P=0.150). Fine root diameter averaged 0.42 mm and ranged from 0.11 to 0.98 mm. Fine root median survivorship did not differ between roots in wet soil and roots in dry soil under field conditions (median lifespan: wet = 109 d; dry= 105 d; P=0.2055; Figure **3.1**). A large portion of the grapevine root system remained apparently unstressed in unirrigated dry soil during the summer months (usually May- October) typical of California summers where precipitation is minimal.

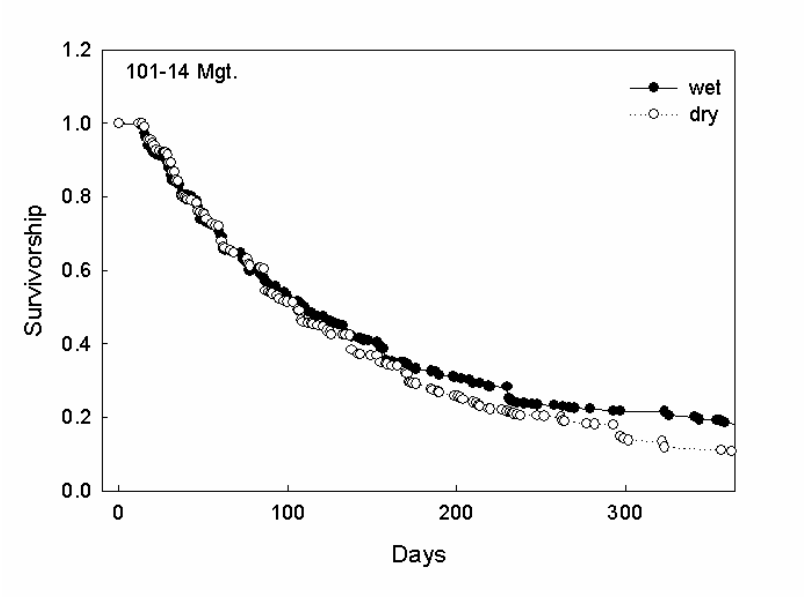

Figure **3.1**: Root survivorship for wet and dry sides of *Vitis vinifera* cv. Merlot vines grafted onto 101-14 Millardet de Gramanet (*V. riparia x V. rupestris*) rootstock in Oakville, CA. Vines were watered on one side only and received 100% Et_c through drip emitters located 50 cm from the trunk of the vine. Data include all roots born in years 2003-2005 (n= 2187). Differences in root survival in wet and dry soil was not significant (P=0.2055).

Greenhouse experiment

Soil moisture content of the dry side pots decreased in all treatments compared to the watered pots (Figure **3.2**). Results of a soil moisture release curve indicated that while the soil began at a near saturated condition (day 0 of experiment), the soil in the dry pots reached about -3.5 MPa by day 14, -9 MPa by day 21, and reached a minimum of about -13 MPa. Soil moisture in the wet soil was significantly higher than that in the dry side in all treatments (P<0.0001) while dry side soil moisture was similar among treatments (P=0.512).

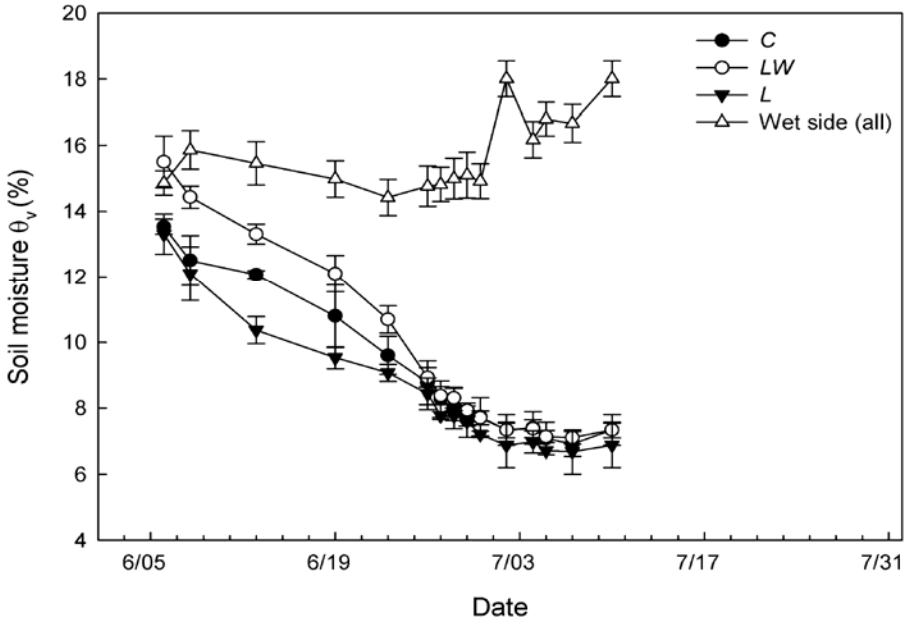

Figure **3.2**: Volumetric soil moisture content (%) for the wet side of the grape vine, 101-14 Mgt. rootstock cultivar (△, average of all three treatments), and the dry side by treatment: *C* (●), plants with a 12-h dark nocturnal period; *LW* (○), plants with 24-h illumination plus supplemental water; and *L* (▼), plants with similar irrigation to that of control but with 24-h illumination (±1 SE). Collectively, three hours after watering, the watered pots of all vines remained wetter than the un-watered pots ($P<0.0001$) while dry-side soil moisture was similar among treatments ($P=0.512$).

Despite dry soil conditions, roots in the dry side of the *C* treatment split-pots had similar survivorship to roots grown in the wet side of the split pots ($P=0.136$), (Figure **3.3**A). In contrast, the treatments with continuous illumination (*LW* and *L*) exhibited shorter lifespans of roots in the dry side of the split pots (Figure **3.3**B, $P<0.0004$ and Figure **3.3**C, $P<0.0001$).

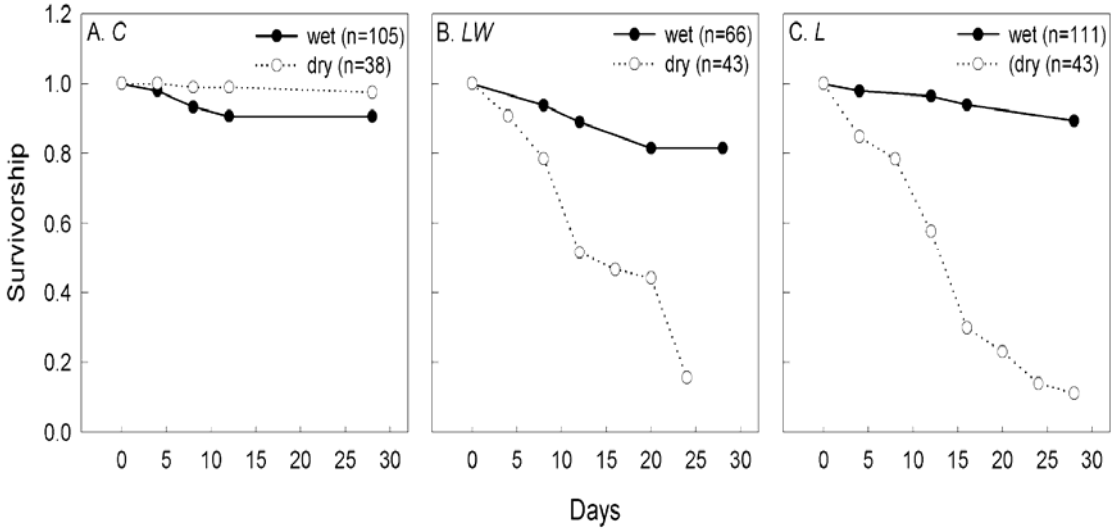

Figure **3.3** Root survivorship on wet and dry sides of rootstock 101-14 Mgt. for A. *C*: plants with a 12-h dark nocturnal period; B. *LW*: plants with 24-h illumination plus supplemental water; and C. *L*: plants with similar irrigation to that of the control but with 24-h illumination. Survivorship of roots in the wet side was significantly higher than that in the dry side in B and C ($P<0.0004$) but not in A ($P>0.50$).

Averaged over the entire study, mid-day leaf water potentials were higher for *C* plants than *L* plants ($P=0.051$) but similar to those of *LW* plants ($P=0.865$). Predawn water potentials of roots in dry soil under 24-hrs of illumination (*L* and *LW*) were nearly twice as negative as for roots of *C* plants ($P=0.034$) (Figure **3.4**).

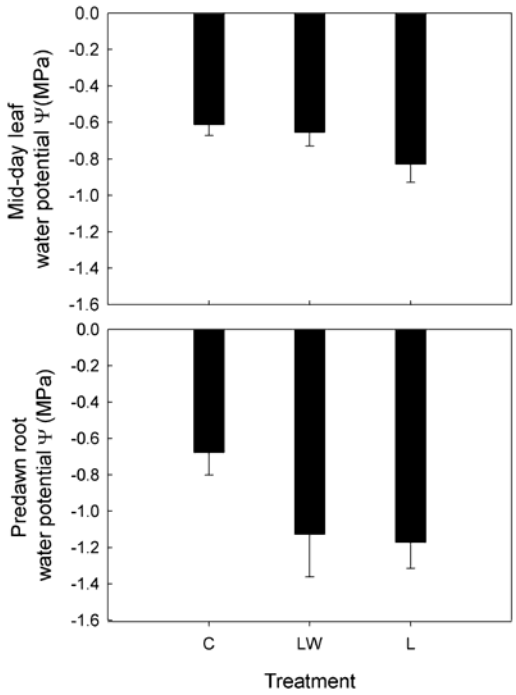

Figure **3.4**: Mid-day leaf water potential and predawn root water potential(MPa) averaged over the entire study of rootstock 101-14 Mgt., for roots in the dry soil with a 12-h nocturnal dark period (*C*); plants with 24-h illumination plus supplemental water (*LW*); and plants with 24-h illumination (*L*) (±1 SE). Leaf water potentials were lower in *L* plants (P=0.051) and similar for *C* and *LW* treatments (P=0.865) and roots under 24-h of illumination (*LW* and *L*) showed lower water potentials compared to the control (P=0.034).

Diurnal Patterns

Changes in plant water potential from midday (13.00 h) to predawn (5.00 h) give some indication of rate of recovery of water potential over the nighttime. Leaf water potential recovery was considered complete for *C* plants when measured near the end of the experiment on July 1 and 2 (Figure **3.5**). Predawn water potential in plants that received supplemental irrigation, *LW*, were not different from those of the control and were also considered to have complete leaf water potential recovery (P=0.416); however, recovery was incomplete for *L* plants compared to the control as indicated by lower predawn leaf

water potentials in the *L* plants (*P*= 0.046)(Figure **3.5**). Roots in dry soil had lower water potentials than roots in wet soil at mid-day for *C*, *L*, and *LW* (all *P*≤0.001) plants. Mid-day and predawn root water potentials in wet soil were similar across light treatments (*P*=0.848). Light treatments strongly affected water potential of roots in the dry soil. In *C* plants, a subsequent rise in root water potential of dry-side roots from 1pm to 5 am to equal those of roots in wet soil at predawn (5am) indicated root water potential recovery is consistent with the movement of water through internal hydraulic redistribution when nocturnal transpiration was minimized. In contrast, predawn root water potential recovery was incomplete for roots in dry soil of plants that received supplemental water plus supplemental light (*P*=0.040) and for roots in dry soil of plants that only received supplemental light (*P*=0.031) (Figure **3.5**).

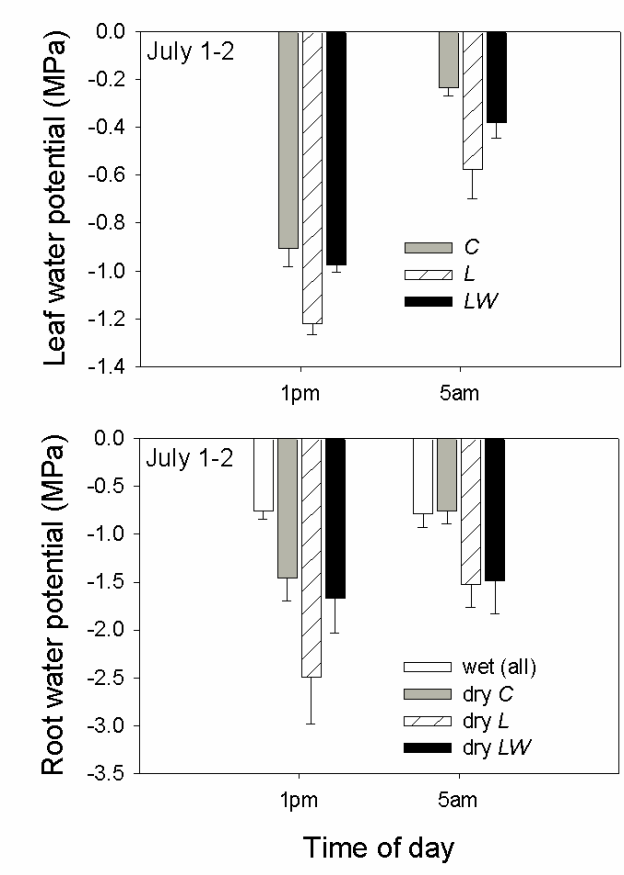

Figure **3.5**: Mid-day (13.00) and predawn (5.00) A. leaf water potentials (MPa) on July

1st and 2nd for rootstock 101=14 Mgt. with a 12-h dark nocturnal period (*C*, hatched bars*;* plants with 24-h illumination plus supplemental water (*LW*) (black bars); and plants with similar irrigation to that of control but with 24-h illumination (*L*, white bars); and B. Mid-day (13.00) and predawn (5.00) root water potentials (MPa) on July 1st and 2nd for roots in wet soil (white bars); and for roots in dry soil in the *C* (gray bars); *L* (hatched bars); and *LW* (black bars). For statistical significance see text.

Over the course of the entire experiment, predawn root water potentials of roots in dry soil remained fairly constant and similar to those in wet soil in the *C* plants (*P*=0.190), but roots in dry soil declined continuously over the experiment in plants exposed to continuous illumination (*P*=0.001) (Figure **3.6**). By the end of the experiment, water potentials of roots in dry soil where plants were illuminated at night had water potentials as low as -2.5 to -3.0 MPa (Figure 3.6b and 3.6c) whereas plants where normal nocturnal hydraulic redistribution was allowed to occur had water potentials of roots in dry soil of about -0.6 MPa (Figure 3.6A).

