PHYSIOLOGICAL AND BIOCHEMICAL RESPONSES OF THREE GRAPE GENOTYPES

TO DEFICIT IRRIGATION

by

SAID ENNAHLI

B.S., Agriculture School of Engineering, MOROCCO, 1998
M.S., Agriculture School of Engineering, MOROCCO, 2001
M.S., University of Georgia, 2003

AN ABSTRACT OF A DISSERTATION

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DOCTOR OF PHILOSOPHY

Department of Horticulture, Forestry and Recreation Resources
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Abstract

This project investigated the physiological and biochemical responses of three grapevine genotypes to a deficit irrigation strategy termed partial rootzone drying (PRD). The principle objectives of the project were to (1) establish if the response to PRD is a unique vine physiological response; (2) to investigate the effect of PRD on berry maturation and composition; (3) effect of PRD on vine water-use efficiency; (4) investigate the hormonal signal involved in the PRD mechanism. In addition, the project investigated the adaptation of these three grapevine genotypes to Kansas climatic conditions. The irrigation project was conducted in both a greenhouse at Kansas State University, Manhattan, KS, and field conditions in an experimental vineyard at the John C. Pair Horticultural Research Center, Haysville, Kansas. The greenhouse study was replicated four times (6 months each run) and the field study ran from the 2005/2006 season through the 2006/2007 season. Application of PRD to vines resulted in a unique physiological response distinct from other established deficit irrigation procedures such as regulated deficit irrigation (RDI); however, the overall physiological responses of the vines were defined by the environmental conditions of the vineyard where the experiment occurred. Reduced water availability, low to medium vigor vines, restricted root development, and high evaporative demand were all factors in the vines response to PRD.

Under these conditions, PRD did maintain the yield at deficit irrigation rates compared to fully irrigated vines, and was responsible for physiological changes in the vine that could have long-term implications for yield stability. The application of PRD resulted in an improvement in

plant water status and maintenance of leaf function as measured by photosynthesis, stomatal conductance, and leaf water potential throughout the season.

PRD did maintain yield in both the 2005 and 2006 seasons. Reductions in vegetative growth or improvements in water-use efficiency were found in the PRD experiments under greenhouse conditions. PRD increased vine stomatal sensitivity to water loss, which in turn led to vines being able to maintain photosynthesis even when soil moisture was low. PRD resulted in extraction of soil water at deeper levels, which, combined with increased stomatal sensitivity to water loss, meant that the vines coped better with water deficits.

PRD causes differences in berry composition at harvest. The development of anthocyanins and phenolics was greater in PRD-treated vines than in control vines; however, berry metabolism (sugar, acids) did not differ in response to irrigation treatments. Therefore, application of PRD under field environments may have positive benefits on vine health and water-use.

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Approved by:

Major Professor
Dr. Houchang, Khatamian

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CHAPTER 1 - Grapevine Responses to Deficit Irrigation: Current Updates

1. Plant water relations:

Both environmental factors and soil moisture availability affect plant water status. A large number of environmental factors that affect stomatal aperture have been investigated (Losch 2004).

To enable plants to respond to decreasing soil moisture availability plants must sense soil drying, reduce stomata opening and vegetative growth in a response appropriate to the reduction in available water. Large sets of data on plants show abscisic acid (ABA) is the most likely hormonal molecule allowing plants to detect, and respond, to drying soil (Liang et al. 1997). Drying soil stimulates production of ABA in roots which travels via the xylem to the leaf where it decreases stomatal conductance, leaf expansion and shoot growth.

When roots sense dryness, the root hormones may decrease leaf stomatal conductance and transpiration before any leaf water potential changes occur in leaves and shoots (Kang and Zhang 2004). Stomata regulate transpiration to allow sufficient carbon gain while preventing leaf water potential (Ψ_l) from becoming too negative.

Increase in xylem ABA is associated with stomatal closure (Stoll et al. 2000b). However, some studies did not observe any change in stomatal conductance while increases in xylem ABA occurred (Trejo and Davies 1994). It is suggested that, in addition to ABA, other factors are controlling stomatal response to decreasing soil moisture.

Stomatal closure is one of the first responses of the plant to water deficit. Regardless of species and genotype, there are differences in stomatal responses to water deficits. As water

availability decreases, large reductions in stomatal conductance (g_s) occur. For a long time, the hypothesis was that photosynthetic rate (A) reduction, caused by drought, was a result of decreases in g_s (Kriedemann and Smart 1971). Many studies have demonstrated that stomatal closure is the dominant factor limiting gas exchange during water deficit (Medrano et al. 2003).

2. Grapevine response to water deficit

It is well established that ABA plays an important role in the response of grapevines to water stress through stomatal closure in leaves (Lovisolo et al. 2002). It also appears likely that root ABA synthesis in response to root drying is the major factor affecting stomatal response in grapevines, although the response can be influenced by osmotic adjustment, xylem hydraulic conductivity, and environmental factors (Cifre et al. 2005). The response of grapevines to water deficits changes in response to the severity of water stress that the vine is experiencing (Cifre et al. 2005). Under mild water stress, decreases in A are the result of stomatal limitations, leading to an increase in water-use efficiency when measured as the ratio of photosynthesis to transpiration or stomatal conductance to water vapor (Cifre et al. 2005). With moderate to severe water stress, non-stomatal inhibition of photosynthesis has been described (Flexas et al. 2004). Under moderate water stress, when photosynthesis is mainly limited by stomatal conductance, a complete recovery of maximum photosynthesis was found to occur just one night after irrigation (Ennahli and Earl 2005). However, if g_s reaches values as low as 50 mmol H_2O m^{-2} s^{-1}, photosynthesis does not recover immediately after irrigation (Quick et al. 1992).

Differences in responses to decreasing soil water availability also exist among cultivars of grapevines. Both isohydric and anisohydric grapevine cultivars exist, with highly variable sensitivities and responses to water deficits (Schultz 2003). An isohydric behavior implies that the same minimum leaf water potential can be reached at midday regardless of soil moisture

level, while anisohydric behavior indicates a strong relation between soil moisture and leaf water potential. The differences between cultivars are not always observed when vapor pressure deficit (VPD) is low, and increases in g_s lead to decreases in Ψ_l in both isohydric and anisohydric varieties. However, VPD is high the water-use strategies of the different cultivars are different. In anisohydric cultivars, under high VPD, decreasing g_s lead to a decline in leaf water potential (Ψ_l), preventing further water loss (Rodrigues et al. 1993). In isohydric cultivars under conditions of VPD, there is no relationship between g_s and Ψ_l with decreases in g_s occurring while Ψ_l remains the same (Bota et al. 2001).

3. Influence of irrigation

Vineyard irrigation in the Midwest of USA has not been widespread because of historically rare occurrences of prolonged drought periods. However, during the past years vineyards were subjected to prolonged droughts, prompting growers to reexamine irrigation. Irrigation increases yield with little effect on sugar concentration (Reynolds et al. 2007). Irrigation increased Chardonnay yields from 20% to 25%, accompanied by only a slight decrease in soluble solids. Increasing water stress via deficits has increased °Brix (Reynolds and Naylor 1994). Irrigation timing also affects sugar concentrations.

Water at the end of stage III (Period of rapid increase in cell size rather than cell number) increased yield in Bacchus and Riesling by 27% without change in sugar concentrations, but irrigation during stage I (period of rapid increase in the cell number) of berry development increased yield but reduced sugar (Reynolds et al. 2007). Conversely, non-irrigated treatments often become water stressed, resulting in low sugar production, low yield, and poor wines (Ginestar et al. 1998). At low levels, irrigation lowers fruit production, and results in poor vegetative growth and fruit composition (low sugar, low or high pH) (Cifre et al. 2005).

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In midsummer, which corresponds generally to stages I and II (berry softening) of berry growth, vines require higher amount of water (Lakso and Pool 2001). At this stage shoot growth and berry development are sensitive to water stress, and drought is detrimental because of decreased vegetative growth (Reynolds and Naylor 1994), fruit set (Matthews and Anderson 1988). Water stress at or following veraison can lower berry weight and yield (Reynolds and Naylor 1994). Fruit composition may also be jeopardized by post-veraison water stress that delays fruit maturity, resulting in both restricted sugar accumulation and poor flavor development (Smart and Coombe 1983).

Excessively irrigated grapevines can increase vigor and, therefore, shaded canopies leading to high levels of water loss, fungal diseases, shading of the fruit in the canopy, reduced air movement, and reduced wine-grape quality (Souza et al. 2005b). For these reasons, application of irrigation water needs to be carefully managed, and research has shown positive benefits can be achieved if a mild water stress is applied through deficit irrigation (Coombe and McCarthy 2000). Enhancment of fruit quality may therefore arise from mild water stress application (Caspari et al. 1997).

In addition to the benefits on grape production if irrigation is managed, efficiency of water use is increased. Water-use efficiency can be defined at a crop level as yield per liters of irrigation water applied (WUEc), or on a leaf scale as the ratio of instantaneous net CO_2 assimilation rate (A) to transpiration (E), otherwise known as instantaneous water-use efficiency (WUEi, Jones, 2004). A second leaf based measure of water-use efficiency, intrinsic WUE (WUE$_l$), takes into consideration differences in WUE independent of specific environmental conditions and is defined as the ratio of A/ g_s (Jones, 2004).

The two major ways in which the wine industry is attempting to improve water-use efficiency is through deficit irrigation applied by either regulated deficit irrigation (RDI) or partial root-zone drying (PRD). RDI involves withholding water from the vine for specific periods (generally, after berry set occurs). PRD involves application of a reduced amount of irrigation water to alternate sides of the vine. RDI often reduces vigor and berry size, although improvements in berry color can be achieved (Coombe and McCarthy 2000). However, RDI is hard to manage, and, if severe stress develops in the water withholding period, substantial yield reductions can occur. As RDI is only applied for few weeks of the season, amount of water saved is very limited.

If managed properly, deficit irrigation, whether applied via drip, sprinklers, or PRD, can have no impact on CO_2 assimilation, despite lower water application than in full irrigation and a reduction in g_s (Souza et al. 2005a). The relationship between photosynthesis and stomatal conductance may not be linear. Photosynthesis generally saturates before stomata are completely open, while the relationship between stomatal conductance (g_s) and transpiration (E) is linear (Cifre et al. 2005). This leaves scope for improvements in WUE_i and WUE_l, as reduced water application can lead to reductions in g_s and E, without negatively affecting A and sugar production in leaves. On a whole plant level, even small improvements in WUE_l can lead to water savings for the plant. This process, together with the fact that a reduction in vegetative vigor results from the application of deficit irrigation, is the basis for application of PRD. In PRD, the root zone of a vine is simultaneously wetted and dried by application of a reduced amount of water to alternative sides of the vine. This irrigation technique stimulates some of the responses associated with drying soil such as reduced vigor and stomatal conductance (Loveys et al. 2000). PRD induces a reduction in stomatal aperture and vegetative vigor without modifying

leaf water status and is associated with a five-fold increase in leaf ABA and increasing sap pH

(Dry et al. 2000, Stoll et al. 2000b).

PRD offers viticulturalists a method by which deficit irrigation application occurs over

the whole season to control vigor and increase WUEc in a controlled and sustained manner. The

major benefit of applying deficit irrigation is that yield is maintained and berry size is not

significantly reduced (Santos et al. 2003).

4. Deficit irrigation: PRD concept

In PRD, water is applied, at each irrigation, but only a part of the root zone receives water

and the other part is allowed to dry. Because only one half of the root system of the vine receives

water, a reduction in the overall requirement for irrigation can be achieved. Non-irrigated roots

in a drying condition produce a hormone that reduces water loss through transpiration and acts as

a natural growth retardant (Stoll et al. 2000a). The nature of the hormonal response is complex.

Most published work refers to chemical signals (hormones) (Sobeih et al. 2004), to hydraulic

responses, and to the interaction of both (Auge and Moore 2002). A potential advantage of the

PRD method is that precise irrigation control is probably less critical for success than it is for

RDI, because plants can always obtain adequate water from the well-watered side of the root

system and the drying side provides the hormonal or hydraulic response to modify vine growth

(Jones 2004).

5. Root hormones: Key element in PRD irrigation management

Understanding the physiological changes that take place under water stress has led to a

better understanding of grape-vine response, in terms of chemical compounds that mediate

responses that happen in the root and to which the leaf responds. It has been well established that

water deficit decreases stomatal conductance, and partial stomatal closure induces a decrease in

transpiration. Therefore dry matter production per unit of water transpired is reduced (Gomez-Del-Campo et al. 2004). Leaf size, yield, and berry size, as well as fruit composition, are also altered in grapes (Gomez-del-Campo et al. 2002).

Traditional explanations for these responses have emphasized the importance of a decline in shoot water status, which accompanies severe soil drying (Gowing et al. 1990). It is now accepted, however, that many of a plant's responses to soil drying can occur in the absence of changes in shoot water status, via chemical changes in the roots. Gowing et al. (1990) split apple roots between two compartments. One part was allowed to dry and the other was irrigated. This restricted leaf growth, due to chemical intervention (Stoll et al. 2000a) including a reduction of cytokinins (Davies et al. 2005, Stoll et al. 2000a).

The physiological changes (e.g., stomatal conductance, shoot growth), linked to chemical changes in the roots, has been studied widely (Comstock, 2002). These observations support the hypothesis that a hormone that travels from roots to shoots is produced, and abscisic acid (ABA) has been extensively studied as the most likely messenger (Vysotskaya et al. 2004).

Since ABA is known as a biochemical compound that is induced by imposing water stress (Comstock 2002), monitoring the level of ABA in a grapevine may be a tool to control proper irrigation, which can maintain crop production and fruit quality. The great challenge is to determine methods that can control water availability to grapevines, and to control the production of root-derived hormones such as ABA, which reduce canopy transpiration and improve WUE (Antolin et al. 2006, Souza et al. 2003). Dry and Loveys (2000) suggested that it is possible to control vegetative growth of grapevines via PRD. Root-derived ABA from dried roots will regulate stomatal conductance, shoot growth, and water supplied from the wetted part of the root. This will ensure stable leaf water status and prevent severe water stress.

Comprehensive data from field and greenhouse studies showed that both growth and transpiration of grapevines could be reduced by PRD (Souza et al. 2003). Similar results were obtained for a diverse range of species (Zegbe et al. 2006).

The effect of PRD can not be sustained for a long period of time without alternating the irrigation for both sides, because of the transient nature of ABA accumulation in roots in dry soil (Loveys et al. 2000). Roots exposed to dry soil for a long time lose their permeability due to suberization and lignification and, therefore, are unable to absorb water when re-watered (Zegbe et al. 2006). The frequency of switching irrigation between rows in PRD will have to be determined according to the soil type and other factors such as rainfall, temperature, and evaporative demand. But in most of the published data concerning grapevines, the PRD cycles were around 10–15 days (Stoll et al. 2000a).

6. Physiological responses to water deficit (case of PRD)

When a certain balance between vegetative and fruit growth is not maintained, irrigation can increase vine growth, which leads to an excessive shoot growth and to more shading, high levels of water loss, fungal diseases, and shading of the fruit in the canopy (Souza et al. 2005b). Shading the clusters can impact negatively both fruit and wine quality and decrease the concentration of different sugars, tartrate, and phenolic and flavour compounds and increase malate, potassium, and pH (Dokoozlian and Kliewer 1995). Excessive shoot and leaf growth has the potential to affect the profitability of vineyards, by decreasing berry quality, increasing disease risk, and escalating management costs. Consequently, vines will require a better trellis design to support increased canopy weight. Productions costs become more expensive as time to manage the vines increases. Many techniques have been developed to minimize vine vigor such as pruning, trellis design, or restricting irrigation application (Dry and Loveys 1998). Restricting

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irrigation is the simplest control method for vineyards. The required infrastructure is generally implemented in situ (pipes, pumps etc), and it is not labor demanding. When meticulously managed, it can provide a long-term solution to the problem of excessive vigor.

Reducing canopy growth improves fruit exposure to sunlight, thus improving fruit quality (Dry 2004). RDI, when applied to a vineyard during a critical period of grapevine growth, results in a reduction in vegetative growth, but is accompanied by a substantial reduction in yield (Poni et al. 1993). However, PRD irrigation, when applied to vineyards, results in shoot growth reduction with no yield reduction (Stoll et al. 2000b). Dry (2004) observed an 18% to 30% reduction in 'Cabernet Sauvignon' shoot growth when subjected to PRD. Similarly, PRD reduced pruning weight by 19% to 29% compared with the control vines, while total leaf area per vine was 25% less. Lateral shoot growth was particularly responsive to PRD irrigation resulting in 55% to 56% reduction in total lateral shoot length per main shoot. PRD reduced the canopy density of treated vines and had a positive impact on berry color by improving cluster exposure to sunlight (Dry, 2004).

6.1. Effect of PRD on yield

Development of grape berries is less sensitive to water stress than vegetative growth (Nagarajah 1989). Hardie and Considine (1976) observed that reduction in vine yield is dependent on the degree of the stress and time of application in relation to fruit development. Water stress when applied to grapevines at all growing stages reduces crop yield. The optimal time to apply water stress to grapevines to reduce shoot growth is between flowering and veraison. However, water stress during flowering and veraison may cause a reduction in vine yield (Mitchell and Chalmers 1982). Although, grape berry weight was the yield component most affected by water stress, as reported by Dry (2004), PRD irrigation did not affected berry

weight (Dry, 2004). It is well established that moderate water application to grapevines results in a higher yield, compared to grapevines under no irrigation or higher volumes of irrigation (Medrano et al. 2003). Neja et al. (1977) observed a reduction in yield associated with either a low or high water application rate, while Kliewer et al. (1983) reported that an increase in water irrigation resulted in an increase yield of 'Carignane' grapevines when compared to non-irrigated vines. Early work on horticulture crops in both greenhouse and field experiments also showed that PRD reduced irrigation water amount by 30–50%, but had no significant yield reduction or even with higher fruit quality (Stoll et al. 2000a).

6.2. Effect of PRD on berry color and ripening

Water status can affect berry composition positively or negatively, depending on the degree of water stress. PRD is associated with an increase in berry quality, believed to be due to increased control over vegetative growth leading to increased light penetration into the fruiting zone (Souza et al. 2005b). Dry (2004) reported that shaded canopies had reduced sugar concentration, pH, anthocyanins, and phenolic compounds. Dry (2004) reported that PRD had the potential to improve 'Cabernet Sauvignon' wine quality by increasing secondary metabolites such as phenolics, which include anthocyanins, tannins, and isoprenoids, which includes cartenoids, and C_{13} – norisoprenoids that affect wine quality (color, astringency, mouth feel, aroma/flavor) . Other PRD experiments found that PRD maintained yield, but berry quality was not improved compared to fully irrigated vines (Souza et al. 2005b).

When PRD irrigation is applied to vines under both pot and field conditions, researchers have shown leaf and xylem abscisic acid (ABA) levels increase in response to drying one side of the root zone (Stoll et al. 2000b). This raises the question of the effect of increased vine ABA levels and the effect that they have on berry physiology. ABA in grape berries is proposed to be

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a promoter of ripening with high levels of ABA occurring in the middle of veraison (Geny et al. 2005).

In PRD irrigated vines, berry ABA concentration increased throughout veraison, and reaches a maximum at the end of this time (Antolin et al. 2006). The increase in berry ABA concentration was earlier and faster in PRD vines irrigated at 50% ETc than in fully irrigated vines. Growth, ripening, and berry composition were advanced in PRD vines compared to vines at 50% Etc. Researchers have suggested that this different response may be mediated by the ABA levels in the berries during ripening (Antolin et al. 2006).

Water stress has also been long associated with increased ABA levels in grapevines (Loveys et al. 2000, Lovisolo et al. 2002). In Chardonnay vines subjected to water deficit stress, ABA levels in berries increased within days of deficits being implemented (Okamoto et al. 2004). Deficit irrigation has been associated with improvements in berry quality largely through reductions in berry size, but it is also associated with reductions in yield (Medrano et al. 2003). Regular irrigation has been reported to be detrimental to the development of berry color (Matthews and Anderson, 1988). Withholding irrigation from field grown 'Carignane' vines resulted in an increase in anthocyanin concentration by 44%, compared with that of irrigated vines (Freeman and Kliewer 1983). PRD irrigation management reduces shoot growth and increase cluster exposure to sunlight, resulting in greater berry color, compared with shaded canopies (Stoll et al. 2000a). Dry (2004) observed an increase of 45% in anthocyanin concentration in PRD-treated 'Cabernet Sauvignon' fruit without any change in berry weight.

6.3. Effect of PRD on berry composition

The degree to which grapevine berries respond to water stress varies. Previous studies suggested that controlling vegetative growth by controlling soil moisture can increase berry

sugar concentration and sugar accumulation rate. Dry et al. (2000) observed that applying water stress during ripening increased sugar concentration by inducing berry shrinkage. They did not observe any increase in sugar concentration when water stress was applied at other stages of development. McCarthy et al. (2000) observed a rapid ripening of vines under RDI compared with non-irrigated or fully irrigated vines. Neja et al. (1977) reported an increase in berry sugar concentration and yield of vines when they were subjected to a reduction in irrigation water early in the ripening phase. PRD water management that resulted in a reduction of canopy size and shoot growth created a balance between crop load and grapevine canopy capable of increased sugar accumulation (Dry, 2004).

6.4. Effect of PRD on berry pH and titratable acidity (TA)

In general, the response of berry pH to soil moisture varies. Berry pH increased with irrigation for 'Carignane' (Freeman and Kliewer 1983), but not for 'Cabernet Franc' (Matthews and Anderson 1988), or 'Cabernet Sauvignon' (Bravdo et al. 1985, Neja et al. 1977). No pH response to irrigation was reported for 'Shiraz' (McCarthy et al. 2000). However, Dry et al. (2000) reported a reduction in pH in PRD irrigated fruits compared with the control vines, and this may have been due to the reduced canopy density. Moderate reduction in titratable acidity (TA) was observed under water deficit measured (Bravdo et al. 1985). In contrast, McCarthy et al. (2000) reported no effect of water stress on berry TA.

7. Conclusions

In summary, current knowledge on physiological responses of grapevines to water stress permits to meticulously implement physiologically based irrigation methods in order to maximize water saving . Partial root drying (PRD) and regulated deficit irrigation (RDI) programs are such tools. Although PRD give a promising hope for water saving, further deep knowledge of the mechanisms that regulate plant carbon assimilation and partitioning under different water regimes is of great interest in the frame of water saving, since these mechanisms play an important role in the regulation of the fragile balance between grape yield and quality. Eventually, this will lead to the description of physiologically based criteria for deficit irrigation scheduling.

