Intensity and duration of deficit irrigation on *Erythroneura elegantula* (Hemiptera: Cicadellidae) on grape (Vitis vinifera)

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Abstract

Intensity and duration of deficit irrigation on *Erythroneura elegantula* (Hemiptera: Cicadellidae) on grape (*Vitis vinifera*)

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Western grape leafhopper (WGLH) (*Erythroneura elegantula* Osborn) is a serious pest of grape (*Vitis vinifera* L.) in many commercial vineyard growing regions of California. WGLH injures vines by removing leaf photosynthetically active area, resulting in the reduction of leaf efficiency, ultimately reducing yield and fruit quality. Regulated deficit irrigation (RDI) is a widely adopted irrigation strategy that reduces irrigation during critical phenological growing points (i.e., berry-set to verasion) to manage vegetative growth and berry size of red varieties. Leafhoppers are known to respond negatively to vine water stress.

In a two year study at a commercial vineyard located in Paso Robles, California, RDI was imposed on Cabernet Sauvignon winegrapes. Two treatments were looked at, intensity of deficit (25% and 50% of the grower's standard irrigations, i.e., close to 1.0 ETc) and, duration of deficit (3 weeks and 6 weeks, starting at berry set). Weekly counts of WGLH nymphs were taken, and then eggs were counted after the end of the second and third generations. Vine water status was monitored with a pressure chamber and stomatal conductance was measured with an LI-6200 CO_2 porometer. Results confirm other studies that have shown that leafhoppers are sensitive to vine water status. In year two of the study, second generation WGLH nymphal density was significantly reduced by RDI but the effect did not last through the third generation, and there was no difference in intensity or duration of the deficit. Second generation WGLH oviposition was also significantly reduced by RDI, and there was no difference in intensity or duration of the deficit. However, oviposition was reduced in the third generation only in the 25% deficit treatment, and there was no difference between deficit duration. One possible

explanation for lower oviposition is leaf epidermal tissue becomes more difficult to penetrate due to a physiological response to thicken leaf cuticle in an attempt to conserve water during times of water stress. This reduction in egg density may in part, explain the reduction in nymphal density.

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Lastly, I would like to express my sincere gratitude to my Graduate Committee Chair, Dr. Michael Costello. He introduced me to the world of plant protection, and believed in me to carry out this project. As I reflect upon my graduate school experience, I realize that I not only was lucky enough to have worked under a premier researcher, but that I gained a friend with whom I hope to collaborate with on many more projects in the future.

Table of Contents

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

INTRODUCTION

Western grape leafhopper (WGLH), *Erythroneura elegantula* Osborn (Hemiptera: Cicadellidae), has long been a damaging insect pest of cultivated grapes (*Vitis vinifera* L.) in California vineyards. Because WGLH is a native insect, it has likely been a pest of commercial vineyards since the mid-19th century. Found throughout the state, it is a primary pest of warmer interior areas of the Central Coast, and in the northern San Joaquin, Sacramento, and Napa valleys. According to data collected by the California Department of Pesticide Regulation (2003) leafhoppers are one of the most chemically treated for insect pests of California vineyards. WGLH nymphs and adults injure vines by piercing into the leaf mesophyll region and sucking out cell contents, thereby reducing leaf photosynthetically active area. These empty cells appear as pale yellowish-white spots or "stippling" (Flaherty et al. 1992). The extent of feeding injury to the vine varies, and depends on population density, vineyard condition, and location of injured leaves (Daane and Costello 1992). Furthermore, Daane and Costello (1992) suggest economic injury level (EIL) is reached at approximately 20 nymphs per leaf; one leafhopper can destroy 1 percent of leaf area, consequently, 20 nymphs per leaf corresponds to 20 percent leaf loss. If populations are left unchecked and feeding injury is allowed to accumulate over the growing season, the reduction in photosynthesis can reduce growth and yield, and ultimately lead to leaf drop (Flaherty et al. 1992). In addition, during harvest, heavily infested vineyards can become an annoyance to harvesting crews as high

populations of leafhoppers can fly into their ears, noses, eyes, and mouths reducing worker productivity, thus, increasing harvest costs. Adult female leafhoppers lay eggs into the epidermal tissue of the leaf, usually on the underside of leaves. The primary natural enemy of leafhoppers is the egg parasite *Anagrus spp.*, and if conditions permit, can keep WGLH populations below EIL (Daane et al. 1995, Costello and Daane 1996).