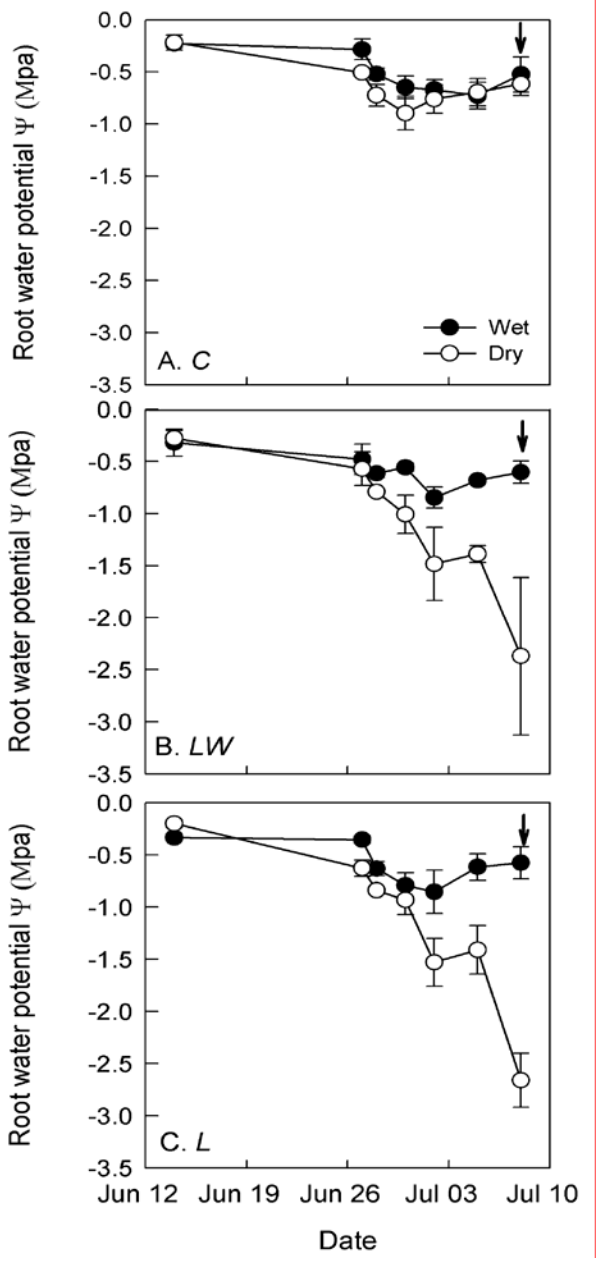

Figure **3.6**: Shifts in predawn root water potential over course of the experiment for rootstock 101-14 Mgt. with A. 12-h dark nocturnal period (control); B. 24-illumination plus supplemental water (light + water); and C. irrigation similar to that of control but with 24-h illumination (light) on both the watered (●) and dry (○) sides of the plant (±1 SE). Light and Light + supplemental water treatments had lower water potentials of roots in dry soil (P=0.001) while the control treatment had similar water potentials in both wet and dry soil (P=0.190). Arrows indicate time point at which roots were sampled for electrolyte leakage.

Patterns of root electrolyte leakage at the end of the experiment (Figure **3.7**) were consistent with patterns of root survivorship (Figure **3.3**) and patterns of predawn root water potential (Figure **3.6**). Roots of plants in the *LW* and *L* treatments exhibited greater electrolyte leakage, an indication of lack of internal water re-hydration and subsequent reduced membrane integrity (P=0.046). Overall, illuminated plants had more than a two-fold increase in electrolyte leakage in dry roots compared to roots grown in wet soil (P=0.002) (Figure **3.7**).

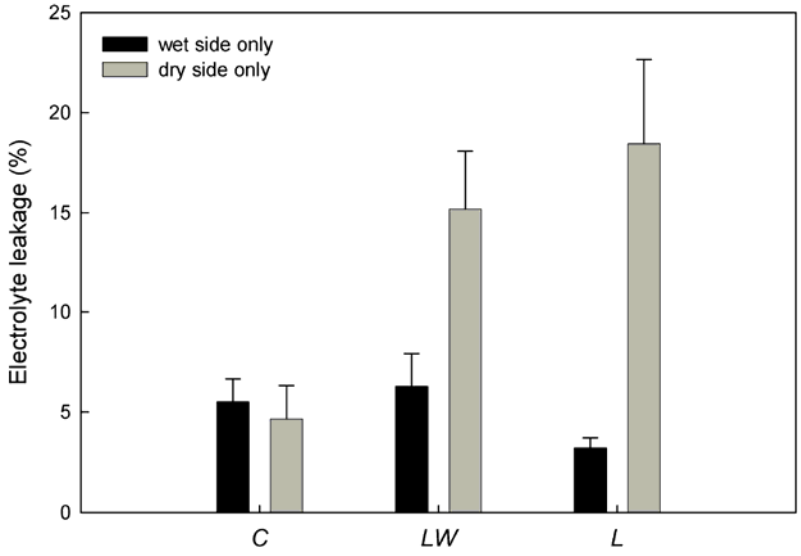

Figure **3.7**: Root electrolyte leakage for roots of rootstock 101=14 Mgt. with a 12-h dark nocturnal period (*C*), plants with 24-illumination plus supplemental water (*LW*), and plants with similar irrigation to that of control but with 24-h illumination (*L*) (%). Black bars represent wet side roots and light gray bars represent dry side roots Statistical differences were found between treatments (P=0.046) and between wet and dry sides of the plant (P=0.002).

Components of water potential

Examination of the components of water potential provides insight into mechanisms of how roots cope with dry soil. Roots in dry soil where hydraulic redistribution was disrupted (*LW* and *L* treatments) had a pattern, although non-significant, of lower osmotic potentials ($\Psi\pi$) compared to *C* roots (Figure **3.8**A, P=0.318). Total water potential (Ψw) was lower for *LW* and *L* treatments compared to *C* treatment (Figure 3.8B, P=0.018). While the *C* plants retained positive root turgor potential ($\Psi\rho$), *LW* or *L* roots exhibited less evidence that they were able to retain positive turgor (Figure 3.8C, P=0.001).

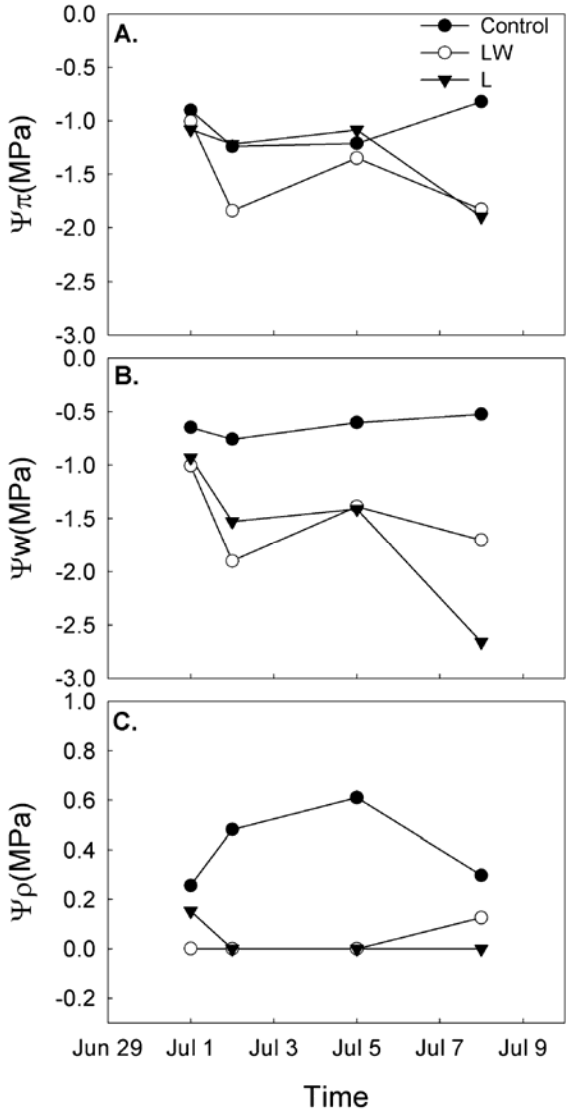

Figure **3.8**: Root predawn osmotic potential, Ψ_π, for roots of rootstock 101=14 Mgt. in the dry soil only (P=0.318) (A.); water potential, Ψ_w (P=0.018) (B.); and turgor potential, Ψ_ρ (P=0.001) (C.) for plants with a 12 hr dark nocturnal period (C) (●); plants with 24-illumination plus supplemental water (LW) (○); and plants with normal similar irrigation to that of control but with 24-h illumination (L) (▼) treatments.

Discussion

Internal hydraulic redistribution requires minimal nighttime transpirational water loss. We successfully devised and implemented a set of treatments using 2 controls, one for length of illumination and another that controlled for increased transpiration due to 24-hrs of illumination but where root available water was not limiting. This allowed for a direct examination of internal water potential recovery of the finest roots of grape. Internal hydraulic redistribution by apparently rehydrating root tissues, when it was allowed to occur under normal nighttime conditions, most likely prevented appreciable first-order root mortality, and electrolyte leakage.

Inhibiting internal hydraulic redistribution by preventing nighttime stomatal closure and maintaining nighttime transpiration with continuous illumination is consistent with the resulting increase in root death, lower root water potentials and more electrolyte leakage by roots in dry soil. Supplemental water to roots in the wet soil did not alter water stress (as indicated by the lower root Ψ) of roots in the dry soil when nocturnal hydraulic redistribution was presumably disrupted. Although a number of studies have already established the phenomenon of hydraulic redistribution within several plant species, few have documented direct changes in internal root water potential (but see Domec et al. 2006; Burgess et al. 1998) an important component for root functioning. Circumstantial evidence of the effects of internal hydraulic redistribution on root lifespan came from a split-pot study that reported a decline in photosynthate allocation to roots in dry soil and a lack of root death in response to drought stress in citrus seedlings (Kosola and Eissenstat 1994).

In grape, we almost certainly observed evidence of similar lifespans of fine roots in wet and dry soil in the field by internal hydraulic redistribution. Despite the potential vulnerability of the fine root system to severe decreases in soil moisture (Smucker and Aiken 1992), localized patches of soil moisture in the irrigated zone and deeper soil layers supported root survivorship and even root production in dry soil (see Chapter 3). Under extremely heterogeneous field conditions, hydraulic redistribution may aid in maintaining water uptake of the entire root system by re-hydrating roots in dry soil

(Houltine et al. 2003). Our work under greenhouse conditions supported this contention. If plants were exposed to 12 h of darkness, we showed that roots in dry soil in a split-pot system coped with water deprivation by only exhibiting moderate water stress during the day and full recovery of root water potential over the night: there was no decrease in lifespan or increase in membrane leakage. In contrast, plants that were subjected to continuous illumination with or without supplemental water not only exhibited lower mid-day and predawn root water potentials in dry soil compared to roots in wet soil, but also had decreased root lifespan, probably a result of the loss of cell turgor and increased membrane leakage as the soil dried.

Although it is known that root desiccation can cause a decrease in membrane stability and therefore an increase in electrolyte leakage (Blum and Ebercon 1981) to our knowledge no studies have evaluated the consequences of decreased root water potential on root vitality. In our study whether or not water flowed out of the root to the soil was inconsequential. Instead, the direct measurement of internal re-hydration of the finest roots confirmed continuous vitality of roots in dry soil with little apparent ill effects as long as sufficient water was available at night. A more detailed look at the decline of root membrane stability with lowering water potential could provide more insight into the length of time and to what level plant membranes cope with decreasing soil moisture.

Species-specific differences in root xylem vessel diameter can influence water flow within the root. We not only studied the finest and youngest roots of the grape root system but we also used a plant known for its large xylem vessels (Salleo Lo Gullo and Oliver 1985). Due to the large vessel size of grape one needs to be cautious about extending our results in grape to species with substantially smaller xylem vessels and greater hydraulic resistance. Nonetheless, we showed that internal hydraulic redistribution apparently occurred in grape despite the known decrease in vessel size and reduction in hydraulic conductivity associated with roots of the finest orders and plants exposed to moderate drought (Lovisolo and Schubert 1998). Understanding variation among species in rates of internal water movement from roots in wet soil to roots in dry soil and its influence on fine root survival may provide additional clues on selection pressures associated with hydraulic architecture.

Ecophysiological Implications

Internal hydraulic redistribution may influence numerous root functions as a direct consequence of increased root persistence in dry soil. The internal transfer of water to portions of the root system in dry soil may allow for enhanced and prolonged nutrient uptake (Matzner and Richards 1996). Moreover, enhanced root growth has been shown to be a consequence of increased photosynthate fixation in plants that perform hydraulic lift (Dawson 1997). Roots of plants that perform hydraulic redistribution may also have a faster recovery response following rain events thus potentially allowing the plant to better compete for water and nutrients (Eissenstat et al. 1999; Burgess et al. 1998). Maintaining roots in dry soil by internal hydraulic redistribution has several beneficial implications on the whole plant such as reduced carbon costs in relation to building new roots and extended nutrient uptake by the root (Eissenstat and Yanai 1997). Extended root lifespan in dry soil may also increase the likelihood of the plant or its mycorrhizal fungi to utilize spatially heterogeneous resources such as water or nutrient patches (Chapin et al. 1987).

While hydraulic redistribution effects on plant success under conditions of water limitation are well recognized, lack of hydraulic redistribution caused by root death, shrinkage or xylem embolisms also may occur (Caldwell, Dawson and Richards 1998; North and Nobel 1997). Hydraulic redistribution may also be limited in plants with high axial resistance due to small root xylem vessels or extensive branching (Tsuda and Tyree 1997). Often referred to as hydraulic segmentation, the xylem of distal roots is commonly more susceptible to cavitation (Tsuda and Tyree 1997) and may therefore show evidence of shorter lifespans during periods of water stress. Root survivorship of high-latitude plants with short periods of darkness or plants that transpire at night (Hultine et al. 2003) may have decreased root survival in dry soil as a result of limited hydraulic redistribution.

Summary

In summary, we found that despite differences in soil moisture, internal hydraulic redistribution of water is the most obvious link to prolonged root lifespan in the finest laterals of the root system. Circumventing hydraulic redistribution by maintaining transpiration during nocturnal hours prevented roots in dry soil from rehydrating at night and resulted in reduced root turgor, increased electrolyte leakage, and decreased root survivorship.

Acknowledgements

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References

Aerts R., Berendse F., Klerk N.M. and Bakker C. (1989) Root production and root turnover in two dominant species of wet heathlands. *Oecologia* **81**, 374-378.

Anderson L.J., Comas L.H., Lakso A.N. and Eissenstat D.M. (2003) Multiple risk factors in root survivorship: a 4-year study in Concord grape. *New Phytologist* **158**, 489-501.

Baker J.M. and van Bavel C.H.M. (1986) Resistance of plant roots to water loss. *Agronomy Journal* **78**, 641-644.

Blum A. and Johnson J.W. (1992) Transfer of water from roots into dry soil and the effect on wheat water relations and growth. *Plant and Soil* **145**, 141-149.

Blum A.and Ebercon A. (1981) Cell membrane stability as a measure of drought and heat tolerance in wheat. *Crop Science* **21**, 43-47.

Boyer J.S. (1995) Measuring the water status of plants and soil. Academic Press, San Diego.

Burgess S.O., Adams M.A., Turner N.C., and Ong C.K. (1998) The redistribution of soil water by tree root systems. *Oecologia* **115**, 306-311.

Burgess S.O., Adams M.A., Turner N.C., White D.A. and Ong C.K. (2001) Tree roots: conduits for deep recharge of soil water. *Oecologia* **126**, 158-165.

Caird M.A., Richards J.H. and Donovan L.A. (2007) Nighttime stomatal conductance and transpiration in C_3 and C_4 plants. *Plant Physiology* **14**, 4-10.

Caldwell M.M. and Richards J.H. (1989) Hydraulic lift: water efflux from upper roots improves effectiveness of water uptake by deep roots. *Oecologia* **79**, 1-5.

Caldwell M.M., Dawson T.E. and Richards J.H. (1998) Hydraulic lift: consequences of water efflux from the roots of plants. *Oecologia* **131**,151-161.