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CHAPTER 2 - Physiological and Biochemical Responses to Partial Root-Zone Drying of Three Grape Cultivars

S. Ennahli, H. Khatamian, and S. Kadir

Abstract:

Physiological and biochemical responses of three grapevine cultivars (*Vitis Vinifera* L., 'Pinot Noir', *Vitis Labruscana* Bailey, 'Concord' and French American hybrid (JS 23.416 x Gewürztraminer), 'Traminette' to partial root-zone drying (PRD) were investigated in potted plants under controlled environmental conditions. The treatments were either full irrigation (FI) where both sides of the root system were irrigated daily to pot capacity (the controls), or PRD where irrigation was withheld on the dried side for 14 days while the wet side was irrigated as FI. PRD and FI treatments did not exhibit any significant differences in leaf water potential (Ψ_l) or photosynthetic rate (A). Stomatal conductance (g_s) was more sensitive to PRD than photosynthesis. PRD reduced stomatal conductance by 41%, 28%, and 47% in Concord, Pinot Noir, and Traminette, respectively, compared to the controls. This resulted in increased water use efficiency (WUE) by 14%, 76%, and 67% in Concord, Pinot Noir, and Traminette, respectively. ABA concentration in the PRD-dry side treatment was higher ($P>0.001$) than both PRD-wet side and the full irrigation treatment. Shoot growth rate of PRD-treated vines was reduced by 36%, 26% and 51% in Concord, Pinot Noir, and Traminette, respectively, compared to fully irrigated vines. The inhibition of g_s and shoot growth that occurred independently of changes in leaf water

status could be due to enhanced accumulation of ABA in roots and shoots, which probably maintained a high water status by reducing water loss under PRD conditions.

Department of Horticulture, Forestry and Recreation Resources, Kansas State University, 2021 Throckmorton Plant Science Center, Manhattan, KS 66502.

*Corresponding author [soukaina@ksu.edu]

1. Introduction

High yield potential of grapes depends on irrigation practices and good viticultural management. The tremendous labor expended in the cultivation of grapevines is a consequence of their inherent tendency to grow vigorously under favorable soil and climatic conditions. Control of vegetative growth reduces canopy density, increases bud fruitfulness, lowers maintenance costs, and improves fruit quality (Stoll et al. 2000). Viticulturists practice various methods to control canopy vigor. These methods entail the use of chemical growth regulators, rootstocks (Novello et al. 1996), pruning practices (Cawthon and Morris 1977) and reduced water supply via irrigation management (Kang and Zhang 2004). While any of these methods may control vegetative growth, the most efficient and practical method may be minimizing water supply.

New methods of irrigation management are being investigated in recent years to reduce water supply (Kang and Zhang 2004). They require the study of plant behavior under drying soil conditions. A new irrigation management technique called partial root-zone drying (PRD) has been developed by Dry and Loveys (1998) and Stoll et al. (2000).

In PRD, water is applied, at each irrigation cycle, where only a part of the root zone receives water while the other part is allowed to dry. Because only one half of the vine root system receives water, a significant reduction in the amount of water supplied can be achieved. The theoretical basis of PRD is that the positive effects of mild water stress and high vine water status are brought together simultaneously via the two parts of the root system (Stoll et al. 2000).

The partial dehydration of the root system due to a water deficit imposed by PRD causes a reduction in root water potential, an increase in xylem sap pH, and a stimulation of root abscisic acid (ABA) synthesis (Wilkinson and Davies 2002). Therefore, partial dehydration decreases stomatal conductance (Hartung et al. 2002) and vegetative growth (Souza et al. 2003). This novel irrigation management, compared to traditional irrigation techniques, has the potential to achieve a substantial reduction in water use and canopy vigor without adversely affecting the yield. Moreover, PRD has been reported to improve grape fruit quality by increasing sugar content due to higher exposure to sunlight, compared with shaded grapes grown under a traditional irrigation system (Dry 2004, Stoll et al. 2000).

It is well established that ABA is synthesized in the roots under PRD irrigation (Dry et al. 2001, Stoll et al. 2000) and transported to the shoots to regulate leaf stomatal conductance. Zhang and Davies (1989) reported that the lower and older leaves produce high amounts of ABA under a prolonged soil drying. As ABA is synthesized in the older leaves, it is transported to upper, younger leaves and buds to reduce water loss. Abscisic acid reduces full stomata opening apertures, therefore reducing water loss, but has little effect on photosynthesis. This substantial reduction in stomatal conductance coupled with little effect on photosynthesis, including photosystem efficiency II (PSII), leads to an increase in crop water use efficiency (WUE) (Kang et al. 2001, Stoll et al. 2000).

PRD has been reported to reduce shoot growth and improve fruit exposure to sunlight (Dry 2004). Dry (2004) observed 18% to 30% reduction in shoot growth, and 19% to 29% in pruning weight, and 25% reduction of total leaf area of 'Cabernet Sauvignon' subjected to PRD, compared with control vines. Lateral shoot growth was particularly responsive to PRD irrigation, resulting in 55% to 56% reduction in total lateral shoot length per main shoot. However, root responses to PRD have received less attention than shoot physiology and yield aspects. Prolonged exposure of a root system to a progressively drying soil may induce changes in root anatomical structures, such as suberization of the epidermis, collapse of the cortex, and loss of succulent secondary roots (North and Nobel 1991). Once these changes occur, roots become hydraulically isolated with lower radial permeability to water and ability to sense soil drying (Zhang et al. 2004). Re-watering, after a long period of soil drying, may improve this situation by inducing new secondary roots (Liang et al. 1996). Apparently such new roots are succulent enough to sense further soil drying, and may also enhance the nutrient uptake from this soil zone. The conclusion is that the increase in the ability to access soil-available resources may be the key mechanism of plant response to PRD. This means root systems of a PRD-treated vine, which may explore more of the soil, may contribute to greater water stress tolerance. Dry et al. (2000) found that, in half-dried pot vines, there was a relative increase in root development in the moist soil layer in the wet side and the deeper layers of the dry side compared to control. They proposed that the part of the root system in the dry soil could survive because of water movement from the 'wet' roots to the 'dry' roots. These results are supported by Dry et al. (2001) who reported that PRD root of field-grown grapevines grew to deeper soil layers and had significantly higher abundance of roots of the 1 mm to 3 mm diameter at the 0.4 m to 0.7 m

depth compared to control vines. However, the authors did not find any significance in the dry weight between the PRD-treated and control vines.

PRD treatment of pot-grown tomato (*Lycopersicon esculentum* Mill.) plants showed an increase in root biomass by 55% as resources (such as water and nutrients) were allocated away from shoots (Mingo et al. 2004). Kang et al. (1998) observed an increase in the root/shoot ratio and in the total root biomass in maize (*Zea mays* L) subject to PRD. However, Stick et al. (2003) reported no significant difference in root/shoot ratios between control and PRD-treated plants of tomato. Tomatoes, maize, and grapevines, however, may differ in the way they store resources because grapevines are perennial plants and may partition significant resources into the wood compared to annual crops.

Comprehensive data sets from field and glasshouse studies, on various crop species, have been established and demonstrate that roots of plants in drying soil have the potential to influence the stomatal conductance in the absence of any water status modification owing to stomatal sensitivity to chemical signals produced in the drying roots (Davies et al. 2000). Importantly, stomatal conductance is well correlated with water use efficiency and shoot vegetative growth. Although substantial evidence is being been published about the effect of PRD on plant physiology, little interest has been given to change in hormonal signals under PRD conditions. The goal of this paper is to investigate the response of grapevines to PRD while monitoring changes of ABA and to evaluate the physiological responses of three different cultivars to PRD.

2. Materials and Methods

Plant Materials. Three grape genotypes obtained from a nursery (Double A Vineyard S, Inc., Fredonia, NY) were planted in divided pots: *Vitis vinifera*, 'Pinot Noir' (grafted on 3309 Couderc), *Vitis labruscana*, 'Concord' and French American hybrid 'Traminette'. The 20 L pots (Nursery Supplies Inc., Orange, CA) were divided in half by a fiberglass sheet sealed to avoid water movement between the two parts as described by Stoll et al. (2000). Each pot had three holes for water drainage. The grapevine root system was split equally into two hydraulically separate compartments that contained soil: sand: peatmoss (1:1:1 by volume), and media was back-filled into gaps around the roots system and around the base of the stem to ensure that the root system was covered. Stems of vines were tied to a supportive stake inserted in the middle of the pot. All pots were watered daily to pot capacity to permit root establishment in both compartments and before PRD treatments were begun. The vines were grown in a temperature-controlled greenhouse at 27ºC ± 2 ºC with a 16/8 hr day / night photoperiod and with natural light of 600 µmol m^{-2} s^{-1}.

Irrigation treatments. The irrigation treatments are control (fully irrigated, FI) where 100% of water was supplied to both sides of each pot, and PRD where 50% of water was supplied to only one side of the root system. Irrigations were alternated between the sides every two weeks. Plants were irrigated twice per day to maintain soil water content close to pot capacity for both control plants and the wetted half of the PRD- treatment. Volumetric soil water content (θ) was measured daily using time-domain reflectometry (TDR) (Soilmoisture Equipment Corp., Santa Barbara, CA). Two 16 cm TDR-type sensors (Delta-T Theta probes, Campbell CS 125, Campbell Scientific, Logan, Utah) were inserted in each half compartment of each pot. Plants were subjected to 5 cycles (14 days each) of PRD. Liquid fertilizer (200 ppm N:100 ppm P:200 ppm K) (Earthgro, Marysville, OH) was applied on a weekly basis after the

commencement of the treatments. The fertilizer was applied to the surface of the soil at the center of each pot close to the main root system.

Gas exchange and chlorophyll fluorescence measurements. Gas exchange and chlorophyll fluorescence were measured on a 14-day basis. Measurements were taken on the youngest fully expanded leaf. Gas exchange measurements were carried out with a Li-Cor 6400 system (LiCor, Lincoln, NE) using a red and blue LED light source (LiCor, Lincoln, NE). Leaves gas exchanges were allowed to reach a steady state before stomatal conductance and photosynthesis were measured. Rate of CO_2 assimilation (A) and stomatal conductance (g_s) were measured at 400 mg/L CO_2 concentration using the system's CO_2 injector (Model 6400-01, LiCor, Lincoln, NE) at a saturating light intensity of 1000 μmol m^{-2} s^{-1}. Leaf chlorophyll fluorescence was measured by a pulse-modulated fluorometer (Fluorescence Monitoring System [FMS-1]; Hansatech Instruments Ltd., Norfolk, U.K.). The FMS-1 requires no dark adaptation of the leaf because it separates actinic light from the fluorescence signal by using modulated fluorometery. Measurements consisted of exposed a leaf section to a LED light source causing excitation of a pulsed fluorescence signal in the absence of actinic light. Four different values were calculated when measuring chlorophyll fluorescence for this experiment; F_o, F_m, F_v, and F_v/F_m. F_o is the initial chlorophyll fluorescence level. F_m represents the maximum fluorescence yield. F_v, or variable fluorescence level, is calculated by simply subtracting F_o from F_m. From the previous values we can calculate F_v/F_m, the PSII efficiency

. Both gas exchange and fluorescence measurements were performed between 1200 and 1500 P.M.

Physiological measurements. Leaf water potential (Ψ_l) was determined, before switching irrigation from the wetted part of the pot to the dry part, using a pressure chamber

(Plant Water Status Console-Model 3005, Soilmoisture Equipment Corp., Santa Barbara, CA). The measurements were performed between 1200 and 1500 P.M. A leaf was excised from the plant and placed in the chamber with a small portion of the cut end of the leaf protruding through the seal. Pressure was applied to the tissue until an endpoint was reached at which water just appeared at the cut end of the leaf blade. When xylem sap was observed using a magnifying glass, the pressure applied to the tissue was equal to the negative of the leaf water potential expressed in megaPascals (MPa).

Shoot measurements for both treated and control plants were taken two weeks after PRD was applied until the end of the experiment. Two shoots per vine were selected and tagged. At the end of the irrigation cycle before alternating sides, shoot length was measured with a ruler, from the tag to the shoot tip. Shoot growth rate expressed in cm length growth per day (cm/day) was calculated as the average increase in shoot length since the previous measurement.

ABA quantification. ABA quantification consisted of sampling leaf tissues every 2 weeks during the course of the experiment. To monitor ABA change during a cycle of PRD, leaf tissues were sampled every 2 days for 3 cycles only. Leaves were stored and transported in liquid nitrogen to the laboratory. Leaves were ground and samples were kept in a freezer (-70 ºC). Quantification was performed using the technique of Mwang et al. (2003).

Biomass. Biomass was harvested after 5 to 6 cycles of PRD. Above-ground plant tissue was separated into stems (including petioles) and leaves. Leaf area was measured using an LI-3100 leaf area meter (Model 3000, Li-Cor, Lincoln, NE). Roots were removed from the pots and gently washed from the media. Fine roots were collected by straining the soil-water mixture several times through a fine mesh screen. Non-root plant debris and other foreign particles were

removed from the roots by hand. Leaves and roots were oven dried at 70 $^{\circ}$C for three days, and biomass is reported on a dry weight per plant basis.

Statistical analysis. Four plants per irrigation treatment and genotype were randomly distributed in the greenhouse in a completely randomized block design. The data were analyzed by the GLM procedure of SAS software (version 8.2, SAS Institute, Cary, NC) to detect significant effects among genotype, time, and their interaction. Means of treatments were separated by the least significant difference (LSD) test at P< 0.05. All data were tested for homogeneity and normality using a residual test.

3. Results

Soil water dynamics: Moisture profiles in the control and PRD-treated plants during the experimental period and during one cycle of PRD are shown in Figure 2.1. At the commencement of PRD, θ of the drying side declined quickly during the first 4 days and became slower thereafter, and was about 10% by the end of the treatment. Volumetric water content of the PRD dry side ranged from $27 \pm 1.35\%$ (mean $\pm$ SE, n = 10) to 15.8% at the 0-20 cm in the soil under the vines. A repeated measure ANOVA showed significant variation in the average soil moisture between FI and PRD treatments (P < 0.0001). Although the θ of the PRD wet side was generally lower than that of the FI pots (which maintained an average θ above 25%), there was no statistical difference between them. The irrigated wet sides in the PRD treatment maintained a θ close to pot capacity, and leaf water potential of the plants was similar to that of FI plants (Figure 2.2).

Leaf water potential: There was no difference in Ψ_l between the PRD and FI treatments at either sample time (P=0.2). There was a significant difference between varieties at day 0 only. Leaf water potential in Pinot Noir and Traminette maintained a constant pattern during the

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duration of the experiment (< -1.05 MPa), except for Pinot Noir, which showed a slight, mild

stress in both treatments on the last day of measurement (Figure 2.2 B,C). In contrast, Concord

under FI exhibited a mild stress during the first 3 cycles of alternative watering, and then Ψ_l

decreased sharply after that to the end of the experiment (Figure 2.2 A). Concord in PRD

treatment showed a sharp decrease after the first cycle. Concord plants averaged a leaf water

potential of -1.19 and -1.30 MPa for FI and PRD treatments, respectively.

Gas Exchange: Figure 2.3 (A, B, C) shows bi-weekly changes of photosynthetic rate (A)

under the FI and PRD treatments. There was a significance difference among the three varieties

but no difference was observed between FI and PRD treatments. Pinot Noir averaged a

significantly higher photosynthetic rate than Traminette and Concord. Concord under the PRD

treatment averaged the lowest A value among all cultivars. Photosynthetic rate for all cultivars

increased sharply during the first cycle then slowly thereafter before decreasing during the last

irrigation cycle. Pinot Noir under the FI treatment exhibited a fluctuating pattern but maintained

a high photosynthetic rate when compared to the other cultivars (Figure 2.3 B).

PRD grapevines had a significantly (P<0.001) lower stomatal conductance on most days

(Figure 2.3 D, E, F). PRD significantly reduced stomatal conductance by 41%, 28%, and 47% in

Concord, Pinot Noir, and Traminette, respectively, compared to the controls. Conductance for all

cultivars exhibited a behavior similar to the photosynthetic rate under the FI treatment but a

slightly different behavior under the PRD treatment. Although PRD reduced stomatal

conductance significantly, as reported above, the average A was reduced slightly (Figure 2.3 A,

B, C). The hyperbolic regression lines fitted for the PRD and FI treatments were not significantly

different (P=0.11) (Figure 2.4). There were no significant differences between cultivars on any

date in intrinsic water use efficiency. However, WUE exhibited a significant difference between

PRD and FI (P<0.0051) (data not shown). PRD increased WUE by 14%, 76%, and 67% in Concord, Pinot Noir, and Traminette, respectively.

ABA dynamics: Bi-weekly abscisic acid content of leaves was significantly higher (P=0.0058) in PRD vines than in control vines during most of measurement days (Figure 2.5 A, B, C). There was no difference between the three cultivars (P=0.50) and effect of date was not observed at any sampling date (P=0.561). To assess the potential effect of root-synthesized hormones with PRD, ABA concentrations in roots were quantified during an irrigation cycle for the Pinot Noir variety because there were no differences among varieties observed in previous replications. After 2 days of PRD treatment, ABA concentration responded to the soil water content decline (Figure 2.5 D). The ABA concentration increased sharply on the dry side of the PRD after 4 days of PRD treatment. There was no difference in ABA concentration between wet side of PRD and FI treatments. ABA concentration in the PRD-dry side treatment was higher (P=0.001) than both the PRD-wet side and the fully irrigated treatment. The PRD dry side had, on average, a total amount of 1.59 nmol g^{-1} in ABA compared to 0.82 and 0.71 nmol g^{-1} in the FI and PRD-wet side, respectively.

Vegetative growth and dry matter production: A significant reduction in shoot growth rate was observed when PRD was compared to the FI control vines (Table 2.1). On average, the shoot growth rate of control vines was 1.12, 0.83, and 1.10 cm/day in Concord, Pinot Noir, and Traminette, respectively compared with an average growth rate of PRD-treated vines of 0.7, 0.6, and 0.53 cm/day in Concord, Pinot Noir, and Traminette, respectively. During the course of the experiment the shoot growth rate of PRD-treated vines was reduced by 36%, 26%, and 51% in Concord, Pinot Noir, and Traminette, respectively, compared to fully irrigated vines.

Plants subjected to decreased water irrigation, as occurred in the PRD treatments, exhibited reduced growth, especially of the leaves and shoots. Thus there was a significant reduction of leaf area in the PRD treatment. Reductions in leaf area were 54%, 34%, and 12% in Concord, Pinot Noir, and Traminette compared to controls, respectively. In contrast, root and shoot biomass remained unaffected by PRD, and biomass of PRD plants reached a maximum size that was similar to that of controls. As expected, there was a significant difference between PRD and FI in leaf area and leaf dry weight. Shoot biomass was similar among irrigation treatments (P=0.286). Cultivars exhibited significantly different shoot biomasses and leaf areas. Concord had higher values for all parameters in both the PRD and FI treatments because of its vigor.

The irrigated sides in the PRD maintained a θ close to field capacity. Volumetric water content of the wet side of the PRD plants was kept close to pot capacity, and leaf water potential (Ψ_l) of the plants was similar to that of FI plants. This is in line with the basic assumption of PRD that Ψ_l could be constantly maintained. A low Ψ_l was often observed during the days when the wet side of the root system had a low θ. Subsequently, maintaining a high θ of the wet side in the PRD treatment may be necessary to maintain a high Ψ_l of the plants. This is supported by recent findings of Zegbe et al. (2006), who found that the amount of water given to the wet side was essential in keeping a high Ψ_l in PRD-treated tomatoes. Marginal reductions in leaf water potentials observed at the end of the experiment were also reported by de Souza et al. (2003), at the end of the growing season in PRD-treated grapevines.

Root biomass was not affected significantly (P= 0.78), and this is in accordance with other trials where a PRD treatment has stimulated root growth while not affecting overall root biomass (Stoll et al. 2000). In pot-grown grapevines, PRD stimulated root development on the

irrigated side of the root-zone (Dry et al. 2000). Kang et al. (2003) suggested that PRD increased

the hydraulic conductance of the roots and that the roots had a greater water uptake capacity than

drip-irrigated trees when average soil water content in the root-zone was the same. An

hypothesis to explain why root biomass did not change in our experiment, despite a reduction in

water supply, can be attributed to the ability of the root to exploit selectively patchily distributed

soil resources under drying soil conditions, a mechanism termed root foraging (Rajaniemi,

2007). Under a progressive soil drying as in PRD, water and nutrient distribution take place and

varies depending on soil characteristics and environmental conditions (soil temperature, VPD,

etc). Roots explore deeper depths in the profile, which is limited in the case of experiments with

potted plants.

In potted, split-root vines, there was no significant change in leaf water potential,

indicating the involvement of a hormonal messenger (Dry et al. 2000). Split-root experiments

with other species have also shown similar reductions in shoot growth without any changes in

leaf water potential (Poni et al. 1992). In contrast, our experiment showed a decrease in leaf

water potential and stomatal conductance (g_s) for the Concord cultivar, and this was presumably

due to chlorosis that developed at an early stage of growth, or to the high level of stress

experienced by vines in this experiment. This confirms the high sensitivity of the Concord

cultivar to water stress.

Furthermore, response of grapevines to PRD is likely to be variable, as different

grapevines are also known to have varying sensitivities and responses to soil water deficits

(Schultz 2003). The relationship between stomatal conductance and leaf water potential varies

within grape cultivars, no strong relationship was reported in some grapevine genotypes and

conditions (Schultz 2003). However, a good relationship between stomatal conductance (g_s) and

leaf water potential and/or water content has been observed in some grapevine genotypes and conditions was reported (Rodrigues et al. 1993). In some cultivars, there is strong evidence linking decreasing conductance with declining water potential, preventing further water loss and possible cavitations interrupting hydraulic systems (Rodrigues et al. 1993). These varieties can be either anisohydric or ishohydric. An isohydric behavior, implies that the same minimum leaf water potential can be achieved at midday irrespective of soil water status. Concord in this experiment shows anisohydric stomatal behavior, which means that there was concomitant decreasing stomatal conductance and decreasing leaf water potential. This fact makes midday leaf water potential a poor indicator of water stress

Stomatal conductance was significantly higher in control vines than PRD-treated vines ($P<0.0001$). However, the large magnitude in stomatal change in control vines, especially in Traminette and Pinot Noir, had little effect on assimilation rate ($P=0.078$). Our experiment resulted in essentially no reduction in assimilation rate for PRD-treated vines compared to control vines (Figure 2.3 A, B, C). When compared, the assimilation rate versus conductance for control and PRD vines (Figure 2.4), no significance difference ($P=0.11$) was observed between the regression lines fitted for the two treatments. These findings concur with studies demonstrating that applying a mild stress can cause only a minor decline in assimilation rate (Jones 1998). No significant changes in assimilation rate, in association with substantial changes in stomatal conductance, may be explained by the fact that assimilation rate and stomatal conductance are under different controls. Jones (1998) reported that the assimilation rate can be enhanced more rapidly than the rate of increase of stomatal conductance when beans (*Phaseolus vulgaris* L.) were subjected to different light intensities. They reported that a further increase in stomatal conductance had no effect on assimilation rate when it reached its saturation level.

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Jones (1998) proposed that stomata in well adapted plants could play an important role in plant

water loss but a relatively small role in the assimilation rate. As a result of reducing stomatal

conductance with a minor effect on the photosynthesis rate, the PRD treatment will ameliorate

the transpiration efficiency and increase water use efficiency without affecting the

photosynthesis rate. Zhang and Kirkham (1995) reported that split-root experiments with annual

crops under partial drying of the root system had no adverse effects on leaf water potential and

assimilation rate. Thus understanding the mechanism that controls stomata aperture will be of

great assistance in the future in water saving. In potted and field grown grapevines, vines

irrigated at 50% normal irrigation by PRD were able to maintain the same yield as fully irrigated

vines, and increased water use efficient by up to 50 % (Stoll et al. 2000). Other research on

grapevines and other horticultural crops found that PRD maintained the yield as in control plants

(Topcu et al. 2007). Therefore, PRD increases significantly WUE. In our study, WUE increased

by 14 %, 76%, and 112% in Concord, Pinot Noir, and Traminette, respectively, compared to

control plants.