Regulated Deficit Irrigation. Regulated deficit irrigation (RDI), is a widely adapted irrigation strategy in California and Australia, and is becoming more common throughout the viticultural regions of the world, and involves applying less than the full water potential requirement to the vineyard (Prichard et al. 2004). By restricting or reducing the amount of water normally required by a vine for a defined period of time, the vine undergoes a water deficit, but only temporarily. This type of irrigation strategy is used to improve vegetative balance and fruit quality in winegrapes (Matthews and Anderson 1988, Dry and Loveys 1998). Among the numerous benefits of regulated deficit irrigation, studies have shown that properly timed RDI strategies promote better fruit quality by producing smaller berries (increasing skin to juice ratio) (Williams 2001), discouraging shoot tip growth (producing a less herbaceous wine) (Matthews et. al. 1990), and reducing the amount of foliage for more light and air penetration, which aids in disease management (Matthews and Anderson 1988).

$ET_c = ET_o \times K_c$. A key method for estimating the irrigation need of a crop is to combine the water lost from the soil (evaporation) with the water lost through leaves (transpiration), into an overall loss, known as evapotranspiration (ET) (Hopkins 1995). Fortunately, in California, ET for a specific crop and location can be easily calculated using data available through a network of weather stations called CIMIS (California

Irrigation Management Information Service) stations. These stations monitor solar radiation, soil temperature, wind speed and direction, air temperature and humidity and precipitation amounts on grass specific to each station's location (Stewart et al. 2011). The grass crop is maintained to very high standards by the California State Department of Water Resources. From data collected by the stations, a reference evapotranspiration (ET_o) is calculated. Because this ET_o is specific to a grass crop, it is necessary to calculate a crop coefficient (K_c) in order to convert CIMIS data to a specific winegrape crop (Stewart 2011). Williams (2001) has demonstrated that in order to obtain K_c, one must first determine the degree of canopy-shaded area (there are several ways to measure shaded area; with a grid, software packages utilizing digital photos, or by simply tape measuring the average width of the shaded area). Therefore, by using the formula $ET_c = ET_o \times K_c$ the full amount of water lost by the vine through evapotranspiration can be accurately estimated. With this knowledge growers can decide what portion of total water loss to replace with irrigation, even more so, researchers and viticulturalists can choose to replace a percentage of full water, essentially creating RDI.

Trichilo et al. (1990) found that a season-wide irrigation deficit on Thompson seedless table grapes increased nymphal mortality on *Erythroneura* spp. at one site in the San Joaquin Valley, but not at another site. Daane and Williams (2003), working with season-wide deficits on Thompson Seedless at the Kearney Agricultural Center in Parlier, found a dramatic and positive correlation with ET_c and variegated leafhopper (*Erythroneura variabilis*) density and dry weight, and number of marked and recaptured adults.

Conversely, Costello (2008) found evidence that a season-wide irrigation deficit is not necessary for producing similar results on leafhopper density; working at two commercial vineyards, Costello found that RDI (50% and 25% of the grower standard irrigation, used as a control) targeted between berry set and veraison (a six week period) lowered second generation nymphal density by about 50% and lowered egg density by 30-50%. Viticulturally, Costello's findings play an important role because imposing water deficits on vines too early in the season (i.e., pre-berry set) or too late (i.e., post-verasion) has shown to negatively affect fruit quality and vine health (Coombe and McCarthy 2000).