Carlisle E.A., Steenwerth K.L. and Smart D.R. (2006) Effects of land use on soil respiration: conversion of oak woodlands to vineyards. *Journal of Environmental Quality* **35**, 1396-1404.

Chapin F.S., Bloom A.J., Field C.B. and Waring R.H. (1987) Plant responses to multiple environmental factors. *BioScience* **37**, 49-57.

CIMIS (2004) California Irrigation Management and Information System http://www.cimis.water.ca.gov/.

Comas, L.H., Eissenstat, D.M. and Lakso, A.N. (2000) Assessing root death and root system dynamics in a study of grape canopy pruning. *New Phytologist* **147**, 171-178.

Cox, D. (1972) Regression models and life tables. *Journal of the Royal Statistical Society* **34**, 187-220.

Dawson T.E. (1997) Water loss from tree roots influences soil water and nutrient status and plant performance In: Flores HL, Lynch JP, Eissenstat DM (eds) *Radical Biology: Advances and perspectives in the function of plant roots.* Current topics in plant physiology, vol. 17 American Society of Plant Physiologists, pp235-250 Rockville, Maryland.

Dawson T.E. (1993) Hydraulic lift and water use by plants: implications for water balance, performance and plant-plant interactions. *Oecologia* **95**, 565-574.

Domec J.C., Scholz F.G., Bucci S.J., Meinzer F.C., Goldstein G. and Villalobos-Vega R. (2006) Diurnal and seasonal variation in root xylem embolism in neotropical savanna woody species: impact on stomatal control of plant water status. *Plant, Cell and Environment* **29**, 26-35.

Donovan L.A., Grisé D.J., West J.B., Pappert R.A., Alder N.N. and Richards J.H. (1999) Predawn disequilibrium between plant and soil water potentials in two cold-desert shrubs. *Oecologia* **120**, 209-217.

Donovan L.A., Richards J.H. and Linton M.J. (2003) Magnitude and mechanisms of disequilibrium between predawn plant and soil water potentials. *Ecology* **84**, 463-470.

Eissenstat D.M. and Yanai R.D. (1997) The Ecology of Root Lifespan. *Advances in Ecological Research* **27**, 1-60.

Eissenstat D. M., Whaley E. L., Volder A. and Wells C. E. (1999). Recovery of citrus surface roots following prolonged exposure to dry soil. *Journal of Experimental. Botany* **50**, 1845-1854.

Eissenstat D.M. (1990) A comparison of phosphorus and nitrogen transfer between plants of different phosphorus status. *Oecologia* **82**, 342-347.

Espeleta J.F., West J.B. and Donovan L.A. (2004) Species-specific patterns of hydraulic lift in co-occurring adult trees and grasses in a sandhill community. *Oecologia* **138**, 341-349.

Hinckley T.M., Lassoie J.P. and Running S.W. (1978) Temporal and spatial variations i the water status of forest trees. *Forest Science Monographs* **20**, 1-72.

Hsiao T.C. and Xu L.K. (2000) Sensitivity of growth of roots versus leaves to water stress: biophysical analysis and relation to water transport. *Journal of Experimental Botany* **51**, 1595-1616.

Huang X., Lakso A.N. and Eissenstat D.M. (2005) Interactive effects of soil temperature and moisture on Concord grape root respiration *Journal of Experimental Botany* **56**, 2651-2660.

Huang B. (1999) Water relations and root activities of *Buchloe dactyloides* and *Zoysia japonica* in response to localized soil drying. *Plant and Soil* **208**, 179-186.

Huang B., Duncan R.R. and Carrow R.N. (1997) Drought-resistance mechanisms of seven warm-season turfgrasses under surface soil drying: II. Root aspects. *Crop Science* **37**, 1863-1869.

Hultine K.R., Williams D.G., Burgess S.S.O. and Keefer T.O. (2003) Contrasting patterns of hydraulic redistribution in three desert phreatophytes. *Oecologia* **135**, 167-175.

Kosola K.R. and Eissenstat D.M. (1994) The fate of surface roots of citrus seedlings in dry soil. *Journal of Experimental Botany* **45**, 1639-1645.

Lovisolo C. and Schubert A. (1998) Effects of water stress on vessel size and xylem hydraulic conductivity in *Vitis vinifera* L. *Journal of Experimental Botany* **49**, 693-700.

Matzner S. and Richards J.H. (1996) Sagebrush (*Artemisia tridentata* Nutt.) roots maintain nutrient uptake capacity under water stress. *Journal of Experimental Botany* **47**, 1045-1056.

McCully M.E. (1999) Root xylem embolisms and refilling. Relation to water potentials of soil, roots, and leaves, and osmotic potentials of root xylem sap. *Plant Physiology* **119**, 1001-1008.

Nardini A. and Pitt F. (1999) Drought resistance of *Quercus pubescens* as a function of root hydraulic conductance, xylem embolism and hydraulic architecture. *New Phytologist* **143**, 485-493.

Nobel P.S. and Cui M. (1992) Shrinkage of attached roots of *Opuntia ficus-indica* in response to lowered water potentials- predicted consequences for water uptake or loss of soil. *Annals of Botany* **70**, 485-491.

North G.B. and Nobel P.S. (1991) Changes in hydraulic conductivity and anatomy caused by drying and rewetting roots of *Agave deserti* (Agavaceae). *American Journal of Botany* **78**, 906-915.

North G.B. and Nobel P.S. (1997) Root-soil contact for the desert succulent *Agave deserti* in wet and drying soil. *New Phytologist* **13**, 21-29.

Ourcival J.M. and Berger A. (1995) Equilibrium between soil water potential and predawn water potential of two pre-saharan shrub species of Tunisia. *Journal of Arid Environments* **30**, 175-183.

Pritchard T.L. (1992) A volume balance approach to quality wine grape irrigation. In: *Viticultural practices* eds. Walker M.A. and Kliewer W.M, pp. 12-23. University of California, Davis.

Querejeta J.I., Egerton-Warburton L.M. and Allen M.F. (2003) Direct nocturnal water transfer from oaks to their mycorrhizal symbionts during severe soil drying. *Oecologia* **134**, 55-64.

Reid J.B., Winfield D., Sorensen I.B. and Kale A.J. (1996) Water deficit, root demography, and the causes of internal blackening in field-grown tomatoes (*Lycopersicon esculentum* Mill.). *Annals of Applied Biology* **129**, 137-149.

Richards J.H. and Caldwell M.M. (1987) Hydraulic lift: substantial nocturnal water transport between soil layers by *Artemisia tridentata* roots. *Oecologia* **73**, 486-489.

Salleo S., Lo Gullo M.A.and Oliver F. (1985) Hydraulic parameters measured in 1-year-old twigs of some mediterranean species with diffuse-porous wood: changes in hydraulic conductivity and their possible functional significance. *Journal of Experimental Botany* **36**, 1-11.

Smart D.R., Carlisle E., Goebel M. and Núñez B.A.(2005) Transverse hydraulic redistribution by a grapevine. *Plant, Cell and Environment* **28**, 157-166.

Smucker, A. J. M. and Aiken, R. M. (1992) Dynamic root responses to water deficits. *Soil Science* **154**, 281-289.

Sperry J.S., Stiller V. and Hacke U.G. (2002) Soil water uptake and water transport through root systems. In: Waisel Y., Eshel A. eds. *Plant Roots: The Hidden Half*. pp. 663-681 Marcel Dekker, New York.

Steudle E. (2000) Water uptake by roots: effects of water deficit. *Journal of Experimental Botany* **51**, 1531-1542.

Taleisnik E., Peyrano G., Córdoba A. and Arias C. (1999) Water retention capacity in root segments differing in the degree of exodermis development. *Annals of Botany* **83**, 19-27.

Tsuda M. and Tyree M.T. (1997) Whole-plant hydraulic resistance and vulnerability segmentation in *Acer saccharinum*. *Tree Physiology* **17**, 351-357.

Williams K., Caldwell M.M. and Richards J.H. (1993) The influence of shade and clouds on soil water potential: the buffered behavior of hydraulic lift. *Plant and Soil* **157**, 83-95.

Winkler, A.J., Cook, J.A., Kliewer, W.M. and Lider, L.A. (1974) *General Viticulture*, University of California Press.

Chapter 4

Consequences of Insect Herbivory on Grape Fine Root Systems with Different Potential Growth Rates

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Abstract

Herbivory tolerance has been linked to plant growth rate where plants with fast growth rates are hypothesized to be more tolerant of herbivory than slower growing plants. Evidence supporting this theory has been taken primarily from observations of aboveground organs but rarely from roots. Grapevines, differing in overall rates of new root production, were studied in Napa Valley, California over two growing seasons in an established vineyard infested with the sucking insect, grape phylloxera (*Daktulosphaira vitifoliae* Fitch). The experimental vineyard allowed for the comparison of two root systems that differed in rates of new root tip production (a "fast grower", *Vitis berlandieri* x *V. rupestris* cv 1103P and slower growing stock, *V. riparia* x *V. rupestris* cv 101-14 Mgt). Each root system was grafted with a genetically identical shoot system (*Vitis vinifera* cv Merlot). Using minirhizotrons, we did not observe any evidence of spatial or temporal avoidance of insect populations by root growth. Insect infestations were abundant throughout the soil profile and seasonal peaks in phylloxera populations generally closely followed peaks in new root production. Our data supported the hypothesis that insect infestation was proportional to the number of growing tips, as indicated by similar percent infestation in spite of a three-fold difference in root tip production. In addition, infested roots of the fast-growing rootstock exhibited somewhat shorter median lifespans (60 d) than the slower -growing rootstock (85 d). Lifespans of uninfested roots were similar for the two rootstocks (200 d). As a consequence of greater root mortality of younger roots, infested root populations in the fast-growing rootstock had an older age structure. While there does not seem to be a trade-off between potential growth rate and relative rate of root infestation in these cultivars, our study indicates that a fast-growing root system may more readily shed infested roots that are presumably less effective in water and nutrient uptake. Thus, differences in root tip production may be linked to differences in the way plants cope with roots that are infested by sucking insects.

Keywords: grape phylloxera**,** root survivorship, root age, *Vitis* rootstock, belowground herbivory, potential growth rate, herbivory tolerance.

Introduction

Belowground herbivory may be a major cause of root turnover in both agricultural and natural ecosystems (Brown and Gange 1989; Eissenstat et al. 2000). Root feeding by insects can cause large changes in plant productivity and plant community structure (Hunter 2001; Dunn and Frommelt 1998; Ingham and Detling 1990). Surprisingly few direct quantitative observations of the effects of root herbivory on root turnover or productivity exist from field observations. Indirect evidence comes from the use of non-selective insecticides, where an increase of from 46 to 125 days in median lifespan was observed for peach tree fine roots (Wells et al. 2002), a reduced rate of new root production was observed in pasture systems (Dawson et al. 2003), and an increase in herb species richness and reduced seedling mortality was realized in old fields (Brown and Gange 1989). Direct evidence from artificial systems indicated that maize roots grown in containers showed a 13.8% reduction in root biomass when plants were infested with 50 western corn rootworm larvae, and 49.5% when infested with 100 larvae (Dunn and Frommelt 1998).

Plant Growth Rate

Defense against herbivory has been linked to plant growth rate (Coley 1988), in the sense that Coley and coworkers (Coley et al. 1985) proposed that slow-growing plants allocate more resources for defense against herbivory than do plants with faster growth rates. Fast-growing plants, on the other hand, would be able to suffer higher levels of damage from herbivores and still maintain an amount of growth commensurate with reproductive success. Controversy still exists concerning the relationship between plant growth rate and susceptibility of plants to insect damage, and most of this research has focused on plant responses to foliar damage. These various investigations have shown that differences in plant growth rates can have both positive (Strauss and Anurag 1999; Cebrian and Duarte 1994), negative (Mutikainen et al. 2002; Hoffland 1996), and no

correlation (Messina et al. 2002, Almeida-Cortez; Shipley and Arnason 1999) with aboveground herbivory.

As an alternative to a response based solely on an evaluation of foliar biomass or photosynthetic area loss to herbivory, loss may also occur in proportion to the number of actively growing tips. Insect herbivores have been hypothesized to preferentially utilize these vigorously growing plant tissues (Price 1991). In this scenario, rapidly growing aboveground plant organs, including leaves and shoots, have a higher probability of herbivore attack in comparison to slower growing plant organs. This also supports the hypothesis that more vigorously growing plants would better tolerate herbivory damage (Fritz, Crab and Hochwender 2000; Woods 1996; Kimberling 1990; Craig, Itami and Price 1989). This would result in a higher absolute consumption of growing tips by herbivores in faster-growing plants, but a proportionally similar amount to that of slower-growing plants (Woods et al. 1996).

Temporal and Spatial Avoidance

Plants may also avoid herbivory. Galling insects, for example, need to be present when the plant organ is at its susceptible stage for successful gall formation (Weis, Walton and Crego 1988). With respect to avoidance and plant vigor, plants with higher potential growth rates may exhibit greater phenological or morphological plasticity (van Schaik, Terborgh and Wright 1993). In the present case of a woody perennial like grape, such plasticity may lead to root or shoot flushes during periods of low herbivore population densities (Mopper and Simberloff 1995; Murali and Sukumar 1993). In addition to temporal avoidance, perennial plant roots may be able to spatially avoid insect herbivory by growing into soil locations not tolerated by the insects.

Consequences of Herbivory on Root Dynamics

We are unaware of any reports that directly observed root herbivory and its consequences on root population dynamics. Our main objective was to contrast the influence of root herbivory by grape phylloxera (*Daktulosphaira vitifoliae* Fitch) on root survivorship and root population dynamics for two genetically distinct rootstocks with different rates of root tip production, but with genetically identical shoot systems. Working with the gall-forming, sucking insect, grape phylloxera, provided us with a novel system because unlike many root-feeding insects that do not produce visible changes in root morphology, phylloxera infestations cause roots to increase in diameter and curl or bend at 90° to 180° angles (Forneck, Walker and Merkt 1996; Riley 1874). These morphological changes allowed us to make brief observations of insect infestations non-destructively and at regular time intervals under field conditions. We did this by using minirhizotron technologies that have presumably minimal impact on plant-herbivore interactions. In addition, gall forming insects such as phylloxera are dependent on the growth of new meristems for survival (Abrahamson and Weis 1987) thus allowing us to directly determine the proportion of actively growing tips in a root system and numbers that are infested. By directly observing roots and their surrounding environment we were better able to determine whether new root production and phylloxera populations were located in the same locations at the same time.

We evaluated our data in a manner that allowed us to test whether it was more consistent with the potential growth rate-defense hypothesis (Coley et al. 1985) in as much as we hypothesized that vines with root systems with higher root tip production would exhibit fewer defense mechanisms compared to root systems with lower root tip production. Support for this hypothesis would be reflected in both higher absolute numbers of roots infested with phylloxera, and a higher percentage of total roots infested. Alternatively, if root infestation were simply proportional to the number of growing tips (Woods et al. 1996), then percent infestation would be similar in fast- and slower-growing root systems. Second, we were able to test the hypothesis that the faster-growing root system would be less tolerant of infestation, leading to more rapid shedding of

infested roots. Third, we were able to examine the hypothesis that the faster growing root system might exhibit greater avoidance of the insects by temporally or spatially producing roots at times or locations of limited insect activity. Lastly, we hypothesized that root herbivores could alter the root system age structure by selectively feeding on the youngest roots of the finest branching orders. In this case, such selective herbivory would cause portions of root systems with herbivory to exhibit older root age structure than portions uninfested with insects, and this might occur to a greater extent in the fast-growing cultivar. We acknowledge that the above responses are not mutually exclusive and that a perennial plant in response to belowground herbivory might employ several strategies.