The closure of stomata under water deficit is controlled by hydraulic and /or non-

hydraulic chemical mechanisms. Hydraulic signaling occurs by changes in xylem sap tension to

reduce plant water status and stomatal conductance in response to soil water deficit (Dodd et al.

1996). However, if plant water status is maintained and stomatal conductance is lowered by

partial closure of stomata as under PRD treatment (our experiment), then it is most probable that

biosynthesis of inhibitory chemicals (hormones) activate the movement of solutes out of guard

cells with the subsequent loss of turgor to close stomata. The partial closure of stomata

associated with PRD irrigation coincides with the drying of the soil surrounding the root system

and maintenance of the leaf water potential (Lovisolo et al. 2002). This suggests that the roots

produce a hormone such as ABA, which increases in concentration in the xylem sap as the soil dries. Root tips can sense quickly any change in soil water content and produce ABA, which acts as a growth inhibitor and stress indicator. Accumulation of ABA in the leaves has the capacity to trigger the exodus of potassium ions (K+) and osmotic loss of water, which subsequently restrict stomatal conductance and water loss through respiration. Dry (2004) showed that a reduction in conductance in response to partial drying of the root system of grapevines was associated with an increase in the concentration of ABA in xylem sap.

Seventy days after implementing the PRD experiments, the differences in total leaf area between the control and PRD-treated vines were significant. Plants subjected to decreased water irrigation, as occurred in the PRD treatment, exhibited reduced growth of all vegetative organs, especially the leaves. Thus there was a significant reduction of leaf area in the PRD treatment, which resulted in 54%, 34%, and 12% of the value obtained in the controls for Concord, Pinot Noir, and Traminette, respectively. Changes in leaf area can be either due to a reduction in total leaf number or to a reduction of leaf expansion or both. However, shoots of PRD-treated vines had fewer leaves and a reduced leaf initiation rather than a reduction in leaf expansion.

Shoot growth rate decreased across all PRD-treatments and all varieties. Irrigation type did affect significantly the shoot growth rate. The maximum shoot growth rate per day for the main shoot during the experiments was 1.23 cm and 0.68 cm per day for FI and PRD of Pinot Noir vines, respectively, a 1.1 cm and 0.71 cm per day for FI and PRD respectively in Traminette, and shoot growth rate values of 1.1 cm and 0.53 cm per day for FI and PRD vines respectively, respectively, in Traminette. But, based on visual observation, leaves of those plants still maintained full turgidity. Similarly, split-root experiments with other species also found that

reductions in shoot growth occurred without any obvious decrease in leaf turgor (Loveys et al. 2002). Restricted water supply from the root can limit shoot growth and its functioning (Davies et al. 2000). Gowing et al. (1990) have shown that the decline in shoot growth was a combination of a decreased rate of leaf initiation and final leaf expansion. Further evidence that the roots controlled shoot responses in such split-root plants was provided by an experiment showing that excising the roots in drying soil allowed growth recovery and stomatal re-opening of apple (*Malus* x *domestica*) seedlings (Gowing et al. 1990). Root excision cannot make more water available to the plant, but it can remove the supply of root-synthesized hormones. Gowing et al. (1990) also observed a reduction in stomatal conductance concomitantly with reduction in shoot growth.

Because leaf water potential could not account for the conductance and growth inhibition responses, our evidence suggests that there is a chemical constraint that is a function of soil moisture availability. PRD produces a chemical (hormone) that is transported in the xylem and causes a reduction in stomatal conductance and vegetative growth (Loveys et al. 2002) and a reduction leaf area expansion (Lecoeur et al. 1995), and a stimulation of root extension (Sharp et al. 1994).

Comprehensive data published on hormones have found that ABA is the primary hormonal regulator associated with changes in leaf physiology under soil drying. The role of ABA in regulating stomatal behavior and shoot growth is well recognized, even though the actual receptors that trigger the signal transduction events have not yet been identified (Wilkinson and Davies 2002) and the mechanistic basis for how ABA might directly inhibit leaf expansion is not well understood. Bacon et al. (1998) hypothesized that an increase of ABA in the vicinity of elongation zones may decrease leaf extension rates by reducing cell wall

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extensibility and/or turgor of these cells. Other work suggests there may be other interacting

factors that may depend on the degree of soil drying, and so the exclusive role of ABA here is

less certain (Sharp and LeNoble, 2002).

In the present study, ABA content of roots in dry soil increased under PRD. Increased

ABA accumulation was detected in shoots of plants under PRD, although the magnitude of

change was not consistent across all days of treatment and it occurred to a less extent in roots

than leaves (Figure 2.5 A, B, and C). This has also been reported in other species (Stoll et al.

2000). In leaves, the biosynthesis of ABA generally increases only when leaf water relations are

significantly interrupted, such as when leaf turgor approaches zero (Hartung et al. 2002). This

could account for the small increases in ABA content in leaves of the PRD treatment when

compared to roots. The small increase in ABA may be due to the import from the root system

alone. Loveys et al. (2002) showed that the fully drying root system of vine increases

significantly the leaf ABA concentration compared to PRD, and this presumably is due to both in

situ synthesis, stimulated by reduced leaf water potential (not observed in our experiments), plus

an import of ABA from the root system. Furthermore, the redistribution of water (Burgess et al.

1998) may contribute to moistening the dried roots in the PRD-treated vines and slowing the

magnitude of stress, therefore reducing the production of root-ABA, which can be translated into

a reduction of leaf –ABA at the end of each PRD cycle. Also hydraulic lift may prolong or

enhance root activity by keeping the roots hydrated, which is in line with our findings.

The increases in ABA content in shoots, even though small in magnitude in some

cases, may be large enough to affect shoot physiological functions. Significant changes in g_s can

occur with only slight changes in leaf ABA content (Stoll et al. 2000). On the basis of evidence

from our experiment and other studies, it is logical to assume that the increases in ABA content

found in the root and shoots of all grapevine cultivars in our study could be associated with the decline in g_s and shoot growth under PRD conditions.

5. Conclusion:

In summary, all three cultivars were able to adapt to PRD by maintaining, photosynthesis, leaf water potential, reducing transpiration rate, and increasing water use efficiency. The inhibition of g_s and shoot growth that occurred independently of leaf water status could be due to hormonal control, which ultimately helped maintain water status by reducing water loss under PRD conditions. Enhanced accumulation of ABA in roots and shoots seem to play an important role in this response. The capability of exploiting such a hormonal response in grapevines would benefit the development of water-saving vines and efficient irrigation programs. This would be an important strategy for conserving water and modifying growth characteristics.
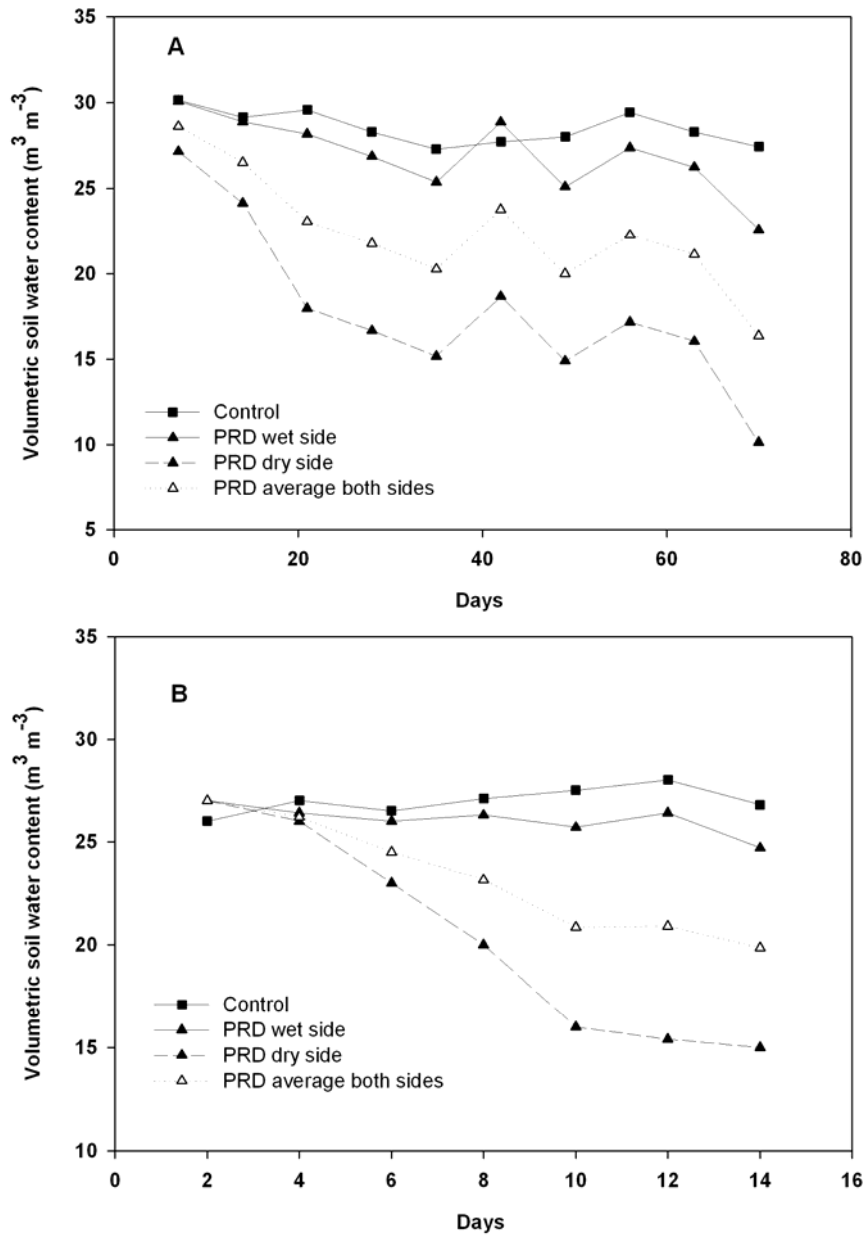
Figure 2.1*: (A) Development of the average soil volumetric water content in pots under full irrifation (FI) and partial root-zone drying (PRD) irrigation treatments (n= 12). (B) The course of volumetric water content during one cycle of PRD treatment.
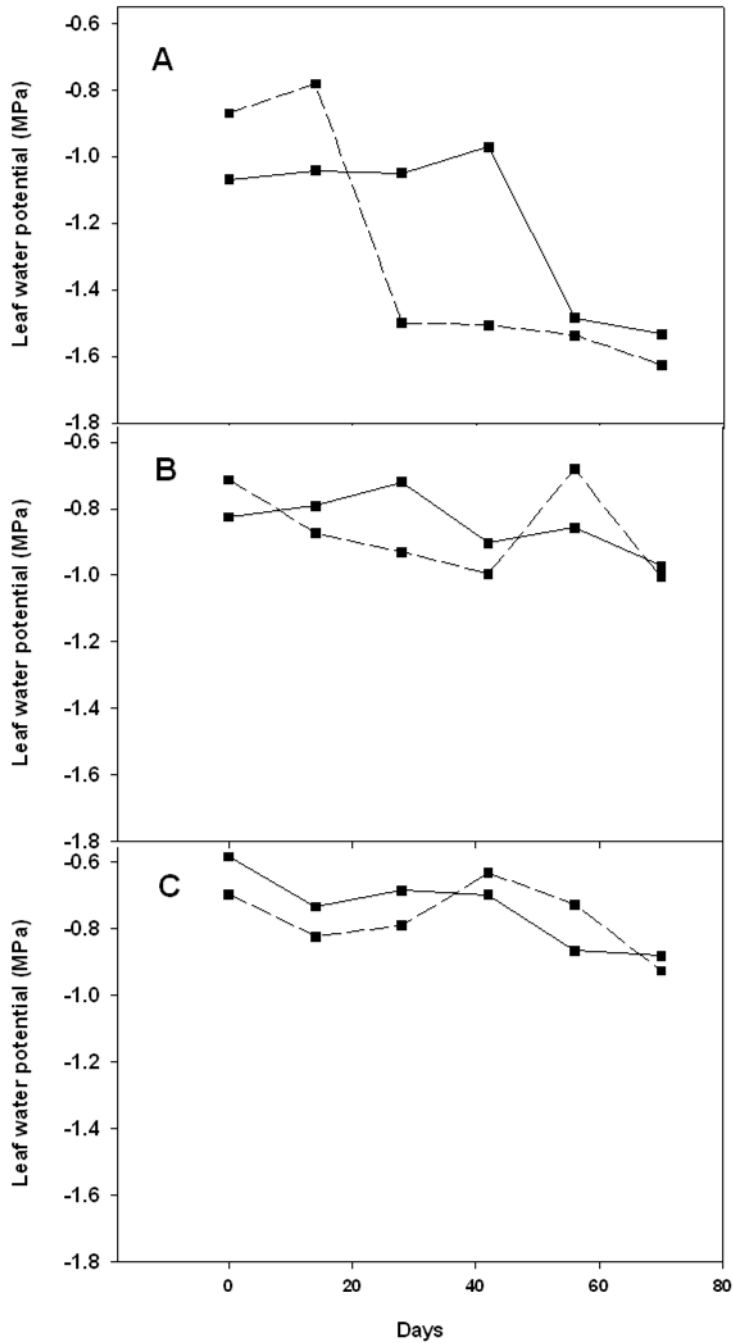
Figure 2.2: The course of leaf water potential (MPa) of potted Concord (A), Pinot Noir (B), and Traminette (C) and as affected by different water regimes (PRD (solid lines), and control (dashed lines)) during the experiment (n=4). X-axis denotes number of days after the commencement of the experiment.
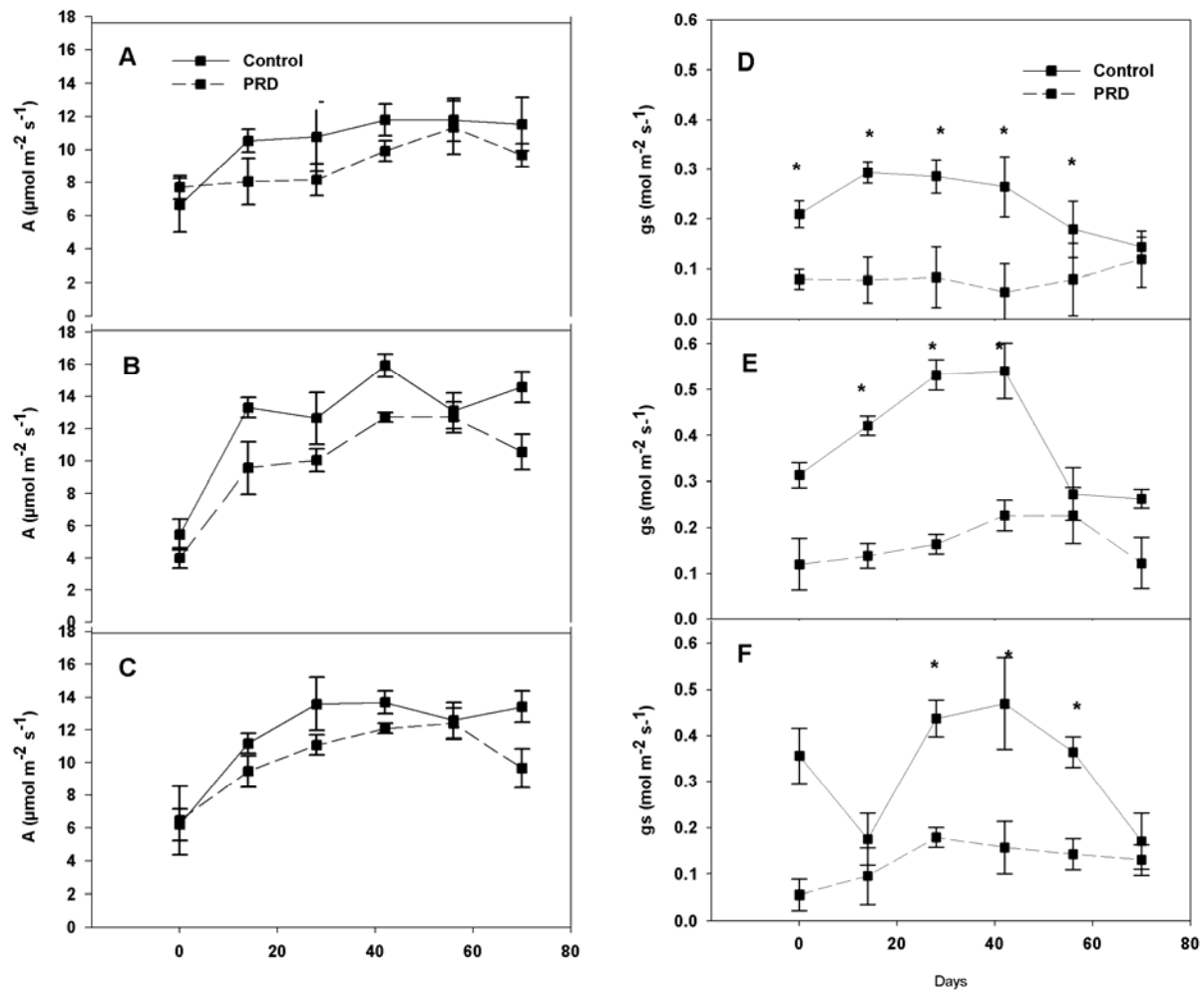
Figure 2.3: Changes in photosynthetic rate in Concord (A), Pinot Noir (B), and Traminette (C) under PRD (dashed lines) and control (solid lines). Changes in stomatal conductance (g_s) in Concord (D), Pinot Noir (E), and Traminette (F) under PRD and FI. Asteriks denote significant differences between treatments. Bars not visible indicate SE smaller than symbol. X-axis denotes number of days after the commencement of the experiment.
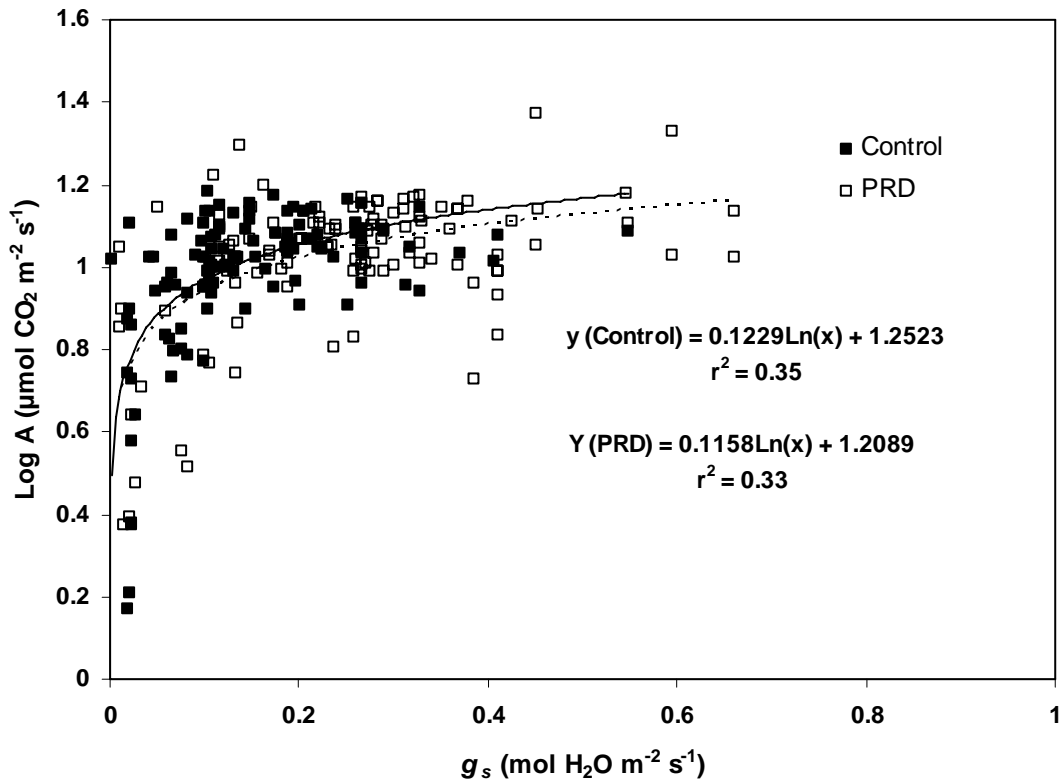
Figure 2.4: Effect of PRD on the relationship between stomatal conductance (μmol H_2O m^{-2} s^{-1}) and assimilation rate (log A, μmol CO_2 m^{-2} s^{-1}) at midday. (All cultivars included because there is no significance difference between cultivars). Regression lines are fitted (PRD: solid line, FI: dashed line).
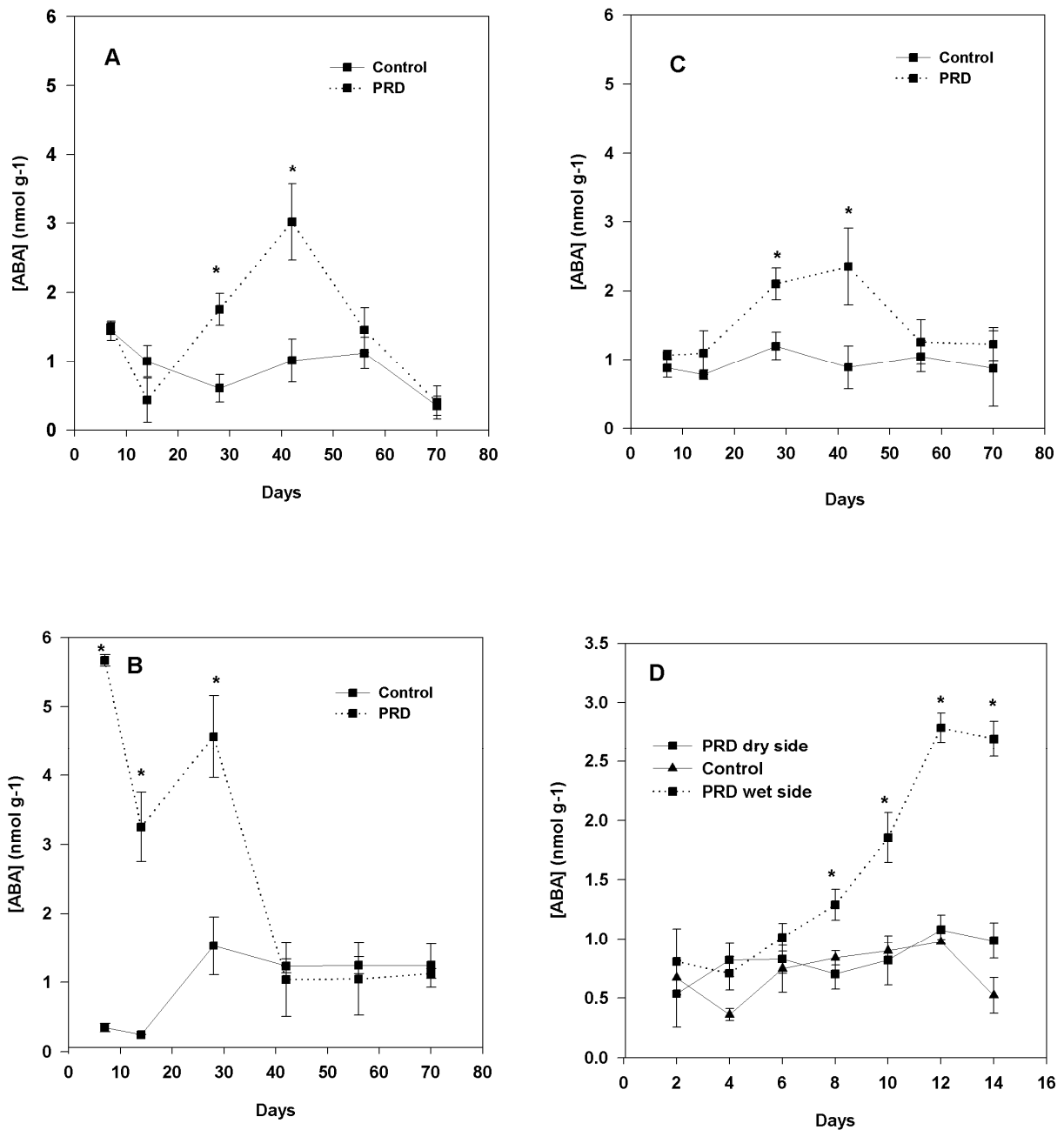
Figure 2.5: Changes in ABA concentration in Concord (A), Pinot Noir (B), and Traminette (C) under PRD (dashed lines) and FI (solid lines), (D) Changes in root ABA concentration of Pinot noir during one cycle of PRD (Closed triangle, control treatment. Closed square and solid line (Wet side of PRD-treated vine), Closed square and dashed line (Dry side of PRd-treated vines). (each point represent a mean ± SE, n= 3). Asteriks denote significant differences between treatments for each cultivar. Bars not visible indicate SE smaller than symbol. X-axis denotes number of days after the commencement of the experiment.