The focus of this work is to confirm previous studies evidencing increased WGLH nymphal mortality and lower oviposition on vines under RDI. As well, this work intends to further provide useful information for growers and vineyard managers improving their ability to control grape leafhopper populations through water or irrigation management and without the use of costly chemical applications while enhancing wine quality at the same time.

This study took place over two growing seasons (2002 – 2003) with the objective of analyzing the potential benefit of decreasing WGLH population density by implementing an RDI program. Similar to Costello (2008), the RDI treatments included a comparison of the grower's standard irrigation (close to 1.0 ET_c) to a 50% deficit (0.5 ET_c) and a 75% deficit (0.25 ET_c). In addition, the deficits were imposed after berry set for either three weeks or six weeks. The aim was to estimate the intensity and duration of RDI necessary to significantly affect leafhopper nymphal and egg density.

CHAPTER 2

MATERIALS AND METHODS

Study Site. For two seasons (2002 – 2003) data for this study were collected at Steinbeck Vineyards, a commercial vineyard located about 15 km east of Paso Robles, California, USA. The vineyard was established in 1992. Our study plot was designed within a portion of a larger block of Cabernet Sauvignon (clone 8 on 5C rootstock) winegrapes. Vines were spur-pruned and trained to a quadralateral cordon system with a single catch wire. The block was developed with north and south row orientation. Vine spacing was 2.1 m in-row and 3.3 m between rows. Soil type was classified as clay-loam. In 2002 the study was located on the southwest corner of the vineyard, but in 2003 we moved it to the northwest corner, in order to achieve better soil uniformity.

Experimental Design. For each year experiments were set up as a randomized complete block- split plot design and treatments were replicated three times. The main plot represented intensity of the deficit; grower standard irrigation as control (approx. 0.8 to 1.0 ETc), 50% of control (0.5 ETc), and 25% of control (0.25 ETc), and the split plot represented the length of time of intensity, i.e., 3 weeks and 6 weeks. Main plot size was six rows by eight vines. In 2002, the three week deficit treatment was imposed between June 21 and July 15, and the six week deficit between June 21 and Aug. 6. In 2003, the three week deficit treatment was imposed between June 21 and July 14, and the six week deficit between June 21 and Aug. 10. We regulated the deficit irrigations using in-line

programmed water-flow timers (Gilmour, Somerset, PA), and applied water amounts were monitored weekly by placing collection containers under the drip emitters.

Nymphal Density. We took weekly counts of WGLH nymphs, sampling 15-20 mature leaves per plot beginning approximately 2 to 3 weeks prior to initiation of the deficits, and directly counting all nymphs per leaf. First generation nymphs were sampled on basal leaves. Second and third generations were taken from leaves located at mid-shoot area (roughly corresponding to the nodes above the vine's fruit zone). It should be noted that a valid effort was given to randomly selecting leaves and counting nymphs on both the upper and under-sides of the leaves.

Oviposition. WGLH eggs were counted after the end of the first, second and third generations. We sampled 20 mature per subplot, brought the leaves back to the laboratory, cut them in half, and counted eggs on the half leaves under a 40x dissecting microscope. Eggs were scored as hatched, parasitized, or live. It was assumed that eggs were parasitized by *Anagrus* spp. In 2002 very few eggs were recorded, and the data are not included in this paper.

Vine Water Status. Using a pressure chamber (PMS Instruments Co., Corvallis, OR), a.k.a. pressure bomb, we measured vine water status weekly. The pressure bomb is an invaluable tool for monitoring winegrape vine water status, due in part, to its ease of operation and relatively reasonable cost. We took five readings per plot between the hours of 1100 and 1400; this is considered the best time of day to check vine water status (Williams, 2001). Only mature fully expanded leaves with full sun exposure (typically the fourth or fifth leaf from the shoot tip) were selected. Using a sharp razor blade and