Materials and Methods

Site and Study System

The experiments were conducted in an established Merlot research vineyard (*Vitis vinifera* cv. Merlot) in Oakville, CA (latitude 38.44N; longitude 122.40W). Soils were Bale (variant) gravely clay loams (fine-loamy, mixed, superactive, thermic Cumulic Ultic Haploxeroll). Climate was characterized by relatively warm, dry growing season conditions (May – Aug. average daily maximum temperature of 30.7°C; average total precipitation of 80 cm as determined from an onsite weather station, (CIMIS 2005). Vines were planted in 1995 using a spacing of 2.4 meters between rows and 2.2 meters between vines and trained to a bilateral cordon with vertical shoot positioning (VSP). The entire experimental vineyard covers about one hectare and is laid out in a completely randomized block design with three irrigation treatments in each of six blocks. Within each irrigation treatment are subplots of eighteen vines (two buffer rows with a central treatment row of 6 vines) of each of two rootstocks (1103P, *V. berlandieri* x *V. rupestris*) and (101-14 Mgt., *V. riparia* x *V. rupestris*). These rootstocks were chosen for our

experiments because of their clear differences in growth rate with 1103P known for being "highly vigorous" (Wolpert et al. 2002) and will be identified as the "fast-growing" rootstock; 101-14 Mgt, is reported to be of "moderate vigor" (Wolpert et al. 2002) and will be referred to as the "moderate-growing" rootstock. All vines were only rooted by the rootstock and exhibited no scion rooting. Rootstock 1103P confers much more vegetative growth on scions than the rootstock 101-14 Mgt, and this was true for this experiment where it produced nearly two times the shoot biomass and greater yields than scions on 101-14 Mgt. As we will show, the total number of new root tips produced is also much higher in 1103P.

The parents of the *Vitis* hybrid rootstocks are all native to North America, as is the insect herbivore, phylloxera (*Daktulosphaira vitifoliae* (Fitch). The native distribution of *V. berlandieri* is mainly Texas and northern Mexico, *V. rupestris* is mainly in the central U.S.A. as far north as Kentucky, southern Missouri and southern Kansas. *Vitis riparia* on the other hand is not only widespread in the Gulf States as far east as Florida, but also along the west coast appearing as far north as British Columbia (Winkler et al. 1974). Grape phylloxera is common throughout this range.

Vines reported in this study were irrigated at 40% of the maximum predicted vineyard evapotranspiration (ET_c). ET_c was determined from a reference evapotranspiration (ET_o) multiplied by a dimensionless crop coefficient K_c that takes into account crop leaf area, reflectance, canopy resistance and evapotranspiration from exposed soil (Hunsaker et al. 2003). The vineyard was irrigated using a micro-irrigation system with one drip emitter located about 50 cm from the trunk of each vine.

Minirhizotron Instillation and Data Collection

In April, 2002 clear cellulose acetate butyrate (CAB) root observation tubes (minirhizotrons) were installed at an angle of 30˚ from the vertical. While CAB may affect the survivorship of roots of some woody species (Withington et al. 2003), it seems improbable that this plastic would differentially affect two closely related cultivars or the

relative sensitivity of roots to phylloxera. One minirhizotron tube was placed through the drip zone and the other was placed 50 cm from the trunk on the opposite side of the vine in an area that was not irrigated. Tubes were 1.5 m in length, 6 cm in outside diameter and had a viewing area of 0.0192 m^2. The bottoms of the tubes were sealed with PVC plugs and the top of the tubes were wrapped with black electrical tape and sealed with rubber stoppers to prevent light penetration. The tops of the tubes were covered with a white aluminum can to prevent radiant heating when not in use. Beginning in June of 2002, a specially designed digital imaging camera (BTC-2, Bartz Technology, Santa Barbara, CA) was used to observe roots every two weeks during the growing season and typically every month after leaf fall and before bud break. A sub-population of roots was sampled at a shorter interval of every three hours for five days to examine short-term changes in root diameter and nodule development. The images were captured directly to a computer using software designed by Bartz Technology (ICAP v.4.1; Bartz Technology, Santa Barbara, CA). All images were analyzed using Win Rhizo Tron MF software (Regents Inc. Quebec, Canada). In addition to monitoring the date of root birth, lifespan, root diameter, root order, number of 1st-order lateral roots (roots with no laterals), number of neighboring roots present at birth and death events of an individual root (index of competition), the date of insect infestation was recorded. Root births were estimated by calculating the date midway between the observation date when a root was first observed and the previous observation date. Similarly, root death was estimated as being midway between the date the root was first observed dead and the previous observation date. Root death was identified by a black and shriveled appearance (Comas, Eissenstat and Lakso 2000) or if the root had disappeared from the window and did not re-appear. Roots that transected more than one minirhizotron observation window vertically within the same minirhizotron observation tube were only counted once. Phylloxera-infested roots were identified as those exhibiting a typical bulbous swelling and with an associated phylloxera population immediately adjacent to the root.

Environmental data collected, including daily maximum air temperature and precipitation, were downloaded daily from a weather station located on site (CIMIS 2005). Volumetric soil water content was estimated approximately every two weeks

using time domain reflectrometry. The minirhizotrons were used as an access tube for soil moisture determination at three depths, 0-30, 30-60 and 60-90 cm, intervals that corresponded with highest root densities, using a Trime soil moisture probe (Mesa Systems Co., Medfield, MA).

Statistical Procedures

Total root production was normalized using a log transformation and analyzed using ANOVA (SPSS Inc. v. 11.0, Chicago, IL). Block and year were not significant and thus removed from the model. Root lifespan data were analyzed with the Cox proportional hazards regression (PROC PHREG; SAS Institute Inc., Cary, NC, USA). This type of analysis allows the influence of all other covariates to be held constant while the "hazard" of an individual covariate is determined (Cox 1972). The "hazard" of a covariate refers to the risk of mortality of a root at any point in time (Allison 1995).

Statistical Analysis System's PROC PHREG (SAS Institute Inc., Cary, NC, USA) uses the partial likelihood method (Cox 1972) to estimate a parameter coefficient of β for each tested covariate, and calculates a chi-square statistic to test the null hypothesis that each β equals zero. A parameter estimate can have either a negative or positive sign depending on the effect it has on the covariate. In this case, a negative sign indicates a decreased hazard of mortality with an increase in the covariate (Wells and Eissenstat 2001). Covariates tested included root diameter, number of daughter roots and the number of neighbor roots present in the window. Effects of year of observation and depth of roots on root population size were analyzed using the GLM procedure (SPSS Inc. v. 11.0, Chicago, IL). Z-tests were used to determine differences and significance values in cumulative grape root mortality between the two rootstocks. Root age-class differences were examined by comparing age distributions of roots in observation windows containing infested roots with adjacent windows containing no infested roots. Age class differences were analyzed using PROC FREQ (SAS Institute Inc., Cary, NC, USA).

Results

The years 2002 and 2003 were typical of weather patterns for Napa Valley, California with cool wet winters and warm summers with no rainfall (Figure **4.1**). Soil moisture generally increased with the soil depth, with 2003 being moister at all three

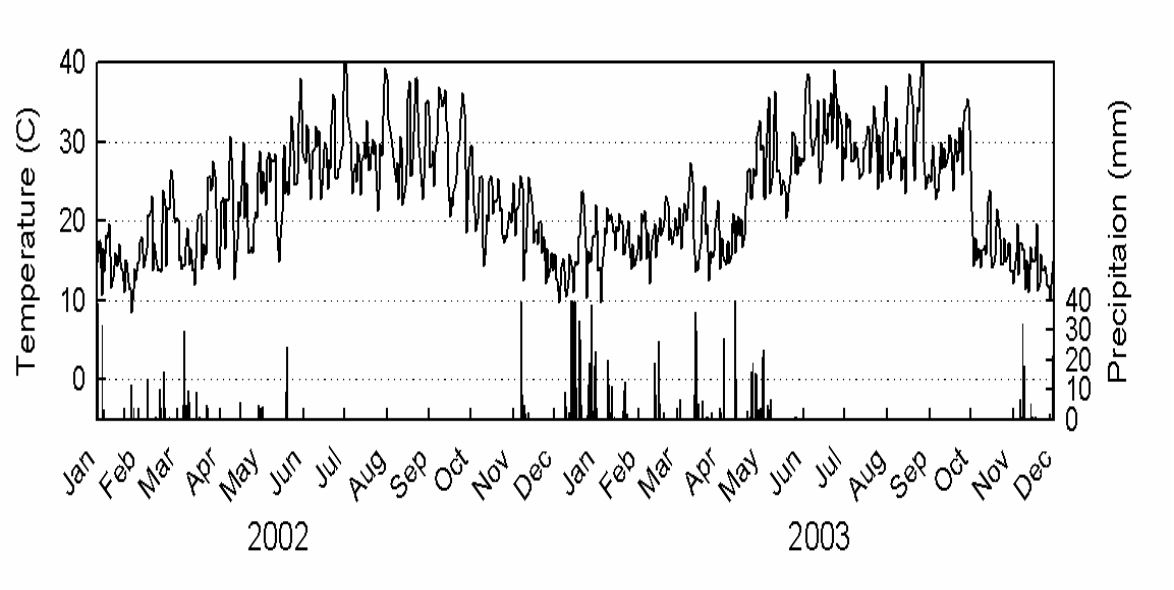

Figure **4.1**: Daily maximum temperatures (line) and precipitation (bars) for Oakville, CA in 2002 and 2003. Total rainfall in 2002 was 94.5 cm and in 2003 was 91.

depths due to greater precipitation amounts in late spring (Table **4.1**; Figure **4.1**). Percent soil moisture only decreased by about 5% of volumetric content over the summer season at all three depths measured, which probably reflect the high clay content of soils at the site (18-36% clay) and its high impedance to root growth as its dries (Table **4.1**) .

Table **4.1**: Soil moisture (%) averaged over both sides of the vine during July, 2002 and July, 2003 at three depths (± 1 SE).

Depth	2002		2003	
	1103P	101-14 Mgt	1103P	101-14 Mgt
0-30cm	25.2 (± 2.0)	22.8 (±2.3)	26.4 (±1.7)	24.3 (±1.4)
30-60cm	26.5 (±1.9)	23.8 (±1.6)	28.7 (±1.6)	26.5 (±1.7)
60-90cm	29.4 (±1.8)	30.2 (±2.2)	30.9 (±1.2)	31.4 (±1.7)

Root Production

The fast-growing root system (1103P) produced three- to four-fold more roots than the slower-growing root system (101-14 Mgt) (P=0.03; Figure **4.2**), and differences in root production between the two root systems were similar in 2002 and 2003. Consistent with the hypothesis that herbivory is proportional to the number of growing tips, we observed a similar percentage of roots infested with phylloxera in the two root systems (P= 0.202). Thus, the absolute number of roots infested was higher in the fast-growing root system (P= 0.004) but the relative number was similar to that in the moderate growing root system in both years of the study.

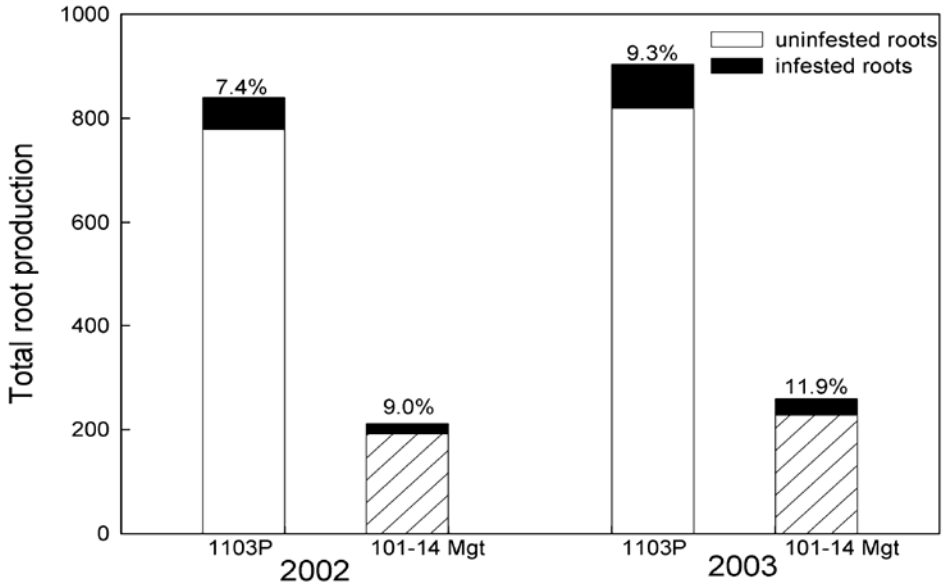

Figure **4.2**: Total annual root production for both sides of the vine (number of roots m^{-2} of viewing surface) for years 2002 and 2003. Shown by the white bars and hatched bars are uninfested roots, of a fast-growing (1103P) and moderate-growing (101-14 Mgt) rootstock. The solid black bars show the number of infested roots. Differences in total root production between the two rootstock cultivars for the two years were significant (*P*= 0.03). Percentages above the bars indicate the percent frequency of infested roots (cultivar effect: *P*= 0.202).

Populations of roots infested with phylloxera reached peaks in mid summer and generally overlapped with periods of peak root production (Figure **4.3**). Peaks in insect activity also corresponded with peaks in seasonal temperature and an extended period of time without rainfall but with irrigation (Figure **4.1**). Thus, there was little evidence for temporal avoidance in root production of either root system in relation to timing of peak insect infestations. There was also little evidence that roots at particular locations in the soil escaped insect infestations. Percent of infested roots by depth was distributed differently for the two rootstock cultivars (rootstock x depth interaction: *P*=0.0251, Figure **4.3**). The majority of infested roots were located in the 60-90 cm depth (56%) for the fast grower while the moderate-growing root system had equal distribution of infested roots at 30-60cm (39%) and 60-90cm (41%) depths. For both root systems, surface roots

(0-30cm) had the lowest percentages of infested roots with 12.5% for the fast growing rootstock and 19.5% for the moderate growing rootstock. We did not observe root diameter (P=0.62) or number of neighboring roots (P=0.21) to affect a root's susceptibility to grape phylloxera infestation in either year.