	Control			PRD		
	Concord	Pinot Noir	Traminette	Concord	Pinot Noir	Traminette
Shoot gr.rate (cm/day)	1.12 **a***	0.83 **ab***	1.10 **b***	0.71 **a***	0.61 **a***	0.53 **ab***
Root biomass (g)	42.34 **a**	39.07 **a**	33.11 **a**	40.96 **a**	38.06 **a**	35.86 **a**
Shoot biomass (g)	50.25 **a**	40.81 **ab**	35.20 **b**	47.22 **a**	36.74 **b**	29.82 **b**
Leaf area (g)	5273 **a***	3667 **ab***	2763 **b***	2412 **a***	2395 **a***	1343 **b***
Leaf dry weight (g)	25.2 **a***	22.3 **a***	35.86 **a***	17 **a***	16 **a***	9.46 **b***

Table 2.1: Mean of shoot growth rate, root and shoot biomass, leaf area, and leaf dry weight for the three cultivars under PRD and FI irrigation treatments. Different letters denote significant differences among cultivars for each treatments, as calculated by Fisher's least significant difference (LSD 5% level). Asteriks denote significant differences between treatment for each cultivar

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CHAPTER 3 - Evaluation of Grapevine Response to Deficit Irrigation in a Two- year Field Study

Abstract

Deficit irrigation strategies are tools for managing grapevine growth and improving fruit quality. This study examines the effects of a newly developed deficit irrigation method termed partial root zone drying (PRD). PRD has not been extensively tested in the U.S. A two-year (2005 and 2006) field study was carried out in *Vitis Labruscana* B, 'Concord', *Vitis Vinifera* L., 'Pinot Noir', and French American hybrid (JS 23.416 x Gewürztraminer), 'Traminette', which received two treatments: partial root zone drying and full irrigation (FI) (control treatment). No significant difference between PRD and full irrigation (FI) treatments at any measurement day in both seasons was observed. Shoot growth in 2006 was greater than in 2005. There was a significant effect between FI and PRD treatment on shoot growth rate in 2006. PRD vines, regardless of the variety, showed a reduction in stomatal conductance as the season progressed in both 2005 and 2006, In 2006, reduction reached values of 56%, 46%, and 47% in Concord, Pinot Noir, and Traminette cultivars, respectively, as compared to control vines. FI-treated vines had the highest photosynthetic rate but it was not significantly different from that of the PRD-treated vines. Midday value of the intrinsic quantum efficiency of photosystem II (PSII) photochemistry remained close to 0.79 without any significant difference between treatments and varieties throughout both seasons. In 2006, PRD increased water use efficiency by 48%, 44%, and 52 % in Concord, Pinot Noir, and Traminette cultivars, respectively. In neither 2005 nor 2006 were there significant differences in yield or its components (p>0.072) between PRD and full irrigation. PRD-treated vines had increased concentrations of berry skin anthocyanins and total

phenols compared to FI-treated vines. During both growing seasons, vine response to PRD deficit irrigation was dependent on the variety and environmental conditions.

Department of Horticulture, Forestry and Recreation Resources, Kansas State University, 2021 Throckmorton Plant Science Center, Manhattan, KS 66506.

*Corresponding author [soukaina@ksu.edu]

1. Introduction:

Given that the water worldwide is scarce (Jury and Vaux 2005), there is significant uncertainty about what the water supply will be for coming generations. With increasing pressures upon the nation's water resources, and irrigated agriculture being the primary user of diverted water globally (Fereres and Soriano, 2007), the adoption of water saving techniques and improvements in water use are essential for the long-term sustainability of the irrigation industry and USA's fresh water supplies. Because the viticulture industry is growing exponentially, and water resources are declining, the increasing demand for vineyard irrigation may be managed if deficit irrigation techniques as means to increase the efficiency of water use (Cifre et al., 2005; Souza et al., 2005a) are adopted. Additionally, precise manipulation of plant–water relations is essential for maximizing the quality of a given crop product, especially in viticulture. Excessive irrigation can negatively affect color and sugar content and cause acidity imbalances in the wine (Esteban et al. 2001). Conversely, inadequate amounts of irrigation water reduce grape yields and can adversely affect quality (dos Santos et al., 2003). Deficit irrigation has been extensively adopted as practice to save water and concomitantly improve fruit and wine quality. At the present time, there are two important irrigation tools, based on physiological knowledge of the

response of grapevines and other crops to water stress: regulated deficit irrigation (RDI) and partial root-zone drying (PRD). In RDI, vegetative and reproductive growth of grapevines is manipulated by withholding or applying less than the full vineyard water use at specific periods of the growing season, thus improving control of vegetative vigor. This optimizes fruit size, fruitfulness, and fruit quality (Dry et al., 2001). However, this technique needs control of water application, which is difficult to achieve in practice because RDI response is dependent on the timing and degree of severity of the water deficits, as well as on crop load (Marsal and Girona, 1997). PRD, a relatively new irrigation technique, has captured the attention of the viticultural industry internationally as a novel irrigation technology that allows control of vegetative growth and transpiration without the severe water stress periods that can occur in RDI (Dry et al., 2001). It economizes on crop irrigation water requirements without a commensurate reduction in yield (Fereres and Soriano, 2007). There have been commercial applications of PRD and the system has already been tested in vineyards located in many environments (Kang and Zhang, 2004).

In PRD, part of the root system is slowly dried and the remaining roots are exposed to wet soil. Roots of the watered side maintain a favorable plant water status, while dehydrating roots induce chemical changes (i.e., hormonal changes) that are transported to the shoots via the xylem. The PRD technique was proposed based on the assumption that roots under a progressive drying soil induce ABA-mediated responses that reduce vegetative growth and stomatal conductance (Loveys et al., 2000; Souza et al., 2003). The wet side of the root system maintains a high water status of the shoot (Kang and Zhang, 2004), while, at the same time, there is an increase in water-use efficiency (WUE) (Antolin et al., 2006; Chaves et al., 2007). In addition, PRD irrigation may have a positive impact on root growth (Wang et al., 2005). PRD induces an

increase in abscisic acid (ABA) concentration (Stoll et al., 2000) and in xylem pH (Stoll et al., 2000) as well as a reduction of cytokinins (Davies et al., 2005).

The frequency of switching irrigation between rows in PRD is dependent on various factors such as soil type, rainfall, temperature, and evaporative demand. In most of the published data on grapevines, PRD cycles are around 10–15 days (Santos et al., 2003).

PRD has been used successfully with several horticultural crops and fruit trees in studies carried out either in pots or under field conditions. The results are variable as a consequence of species differences, soil type, climatic conditions, and agronomic practices. The debate in the literature over the effects and underlying causes of PRD functioning is still intense (Chaves et al., 2007).

Numerous field trials, plus industry adoption, have proved that PRD is an effective method of improving water use efficiency. PRD resulted in no yield decreases (Kirda et al., 2005), no reduced vegetative growth (Santos et al., 2003), no change in fruit quality or higher fruit quality(Antolin et al., 2006; Souza et al., 2005a), and higher water use efficiencies (Kriedemann and Goodwin, 2003).

In this study, three different grapevine cultivars were grown under Midwestern field conditions to: (1) study the phenotypic variation in response to PRD, (2) investigate the effects of PRD on crop water relations, gas exchange, vegetative growth, yield, and fruit quality (3) study the potential of PRD to save water for grapevines under field conditions, (4) evaluate the feasibility of applying deficit irrigation under Midwestern climatic conditions.

2. Materials and Methods

Plant Materials: Research was conducted during two seasons (2005 and 2006) at the John C. Pair Horticultural Research Center of Kansas State University in Wichita, Kansas (Latitude,

37 °, 31 min. and 15.3 sec. North , Longitude 97°, 18 min. and 56.2 sec. West) on a Canadian

fine sandy loam soil. Each plot consisted of three grapevines spaced 2.4 m between rows and 2

m within rows. Vines consist of three grape genotypes, *Vitis Vinifera* L., 'Pinot Noir', *Vitis*

Labruscana B., 'Concord' and French American hybrid (JS 23.416 x Gewürztraminer),

'Traminette'. Pinot Noir vines were trained to a low cordon system, while Traminette and

Concord vines were trained to a high-cordon system, where fruiting canes are trained upwards

from the trunk, and supported by securing them to a number of trellis wires running the length of

the row of vines. Vines were irrigated regularly during the growing season via a subsurface and

drip irrigation system with two drip lines installed one at each side of the vines. Each plot was

covered with black polyethylene (6.0 m x 1.5 m) to avoid rainfall infiltration. The three varieties

were selected from 24 cultivars being evaluated at the same vineyard because, in addition to

producing white and red wine types, they are the most promising varieties in the wine region.

They also contrast in precocity. Traminette starts vegetative growth earlier than Pinot Noir and

Concord. They also differ in cold hardiness, Concord is more cold-hardy than Traminette and

Pinot Noir.

Irrigation and Experimental Design: The irrigation treatments were PRD and full

irrigation (FI). The PRD treatment consisted of irrigating half of the root system while the other

part was allowed to dry. The irrigation system consisted of two lines which were installed one at

each side of the vines positioned 30 cm from the trunk. Irrigation water was applied with buried

and above ground emitters (3 L/h), two per vine, positioned 30 cm from the vine trunk (emitters

were on the outside of both sides of the rows). Water was applied on both sides of the root

system. Water was monitored with flow meters placed in each irrigation line. The wet and dry

sides were switched every 10-21 days. The full irrigation (FI, control) treatment consisted of

100% of water applied to both sides of the vine via drip emitters. Water was supplied twice per week from the beginning of berry development (June) until the middle of July, then 3 times a week from the middle of July to harvest date. Water was supplied based on the soil matric potential. Soil matric potential was measured using portable tensiometers (Soilmoisture Equipment Corp., Santa Barbara, CA, USA). Each tensiometer unit consisted of thermally isolated tube with a porous ceramic tip on the one end and a "null knob" sealing cap and vacuum gauge on the other. When the tensiometer is filled with water and the ceramic tip is inserted into the soil, water can move in and out of the probe through the connecting pores in the porous tip. In drying soil, this creates a vacuum inside the probe that registers as soil suction on the gauge. Soil suction was measured in centibars.

Cumulative rainfall during the experimental period (mid-June until the end of August) was 447 mm in 2005 growing season and 147 mm in the 2006 growing season. Figure 1 shows monthly rainfall and the mean air temperatures at the experimental research station site during the 2005 and 2006 growing seasons.

Gas Exchange and water relations: Photosynthetic CO_2 assimilation (A) and stomatal conductance (g_s) were measured with an open gas exchange system (LI-6400, Li-Cor Inc., Lincoln, NE, USA) on 3 sun-exposed and fully expanded leaves of 3 individual vines per plot for each cultivar during the growing seasons of 2005 and 2006. Red-blue light-emitting diodes maintained incident irradiance at saturating levels (1000 $\mu mol\ m^{-2}\ s^{-1}$). The flow rate of air through the sample chamber was set at 400 $\mu mol\ s^{-1}$, and leaf temperature was maintained at 27 $\pm 0.6°C$ by the chamber thermoelectric coolers. The sample chamber CO_2 concentration was adjusted to 400 $\mu L\ L^{-1}$ with the system's CO_2 injector (Model 6400-01, LI-COR). Although the relative humidity was not controlled directly, vapour pressure deficit (VPD) varied between 1.5

and 2.0 kPa. After the leaf was enclosed in the cuvette, it was allowed to reach steady-state

values (e.g., when the coefficients of variation of CO_2 and H_2O inside the chamber were < 5%),

which occurred within 1-4 min. To assess the trade-off between CO_2 uptake and water loss, we

calculated instantaneous water-use efficiency (WUE) as ratio between photosynthetic rate and

stomatal conductance (A/g_s). Gas exchange was expressed on a one-sided leaf area basis for all

cultivars. Grapevines leaves were larger than the cuvette, so the default area of 6 cm^2 was used

for all calculations. Equations for calculating A [μmol (CO_2) m^{-2} s^{-1}], g [mol (H_2O) m^{-2} s^{-1}],

substomatal carbon dioxide concentration [Ci, μmol (CO_2) mol^{-1} (air)], and vapor deficit

pressure (VPD) (kPa) are given in the LI-6400 users manual (LI-COR Biosciences, Lincoln, NE,

2002) after von Caemmerer and Farquhar (1981).

For the determination of chlorophyll fluorescence, readings were taken with a

Fluorescence Monitoring System (FMS, Hansatech Instruments Ltd. Nortfolk, England), using

leaves submitted to ambient light (when the photosynthetic apparatus was at full capacity). The

optic fiber was maintained at a constant distance of 2 cm at an angle of approximately 60º in

relation to the adaxial surface of the leaf. The light pulses had a duration of 0.8 s and an intensity

of 1000 μmol.m^{-2}.s^{-1}. Upon the application of a saturating flash, fluorescence raises from the

ground state value (Fo) to its maximum value, Fm. In this condition, the first electron acceptor of

PSII, is fully reduced. This allows the determination of the maximum quantum efficiency of

photosystem II (PSII) primary photochemistry, given by Fv/Fm = (Fm-Fo)/Fm. In healthy

leaves, this value is always close to 0.8, independently of the plant species studied. A lower

value indicates that a proportion of PSII reaction centers are damaged, a phenomenon called

photoinhibition, often observed in plants under stress conditions. Upon the application of a

second saturation flash in the presence of actinic light, the maximum fluorescence obtained

(Fm') is lower to that observed in the dark (Fm), the difference between Fm and Fm' reflects fluorescence quenching due to heat dissipation. The intrinsic quantum efficiency of PSII photochemistry [(Fv/Fm) = (Fm - Fo)/Fm], according to van Kooten and Snel (1990), is known to be linearly correlated with the level of PSII photo-inactivation in grapevine leaf (Hendrickson et al., 2003). Other parameters were calculated such as the effective quantum yield of PSII defined as [ΦPSII = (F'm - FS)/F'm], according to Genty et al., (1989), and thylakoid linear electron flux through PSII defined as (ETR = ΦPSII x PAR x 0.45 x 0.85), where ΦPSII is the fraction of absorbed PPFD absorbed by the antennae of photosystem II (Loreto et al., 1994), PAR corresponds to the flux density of incident PPFD [μmol(quantum) m^{-2} s^{-1}]. A value of 0.45 was used to correct for the proportion of light absorbed by PSII (Earl and Ennahli, 2004), and a factor of 0.85 was used in the ETR formula assuming that 85 % of the incident quanta are absorbed by the grape leaves (Schultz, 1996).

After performing gas exchange measurements, leaf water potential was measured biweekly with a Scholander-type pressure chamber (Model 1000, PMS Instrument Co., Corvallis, OR, USA) during the growing season until harvest. Individual leaves were excised and placed in plastic bags containing moist paper towels and measurements were performed in situ.

Vegetative growth: Two shoots per vine were randomly selected and tagged below the apical. Shoot length was measured at the end of each irrigation cycle, before alternating the irrigation cycle from the node above the tag to the shoot tip. Shoot growth rate was expressed in cm length growth per day (cm/day) and was calculated as the average increase in shoot length since the previous measurement. Light at the cluster zone where clusters are more concentrated was measured on sunny days at mid-day using a Sunflek Ceptometer (Model SF-40, Delta T Devices Ltd, Cambridge, UK) inserted horizontally at the cluster zone along the row. The values

of incident photosynthetic photon flux density (PPFD) were expressed in percentage of a reference PPFD, measured over the canopy top.

Fruit Maturity and Composition: Every two weeks, berries were collected from veraison until harvest date where berries become soft and take on the colors characteristic of their specific varieties. Two sets of 50 berry samples were collected randomly from each vine. Outer and inner berries from each grape cluster were taken. One set was immediately weighed and processed for berry maturity, and the second set was stored at –20 ^{o}C for later chemical analysis. Maturity analysis consisted of squeezing 50 fresh berries and centrifuging the resulting juice at 3500 rpm for 10 minutes. Juice was analyzed for total soluble solids concentration (SSC) using a hand-held refractometer (PR-32 Palette, ATAGO Co., Ltd., Tokyo, Japan). pH was measured with a pH meter (Accumet AB-15, Fisher Scientific, Pittsburgh, Pennsylvania, USA), and titratable acidity was done by titration with NaOH as recommended by Office International de la Vigne et du Vin OIV (OIV, 1990). It consists of titrating 5 ml of sample juice with 0.1 N NaOH (phenolphtalein is used as an indicator of the titration end point).

Two sets of 25 berries each were taken from the second frozen fifty-berry set, and homogenized. To calculate the concentration of total anthocyanin and phenolic content, one gram of slurry was extracted in 50% (v/v) EtOH (pH 2) for one hour and then centrifuged at 3500 rpm for 5 minutes. An aliquot of the ethanol extract was diluted 1:11 in 1 M HCl, and left to stand for 3 h. The absorptance of aliquots of the diluted HCl extract solution was determined by using a spectrophotometer (Thermo-Jarrel Ash Inc., Franklin, MA) at 520 nm and 280 nm for anthocyanins and phenolics, respectively.

ABA quantification: ABA quantification consisted of sampling leaf tissues every measurement day for the course of the experiment. Leaves were stored and transported in liquid

nitrogen to the laboratory. Leaves were ground and samples were maintained in a freezer (-70 $^\circ$C). Quantification was performed using the Mwang et al. (2003) technique.

Yield components and fruit quality and composition: At harvest, 50-berry samples from random clusters in each cultivar plot were collected. Berry weights were determined on these samples. Yield and cluster number were determined on a per vine basis. In 2006, harvest dates were 10 August for Concord and 31 July for Pinot Noir and Traminette. Heavy bird damage occurred in Pinot Noir cultivars, which caused 90% in yield loss of many replications in 2006. Each vine for which data were obtained was pruned in the dormant season, and weights of cane prunings were collected using a dairy scale as estimates of vine size.

Statistical analysis: Factorial analyses of variance (ANOVA), with year, sampling time and/or treatments as main factors, were used to test the main effects and factor interactions on the physiological, biochemical, and growth parameters evaluated. For multiple comparisons of treatments, standard errors and Fisher least significant differences (LSD) were reported.

Statistically, significant differences were assumed for $P < 0.05$ and statistical data analyses were performed with Statistica (SAS version 8.02 USA).

3. Results:

Environmental conditions, plant water status, and vegetative growth: There were large differences in rainfall distribution and temperatures during the growing seasons of 2005 and 2006 (Figure 3.1). The higher temperatures in the summer 2005 led to higher negative values of midday water potential (Ψ_l) for all watering regimes and cultivars, compared with 2006, which had moderate temperatures except late in the growing season. During both seasons, PRD vines maintained water potential values close to those of FI-treated vines. No significance difference between PRD and FI treatments at any measurement day in both seasons was observed. The FI-

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treated vines generally showed higher Ψ_l (more negative values) throughout the growing season in 2005 and slightly lower Ψ_l in 2006. However, PRD-treated vines showed a progressive decline in Ψ_l from July onwards, with higher Ψ_l values (more negative values) observed in 2005 compared with 2006 (Figure 3.2).

Shoot growth in 2006 was greater than in 2005. There was a significant effect between FI and PRD treatment on shoot growth rate in the 2006 growing season. In 2006, in the FI-treated vines, the shoot growth rates of Concord, Pinot Noir, and Traminette were 1.37 cm, 1.14 cm, and 1.17 cm per day respectively, compared with an average daily shoot growth rate of 1.21, 0.67, and 0.82 cm per day for Concord, Pinot Noir, and Traminette, respectively, in the PRD-treated vines. Concord had the largest shoot growth rate, while Pinot Noir had the smallest growth rate. Traminette was intermediate.

PRD vines, regardless of the variety, showed a reduction in stomatal conductance as the season progressed in both 2005 (Figure 3.3, D,E, and F) and 2006 (Figure 3.4 D,E, and F). Reduction reached values of 56%, 45%, and 47% in Concord, Pinot Noir, and Traminette, respectively, as compared to control vines in 2006. In 2005, the PRD treatment reduced stomatal conductance by 36%, 20%, and 46%, Concord, Pinot Noir, and Traminette, respectively. There was no significance difference among varieties in both seasons. FI-treated vines had the highest photosynthetic rate, but they were not significantly different from that of PRD-treated vines. In 2005, the FI-treated vines had an average photosynthetic rate of 13, 15, 15 μmol CO_2 s^{-1} in Concord, Pinot Noir, and Traminette, respectively, compared to PRD-treated vines, which had photosynthetic rates of 12, 14, and 14 μmol CO_2 s^{-1}, respectively. In 2006, the average A values were 13, 15, and 15 μmol CO_2 s^{-1} in Concord, Pinot Noir, and Traminette, respectively, compared to PRD-treated vines, which had A values of 12, 16, and 14 μmol CO_2 s^{-1},

respectively. Pinot Noir exhibited a higher photosynthetic rate than Concord and Traminette in two out of five measurement dates in 2005 and in one out of five measurement dates in 2006. PRD reduced intercellular CO_2 (C_i). The reduction was observed clearly in 2006, but there was no significant difference between irrigation treatments and varieties. Under dry conditions (that is, the 2005 season) estimates of C_i based on leaf gas exchange measurements are generally considered to be unreliable under conditions of mild stress when g_s is low as occurred in PRD-treated plants (Ennahli and Earl, 2005). C_i was overestimated due to patchy stomatal behavior induced by moderate mild stress (Ennahli and Earl, 2005). The midday value of ΦSII (Fv/Fm) remained close to 0.8 without any significant difference between treatments and varieties throughout both seasons (Figure 3.5). But the Concord variety showed a slight decline at the end of the dry season of 2005. Intrinsic water use efficiency, calculated as the ratio of photosynthetic rate to stomatal conductance rate, was higher in PRD-treated vines compared to controls. In 2005, the PRD treatment increased the intrinsic WUE by 33%, 26%, and 47% in Concord, Pinot Noir, and Traminette, respectively. In 2006, PRD increased WUE by 48%, 44%, and 52 % in Concord, Pinot Noir, and Traminette, respectively.