leaving enough length of petiole to reach through the chamber insert, leaves were carefully excised. Immediately following the cut (within 30 seconds), the entire leaf was placed into the chamber with the petiole placed through the chamber insert gland, facing upward. The chamber was then tightly secured to assure that no gas would escape during pressurization. We then slowly applied compressed nitrogen to the chamber until the chamber pressure equaled that of leaf pressure. A magnifying glass was used to see the bubbling liquid (sap) exuded at the top of the petiole; it is at this point the leaf is said to have reached zero water potential (Williams 2001). Furthermore, Williams (2001) describes measuring water potential as a balance of pressure, i.e., the pressure required to push sap to the surface of the cut petiole is the original leaf water potential, measured in (-) bars. A higher (-) bar reading on the pressure bomb effectively represents a higher degree of water stress, or more accurately, a more negative water potential. Our pressure bomb reading were recorded in bars, and were later converted to megapascals (-1MPa = 10 bars).

We also took weekly measurements of stomatal conductance (mmhos $CO_2/m^2/sec$), using a LI-6200 (LI-COR, Lincoln, NE), taking five readings per subplot and selecting leaves for measurement which were mature and in full sun.

Pressure bomb, stomatal conductance and leafhopper nymphal count data were log 10 transformed and analyzed by repeated measures analysis of variance, with mean separation by orthogonal contrasts (SAS 2008). Leafhopper egg counts were analyzed by ANOVA, with mean separation using Tukey's Honestly Significant Difference. Differences were considered statistically significant at $p<0.05$.

CHAPTER 3

RESULTS

APPLIED WATER. Water applied in each year and a comparison with estimated ET_c is shown in Table 1. In each study year, water applied in the control treatment was at just over 90% of estimated ET_c.

LEAF WATER POTENTIAL. Leaf water potential at Steinbeck's did not differ significantly among intensity nor duration treatments in either 2002 or 2003 (Figs. 1-4).

STOMATAL CONDUCTANCE. In 2002 the three week deficit duration did not show separation from the control until the third week, and there appeared to be an extended period of difference after the deficit ceased (lag time) for the next weeks (Fig. 5). For the first three weeks (27 June-11 July), there was a significant difference in the repeated measures ANOVA ($F=6.83$, $df=2,42$, $p=0.05$), with contrasts showing a difference between the deficit treatments and the control by 12.96% (repeated measures ANOVA $F=36.26$, $df=1,42$, $p<0.01$), but not between deficit treatments ($F=1.73$, $df=1, 42$, $p=0.19$). For the subsequent three weeks (18 July-1 Aug), the repeated measures ANOVA for intensity was significant ($F=6.83$, $df=2, 42$, $p=0.05$), with the deficits 18% lower than the control ($F=36.26$, $df=1, 41$, $p<0.01$). There was a significant effect of time ($F=6.57$, $df=1, 42$, $p=0.01$), with the six week duration 12% lower than the three week duration. For the post-six week deficit period (8 Aug-29 Aug), there was significant

interaction between intensity and time ($F=5.26$, df=2, 42, $p<0.01$) with no effect from the three week duration ($F=0.32$, df=1, 22, $p=0.73$), but a 14.75% difference between deficits and control for the six week duration ($F=12.35$, df=1,22, $p<0.01$).

In 2003 for the three week deficit duration (Fig. 7), there was a significant difference among treatments for the first three weeks (25 June-14 July) (repeated measures ANOVA $F=12.58$, df=2,40, $p=<0.01$), with the contrast between the control and the deficits significant ($F=25.07$, df=1,40, $p<0.01$), and the deficit treatments lowered by 26.2% compared to control, but no difference between the 50% and 25% deficits ($F=0.10$, df=1,40, $p=0.75$). For the subsequent three weeks (23 July-6 Aug) there was a significant difference (repeated measures ANOVA $F=6.50$, df=2,31, $p<0.01$), with deficit treatments 10.2% lower than control (contrast $F=12.20$, df=1,31, $p<0.01$), but there was no difference between the 50% and 25% deficits (contrast $F=0.8$, df=1,32, $p=0.38$). For the next four weeks (13 Aug-3 Sept) there was no significant difference among treatments ($F=0.80$, df=2,31, $p=0.38$). For the six week deficit duration (Fig. 8), there was a no significant difference among treatments beyond the initial three week period of 25 June-14 July, nor was there any effect of deficit duration.