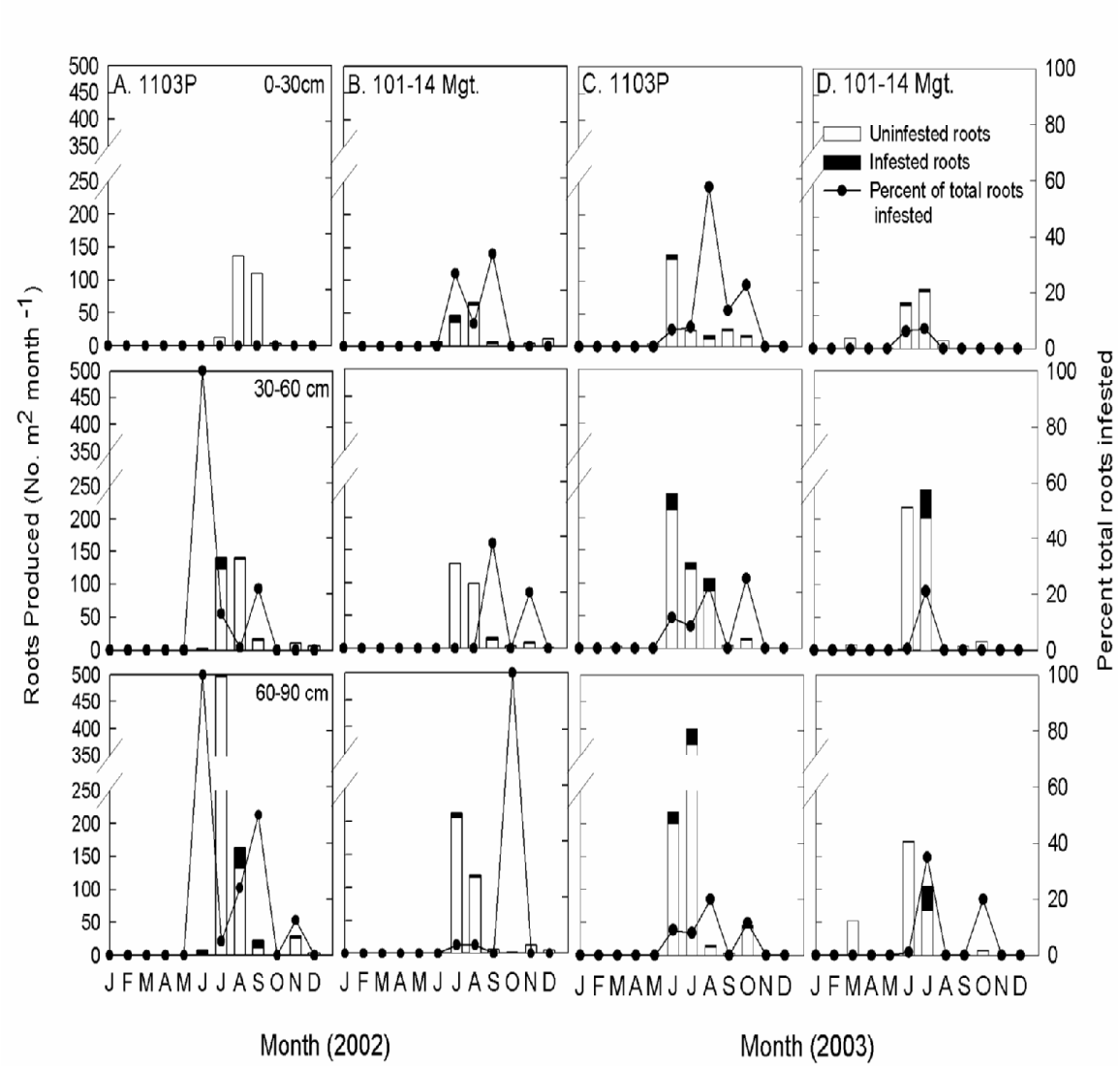

Figure **4.3**: Monthly patterns of root production and percent infestation on the irrigated side of the vine for the fast-growing root system, 1103P (A. and C.) and the moderate-growing root system, 101-14 Mgt (B. and D.) for 2002 (A. and B.) and 2003 (C. and D.) at 0-30, 30-60, and 60-90cm depth intervals (upper, middle and bottom row of plots, respectively). Shown by the histogram bars is monthly new root production by depth interval of uninfested roots (unshaded) and infested roots (shaded) (mean of 6 plots with 1 tube per plot). Total monthly root production is indicated by the sum of the two bars.

Data are expressed per square meter of viewing surface of minirhizotron (total viewing surface per plot over a 30-cm vertical depth = 63 cm^2). The line graph represents the monthly percentage of total new roots that were infested over the year.

Percentages of root mortality associated with infested roots were highest immediately following tube installation, but declined to more constant values shortly afterward (Figure **4.4**). By October of the first year of the study, approximately twice the percentage of total roots associated with phylloxera infestation died at each sampling date for the more vigorous root system, (1103P), compared to that of the less vigorous root system (101-14 Mgt). Statistically significant differences were detectable between rootstocks for nearly all sampling dates ($P < 0.05$).

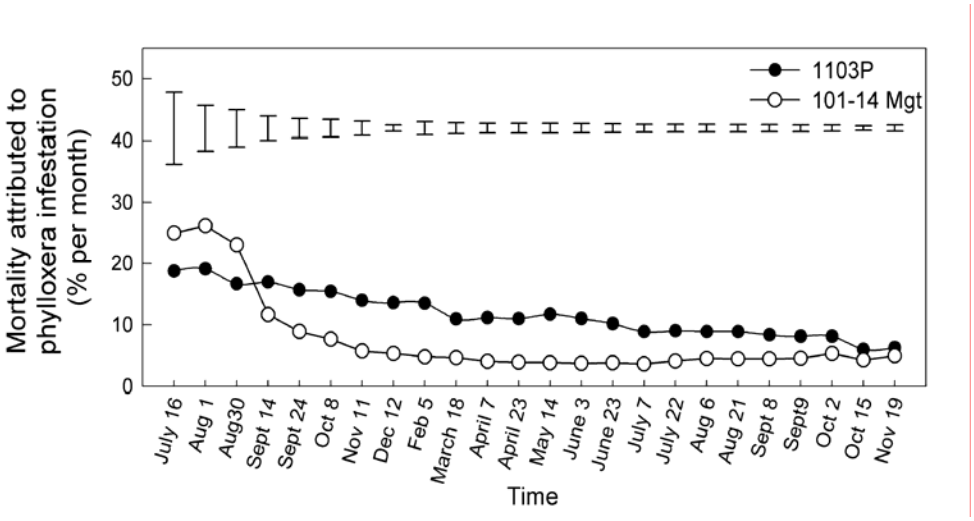

Figure **4.4**: Percentage of total grape root mortality observed from the beginning of the experiment (June, 2002 until November 2003) the date indicated that was attributable to phylloxera for a rootstock with fast growth (1103P) and moderate growth (101-14 Mgt) Thus, the data reflect the cumulative infested root mortality divided by the cumulative total root mortality over the course of the experiment. Observations were combined for minirhizotron observation tubes located on the irrigated and the non-irrigated sides of the vine. Vertical bars represent pooled standard errors. Significant differences observed between rootstocks are indicated by an asterisk ($P < 0.05$).

Root Mortality

Cumulative root mortality observed from the beginning of the experiment to month reported ranged from 18.8% to 6.0% and 25.0% to 3.6% for the fast-growing root system and moderate-growing root system, respectively. A two-fold increase in root mortality attributable to phylloxera infestation initially occurred for roots on the irrigated side of the vine. Too few roots were born on the non-irrigated side (dry soils) that were visible through the minirhizotron windows to permit a direct statistical comparison between the irrigated and non-irrigated roots. Nonetheless, the observed mortality patterns for non-irrigated versus irrigated roots were nearly identical to those shown in Figure **4.4**.

Root Survivorship

Root survivorship of uninfested roots was similar for the root systems of both rootstocks ($P = 0.991$) but infested roots died more quickly in the fast-growing rootstock, 1103P ($P= 0.022$, Figure **4.5**). According to hazard ratios, roots deeper in the soil had 14% and larger diameter roots had 37% lower risks of mortality ($P=0.001$). The more roots present at the time of birth decreased risks of individual root mortality by 3% ($P < 0.001$) while the opposite was true for the number of roots present at time of death by 5% ($P < 0.001$).

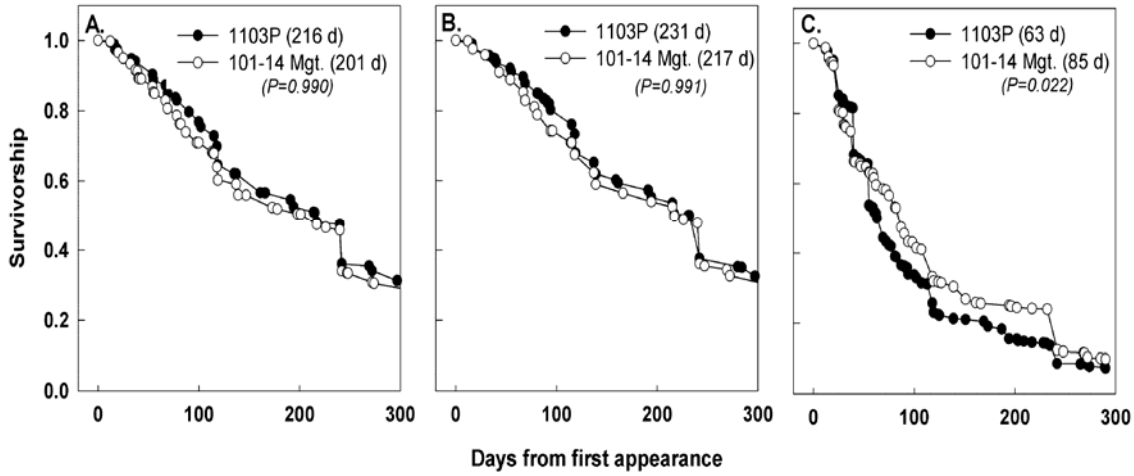

Figure **4.5**: Root survivorship in a vineyard in Oakville, California for A. total, B. uninfested, and C. phylloxera-infested roots of a rootstock with high- (1103P) and moderate-growth (101-14 Mgt) rates. Median lifespans are indicated in parentheses. Data are for all roots observed through minirhizotron windows located in both irrigated plus non-irrigated soils in 2002 and 2003. The rootstock of moderate growth rate, which produced fewer roots, had significantly higher root survivorship (P= 0.022). Differences in survivorship between uninfested (Fig 4.5B) and infested (Fig 4.5C) roots were significant for both root systems (P<0.001).

Infestation Rates

Roots that became visibly infested with phylloxera usually did so within two weeks following their birth for both root systems (7.5 ± 1.5 SE days). Once infested, root survivorship of phylloxera-infested roots was considerably lower than that of uninfested roots (contrast Figure 4. 5B with 4.5C; P<0.001).

Regardless of the population size of the infestation, if a fine lateral root became infested with phylloxera, and a nodosity was formed, the median lifespan was about 60 and 85 days on the high- and moderate-growing rootstocks, respectively. Uninfested roots, on the other hand, were not significantly different between the two rootstocks (P=0.991) and had a lifespan of greater than 200 days (Figure 4.5).

Root Age Structure

Infested and uninfested roots in June, 2003 displayed a bi-modal age structure with the majority of roots falling into either a "young" age class (<100 days) or an "old" age class (>300 days, Figure **4.6**). For the entire root population, similar age structures were observed for both rootstocks (Figure **4.6 A**); however, for the fraction of the population infested with phylloxera, the fast-growing root system exhibited an older root age structure than that of the moderate-growing root system (Figure **4.6 C**; $P<0.001$).

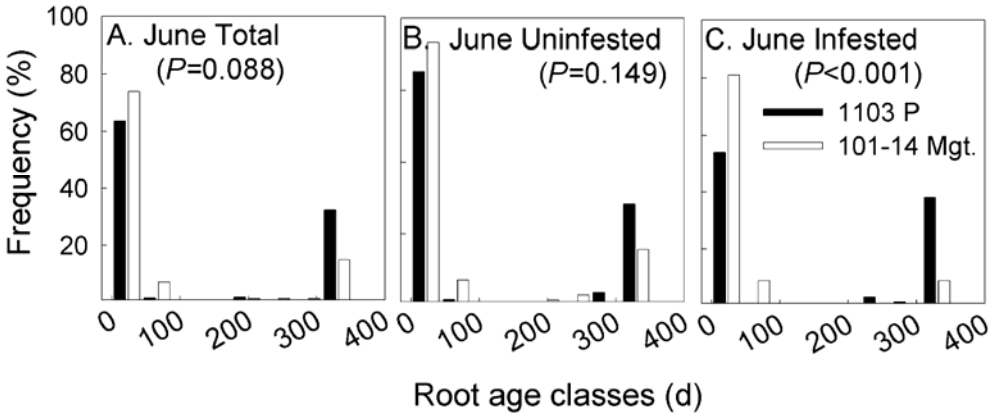

Figure **4.6**: Root age structure of A. total, B. uninfested, and C. infested fine roots over the growing season in 2003 for the rootstock of high (1103P; shaded) and moderate (101-14 Mgt; (unshaded) rates of root production. Age structure is indicated by the relative frequency of roots in a given age class.

Among roots less than 100 d old, no significant age class differences were observed between rootstocks. Consequently, the data were combined for the two rootstocks to evaluate the effects of phylloxera infestation on root age structure of this "younger" root population (Figure **4.7**). The age structures of infested and uninfested roots were similar in the 100d age class during June and July; however, significant differences in root age structure developed in August ($P < 0.001$) and September ($P < 0.002$). Populations of

uninfested roots alive in August and September had an older age class distribution than infested roots, although the majority of roots were still younger than 60 days.

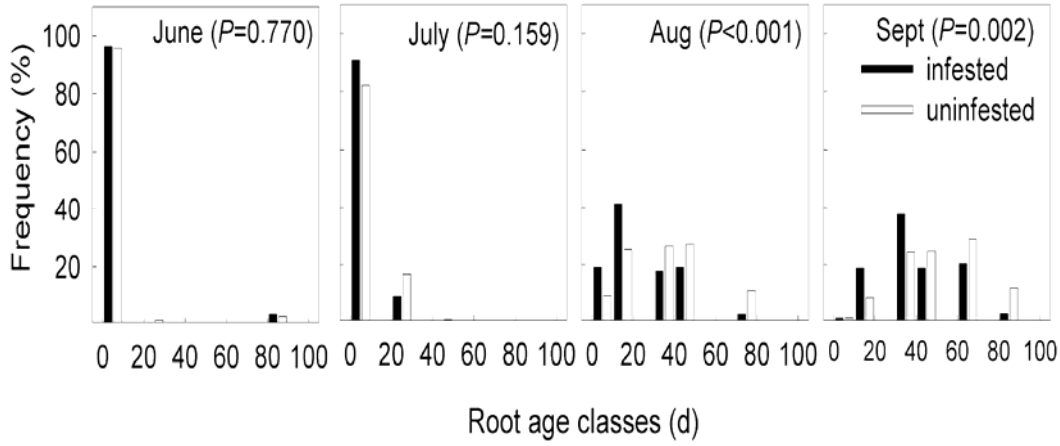

Figure **4.7**: Root age structure of roots less than 100 days old from June through September of 2003 for both rootstocks combined. Age classes are in 10-d intervals (e.g., the large June age class represents infested and uninfested roots that are 0 to 10 d old. . The 80-90 day-old age class in June represent a very small fraction of roots born in the early spring of 2003. The probability of a significant difference in age structure between infested and uninfested roots is indicated in parentheses.

Discussion

Trade-offs between plant growth rate and herbivory defense have been proposed for foliar herbivory (Cebrian and Duarte 1994; Coley 1988) but this is the first study to our knowledge to report on the relationship between growth rate of root systems and root herbivory tolerance. In grape, we found that root systems with a three-fold difference in root tip production (Figure 4.2) exhibited similar proportional rates of infestation by phylloxera, an indication of similar defenses to the insect. However, roots of the faster-growing root system that became infested with phylloxera had shorter lifespans than

those of the moderate-growing root system (Figure 4.5), suggesting differences in how woody perennial plants and resistant rootstocks of different growth potential might tolerate a root-feeding insect.