The reduction in vegetative growth observed in PRD-treated vines, compared to the control vines, resulted in a more open canopy as indicated by the increase in the PPFD received by the clusters in these vines (data not shown). The increase in light intensity inside the canopy of the PDR-treated vines was a consistent finding during the two seasons. In 2005, light intensity inside the canopy increased by 12%, 23%, and 21% in Concord, Pinot Noir, and Traminette, respectively, compared to control vines. In 2006, light intensity showed an increase of 32%, 41%, and 33% in Concord, Pinot Noir, and Traminette, respectively, above the bunch zone, compared to the fully irrigated control vines.

In neither year were there any differences in yield or its components (p>0.072) between PRD and the conventional irrigation. As for yield components, the number of clusters per vine was independent of soil moisture availability, and Pinot Noir had a higher number of clusters per vine than Concord and Traminette (Table 3.1). Fruit °brix, pH, titrable acidity, and pruning weight were not affected by either irrigation treatment in both growing seasons. Berry weight showed a varietal difference in both 2005 and 2006. Concord had a, higher weight compared to Pinot Noir and Traminette. In 2005, yield and °Brix showed a varietal difference. In general, the PRD treatment reduced slightly the pH and resulted in a higher content of total soluble solids in both seasons in the cultivars compared to the FI treatment. Pinot Noir exhibited slightly higher values of °Brix than Concord and Traminette in both seasons, and the values were higher in 2005 than 2006. Although no statistical difference was observed in pruning weight between treatments, Concord had a higher pruning weight in 2006 compared to Pinot Noir and Traminette, but not in 2005.

Berry composition at harvest changed with treatments. The PRD treatment induced an increase in concentration of total anthocyanins and total phenolics in comparison to levels found in FI-treated vines (Table 3.1). In Pinot Noir, anthocyanin accumulation was higher than that measured in Traminette.

4. Discussion

The present results corroborate other results and show that partial root-zone drying is an efficient method of deficit irrigation, because it reduces vigor and transpiration. It also has a positive effect on light interception in the cluster zone and on berry quality. We demonstrated that the physiological responses to PRD were similar among the three cultivars. However, most marked effects on physiological parameters were observed in Concord than in Pinot Noir and

Traminette in the dry season of 2005. This can be explained by its high sensitivity to water stress (Naor and Wample, 1994). Consistent with the evidence from other split root procedures (Davies et al., 2000; Zhang and Kirkham, 1995), water potentials of PRD vines did not differ from those of well-watered vines (Figure 3.2). There were significance differences among varieties in Ψ_l in 2005. These differences may be due to a genetic variance in response to a mild-water stress and heat stress, which occurred in 2005. In the summer of 2006, which had more moderate temperatures, there was no significance difference among varieties. These data provide evidence that the root system in the well-watered soil portion could supply adequate water to above ground biomass to prevent leaf water deficit and to maintain physiological and metabolic functions. The results also indicate that the PRD treatment did not impose a hydraulic limitation on these growth and physiological parameters. In contrast, drying the whole root system, where irrigation input is either reduced or withheld completely for specified periods of time, results in a decreased leaf Ψ (El-Ansary et al., 2005). These results support the hypothesis that a root-synthesize chemical (hormone) and not a leaf-sourced chemical (hormone) may be responsible for triggering the growth rate reduction in the PRD treated vines.

Field trials over two seasons provided evidence that PRD treatment did not affect photoassimilation rate while reducing stomatal conductance (Figures 3.3 and 3.4). This can be explained by the fact that the dynamics of the assimilation rate and stomatal conductance are different. Jones (1998) proposed that in well-adapted vines, stomata could play an important role in limiting water loss with relatively small role in determining the photosynthetic rate. Stomata remained partially closed on the PRD-treated vines compared to FI-treated vines, even though the leaf water potential was not affected. This implied that an additional mechanism was responsible for the stomatal response. This may be due to a prolonged effect of increased ABA

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in the leaves, which promotes the closing of opened stomata and inhibits the opening of closed stomata (Mishra et al., 2006). The stomatal closure caused a decrease in internal leaf CO_2 (C_i) due to reduced CO_2 availability.

A decrease in the ratio of Fv/Fm has been attributed to the inactivity of the PSII reaction centers due to the degradation of the D1 and D2 proteins responsible for the transfer of water electrons to chlorophyll a associated with the PSII reaction center (Lazár, 1999). Thus, the decline in the Fv/Fm ratio could be related to damage to the protein structures of the photochemical complexes of the thylakoid membranes. Therefore, the capacity of the plastoquinone complexes, responsible for the photochemical transport of electrons between photosystems I and II, to carry out oxireduction reactions may have been affected. However, the electron transport rate (ETR) of PRD-treated vines did not differ from FI-treated vines (data not shown), suggesting that the decline in Fv/Fm observed in the Concord variety in 2005 (Figure 3.5) was not significant and no damage was associated with photosynthetic apparatus. Although PRD-treated vines under the dry summer conditions of 2005 exhibited a reduction in C_i and g_s, there was no photoinhibition of photosynthesis at the level of the chloroplast. Similar findings were observed by Ennahli and Earl (2005) on cotton. The high tolerance of the photochemical apparatus of grapevines leaves to environmental stress including mild stress and high temperatures has been reported (Kadir, 2005).

Water use efficiency is reported to be affected by irrigation treatments and differences among cultivars (During, 1987). In both season there was no varietal effect on WUE but there was a significant irrigation effect. The minor change in assimilation rate associated with considerable reduction in stomatal conductance and transpiration increased water use efficiency in PRD-treated vines, on average, by more than 40% compared to control vines. Soil water

availability affected the seasonal transpiration efficiency, i.e. the ratio of net photosynthesis to water transpired over the growing season (Farquhar and Richards, 1984). For the deficit irrigation treatment (PRD), there was an increase in water use efficiency in the three cultivars in both seasons, when compared with the fully irrigated treatment. In addition, in most instances, the intrinsic water use efficiency (A/g_s) was different between PRD and FI-treated vines (Figure 3.3 and 3.4).

The results show that deficit irrigation treatment promoted an increase in water use efficiency as compared with full irrigation, in the short-term, as expressed by the A/g_s ratio or in the long-term as would have expected by the ratio of pruning weight and irrigation amount., since there was no significant differences in pruning weights between PRD and FI while irrigation was reduced along the growing. It was also obvious that the response to deficit irrigation varied with the grapevine variety and with the environmental conditions of the particular year. Differences between treatments were more marked in the dry year. In the dry year (2005), PRD induced lower (more negative) leaf water potentials than in the FI treatment. This resulted from reduced leaf area and higher midday stomatal closure in PRD than in FI. This suggests that stomatal closure in PRD vines had an effect on plant water status and on vegetative growth. The differences in stomatal control of water use efficiency between these varieties may be linked to the presence or absence of an interaction between hydraulic messages and root-to-shoot chemicals (hormones) (Tardieu and Simmoneau, 1998).

The role of the plant hormone abscisic acid (ABA) in regulating stomatal aperture and consequent plant water shortage has been studied widely in grapevines, both in pots and under field conditions (Stoll et al., 2000). Leaf-ABA increased in vines of 2005 compared to 2006. This may be due to the dry conditions that induced lower leaf water potentials. Hartung et al.

(2002) found that biosynthesis of ABA generally increases only when leaf water relations are significantly interrupted. (such as leaf water relations, leaf relative water content, and leaf stomatal conductance) This could explain the lower concentration of ABA in leaves for both the PRD- and FI-treated vines in 2006. The large increase in ABA concentration may be due mostly to an import from the root system. Loveys et al. (2002) showed that, after fully drying a root system of a vine, increases of leaf ABA concentration occurred in PRD-treated vines, and this presumably was due to in situ synthesis, stimulated by a reduced leaf water potential (which was observed in 2005), plus an import from the root system. However, in 2006, we should be aware that the hydraulic redistribution of water from the wet to dried part of the root system (Burgess et al., 1998) may contribute to dilution of root- ABA in the dry roots, which slowed the magnitude of stress and maintaining relatively higher water potential. Therefore, this reduced the production of root-ABA, which results in reduction of ABA in the leaf at the end of each PRD cycle.

Shoot growth rate was also reduced by the PRD treatment relative to that of controls in 2006 and 2005 season, without any effect on leaf water potential. Similarly, split-root experiments with other species also found that reductions in shoot growth occurred without any obvious decrease in leaf water potential (Ismail et al., 2002). PRD is generally associated with a reduction in vegetative growth and vigor (Santos et al., 2003). Restricted water supply from the roots can limit shoot growth and functioning (Davies et al., 2000). The growth rate of PRD-treated vines was lower than that of the FI-treated vines in 2005. In our study as well as that of Gu et al. (2004), shoot growth was measured but not leaf area. Vegetative reduction could be more marked in leaf area than in shoot length (Santos et al., 2003). The first PRD experiment (season 2005) was done on a vigorous vineyard and the soil water content prior PRD

implementation was higher, and it may account for the minor difference between PRD and FI treatments in vegetative growth. If soil water content was lower on the dry side of the PRD-vines under different soil and other climatic condition, as in 2006 growth season, it is more likely that the effect of PRD treatment would have been more pronounced. Our study and previous split-root studies indicate other factors than water may be involved in the growth inhibition, most probably, a regulatory mechanism not related to water potential controlled the shoot physiological responses in grapevines under the PRD treatment. Because leaf water potential could not account for the conductance and growth inhibition responses, evidence pointed toward a potential chemical constraint as a function of soil moisture availability. In addition, it has been demonstrated that shaded canopies, which result from an increase in growth over the season, may modify the ratio of red to far-red (R/FR) and PAR, which results in more internode elongation, and, thus, substantial shoot extension (Kurepin et al., 2007).

In our study, PRD irrigation maintained yield similar to that in the FI treatment. Other authors have obtained similar results, when they have compared PRD with FI treatments (Tang et al., 2005). The maintenance of yield in the PRD treatment resulted in increases in WUE. There are several possible reasons for the water saving and yield maintenance: 1) Partial stomatal closure may have reduced water use and at the same time maintained crop net photosynthesis, as stomatal resistance together with boundary layer resistance are the major controlling resistances for transpiration, while for CO_2 uptake mesophyll resistance is often the greater controlling resistance (Jones, 1992). However, it is unclear how effective partial stomatal closure is with respect to water saving as the transpiration of field crops is coupled to radiation (Jarvis and McNaughton, 1986). 2) A vegetative leaf area reduction in the PRD treatment may have caused less transpiration, but it appears high enough to maintain crop photosynthesis and crop growth

rate. In this way, more photosynthate can be used for fruit development as berries act as strong sinks. 3) The PRD treatment may promote root growth which maximizes the absorption of available water in the soil profile. Although we did not measure root growth, several studies have shown that PRD increases root depth and root density (Kang and Zhang, 2004), and higher root hydraulic conductivity was found locally by restriction of water supply (Poni et al., 1992). In addition, Smart et al. (2005) showed that grapes (*Vitis riparia* x V. *Berlandieri* cv 420 A) can redistribute water from a restricted zone of available water, laterally across the high resistance pathways of the trunk and into roots and soils on the non-irrigated side. This water redistribution into roots under the PRD condition may extend root longevity, increase the vine's water capacitance during periods of high transpiration demand, and enhance nutrient acquisition, all of which maintain shoot water status and better fruit development without detrimental effect on yield.

In both seasons, PRD resulted in a reduction in vegetative growth and improved canopy microclimate by improving light interception which led to improvement in fruit quality. Loveys et al. (2000) showed that improvements in berry composition are due to changes in metabolism such as increase in anthocyanin concentration (Dry, 2004) rather than an effect on berry size. Studies with a range of different *Vitis vinifera* varieties found PRD did not affect berry composition with respect to sugar accumulation (°Brix), pH, or titrable acidity (Antolin et al., 2006; Gu et al., 2004). In field-grown grapevines (*Vitis vinifera* cv. Castelao) PRD did not have any effect on yield, growth, and quality of grape berries compared to fruit of fully irrigated vines (Souza et al., 2005a). There was generally no difference in color at harvest (visual observations), total phenolics, and pH across the treatments, although pH was lower in PRD vines in 2005 than 2006. Increased light penetration into the canopy and a higher level of sun exposure lead to

improvement of some berry characteristics, such as higher concentrations of total anthocyanins in PRD vines (Souza et al. 2005b). Other researchers conclude that a better light penetration with PRD vines was not the only reason for increased anthocyanin concentrations with PRD vines. These vines also have more leaf area than vines drip irrigated with the same amount of water (Antolin et al., 2006).

Anthocyanins, which account for more than half of the overall of the total polyphenols, are directly responsible for the increase in polyphenols in red grape varieties (Ryan and Revilla, 2003). In both seasons, the total anthocyanin concentration was significantly higher (P<0.0001) in PRD-treated vines relative to control vines. Because there was no difference in berry weight of PRD-treated vines when compared to controls vines, it can be suggested that the changes in anthocyanin concentration were due to the irrigation treatment and not due to changes in the ratio of berry skin surface to the total berry volume. Summer temperatures may have affected the concentration of anthocyanins in the grape berries. The smaller concentration noticed in the summer 2005 can be due to the inhibition by the higher temperatures that prevailed during the whole growing season as compared to growing season of 2006. Laleh et al. (2006) observed that an increase in temperature is associated with a substantial degree in anthocyanin destruction. They observed a 60% destruction of anthocyanins in *Berberis orthobotrys* at temperatures above 15 °C. Mori et al. (2007) suggested that the decrease in anthocyanin accumulation under high temperatures resulted from factors such as anthocyanin degradation as well as the inhibition of mRNA transcription of the anthocyanin biosynthetic genes. The PRD treatment increased total phenolics in both seasons as compared to control vines. The increase in the concentration of total phenolics due to PRD was found to be due, in part, to an increased contribution of seed-derived proanthocyanidins and flavan-3-ol monomers (Loveys et al., 2002).

It is suggested that the main reason responsible for the increase of the total anthocyanins and phenolics in PRD vines is the better microclimate at the cluster zone as well as the percentage of exposed clusters (Santos et al., 2003).This suggests that the main impact of the type of irrigation was via the light interception and the overall microclimate in the cluster zone, which affected the vigor of the vines. The effect of light microclimate and canopy structure on fruit composition has been extensively studied (Downey et al., 2006). An increase in anthocyanin concentration in response to reduced canopy density and greater bunch exposure has been discussed Dokoozlian and Kliewer (1996). Dokoozlian and Kliewer (1996) found that shading impacted berry development and reduced the skin anthocyanin content. However there was also a slight decrease in carbon assimilation in these grapevines, which resulted in lower concentrations of other metabolites such as sugars and organic acids. This may explain why titratable acidity (TA) and pH are generally higher in FI-treated vines than PRD-treated vines, but differences were not statistically significant in our study.

5. Conclusion:

In conclusion, our results demonstrated that PRD can be used as an irrigation management strategy to save water. PRD can be used successfully to control sink-source relationships, maintaining yield, improving fruit quality, and in increasing WUE in relation to fully irrigated crops. This study reveals physiological differences between PRD-treated vines and FI vines (the deficit irrigation receiving equal amount of water as PRD, but distributed by the two sides of the root system). These differences include reductions in stomatal conductance in plants grown under the PRD treatment as compared to plants grown under the FI treatment, reduction in vegetative growth in the PRD vines when compared to FI-treated vines, and an increase in water use efficiency. Vegetative growth inhibition occurs in spite of similar plant water status in PRD plants, suggesting that phytohormones such as ABA control the physiological responses. No significant differences in photosynthetic rates or chlorophyll fluorescence parameters were found between the plants grown under the PRD and FI treatments. Vegetative growth reduction in the PRD treatment as compared to FI treatment led to an increase in light interception. Thus the clusters were exposed to more solar radiation, which may lead to some potential to improve fruit quality. The PRD treatment increased concentrations of berry skin anthocyanins and total phenols, compared to berries of plants grown under the FI treatment. Vine response to PRD was dependent on the variety and the environmental conditions during the growing season.
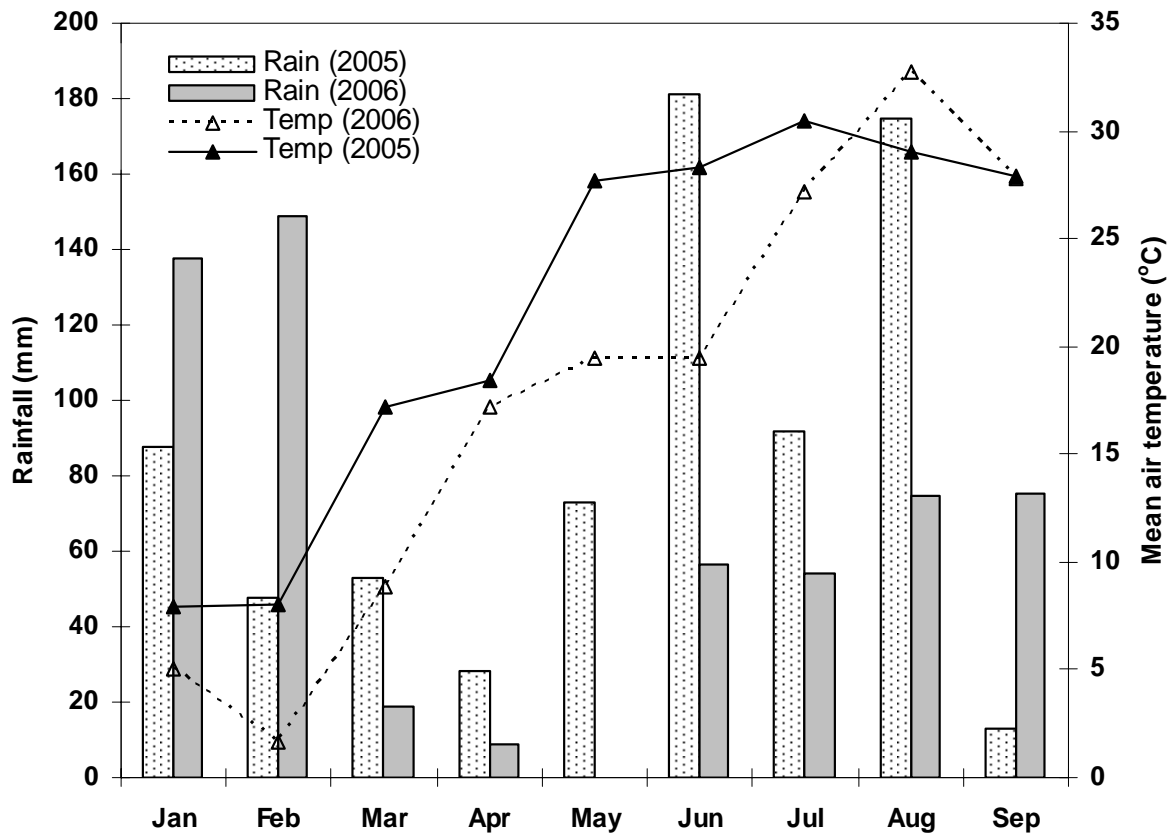
Figure 3.1: Total rainfall (bars) and monthly mean air temperature (lines) at the John C. Pair Horticultural Research site, during 2005 and 2006 season.
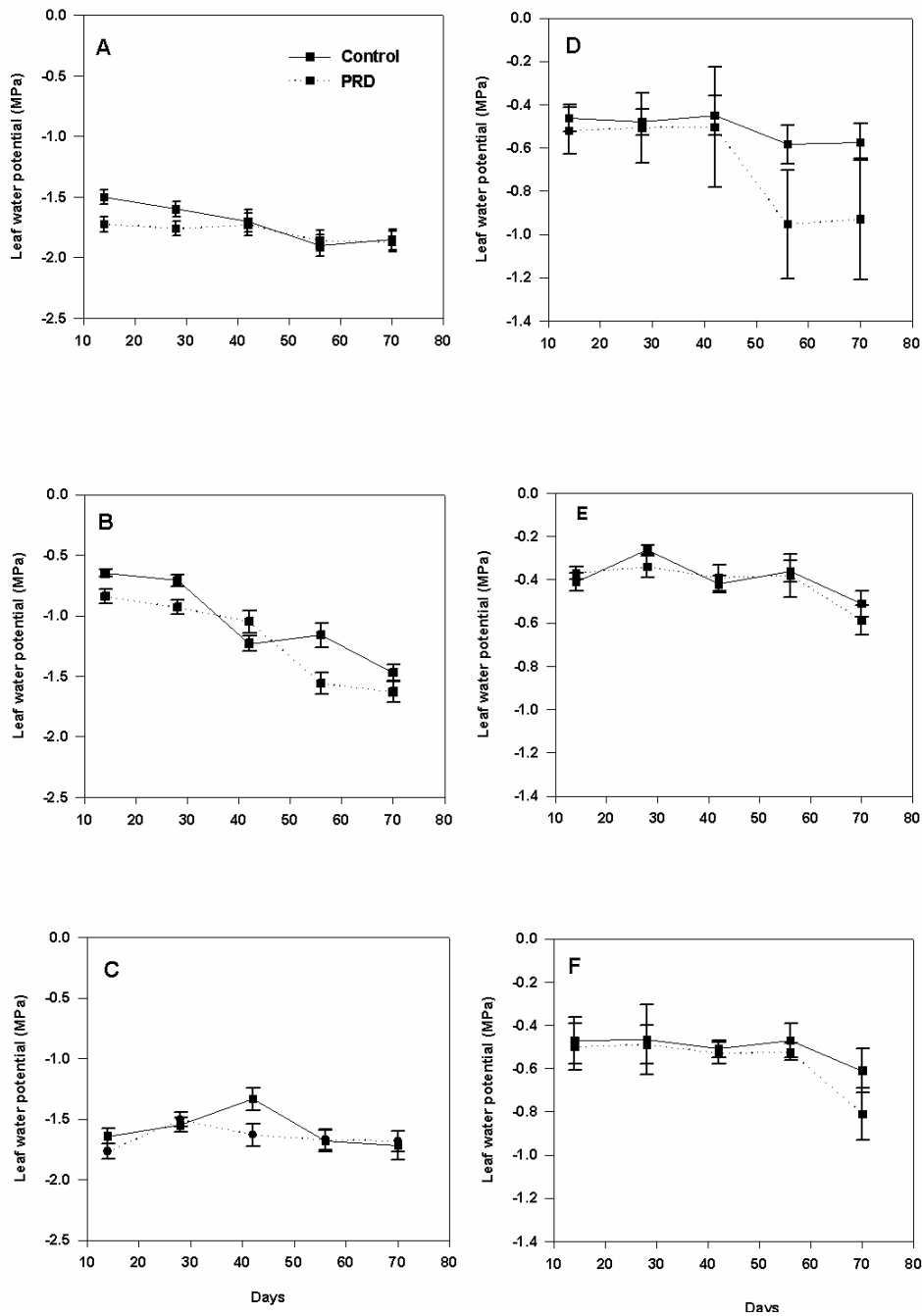
Figure 3.2: Seasonal evolution of midday leaf water potential for full irrigation (closed symbol and solid line) and PRD (closed symbol with dashed line) in Concord (A), Pinot noir (B), and Traminette (C) in 2005, and in Concord (D), Pinot noir (E), and Traminette (F) in 2006. Each point represents the average of 9 measurements with SE. Bars not visible indicate SE smaller than symbol. Asterisks demote significant difference among treatments at p <0.05. X-axis denotes number of days after the commencement of the experiment.
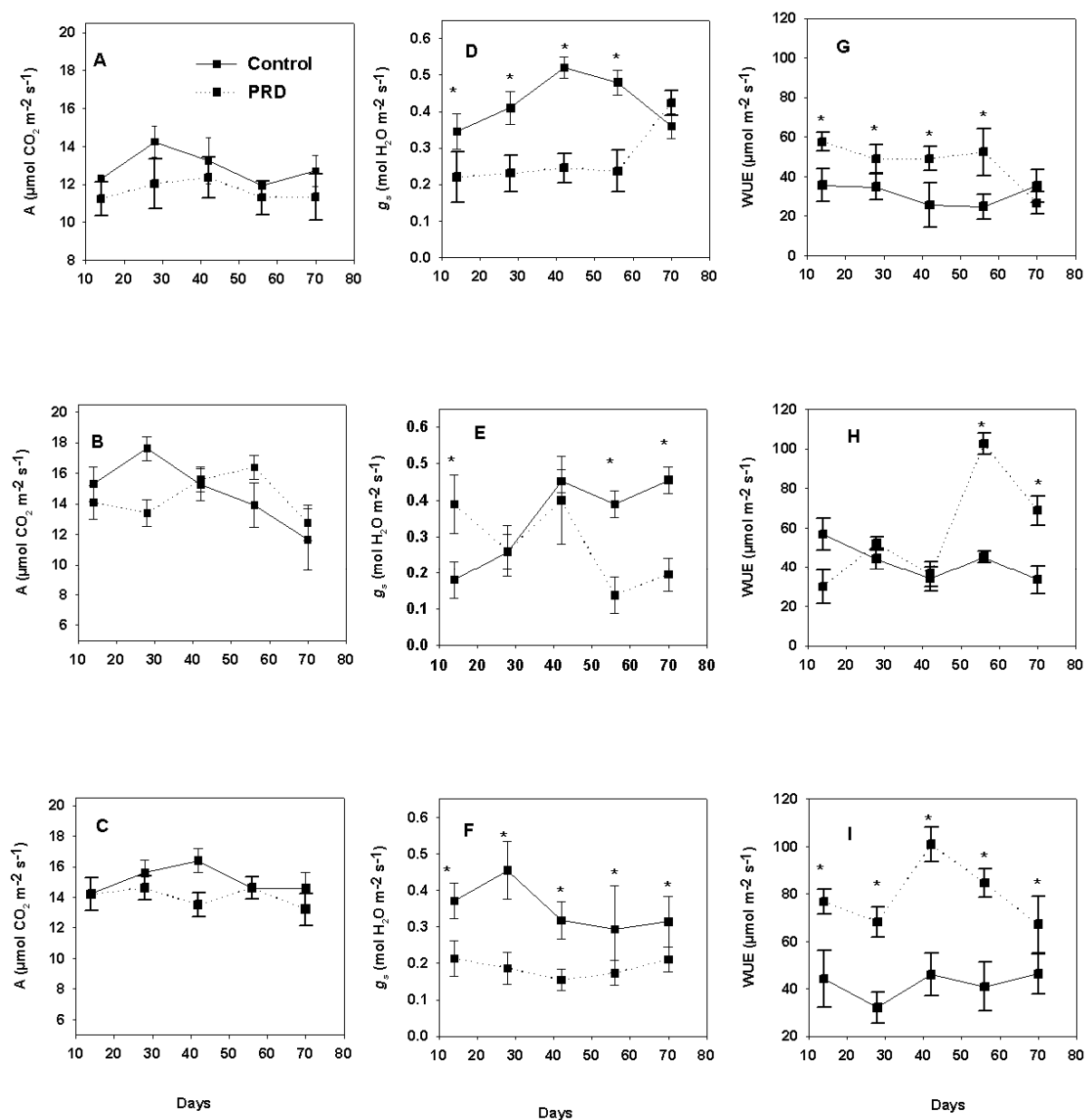
Figure 3.3: Evolution of photosynthesis (A), Conductance (g_s), and intrinsic water use efficiency (WUE) for PRD (dashed lines) and full irrigation (solid lines) in Concord (A, D, G),Pinot Noir (B, E, H), and Traminette (C, F, I) during the course of the experiment in 2005. Asteriks denote significant differences between treatment for each cultivar. Bars not visible indicate SE smaller than symbol. X-axis denotes number of days after the commencement of the experiment.
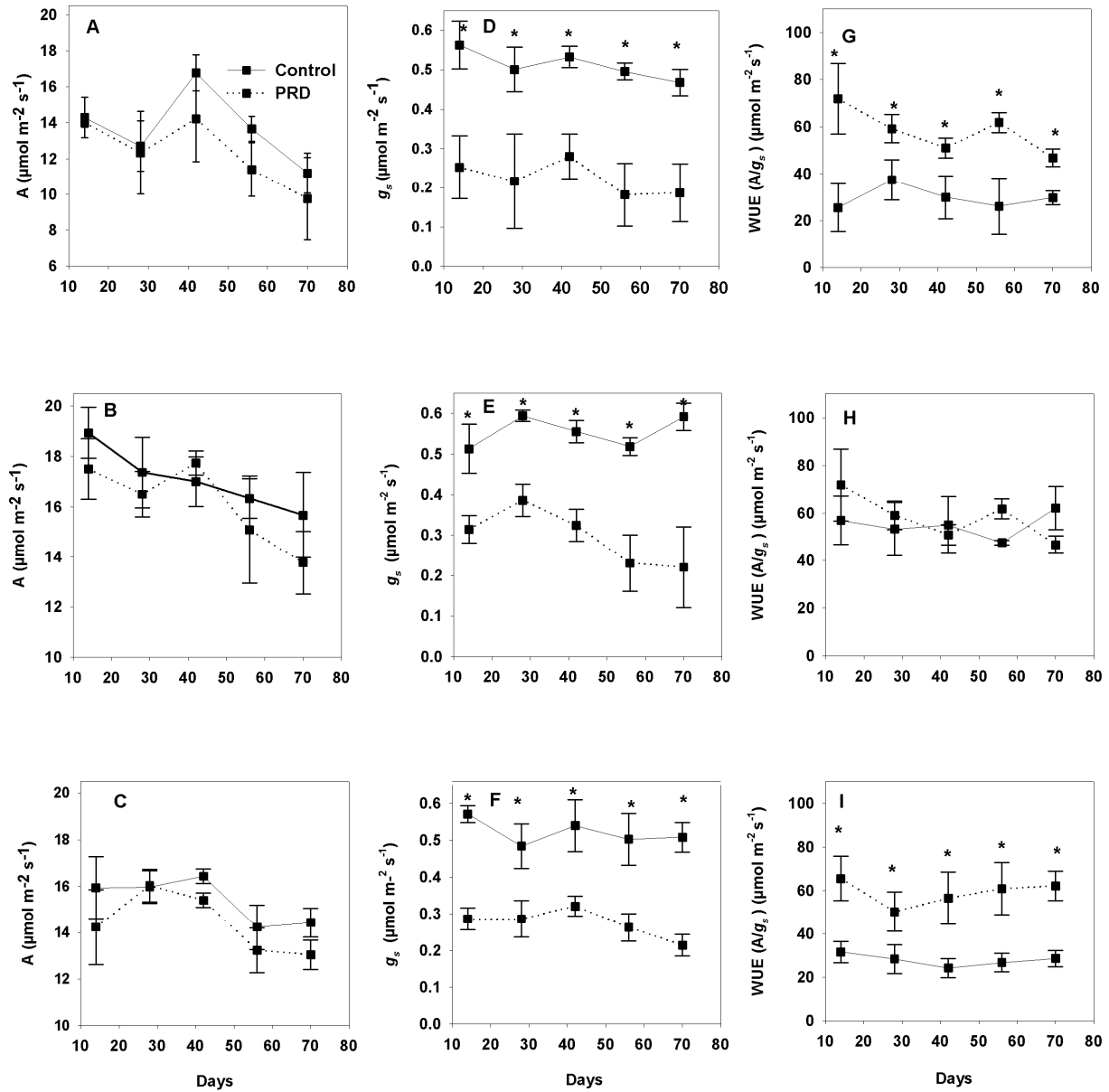
Figure 3.4: Evolution of photosynthesis (A), Conductance (g_s), and intrinsic water use efficiency (WUE) for PRD (dashed lines) and full irrigation (solid lines) in Concord (A, D, G),Pinot Noir (B, E, H), and Traminette (C, F, I) during the course of the experiment in 2006. Asteriks denote significant differences between treatments for each cultivar. Bars not visible indicate SE smaller than symbol. X-axis denotes number of days after the commencement of the experiment.
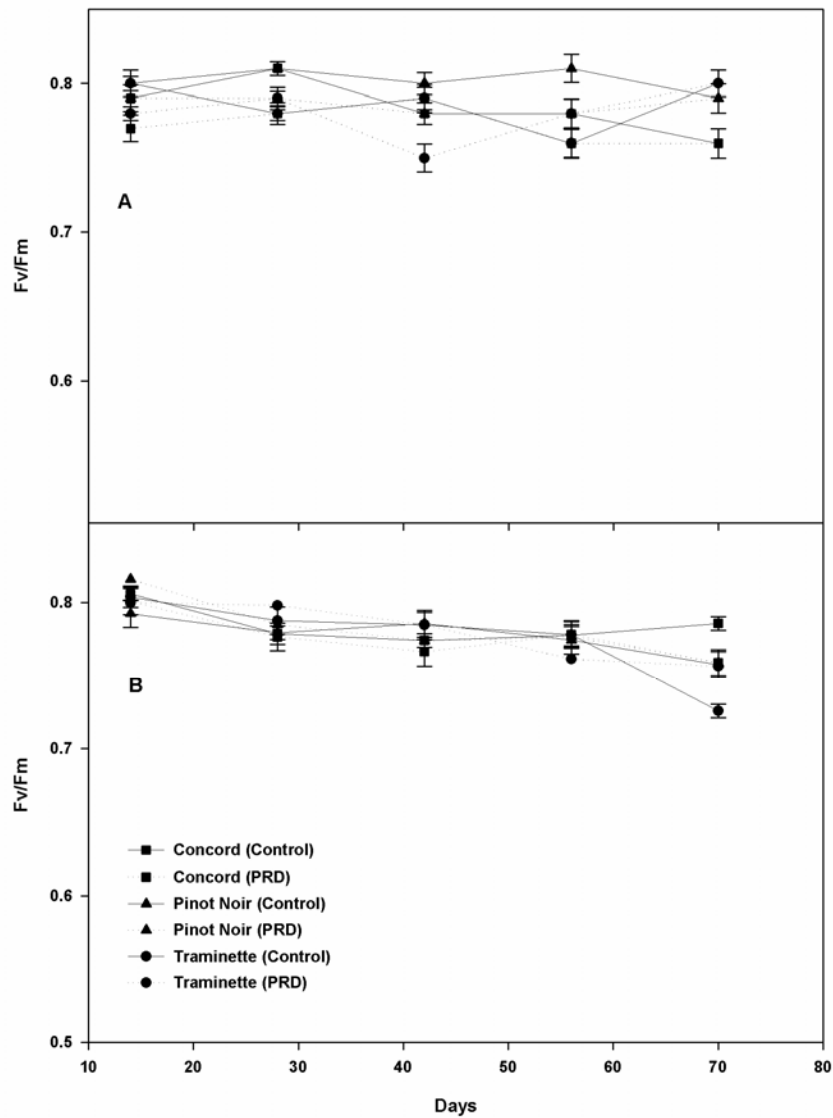
Figure 3.5: Seasonal trends of quantum efficiency (Fv/Fm) for PRD (open symbol) and full irrigation (closed symbol) in Concord (□),Pinot noir (Δ), and Traminette (O) in 2005 (A) and 2006 (B). Bars not visible indicate SE smaller than symbol. X-axis denotes number of days after the commencement of the experiment.

| Variable	Control			PRD			
	Concord	Pinot Noir	Traminette	Concord	Pinot Noir	Traminette	Sign.
2005							
TSS (Brix)	17.0	20.6	18.6	19.4	21.1	21.0	n.s
TA (g/L)	4.5	4.9	5.1	5.1	4.5	4.9	n.s
pH	3.6	3.4	3.6	3.4	3.5	3.4	n.s
Anthocyanins (mg/berry)	0.3	0.6	N.A	0.86	1.1	N.A	**<0.001**
Phenolics (AU/g)	1.2	1.5	1.5	1.1	1.5	1.2	n.s
# of cluster	83	90	78	92	131	124	n.s
Pruning weight (Kg/vine)	7.3	7.3	5.6	7.7	5.1	5.9	n.s
Yield (kg/vine)	14.6	9.7	20.3	19	9	34	n.s
Berry weight (g)	3.2	1.0	1.6	3.5	1.2	2	n.s
2006							
TSS (Brix)	15.0	15.3	16.1	15.2	19.6	18.6	n.s
TA (g/L)	5.3	5.7	4.5	5.3	4.8	5.1	n.s
pH	3.3	3.4	3.2	3.3	3.4	3.1	n.s
Anthocyanins (mg/berry)	0.53	1.02	N.A	1.5	1.8	N.A	**<0.001**
Phenolics (AU/g)	1.1	1.6	1.4	1.2	1.4	1.3	**<0.002**
# of cluster	N.A	N.A	N.A	N.A	N.A	N.A	N.A
Pruning weight (Kg/vine)	4.0	2.8	3.05	4.0	1.8	3.4	n.s
Yield (kg/vine)	14.1	10.9	30.6	25.3	13.1	41.5	n.s
Berry weight (g)	2.7	1.6	1.7	2.6	1.6	1.6	n.s

Table 3.1: Yield and fruit composition at harvest from Concord, Pinot Noir, and Traminette subjected to PRD and full irrigation.

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CHAPTER 4 - Seasonal and Diurnal Gas Exchange and Chlorophyll Fluorescence in Three Grapevine Cultivars under Field Conditions

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Abstract

Seasonal variation in rainfall and soil water availability may be important controls on the leaf gas exchange, growth, and phenology of grapevines, especially in areas that have pronounced seasonal variation in rainfall, soil moisture, and temperature. This study was conducted to examine the responses of three wine grape cultivars (American cultivar, European cultivar, and an American-European hybrid) to high temperatures, and to assess the potential for adoption of the European cultivars in south central Kansas. Pinot Noir had higher gas exchange parameters along with higher water potentials and chlorophyll fluorescence, while Concord had the lowest values in all three seasons and experienced a moderate stress by the end of the season. The dry condition in 2005 caused water potentials to decline sharply compared to values recorded in 2006. Pinor Noir and Traminette had maximum water potentials of -1.17 MPa and -1.1MPa, respectively, compared to -1.52 MPa in Concord. Photosynthetic rate decreased slightly during the summer with a slight recovery at the beginning of September, and did not differ across the seasons among cultivars. Stomatal conductance followed the same pattern. However, in both 2004 and 2005, stomatal conductance dropped sharply late in the season because of frequently occurring high temperatures that were greater than 32 ºC. Maximum morning values of Fv/Fm were within the optimal range. The diurnal course of Fv/Fm showed a rapid decrease in the morning. Then it became relatively constant and then increased again in the early evening.

Our results, thus, suggest the presence of marginal photoinhibition during mid-summer in Concord cultivars, because no full recovery of photochemical efficiency early in the evening was observed. The European cultivar (Pinot Noir) and the French–American hybrid (Traminette) are more adapted to field conditions in Kansas than Concord

Department of Horticulture, Forestry and Recreation Resources, Kansas State University, 2021 Throckmorton Plant Science Center, Manhattan, KS 66506.

*Corresponding author [soukaina@ksu.edu]

1. Introduction:

Cultivated grapevine is a heterogeneous species with an estimated 10–20 000 cultivars (Schultz 2003) grown from the cool temperate 50° north latitude, through the dry Mediterranean-type climates, to the tropics. The diversity of this species with respect to its tolerance to drought seems large, yet it has been generally classified as 'drought avoiding' (Smart and Coombe 1983) or as 'pessimistic' following the ecological classification of Jones (1980) into 'pessimists' and 'optimists'. The principal difference in strategies would be that 'pessimists' modify their growth and physiology to conserve current resources and to control their demand for future resources whereas the 'optimists' use all the resources available to them in expectation of more arriving.

Seasonal variation in rainfall and soil water availability may be important controls on the leaf gas exchange, growth, and phenology of grapevines, especially in areas that have pronounced seasonal variation in rainfall, soil moisture, and temperature (Borchert 1994). Drought that develops during the growing season can cause declines in photosynthesis and stomatal conductance (Schultz 2000), and trigger important phenological events such as leaf

shedding, flowering (Schultz 2000), and fruit maturity (Esteban et al. 2001) and decrease in yield. The rapidly falling yields in the *V. Labruscana* cultivars been attributed to changes in seasonal and diurnal weather patters and, specifically, to frequency of drought events during the fruit maturation phase (stage III) of grape berry development (Ontario Grape Growers Marketing Board (OGGMB), 2002).

Drought stress occurring during the growing season in response to low soil moisture and high temperatures and irradiation may lead to poor production, poor vegetative growth, increases diseases pressure (erineum mite and European spider mite), poor fruit composition (low sugar, low or high pH; impeded nutrient uptake), and poor wine quality (low acid and atypical aging) (depending on when in the season it occurs) (Cifre et al. 2005, Escalona et al. 2003). In midsummer, which corresponds generally to stages I and II of berry growth, vines require the greatest amount of water (Lakso and Pool 2001). At this stage, shoot growth is rapid and both shoot growth and early berry development are sensitive to water stress, and drought is detrimental because of decreased vegetative growth (Reynolds and Naylor 1994), fruit set, and overall yield (Matthews and Anderson 1988). Following the lag phase of berry development, water is required to maintain the fully developed canopy and allow subsequent cell expansion in developing berries. Water stress at or following veraison can lower berry weight and yield (Reynolds and Naylor 1994). Fruit composition may also be jeopardized by post-veraison water stress that delays fruit maturity, resulting in both restricted sugar accumulation and poor flavor development (Smart and Coombe, 1983). Water stress near harvest may reduce dry matter production and cause early leaf abscission (Fanizza and Ricciardi 1990). In climates with cold winters, carbohydrate reserves help prevent winter damage due to cold temperatures (Wolf 1988).

Grapevines are often grown in areas where plant water deficits develop and constrain vine growth, especially during the establishment of young vines. Grapes grown in Midwestern regions of the USA may experience cold winters and warm, dry summers. Following may be a spring season with a water-saturated soil profile. The soil progressively dries down during the summer season. Concomitantly, temperatures differ dramatically during the seasons, and those over 35 °C are not uncommon in Kansas during the summer. Therefore, the growing season is often characterized by low soil moisture and high vapor pressure deficit (Kadir 2005). It is generally thought that grapevines respond to adverse climatic conditions by a mechanism of drought avoidance rather than tolerance (Williams et al. 1994). An immediate response of grapevine is the decline in the photosynthetic activity, which is closely associated with a decline in stomata conductance (g_s) on both a diurnal and seasonal basis (Flexas et al. 1998). Summer ambient air temperatures and vapor air pressure deficit both can affect stomatal opening in grapevines (Escalona et al. 2003). Grapevine cultivars show different stomatal responses to water deficit (Escalona et al. 2003) and, consequently, have different rates of photosynthesis. Extensive reviews on stomatal function and response to environmental factors have been well published (Lovisolo et al. 2002). Stomata respond to a number of environmental variables: temperature, photosynthetic photon flux density, vapor pressure deficit, carbon dioxide concentration, water stress, and a host of tissue cellular and sub-cellular processes involving solute membrane characteristics, hormones, etc. The relations among the factors involved are complex and dynamic.

Grapes are being considered as a high crop value. Before Prohibition, Kansas was among the first 10 states in terms of grape production (Schueneman 1982), but declined after prohibition. However, in the past two years, grape cultivation and winemaking have resurged in

Kansas. Kansas provides optimal conditions such as enough sunlight, irrigation, fertile soils, and a dry climate. Although most commercial grape cultivars are American-type that are more sensitive to high temperatures and heat stress, there is always a need for new cultivars, such as the European types, which might have potential in some areas in the state. An example is Pinot Noir, a variety originating from Burgundy in France, which is the most expensive category of table wine produced in North America (Haeger and Storchmann 2006). Considering the importance of introducing new cultivars into Kansas, it is essential to evaluate responses of different cultivars to field conditions. Genotypes within grape species differ in response to high temperature that may develop during the growing season. Kadir (2005) found that the European cultivars are relatively less sensitive to high temperature than that of the American cultivars. Thus, there is a substantial genetic variability for the temperature traits. Therefore, this study was conducted to examine the responses of three wine grape cultivars (American cultivar, European cultivar, and an American-European hybrid) to high temperatures, and to assess the potential for adoption of the European cultivars in south central Kansas where summer temperatures are between 37 °C and 40 °C.

2. Materials and Methods

Plant Materials: The research was conducted during three seasons (2004-2006) under field conditions at the John C. Pair Horticultural Research Wichita, Kansas (Latitude, 37 °, 31 min, 15.3 sec North, Longitude 97º, 18 min, 56.2 sec West) on a Canadian fine sandy loam soil (Mollisols). Each plot consists of three grapevines spaced 2.4 m between rows and 2 m within rows. Vines consisted of three grape genotypes (*Vitis Vinifera* L, 'Pinot Noir'), *Vitis Labruscana* B, 'Concord,' and French American hybrid 'Traminette'. Pinot noir vines were trained to a low-cordon system, while Traminette and Concord vines were trained to a high-cordon system. The

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three varieties were selected from 24 cultivars being evaluated on the same vineyard, because, in addition to producing different wine types (white versus red), they are the most promising varieties in the wine region, and they contrast in precocity (Traminette starts vegetative growth earlier than Pinot noir or Concord) and in cold hardiness (Concord is the most cold-hardy).

Irrigation and Experimental Design: The irrigation system consisted of two lines, which were installed one at each side of the vines positioned 30 cm from the trunk. Irrigation water was applied with buried- and above-ground emitters (3 L/h), two per vine, positioned 30 cm from the vine trunk (out to both sides of the rows) and distributed on both sides of the root system. Water was monitored with flow meters placed in each irrigation line. Water was supplied twice per week from the beginning of berry development (June) until July, then 3 times a week from July to harvest. Cumulative rainfall during the growing season (mid-June until the end of August) was 194, 447, and 147 mm in 2004, 2005, and 2006, respectively. Figure 4.2 shows monthly rainfall and the mean air temperature at the experimental research station site during the 2005 and 2006 growing seasons.