According to the plant vigor hypothesis established from aboveground studies, insect infestation level may be related to the number of young growing tissues (Woods 1996). The two root systems we examined had similar percent infestation of the finest roots. The root system of the cultivar that produced many more root tips (1103P, Figure 4.2) had more infested roots than that of the cultivar with the slower-growing root system (101-14 Mgt, Figure 4.3) but the proportion infested was similar. Therefore, the number of meristematic tips, and perhaps the insect's ability to find them, appears to be the driving factor in herbivory damage by the galling insect in this system.

Root lifespan of infested roots of the faster-growing cultivar, on the other hand, was shorter than infested roots of the slower growing rootstock (Figure 4.5). This led to higher total root mortality attributed to phylloxera in the faster-growing root system after the initial root establishment phase (Figure 4.4). Thus, fine roots of the grape rootstock of higher apparent growth rate seemed less tolerant of infection by a sucking insect like phylloxera.

Differences in root longevity between the rootstock also have important implications for the insect. The greater longevity of infested roots in the slower-growing rootstock would allow for longer lifespans of the phylloxera, possibly leading to greater egg production and subsequently higher insect populations.

The high rates of root mortality in the beginning of the study (Figure 4.4) may have been a consequence of the large new root flush following the disturbance of the root system caused by minirhizotron installation (Smart et al. 2005). It is possible that insect populations increased as a consequence of increased carbohydrate availability during these root flushes or the high concentration of very young, susceptible new root meristems. Similar root flushes can be caused by nematode attack in natural systems or root pruning in agricultural systems (Murray and Clements 1998). Nonetheless, during 2003 the percentage of roots succumbing to insect infestation appeared to be stabilizing in both root systems with fine root mortality attributable to the insects at only about 5-

10% (Figure 4.4). As a consequence of shorter root lifespans, insect-related root mortality in the faster-growing rootstock was twice that of the moderate-grower in spite of the similar percentage of infestation.

Laboratory studies have indicated that phylloxera feeding on nodosities of *V. vinifera* roots is rarely tolerated and results in root death (Kellow, Sedgley and Van Heeswijck 2004). Our field study indicated that the rootstock that produced fewer roots (101-14 Mgt) had a lower risk of root mortality caused by phylloxera infestation as compared with the rootstock that produced more roots (1103P). In both cases, infested roots sustained relatively long median lifespans (63-85 d; Figure 4.5) similar to the (90 days) found under optimal laboratory study conditions (Forneck,Walker and Merkt 1996).

Previous research has suggested several causes for decreased lifespan of phylloxera-infested roots. One theory suggests that the phylloxera insect may serve as a vector for introduction of fungal or bacterial pathogens (Omer and Granett 2000; Granett et al. 1998). Fungal opportunists may enter the root upon damage by grape phylloxera probing and be the ultimate cause of root death. Root swelling may also be linked to the main cause for the increase risk of mortality in roots (Kellow,Sedgley and Van Heeswijck 2004). However, we observed little change in root diameter of insect-infested roots after initial swelling for either root system.

Unlike aboveground plant structures, roots reside in an often extremely heterogeneous environment in regards to oxygen, moisture, temperature and physical impedance, all of which may influence root–insect interactions. Nonetheless, we did not see any evidence of temporal or spatial avoidance of insect herbivory by the root systems. Root growth was primarily concentrated at depths of 30-90 cm which was also the location where phylloxera populations were most abundant and where temperature and moistures conditions should be more stable. Both root systems had readily visible insect populations at times of high root abundance and regardless of soil depth. Because the insect does not apparently cause high rates of root mortality in germplasm where the vine and insect coevolved, there may have been little selection pressure for growth mechanisms that would allow for avoidance of the insect.

Root system function is tightly coupled to root age (Volder et al. 2005). In woody perennial plants where root systems undergo seasonal flushes of new root cohorts, the age structure of the finest roots is dynamic (Wells and Eissenstat 2003). High levels of nutrient uptake potential and metabolic activity are associated with very young roots (Volder et al. 2005; Comas, et al. 2000). Thus, selective mortality of the youngest roots that alters a root systems age structure towards an older age distribution may diminish the ability of roots to acquire water and nutrients. The higher mortality rate of infested roots for the cultivar that produced more new roots led to a somewhat older infested root population than that of the moderate-growing root system (Figure 4.6C). Because infested roots represented only a small fraction of the total root population, the age structure of the total root population (infested plus non-infested roots) was similar for the two root systems (Figure 4.6A). Nonetheless, insects infested much larger percentages of newly formed roots for both root systems (Figure 4.7). This resulted in proportionally fewer roots reaching older age classes in root populations infested with insects as compared with uninfested roots. Infestation in August, for example, caused relatively fewer roots to reach ages older than 40 days, and resulted in a greater percentage of roots in the 10 and 20 d old category (Figure 4.7). Because roots older than 40 d are not likely to show high metabolic activity and nutrient uptake capacity (Volder et al. 2005; Comas, et al. 2000), this effect on root age structure was not likely to have a large impact on nutrient and water acquisition of the overall plant. Thus, a shift to an older average age structure of roots over 40 d old may not necessarily result in large decreases in overall performance of the root system.

Summary

In summary, we found that grape phylloxera infested rootstocks with different growth rates (new root production) in proportion to the number of growing tips. The rootstock with inherently more new root production was also observed to be less tolerant of insect feeding at the local level as indicated by more rapid mortality of infested roots. We

observed no evidence of temporal or spatial avoidance of the insect in either root system cultivar.

The overall impact of phylloxera on these root systems and aboveground production is still in question. While we observed appreciable reductions in root survivorship of infested compared to uninfested root populations, the cumulative loss of only 4-6% of the total roots attributable to insect infestation (Figure 4) suggests only a small decrease in root biomass. Moreover, it is unlikely that a reduction of "middle age" classes of roots (40 to 60 d old) in infested root populations substantially affected the plant's ability to take up water and nutrients (Comas et al. 2000; Volder et al. 2005). Not known is the amount of carbon loss caused by direct feeding of the insects or in gall formation and maintenance, which might be lower in the faster-growing rootstock cultivar if it sheds phylloxera-infested roots more rapidly.

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References

Abrahamson, W.G., and Weis, A.E. (1987) Nutritional ecology of arthropod gall-makers. In: F. Slansky and G. Rodriguez (eds.) *Nutritional Ecology of Insects, Mites and Spiders.* Pp.235-258. New York: Wiley.

Allison, P.D. (1995) Survival analysis using the SAS system: a practical guide. SAS Institute, Cary, North Carolina, USA.

Almeida-Cortez, J.S., Shipley, B. andArnason, J.T. (1999) Do plant species with high relative growth rates have poorer chemical defenses? *Functional Ecology* 13, 819-827.

Brown, V.K. and Gange, A.C. (1989) Differential effects of above- and below-ground insect herbivory during early plant succession. *Oikos* 54, 67-76.

Cebrian, J. and Duarte, C.M. (1994) The Dependence of herbivory on growth rate in natural plant communities. *Functional Ecology* 8, 518-525.

Coley, P.D., Bryant, J.P. and Chapin F.S. III (1985) Resource availability and plant antiherbivore defense. *Science* 230, 895-899.

Coley, P.D. (1988) Effects of plant growth rate and leaf lifetime on the amount and type of anti-herbivore defense. *Oecologia* 74, 531-536.

Comas, L.H., Eissenstat, D.M. and Lakso, A.N. (2000) Assessing root death and root system dynamics in a study of grape canopy pruning. *New Phytologist* 147, 171-178.

Cox, D. (1972) Regression models and life tables. *Journal of the Royal Statistical Society* 34,187-220.

Craig, T.P., Itami, J.K., and Price, P.W. (1989) A strong relationship between oviposition preference and larval performance in a shoot-galling sawfly. *Ecology* 70, 1691-1699.

Dawson, L.A., Grayston, S.J., Murray, P.J., Cook, R., Gange, A.C., Ross, J.M., Pratt, S.M., Duff, E.I., and Treonis, A. (2003) Influence of pasture management (nitrogen and lime addition and insecticide treatment) on soil organisms and pasture root system dynamics in the field. *Plant and Soil* 255, 121-130.

Dunn, J.P. and Frommelt, K. (1998) Effects of below-ground herbivory by *Diabrotica virgifera virgifera (Coleoptera)* on biomass allocation and carbohydrate storage of maize. *Applied Soil Ecology* 7, 213-218.

Eissenstat, D.M., Wells, C.E., Yanai, R.D., and Whitbeck, J.L. (2000) Building roots in a changing environment: implications for root longevity. *New Phytologist* 147, 33-42.

Forneck, A., Walker, M.A. and Merkt, N. (1996) Aseptic dual culture of grape (*Vitis* spp.) and grape phylloxera (*Daktulosphaira vitifoliae* Fitch). *Vitis* 35, 95-97.

Fritz, R.S., Crabb, B.A., and Hochwender, C.G. (2000) Preference and performance of a gall-inducing sawfly: a test of the plant vigor hypothesis. *Oikos* 89, 555-563.

Granett, J., Omer, A.D., Pessereau, P. and Walker M.A. (1998) Fungal infections of grapevine roots in grape phylloxera-infested vineyards. *Vitis* 39-42.

Hoffland, E., Neimann, G.J., Van Pelt, J.A., Prueveen, J.B.M., Eijkel, G.B., Boon, J.J. andLambers H. (1996) Relative growth rate correlates negatively with pathogen resistance in radish: the role of plant chemistry. *Plant, Cell and Environment* 19, 1281-1290.

Hunsaker, D.J., Pinter, P.J.,Jr., Barnes, E.M. and Kimball, B.A. (2003) Estimating cotton evapotranspiration crop coefficients with multispectral vegetation index. *Irrigation Science* 22, 95-104.

Hunter, M.D. (2001) Out of sight, out of mind: the impacts of root-feeding insects in natural and managed systems. *Agricultural and Forest Entomology* 3, 3-9.

Ingham, R.E. and Detling, J.K. (1990) Effects of root-feeding nematodes on above-ground net primary production in North American grassland. *Plant and Soil* 121, 279-281.

Kellow, A.V., Sedgley, M. and Van Heeswijck, R. (2004) Interaction between *Vitis vinifera* and grape phylloxera: changes in root tissue during nodosity formation. *Annals of Botany* 93, 581-590.

Kimberling, D.N., Scott, E.R. and Price, P.W. (1990) Testing a new hypothesis: plant vigor and phylloxera distribution on wild grape in Arizona. *Oecologia* 84, 1-8.

Messina, F.J., Durham, S.L., Richards, J.H. and McArthur, E.D. (2002) Trade-off between growth and defense? A comparison of sagebrush populations. *Oecologia* 131, 43-51.

Mopper, S. and Simberloff, D. (1995) Differential herbivory in an oak population: the role of plant phenology and insect performance. *Ecology* 76, 1233-1241.

Murali, K.S. and Sukumar, R. (1993) Leaf flushing phenology and herbivory in a tropical dry deciduous forest, southern India. *Oecologia* 94, 114-119.

Murray, P.J. and Clements, R.O. 1998 Transfer of nitrogen between clover and wheat: effect of root herbivory. *European Journal of Soil Biology* 34, 25-30.

Mutikainen, P., Walls, M. Ovaska, J. Keinänen, M., Julkunen-Tiitto,R, and Vapaavuori E. (2002) Costs of herbivore resistance in clonal saplings of *Betula pendula*. *Oecologia* 133, 364-371.

Omer, A.D. and Granett, J. (2000) Relationship between grape phylloxera and fungal infections in grapevine roots. *Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz* 107, 285-294.

Price, P.W. (1991) The plant vigor hypothesis and herbivore attack. *Oikos* 62, 244-251.

Riley, C.V. (1874) The grape phylloxera *Phylloxera vastatrix* Planchon. In: Regan and Carter (eds).*6th Annu. Rep. Noxious, beneficial, and other insects of the state of Missouri*, pp.30-65. 9th Annu. Rep. State Board Agric. State of Missouri. Jefferson City, MO:

Smart, D. R., Bauerle, T.L., Goebel, M., Carlisle, E. and Stockert, C. (2005) Artifacts of grapevine root proliferation following installation of minirhizotron observation tubes. *Acta Horticulturae.*

Strauss, S.Y. and Agrawal, A.A. (1999) The ecology and evolution of plant tolerance to herbivory. *Trends in Ecology and Evolution* 14, 179-185.

Van Schaik, C.P., Terborgh, J.W. and Wright, S.J. (1993) The phenology of tropical forests: adaptive significance and consequences for primary consumers. *Annual Review of Ecology and Systematics* 24, 353-377.

Volder, A., Smart, D.R., Bloom, A.J. and Eissenstat, D.M. (2005) Rapid decline in nitrate uptake and respiration with age in fine lateral roots of grape: implications for root efficiency and competitive effectiveness. *New Phytol*ogist 165, 493-502.

Weis, A.E., Walton, R. and Crego, C.L. (1988) Reactive plant tissue sites and the population biology of gall makers. *Annual Review of Entomology* 33, 467-486.

Wells, C.E. and Eissenstat, D.M. (2001) Marked differences in survivorship among apple roots of different diameters. *Ecology* 82, 882-892.

Wells, C.E., Glenn, D.M. and Eissenstat, D.M. (2002) Soil insects alter fine root demography in peach (*Prunus persica*). *Plant, Cell and Environment* 25, 431-439.

Wells, C.E. and Eissenstat, D.M. (2003) Beyond the roots of young seedlings: the influence of age and order on fine root physiology. *Journal of Plant Growth Regulators* 21, 324-334.

Winkler, A.J., Cook, J.A., Kliewer, W.M., and Lider, L.A. (1974) *General Viticulture*, University of California Press.

Withington, J.M., Elkin, A.D., Bulaj, B., Olesiński, J., Tracy K.N., Bouma T.J., Oleksyn J., Anderson L.J., Modrzyński J., Reich P.B., and Eissenstat D.M. (2003) The impact of material used for minirhizotron tubes for root research. *New Phytologist* 160, 533-544.

Wolpert, J., Walker, M.A., Weber, E., Bettiga, L., Smith, R., Verdegaal, P., McGourty, G., and Ingels, C. (2002) Field evaluation of winegrape rootstocks. Napa, CA:

American Vineyard Foundation, California Rootstock Commission, Viticulture Consortium.

Woods, J.O., Carr, T.G., Price, P.W., Stevens, L.E. and Cobb, N.S. (1996) Growth of coyote willow and the attack and survival of a mid-rib galling sawfly, *Euura* sp. *Oecologia* 108, 714-722.

Chapter 5

Synthesis

Using a system where genetically identical shoots were grafted to genetically diverse root systems, we demonstrated that fast- and slow-growing root systems may respond differently to low resource availability such as water, making it difficult to accurately determine root to shoot proliferation strategies. Indeed the response differences we found in two root systems that differ in their potential growth rate offers support for our original hypothesis that root production and partitioning between wet and dry soil are specific responses to localized soil moisture. We were unable to find any differences in tolerance of dry soil between the two root systems as demonstrated by the similarity in root lifespan between both root systems. Overall not only do our results contribute to our understanding of the diverse root foraging responses to heterogeneous soil moisture that may occur in ecological communities but adds to our understanding of the theories behind the potential growth rate of plants (Grime 1977). Moreover, our results can also be applied to our understanding of cultivar behavior in horticultural based systems. Future cultivar breeding programs need to consider root system growth rate in conjunction with the available resources located in the planting environment.