Gas exchange and water relations: Photosynthetic CO_2 assimilation (A) and stomatal conductance (g_s) to water vapor were measured with an open gas exchange system (LI-6400, Li-Cor Inc., Lincoln, NE, USA) on 3 sun-exposed and fully expanded leaves of 3 individual vines per plot for each cultivar during the growing seasons of 2004, 2005, and 2006. Red-blue light-emitting diodes maintained incident irradiance at saturating levels (1200 μmol m^{-2} s^{-1}). The flow rate of air through the sample chamber was set at 400 μmol s^{-1}, and leaf temperature was maintained at 28 ± 0.8°C by the chamber thermoelectric coolers. The sample chamber CO_2 concentration was adjusted to 400 μL L^{-1} with the system's CO_2 injector (Model 6400-01, LI-COR). Although the relative humidity was not controlled directly, vapor pressure deficit (VPD)

varied between 1.5 and 2.0 kPa. After the leaf was enclosed in the cuvette, leaves were allowed to reach steady-state values (e.g., when the coefficients of variation of CO_2 and H_2O inside the chamber were < 5%) within 1-4 min. To assess the trade-off between CO_2 uptake and water loss, we calculated instantaneous water-use efficiency (WUE) as the ratio of photosynthesis rate to transpiration rate (A/E). Gas exchange was expressed on a one-sided leaf-area basis for all cultivars. Grapevines leaves were larger than the cuvette, so the default area of 6 cm^2 was used for all calculations.

Leaf chlorophyll fluorescence was measured by a pulse-modulated fluorometer (Fluorescence Monitoring System [FMS-1]; Hansatech Instruments Ltd., Norfolk, U.K.). The FMS-1 requires no dark adaptation of the leaf because it separates actinic light from the fluorescence signal by using modulated fluorometery. Measurements consisted of exposed a leaf section to a LED light source causing excitation of a pulsed fluorescence signal in the absence of actinic light. Four different values were calculated when measuring chlorophyll fluorescence for this experiment; F_o, F_m, F_v, and F_v/F_m. F_o is the initial chlorophyll fluorescence level. F_m represents the maximum fluorescence yield. F_v, or variable fluorescence level, is calculated by simply subtracting F_o from F_m. From the previous values we can calculate F_v/F_m, the PSII efficiency

Leaf water potential (Ψ_l) measurements were conducted with a Scholander-type pressure chamber (Model 1000; PMS Instrument Co., Corvallis, OR, USA) during 2005 and 2006 until harvest. Measurements were taken at 0700 h, midday at 1200–1400 h, and at 1900 h immediately following leaf gas exchange measurements in the field. Individual leaves were excised and placed in plastic bags containing moist paper towels and measurements were performed in situ.

Data were collected based on days which correspond to specific berry growth stage (Figure 4.1).

Statistical analysis: Factorial analyses of variance (ANOVA), with year, sampling time and/or treatments as main factors, were used to test the main effects and factor interactions on the physiological and biochemical parameters evaluated.

Results and Discussion:

Seasonal changes in environmental conditions within the experimental site are shown in Figure 4.2. Mean daily air temperature fluctuated around 25 °C in June–August in 2005, and it became cooler in September. Periods of heavy rain occurred at the beginning of June, followed by a scattered rainfall until early August when more frequent rainfall occurred. In 2006, June and July averaged a temperature of 15 ºC and 20 ºC, respectively. Maximum temperature of the summer season occurred in August with an average of 32 ºC before temperatures became cooler in September. Rainfall was two times less than in 2005, and most rain occurred at the end of August and in early September.

Leaf water potential (Ψ_l) has long been used as an index of water stress, because it reflects a combination of many factors such as vapor pressure deficit, leaf intercepted radiation, soil water availability, internal plant hydraulic conductivity, and stomatal regulation. Midday and early morning leaf water potentials were measured only in 2005 and 2006. The higher temperatures in the summer of 2005 led to higher midday water potentials (more negative values) (Ψ_m) for all cultivars (Figure 4.3 B). The season of 2006 was marked by relatively warm temperatures until late August. This resulted in relatively lower Ψ_m potentials. They can be explained by lower vapor pressure deficits due to warm temperatures. At the end of the season, Concord had the highest water potential, but it was not significantly different from Pinot Noir

and Traminette. Grapevine cultivars did not exhibit any differences in water potential in 2006 across the season. In 2005, Concord and Traminette showed a higher midday potential late in the late season compared to Pinot Noir (Figure 4.3 B). Maximum midday water potential recorded in Concord, which experienced a moderate stress, are in agreement with other studies (Naor and Wamble 1994). The dry condition in 2005 caused water potentials to decline sharply compared to values recorded in 2006. Pinot Noir and Traminette exhibited a mild stress, while Concord exhibited a moderate stress in 2005. Pinor Noir and Traminette had maximum water potentials of -1.17 MPa and -1.1MPa, respectively, compared to -1.52 MPa in Concord. We conclude from our study that Concord is the most sensitive among the varieties. Naor and Wamble (1994) found that leaf water potential of irrigated Concord vines decreased from -1.0 MPa to -1.6 MPa during the growing season under field conditions. However, leaf water potential is variable, depending on the local climate and is related to VPD and ambient temperature (Williams and Baeza, 2007). The diurnal fluctuations of Ψ_l in response to VPD have been reported in previous studies conducted on grapevines (Smart and Coombe 1983). We believe that the high temperatures had the most impact on Ψ_l. Grapevine is a water-stress-avoiding species (Chone et al. 2001), and stomatal conductivity is regulated to control leaf water deficit. It maintains midday leaf water potential at a constant value (Naor 1998). Diurnal time courses of leaf water potential were typical, declining during the day and with partial recovery in the late afternoon at about 19:00 h (Figure 4.4). The differentials between leaf water potentials in the morning and midday were highest at peak temperatures and changed with cultivars ($P<0.0001$). Midday values were lower than early morning values for the three species ($P<0.001$). Leaf water potential of vines decreases to maintain transpiration (Tognetti et al. 2004).

Measurement of early morning water potential on vines provides a good estimate of the soil moisture status within the vineyard (Figure 4.3 A), if one assumes that vines are in equilibrium with soil water potential (Tognetti et al. 2007). Caution is needed with this hypothesis because differences in water potential observed early in the morning may not necessarily reflect the water status of vine later in the day, because water potential may come into the equilibrium with the wettest portion of the soil in the plant root system (Ameglio et al. 1999).

The seasonal variations of A and g_s in response to environmental conditions are similar, and this results in the close coupling of gas exchange components (Moriana et al. 2002). Photosynthetic rate decreased slightly during the summer with a slight recovery at the beginning of September (Figure 4.5). Photosynthetic rate at the beginning of summer did not differ across the seasons among cultivars. The soil volume wetted during the spring, resulting from rainfall events, occurred at the beginning of summer of 2004 and 2005, and the rainfalls wetted the entire root system. This avoided restriction of canopy gas exchange due to chemical signals (hormones) produced in roots under drought (Wahbi et al. 2005). Thus photoassimilation rates in the spring were higher than those observed during the rest of the season. Pinot Noir always had the highest photosynthetic rate followed by the hybrid Traminette and then Concord. Stomatal conductance followed the same pattern. However, in both 2004 and 2005, stomatal conductance dropped sharply late in the season because of frequently occurring high temperatures that were greater than 32 ºC. In 2004, g_s of Concord, Pinot Noir, and Traminette decreased from 0.28, 0.37, and 0.47 mol H_2O m^{-2} s^{-1} to 0.05 mol H_2O m^{-2} s^{-1}, respectively, while in 2005, only Concord showed a sharp decrease late in July. Its stomatal conductance decreased from 0.30 to 0.13 mol H_2O m^{-2} s^{-1} and then recovered partially to 0.20 mol H_2O m^{-2} s^{-1} in early

September. Cultivars exhibited differences in stomatal conductance (P<0.0001) in the month of July in both 2005 and 2006. Concord had a lower g_s compared to Pinot Noir and Traminette. The 2006 growing season was marked with moderate temperatures. In 2006 no significant differences in photosynthetic rate and g_s among varieties were observed, although Pinot noir had higher values for these parameters than did Traminette and Concord. The decline observed at the end of July was less than that observed in 2004 and 2005.

Many factors have been implicated in the decrease of g_s during the day (Correia et al. 1995). In our experiment, the sharp decrease in g_s late in the season may be associated with the increase in VPD. The decrease in Ψ_l observed late in the season (Figure 4.3) in all years may also have affected the diminution in g_s through an increased stomatal sensitivity to abscisic acid (Tardieu and Davies 1993). However, Correia et al. (1995) found that: (i) changes in g_s normally preceded changes in Ψ_l; (ii) plants under water stress had higher values of Ψ_l in the afternoon than well-watered plants; (iii) g_s decreased although Ψ_l remained constant after mid-morning. Kriedemann and Smart (1971) did not find a reduction in g_s as leaf water potential decreased until it reached -1.3 MPa, when g_s sharply decreased.

Summer temperatures not only decreased A and g_s but also changed the diurnal patterns of gas exchange (Figure 4.6). Well-watered plants in a cool climate show a dome-shaped diurnal course of A, paralleling the course of global radiation (Tenhunen et al. 1987). Under hotter and drier conditions, as at our experimental site, this pattern changed. In June, the diurnal course of A followed the typical pattern with a midday depression. This has also been reported for oak by Epron et al. (1992). In July, August, and September, A was greatest in the morning and did not recover to the morning values following the midday depression. The same afternoon decline in A was observed in the species of Strawberry Tree (*Arbutus unedo*) (Tenhunen et al. 1982). Two

factors are implicated in the reduction of A in the afternoon for similar photon flux density

(PFD) conditions. First, there is greater sensitivity of leaf gas exchange to air temperature and

VPD under summer conditions (Tenhunen et al. 1987). Thus there are higher losses by

photorespiration caused by an increase in ambient temperature (Zufferey et al. 2000). Second, g_s

decreases partly due to an increase in VPD (Düring 1976). This is characteristic of semi-arid

environments. An increase in VPD is correlated with an increase in temperature from morning to

afternoon (Grimes et al. 1987). Many other environmental variables, such as irradiance and

temperature may interact with soil water depletion and cause a reduction in photosynthesis and

alteration of the relationship between A and g_s (Epron and Dreyer 1990). At the molecular level,

the decrease in photosynthetic rate observed in our study can be explained by the decrease in the

Rubisco activation state. It has been reported that the activation state of Rubisco decreases at

high temperature (Kobza and Edwards 1987). Crafts-Brandner and Salvucci (2000) showed that,

when leaf temperature exceeded 35 ºC, the photosynthetic rate in cotton was lower than that

expected from Rubisco kinetics. The decrease in photosynthesis is ascribed to a decrease in the

Rubisco activation state (Crafts-Brandner and Salvucci 2000, Salvucci and Crafts-Brandner

2004a). Inactivation of Rubisco at high temperatures may involve a decrease in activity of

Rubisco activase (Crafts-Brandner and Salvucci 2000, Salvucci and Crafts-Brandner 2004a) and

an increase in the synthesis of xylulose-1,5-bisphosphate, which inactivates Rubisco (Salvucci

and Crafts-Brandner 2004b).

Intrinsic water use efficiency (A/ g_s) increased as g_s decreased (Figure 4.7). A/ g_s as a

function of g_s, followed the same pattern in 2004 and 2005. A similar response in grapevines was

reported by Cuevas et al. (2006) and Cifre et al. (2005). Under the field conditions, vines

experienced water stress mainly at the end of the season ($g_s < 50$ mmol m^{-2} s^{-1}). Water stress

causes both A and g_s to decrease and the g_s response is more sensitive. An increase in A/ g_s is a mean to offset the decrease in A as stomata close. When g_s is higher than 300 mmol m^{-2} s^{-1}, differences between A/ g_s in the morning, at midday, and in late afternoon tend to be less (Figure 4. 7). As water stress increased, differences in A/ g_s in the morning, midday, and afternoon became more evident.

Fv/Fm is a good index of photoinhibition in grapevines, and its decrease is generally attributed to damage in PSII (Nunez-Olivera et al. 2006). In our study, maximum morning values of Fv/Fm were within the optimal range reported by Björkman and Demmig (1987) and they occurred early in the summer in all three years. This suggested that there was relatively low stress during these periods (Figure 4.8). Indeed, many studies have described reductions in photochemical efficiency and low photosynthetic rates during summer droughts (Valladares and Pearcy 1997). Conversely, predawn values of Fv/Fm in July in every year were lower than 0.78, with a minimum values of 0.67 ± 0.02 for Pinot Noir, 0.62 ± 0.02 for Traminette, and 0.57 ± 0.01 for Concord. These values are in agreement with previous studies on Mediterranean vegetation, which also reported lower Fv/Fm values during the growing seasons (Oliveira and Peñuelas 2001). The diurnal course of Fv/Fm showed a rapid decrease in the morning. Then it became relatively constant and then increased again in the early evening. Our results, thus, suggest the presence of marginal photoinhibition during mid-summer in Concord cultivars, because no full recovery of photochemical efficiency early in the evening was observed. Persistent reductions in PSII efficiency are attributed predominantly to the retention of de-epoxidized components of the xantophyll cycle (zeaxanthin and antheraxanthin) and their sustained presence for energy dissipation activity. This may help to protect PSII from over-excitation and photodamage (Kyparissis et al. 2000). Nevertheless, sustained reductions of

Fv/Fm values may also result from accumulation of non-functional PSII reaction centers and partial photoinactivation of PSII (Niyogi 1999). In mid- summer, the relatively high assimilation rates of Concord varieties favor the hypothesis that the observed photoinhibition was due to photoprotection rather than photodamage.

Higher net photosynthetic rates in Pinot Noir and Traminette than in Concord early in the summer would create a higher photochemical sink for the electrons, which protect the photosynthetic apparatus against the accumulation of excessive excitation energy. In addition, the high values of Fv/Fm measured throughout the day, and their almost complete recovery in the evening suggest that no photoinhibition or chronic damage occurred to vines in this study. A transient decrease in PSII efficiency did occur. But that is a general feature of photosynthesis in natural environments, and it is thought that a down-regulation maintains a balance between light-driven electron flow and requirements for reducing power. These findings suggest that the vines in this experiment were well adapted to their environment and that the environmental conditions that they experienced during the experiment were not so adverse as to cause photoinhibition. Absence of photoinhibition has been frequently found in field-grown vines (Flexas et al. 1998).

4. Conclusion:

The main conclusion from this trial is that, even when vines were well supplied with water, A, and A/ g_s decreased from morning to afternoon. This was partly due to the reduction in A and g_s. Value of these measurements increased as water stress increased. In summer, regardless of soil water content, a relative water stress developed in the vineyard late of the growing season as a consequence of higher ambient temperatures and VDP. Pinot Noir had higher gas exchange parameters along with higher water potentials and chlorophyll fluorescence, while Concord had the lowest values in all three seasons and experienced a moderate stress by