Based on our root dynamics results we recognized the potential importance of hydraulic redistribution on root survival in dry soil. While the ideas that roots in wet soil support those in dry soil seems somewhat intuitive we were the first to demonstrate the importance of internal movement of water on root survivorship, and viability. By placing plants under 24-hrs of illumination we prevented internal hydraulic redistribution and thus prevented roots in dry soil from rehydrating at night which resulted in reduced root turgor, increased electrolyte leakage, rapidly declining root water potentials and ultimately rapid death of roots in the dry soil.

We were also able to show that plant growth potential plays a role in total phylloxera infestation on grapevine roots where the greater the number of growing

meristems supporting a larger population of insects. Trade-offs between plant growth rate and herbivory defense have been proposed for foliar herbivory (Cebrian and Duarte 1994; Coley 1988). We supported the plant growth rate hypothesis by demonstrating faster-growing plants were less tolerant to herbivory than slower-growers as indicated by more rapid root mortality of infested roots. However, avoidance was not evident in either root system. Insects infested high percentages of newly formed roots for both root systems. This resulted in proportionally fewer roots reaching older age classes in root populations infested with insects as compared with uninfested roots.

Practical implications

Ultimately this research might influence future ways we think about wine grape irrigation practices. Text book examples claim two peaks in root production, one in the spring and a second flush in the fall after harvest (Williams and Mathews 1990). To our knowledge many vineyard managers base their irrigation and fertigation schedules on this bi-modal root growth assumption. However, this widely accepted view in timing of root growth may need to be revisited in that we found high yearly variability in relative root production in both root systems (Figure **C.1**; Figure **C.2** ; Figure **C.3;** Figure **C.4;** Figure **C.5**). Root flushes were observed most often during the warmest portion of the growing season (May-July) regardless of irrigation percentage or seasonal variation, no relationship was detected between root production and localized water stress. We found a similar observation was recently reported in the tree species *Fagus sylvatica* under drought stress conditions (Mainiero and Kazda, 2006).

Future research

Root physiology

There are many possible avenues for possible future research. Perhaps the area of most need lies in the coupling of root production, growth and mortality responses to abiotic stressors with corresponding root physiological changes. Physiological and structural changes in root tissues influence the ability of roots to take up and transport water during episodes of drought. While root anatomy can influence water flow in the root, the physiological processes and the plasticity of those processes can greatly affect individual cellular responses to stress. Root physiological adjustment to water stress is an intricate process; and an interesting adjustment in root physiological function can occur during periods of soil moisture depletion when it is unlikely that the entire root system experiences equal levels of drought stress. On a whole plant level, spatial variation in water availability at the root level influences transpiration and stomatal closure. The ability of roots to take up water and thus support plant growth and function is influenced by these physiological responses to drought stress. One interesting avenue concerns the importance of xylem anatomy on water flow. Often dependent on the species, xylem anatomy has been associated with the high axial water conducting efficiency of the roots (Sperry et al. 1994). Studies have shown that at least in maize, it can be difficult to link a specific developmental stage to ultimate transport efficiency (Mistríková and Kozinka 1989). Long xylem conduits and therefore fewer pit membranes are linked with efficiency in water flow (Pate et al. 1995). Few studies are available in this area but some evidence suggests that roots of greater depths (2 meters) have larger and longer xylem conduits (Pate et al. 1995). Sampling roots of known age and order for physiological and structural changes in response to water deficit will further our understanding of how root systems function in response to heterogeneous soil moisture.

Hydraulic redistribution and root cavitation

Greater differences in water potential within the root system suggest greater axial resistance. An explanation for this resistance is xylem cavitation. If a substantial drop in root water potential occurs, then this should be an indication of water cavitation in the xylem of root orders less distal from the main framework roots. During periods of water stress embolisms can render some roots inoperable. Determining the water potential threshold at which xylem embolisms are refilled is an interesting topic for future exploration. By examining two root systems that differ in their response to water stress we can provide further evidence of the mechanisms that control root lifespan. Roots provide the best place to examine cavitation events since they represent the lowest threshold tensions within a plant (McCully, Huang, and Ling 1997).

A closer look at PRD

Changes in the water potential surrounding a growing root influence the relative water content and turgor of that root, which subsequently influences ABA production and transport to the shoot (Zhang and Davies 1987). In grape, attempts were made to differentiate chemical signals within a root system by using PRD (partial rootzone drying) techniques (Stoll, Loveys, and Dry 2000). A 10-14 day cycle resulted in a 10-fold increase in ABA content of the roots in dry soil in the field. Under well-watered conditions, root cells maintain low levels of ABA (Masia et al. 1994). The information we present here on prolonged root viability in dry soil by hydraulic redistribution provides reason to revisit some previous research on PRD. Measurements of root water potential coupled to root ABA production would provide an interesting avenue of future research and allow us to better determine the most efficient ways to irrigate grape vines in dry climates.

Appendix A

Stomatal conductance

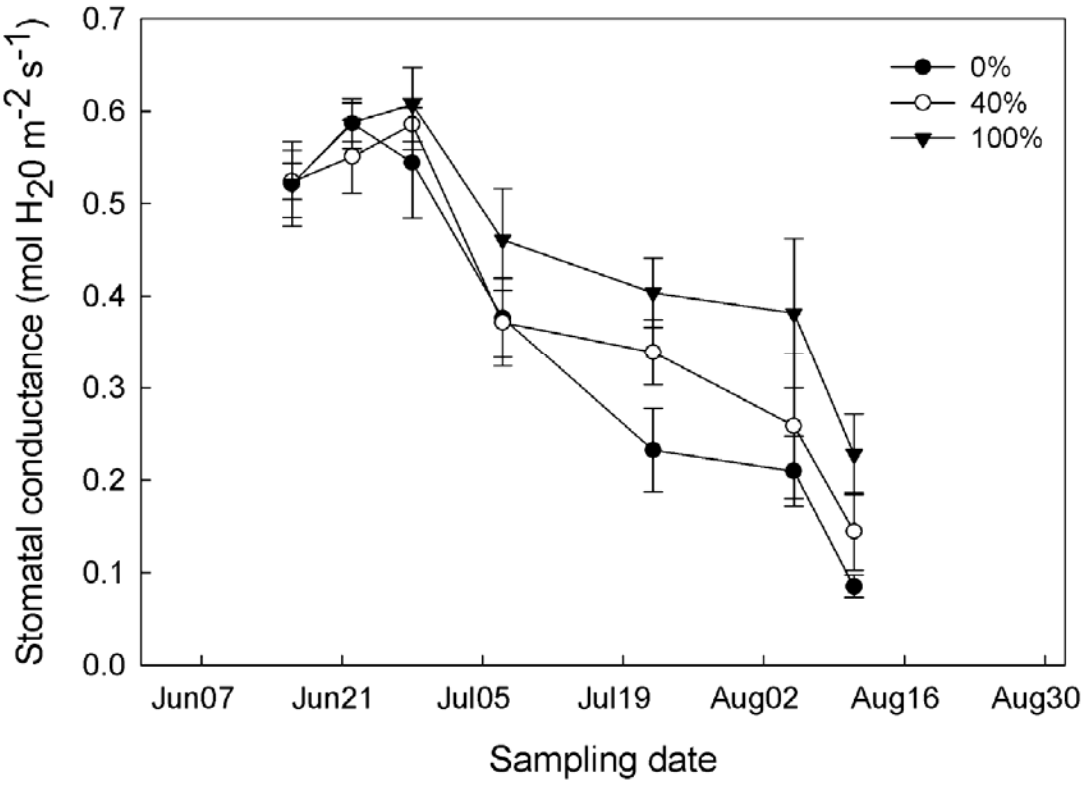

Figure **A.1**: Stomatal conductance (mol H_2O m^{-2} s^{-1}) of 'Merlot' grape vines under different levels of irrigation in Oakville, CA, over the stressed portion of the growing season (July- August) in 2004. Irrigation treatments were 0% (●), 40% (○) and 100% (▼) replacement of evapotranspiration (Et_c) (± 1 SE) (P<0.001). Data for vines on root systems associated with higher shoot vigor and lower shoot vigor were combined.

Appendix B

Photosynthesis

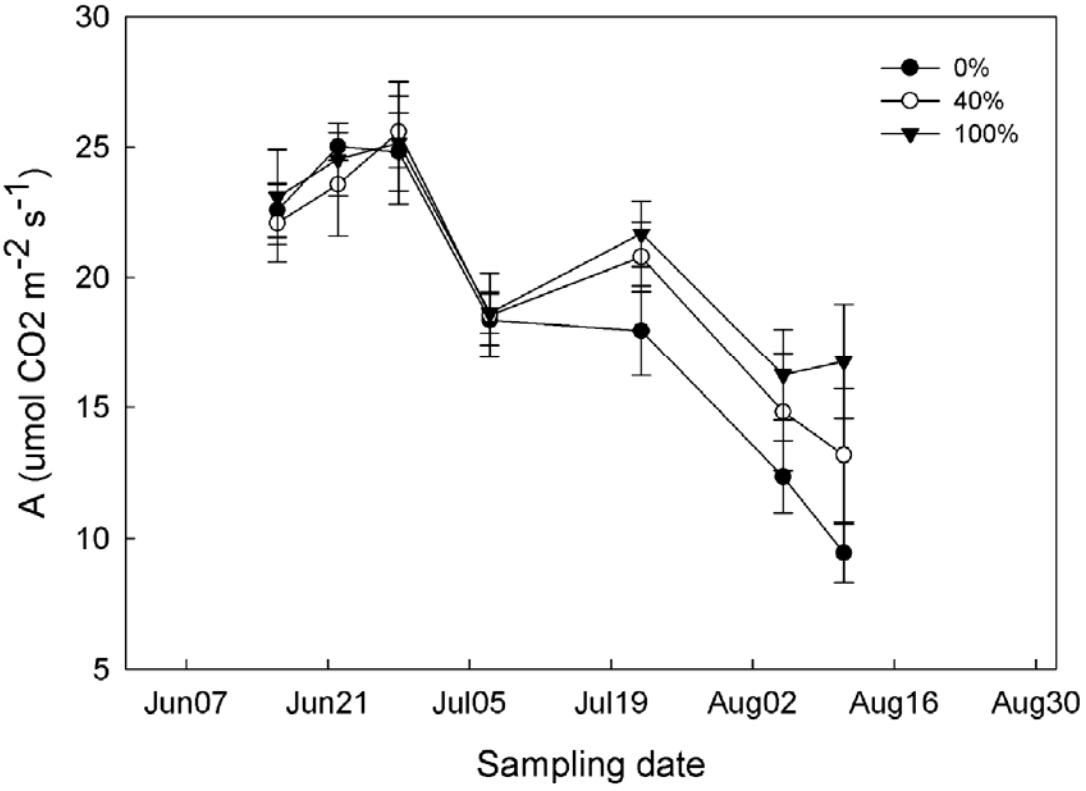

Figure **B.1**: Photosynthesis (MPa) of 'Merlot' grape vines under different levels of irrigation in Oakville, CA, over the stressed portion of the growing season (July- August) in 2004. Irrigation treatments were 0% (●), 40% (○) and 100% (▼) replacement of evapotranspiration (Et_c) (± 1 SE) (P=0.022). Data for vines on root systems associated with higher shoot vigor and lower shoot vigor were combined.

Appendix C

Root Periodicity

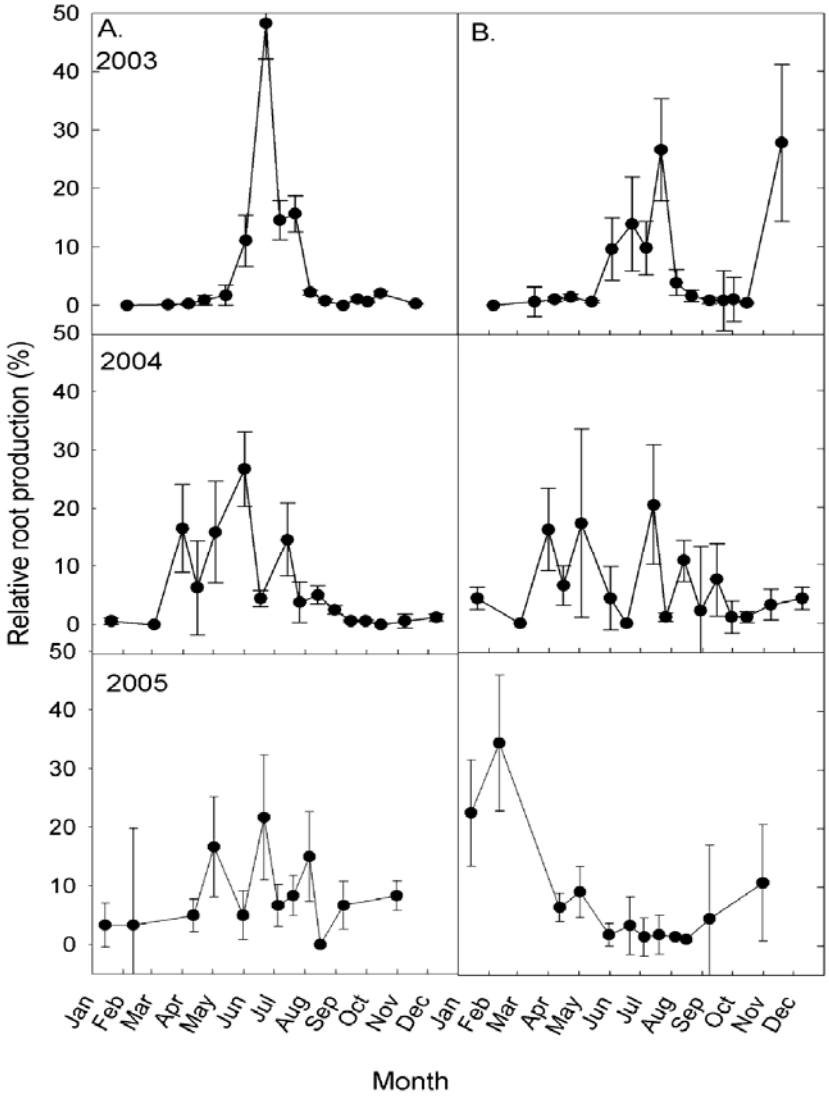

Figure **C.1**: Relative root production (percent of annual root production occurring on a particular date) in a Merlot vineyard on two rootstocks over three years under deficit irrigation (100% ET) in the soil region beneath the drippers. Bar indicates ±1 SE.