the end of the season. The European cultivar (Pinot Noir) and the French–American hybrid (Traminette) are more adapted to field conditions in Kansas than Concord. Concord is reported to be sensitive to water stress, which we also observed in our experiments. Pinot Noir is well known to be very sensitive to cold. During the three years, it has been observed that the survival of Pinot Noir vines was lower than that of Traminette and Concord (data collected by others). Therefore, we recommend Traminette as a potential candidate for field conditions in the Midwestern part of the USA.
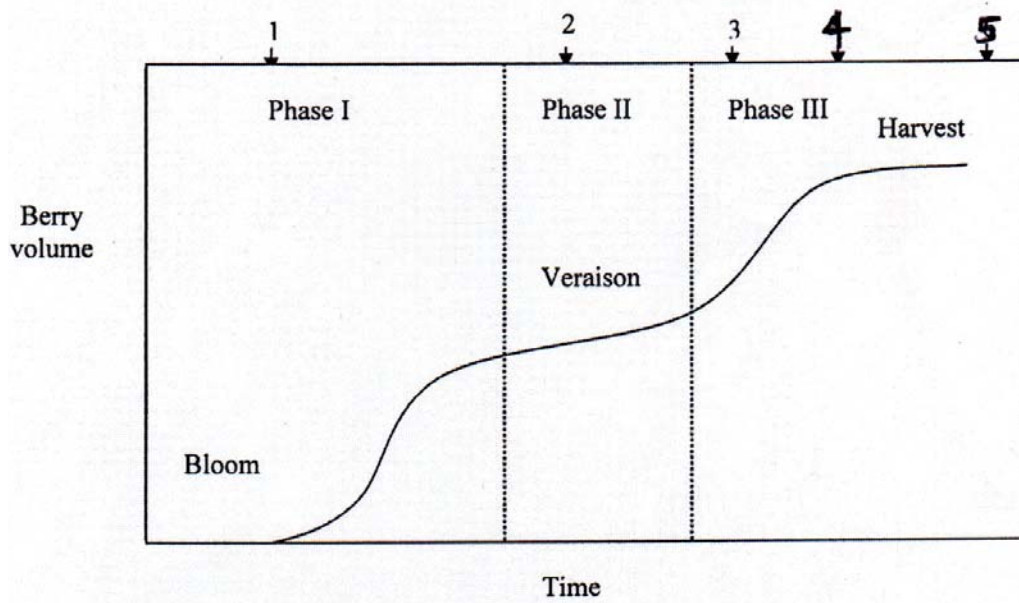
Figure 4.1: Experimental calendar and irrigation schedule superimposed on theoretical curve of berry growth. Arrows at the top indicate approximate periods of gas exchange measurements: 1: fruit set, 2-pre-veraison, 3- post-veraison, 4-pre-harvest, 5- post harvest.
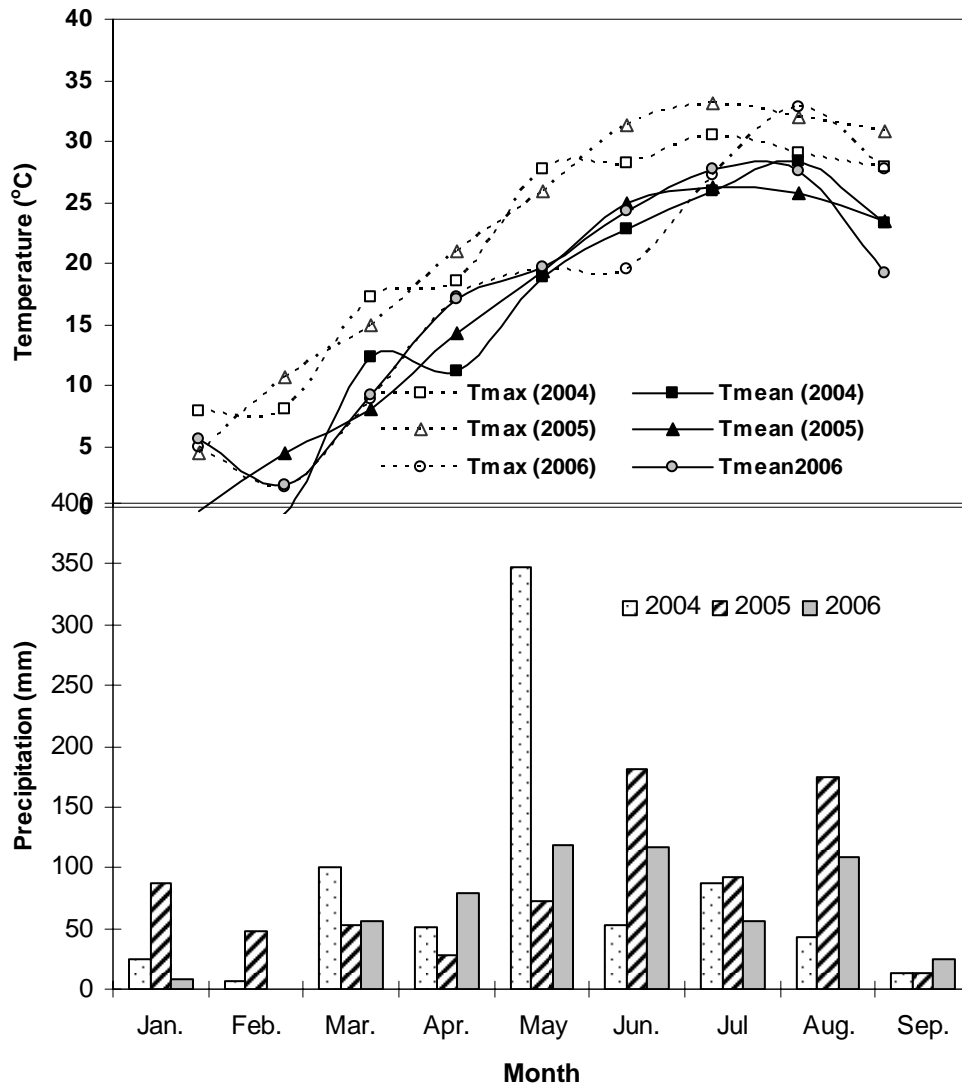
Figure 4.2: Total rainfall (bars) and monthly mean air and maximum air temperature (lines) at the John C. Pair Horticultural Research site, during 2004, 2005, and 2006 growing seasons.
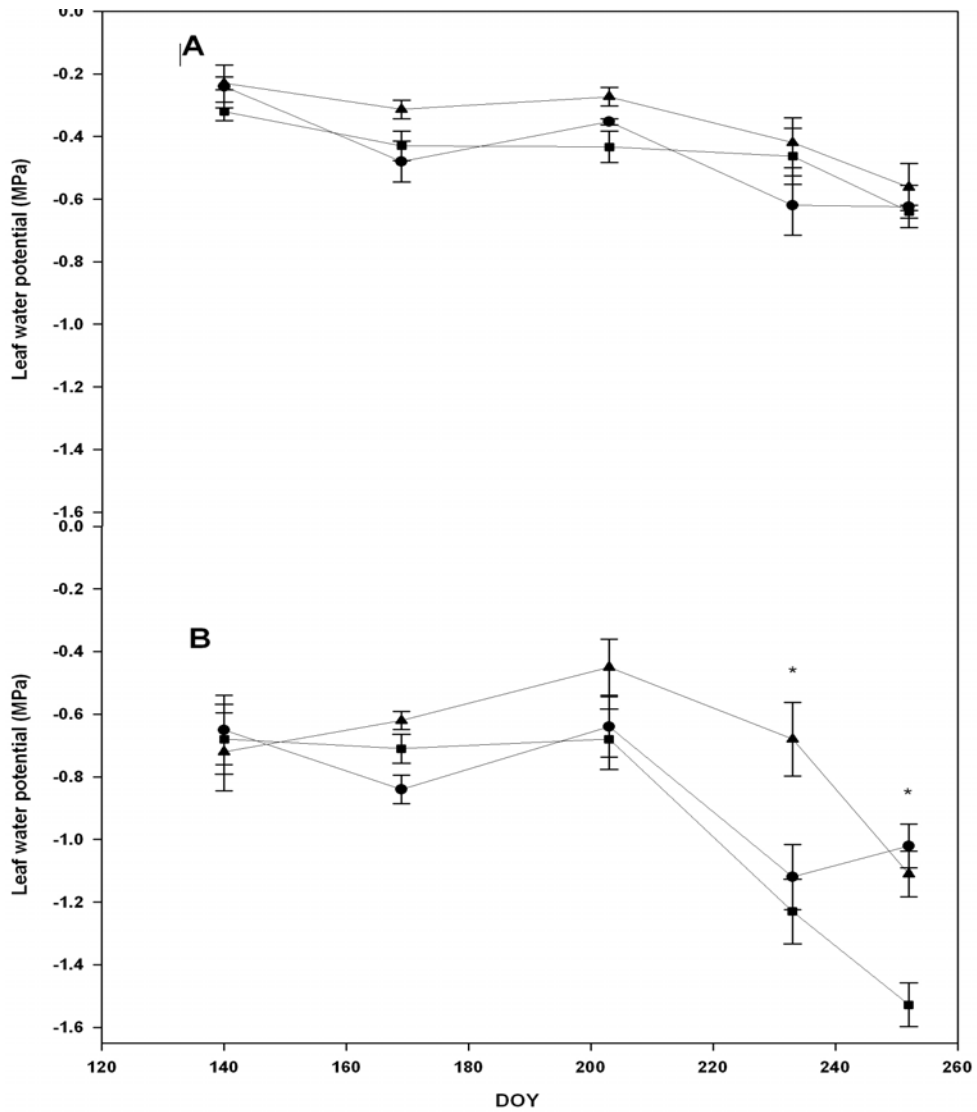
Figure 4.3: Seasonal pattern of early morning and midday leaf water potential of grapevines cultivars concord (square), Pinot noir (triangle), and Traminette (circle) in 2005 growing season. Bars not visible indicate se smaller than symbol. Asterisks denote significant difference among varieties at p<0.05. DOY: Day of the year. Asteriks denote significant differences between cultivars.
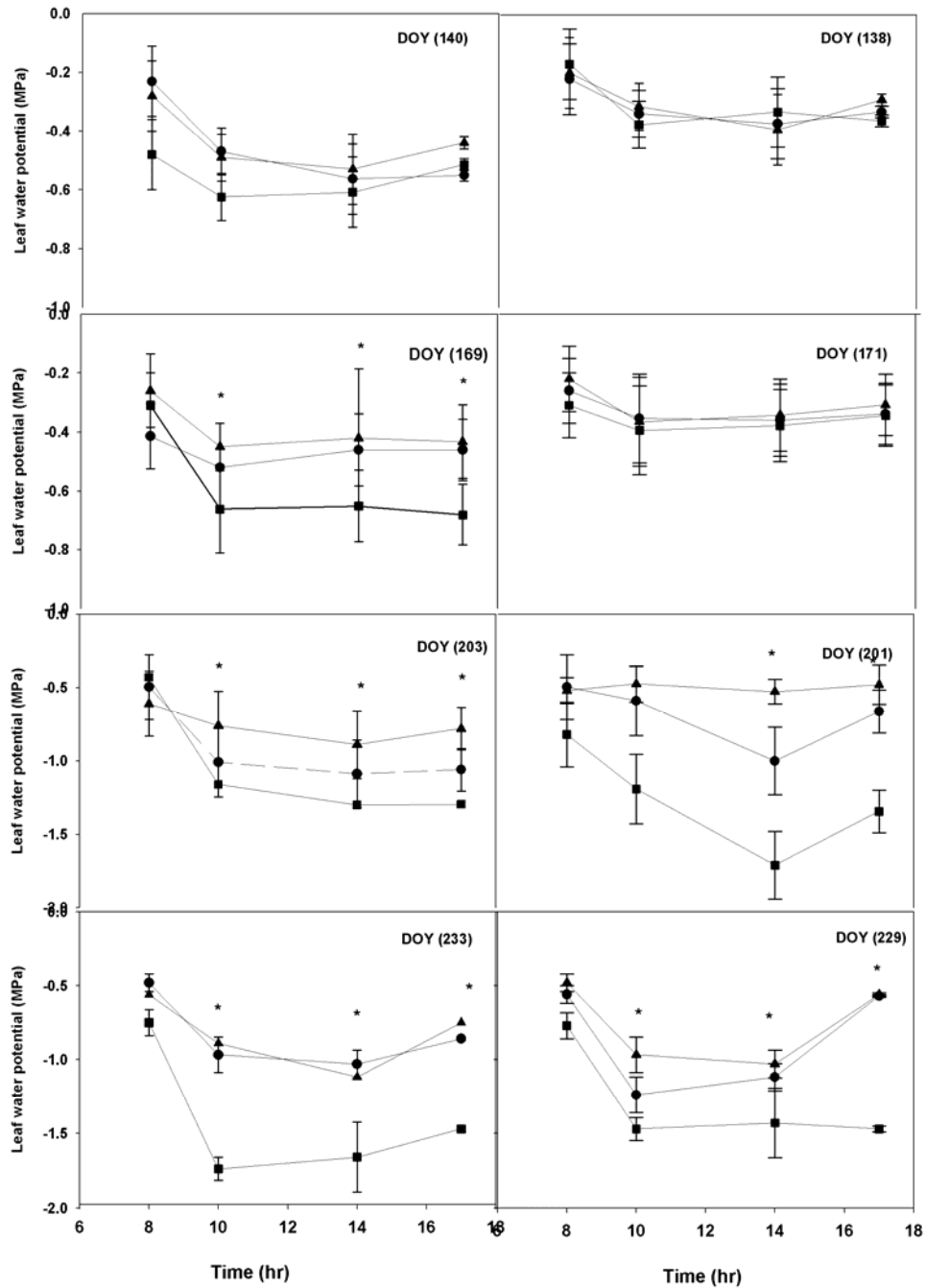
Figure 4.4: Diurnal course of leaf water potential measured at different time (DOY) in grapevines cultivars concord (square), Pinot noir (triangle) and Traminette (circle) in 2005 (A) and 2006 (B) growing season. Bars not visible indicate se smaller than symbol. Asterisks denote significant difference among varieties at p<0.05. DOY: Day of the year Asteriks denote significant differences between cultivars.
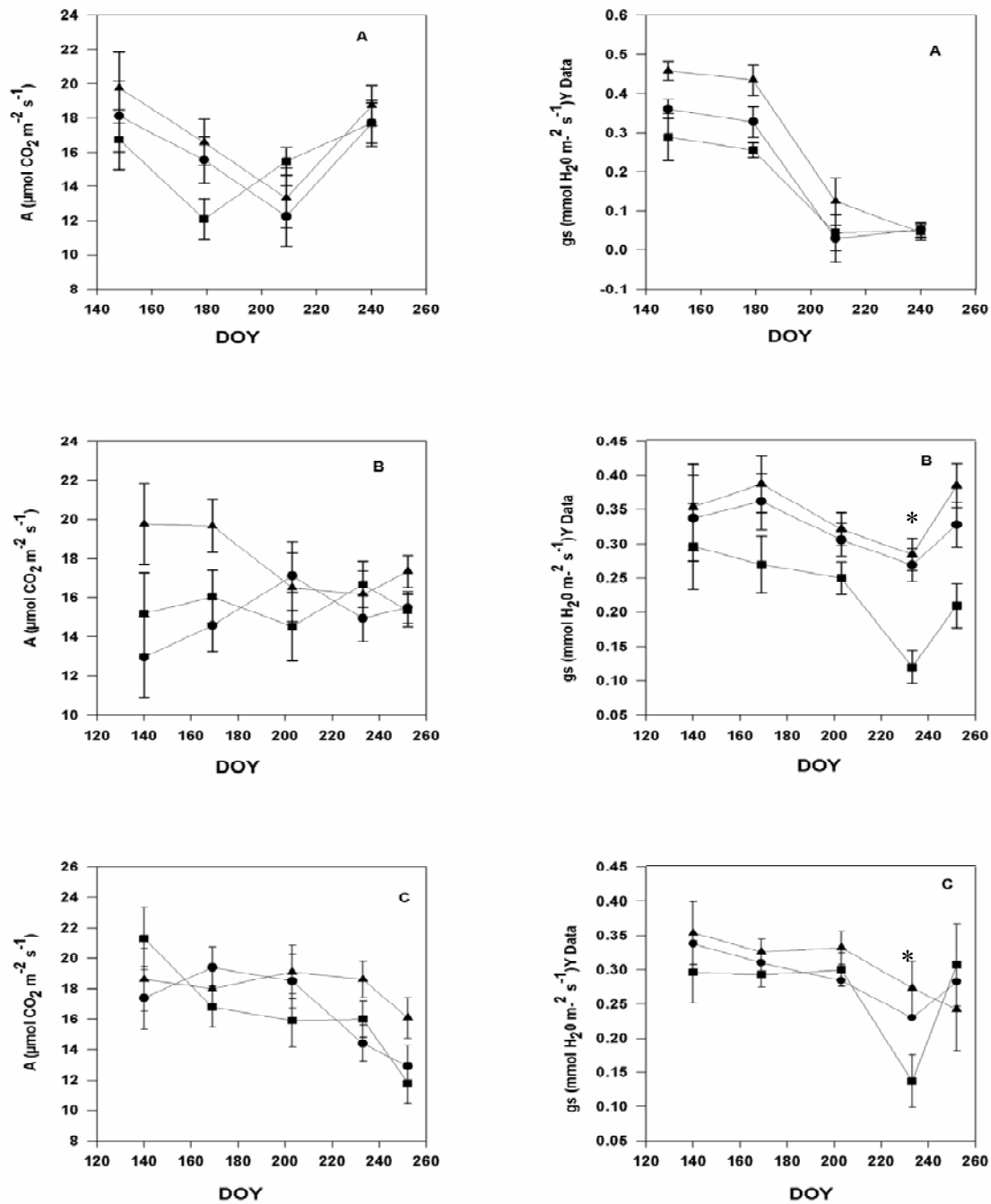
Figure 4.5: Seasonal course of photosynthesis, and conductance in grapevines cultivars concord (square), Pinot noir (triangle) and Traminette (circle) in 2004 (A), 2005 (B), and 2006 (C) growing seasons. Bars not visible indicate se smaller than symbol. DOY: Day of the year. Asteriks denote significant differences between treatment for each cultivar.
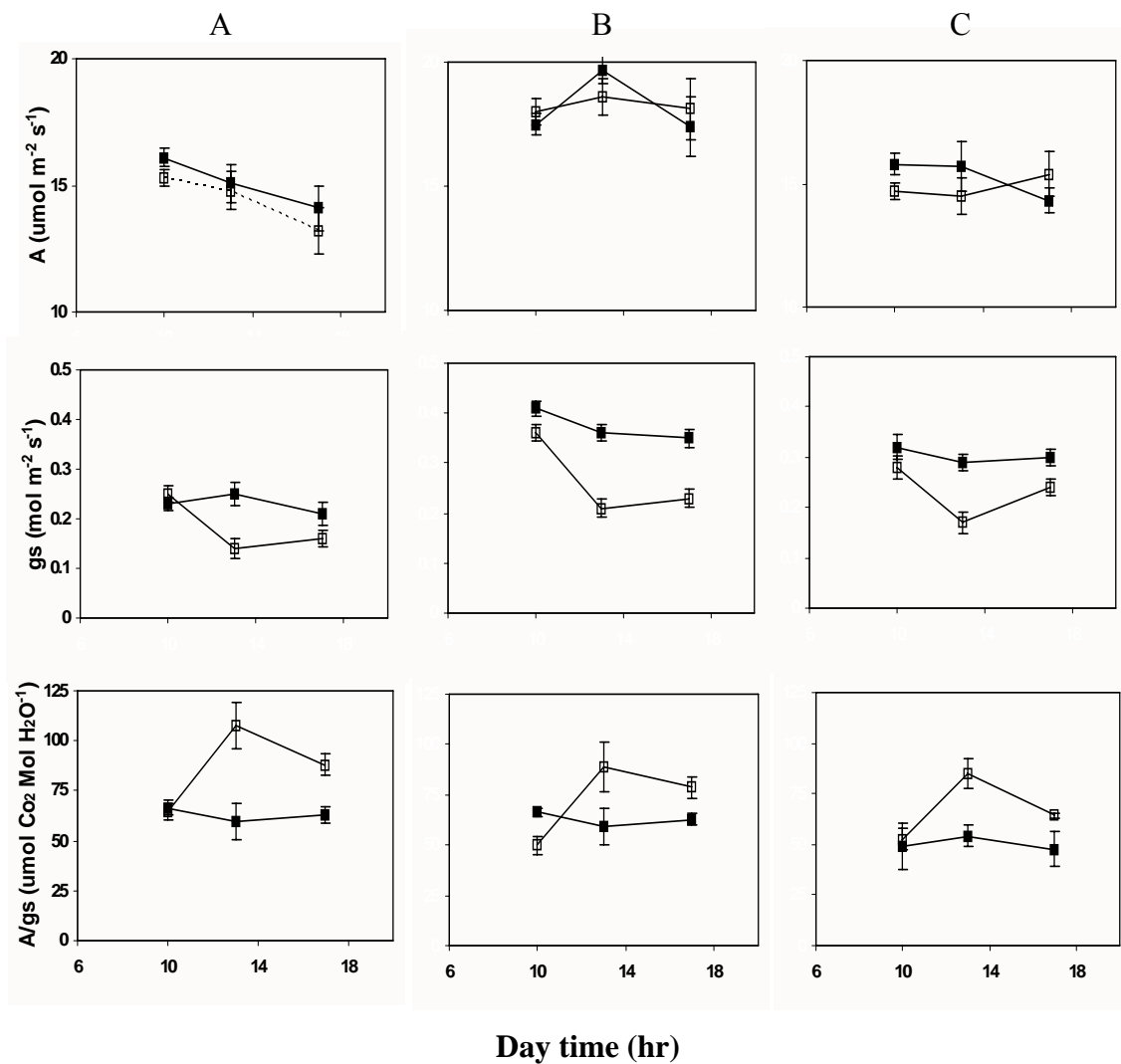
Figure 4.6: Diurnal course of photosynthesis (A), stomatal conductance (gs), intrinsic water use efficiency (A/gs) in Cultivars Concord (A), Pinot noir (B), and Traminette (C), respectively on 10 am, 01pm, and 17 pm of August 15 2006 for PRD (open symbol) and Full irrigation (closed symbol)
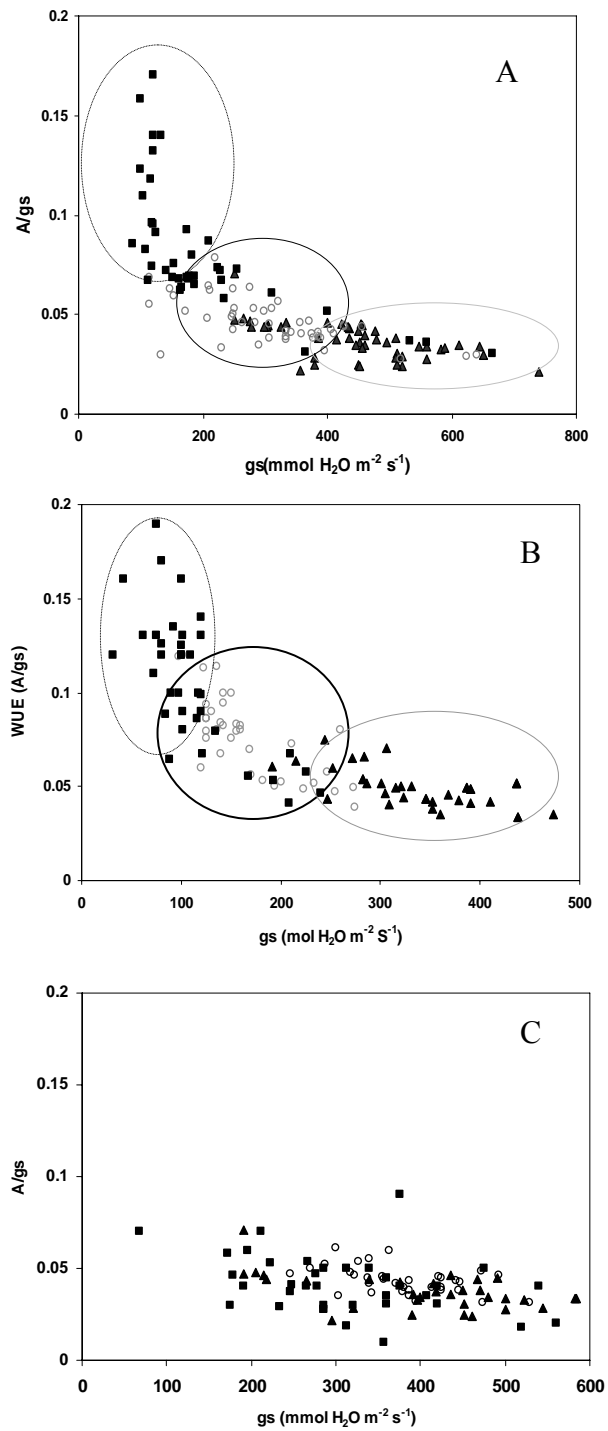
Figure 4.7: The relationship between intrinsic water use efficiency (WUE) and stomatal conductance (g_s) at 07:00 am (close triangle), midday (close squares), and 19:00h (open circles) in 2004 (A), 2005 (B), and 2006 (C) growing season.
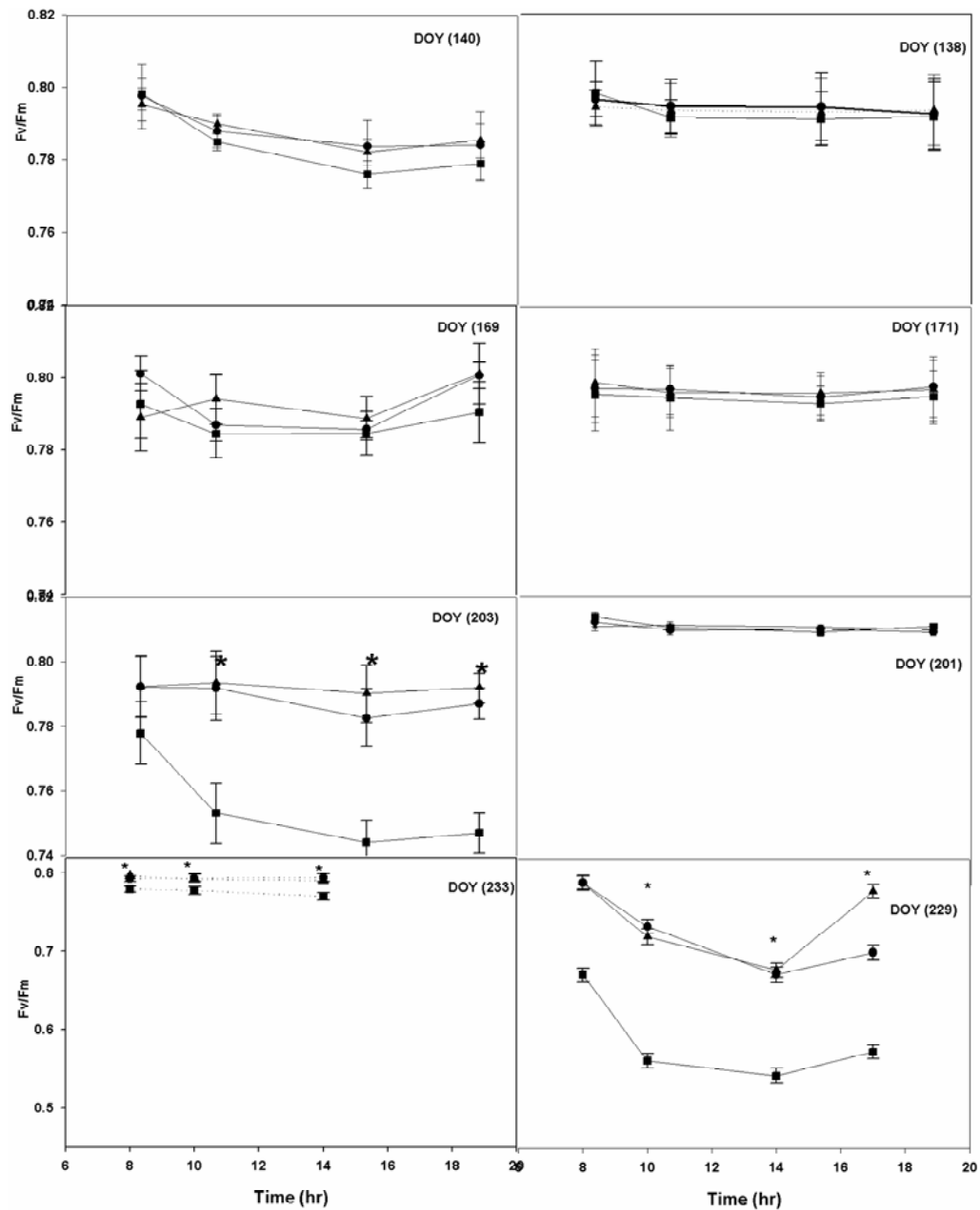
Figure 4.8: Diurnal course of quantum efficiency (Fv/Fm) measured at different time (DOY) in grapevines cultivars concord (square), Pinot noir (triangle) and Traminette (circle) in 2005 (A) and 2006 (B) growing season. Bars not visible indicate se smaller than symbol. Asterisks denote significant difference among varieties at p<0.05. DOY: Day of the year.

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GENERAL CONCLUSIONS

Alternating the wet and dry zones was shown to be vital to reduce excessive vegetative growth. PRD resulted in a substantial reduction of stomatal conductance and transpiration while maintained photosynthetic rate at optimum range. These physiological responses occurred without any changes in plant water status. This suggested that root-derived hormonal signal (ABA) might be involved in the control of these physiological processes.

Results of this study support the idea of importance of root-derived ABA as a chemical signal in grape cultivars. Although precise mechanisms still requires elucidation, one avenue is to apply exogenous ABA at PRD-treated vines and monitor the physiological changes in response to alternating irrigation at both sides of the root system.

Results suggest that the improvement of PRD vines being able to more consistently maintain water-use and water status, particularly under low soil moisture conditions may be due to a combination of factors. These factors include increased root development allowing improved soil moisture extraction, increased ABA production leading to the vines regulating vine water loss via subtle improvement in stomatal control leading to PRD vines being able to more consistently maintain plant water-use irrespective of irrigation events and vineyard environmental conditions.

Changes in shoot growth affects canopy structures, thereby influencing the light penetration into the canopy. Results of this study and others on grapevines using PRD showed that, due to a reduction in shoot growth, PRD-treated vines had significantly higher light

penetration inside the canopy. This has resulted in a significant increase in anthocyanines concentration thus enhanced fruit quality.

It was demonstrated in this study and others that PRD improves the water use efficiency. The adoption of the PRD irrigation techniques by the industry can be reached when further investigations to better decorticate the mechanism of PRD techniques from the hormonal signaling to the whole plant physiology response are conducted

The future directions leading out of this project should be based around several areas. These include varietal differences to deficit irrigation, effect of soil type and evaporative demand on vine responses to deficit and PRD irrigation, assessment of appropriate level at which to apply deficit irrigation and determining how often the side of the vine irrigated should be alternated to sustain the ABA signal.