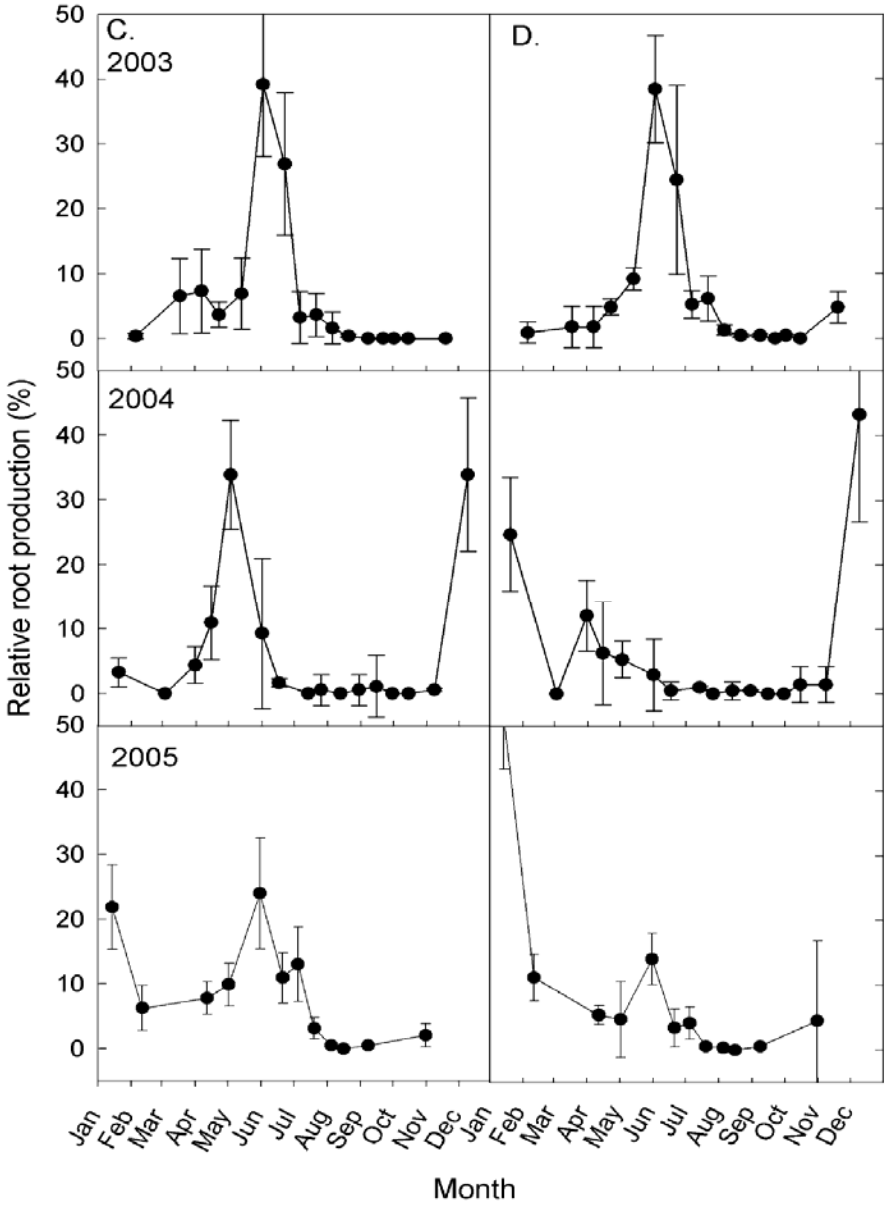

Figure **C.2**: Relative root production (percent of annual root production occurring on a particular date) in a Merlot vineyard on two rootstocks over three years under deficit irrigation (100% ET) in the dry soil region. Bar indicates ±1 SE.

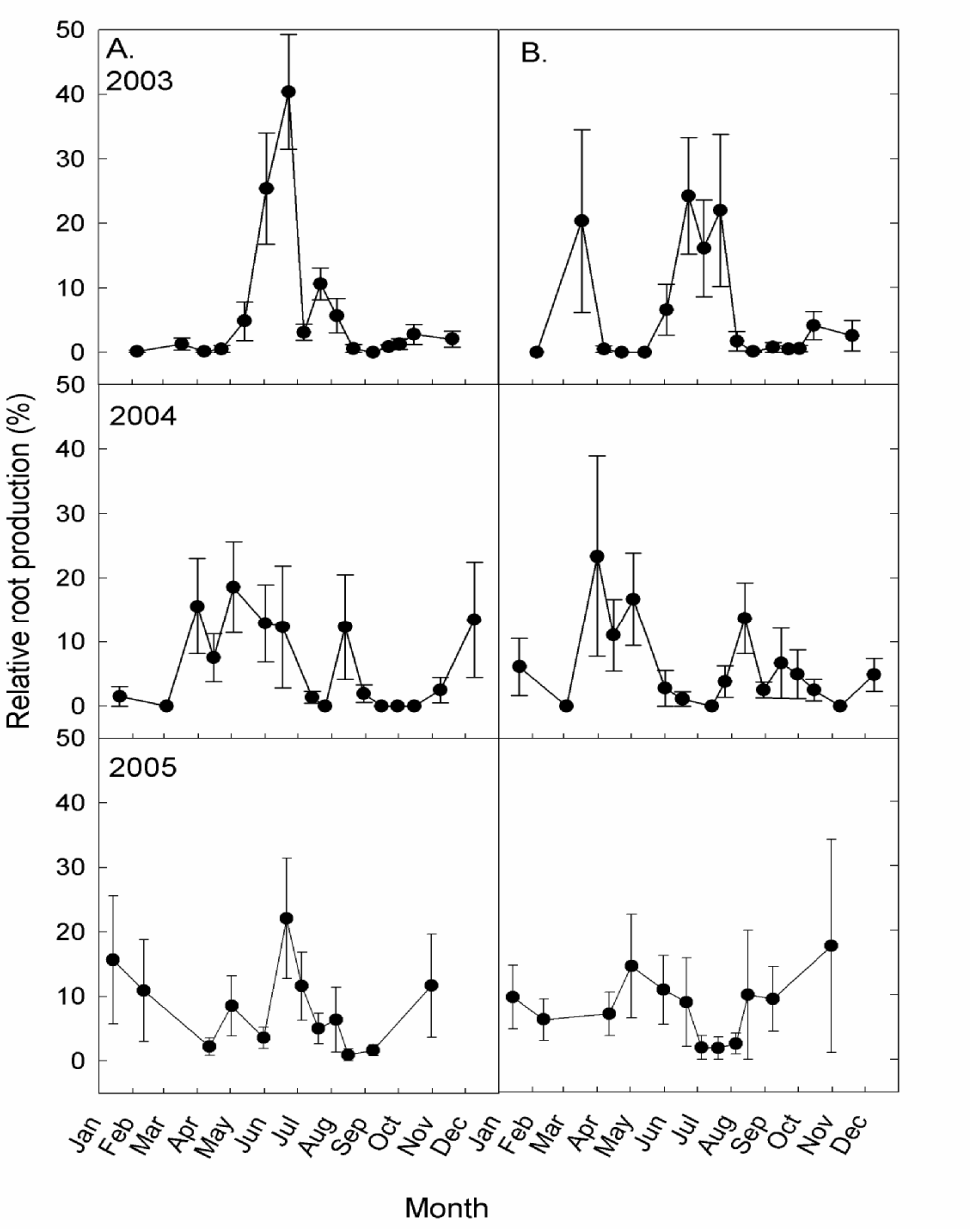

Figure **C.3**: Relative root production (percent of annual root production occurring on a particular date) in a Merlot vineyard on two rootstocks over three years under deficit irrigation (40% ET) in the soil region beneath the drippers. Bar indicates ±1 SE.

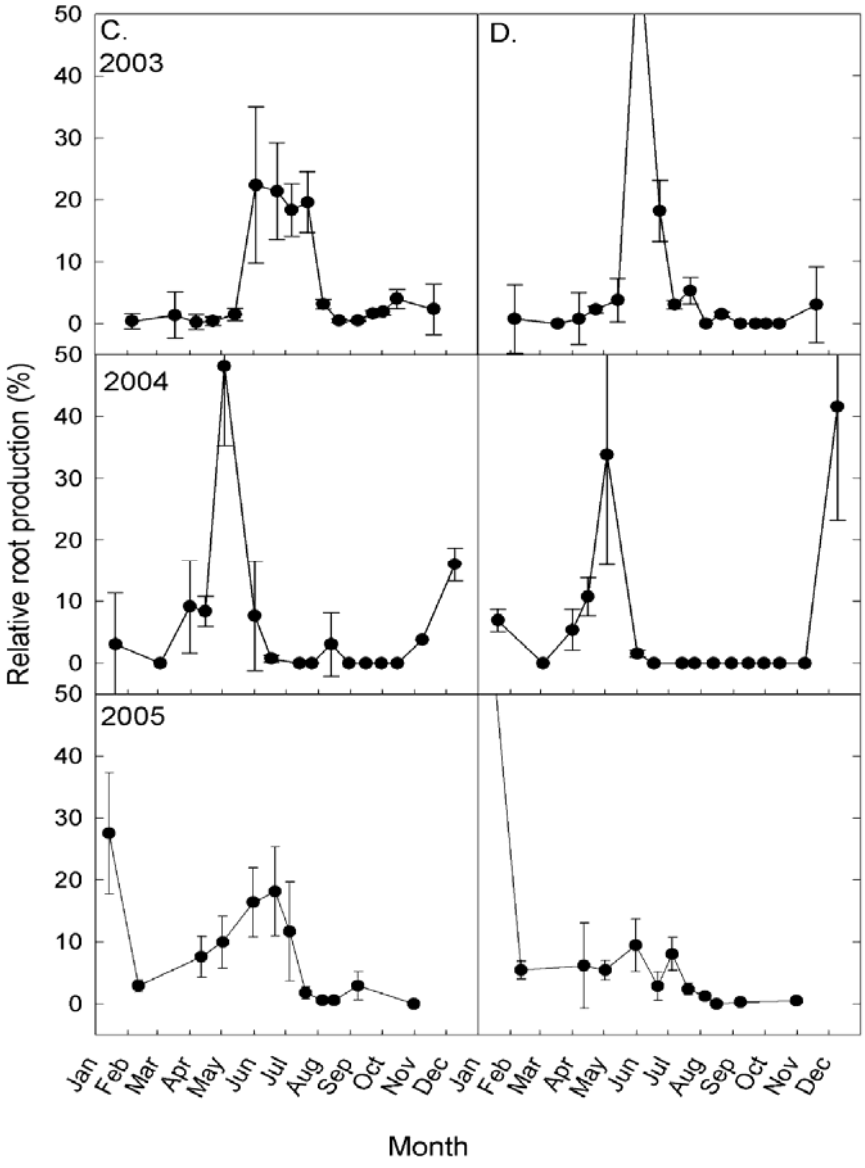

Figure **C.4**: Relative root production (percent of annual root production occurring on a particular date) in a Merlot vineyard on two rootstocks over three years under deficit irrigation (40% ET) in the dry soil region. Bar indicates ±1 SE.

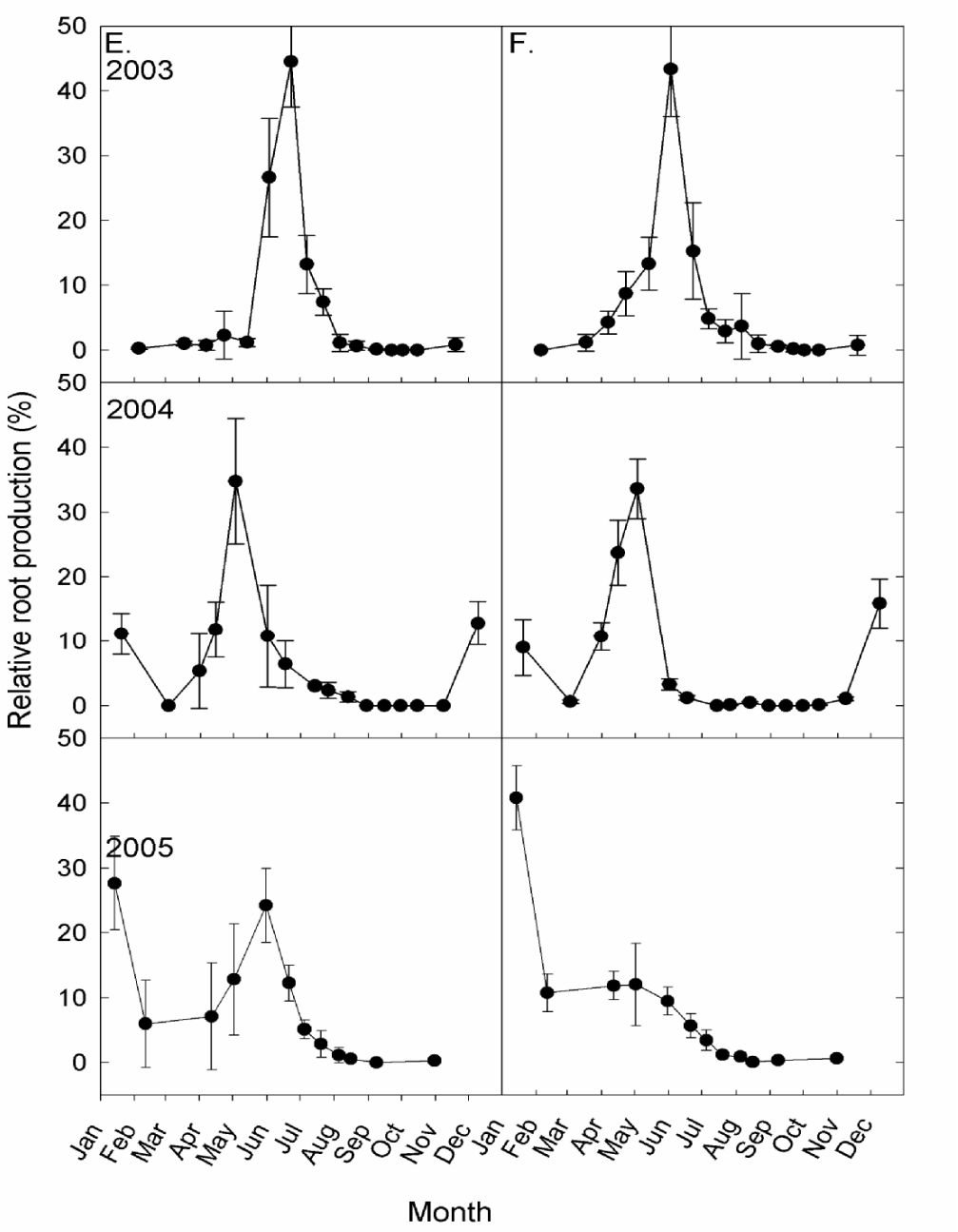

Figure **C.5**: Relative root production (percent of annual root production occurring on a particular date) in a Merlot vineyard for two rootstocks E. 1103P and F. 101-14 Mgt. over three years under deficit irrigation (0% ET) in the dry soil region. Bar indicates ±1 SE.

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T.L. Bauerle (2006) Grapevine Root Dynamics. *American Wine Society Journal* **38**:24-28.

D.R. Smart, C. Stockert, **T.L. Bauerle**, E. Carlisle, and M. Goebel. (2005) Artifacts of grapevine root proliferation following installation of minirhizotron observation tubes. *Acta Horticulturae* (ISHS) **689**:153-158.

W.L. Bauerle, T.H. Whitlow, T.L. Setter, **T.L. Bauerle**, and F.M. Vermeylen (2003) Ecophysiology of *Acer rubrum* L. seedlings from contrasting hydrologic habitats variation in growth, gas exchange, tissue water relations, abscisic acid, and carbon isotope discrimination. *Tree Physiology* **23**:841-850.