LEAFHOPPER NYMPHAL DENSITY. In 2002, there was no distinct separation among generations; we estimated the second generation to have been between 18 July and 15 August, and the third generation to be 22 August through 12 September (Figs. 9 and 10). For the second generation, there was no intensity effect ($F=2.84$, df=2,6 $p=0.17$, no time effect ($F=0.11$, df=1,6 $p=0.29$, and no time x intensity interaction ($F=4.47$, df=2, 6, $p=0.06$). For the third generation there was no difference either for intensity ($F=3.79$, df=2,6 $p=0.12$), time ($F=0.61$, df=1,6, $p=0.46$) nor interaction ($F=0.21$, df=2, 6, $p=0.81$).

In 2003 there was much clearer separation between generations; we estimated the second generation to be between 30 July and 3 September, and there was a significant effect of intensity ($F=11.22$, $df=2,6$, $p=0.02$) but not of time ($F=0.01$, $df=1,6$, $p=0.91$) and no interaction ($F=1.82$, $df=2,6$, $p=0.24$). Orthogonal contrasts show that the deficit irrigation treatments (combined) had leafhopper density 44% lower than the control ($F=26.11$, $df=1,6$, $p<0.01$), but there was no difference between the 0.5 deficit and 0.25 deficit treatments (Figs. 11 and 12). However, the effect of the deficit treatments did not last into the third generation, with no difference in intensity ($F=2.29$, $df=2,6$, $p=0.21$), time ($F=0.40$, $df=1,6$, $p=0.55$) and no interaction ($F=0.29$, $df=2,6$, $p=0.76$).

LEAFHOPPER EGGS. Leafhopper egg density was extremely low in 2002, and there were not sufficient numbers to analyze. Therefore, the only data we have for leafhopper egg density is from Steinbeck's in 2003. For the second generation, the ANOVA for deficit intensity was significant ($F=6.32$, $df=2, 359$, $p=0.05$), with the deficits different from the control by 45% and no interaction between deficit intensity and time ($F=1.94$, $df=2, 359$, $p=0.14$) (Figs 13 and 14). For the third generation, the ANOVA for deficit intensity was significant ($F=8.45$, $df=2, 359$, $p=0.04$), with the 25% deficit lower than the 50% deficit by 16%, but the 50% deficit did not differ from the control, and there was no interaction between deficit intensity and time ($F=1.03$, $df=2,359$, $p=0.35$) (Figs 13 and 14).

CHAPTER 4

DISCUSSION

This study adds to the body of work that has provided evidence that *Erythroneura* spp. leafhopper density is affected negatively by water stressed vines (Trichilo et al. 1990; Daane and Williams 2003; Costello 2008). This study also confirms a finding by Costello (2008) in that *Erythroneura* spp. leafhopper oviposition can be reduced with properly timed irrigation deficits. In addition, results showed little difference between irrigation deficit intensity or duration on leafhopper nymphal density.

Several theories have surfaced in an effort to explain why leafhopper nymphs respond negatively to water stressed vines. Trichilo et al. (1990) introduced the concept of altered microclimates and leafhopper preference existing due to water status. Trichilo et al. (1990) also suggested leafhoppers prefer a cooler, well-watered vine over a stressed vine, and therefore, being highly mobile, adults tend to fly away from water stressed vines in search of more thoroughly watered vines; however, they do not speculate as to what it is about the well watered vines that improves leafhopper performance on these vines. Still, there is little doubt that adult leafhopper movement explains why in the current study we did not see an effect on nymphal density past the 2nd generation. Our deficit treatments reverted back to the standard grower irrigation (0.8 to 1.0 ET_c) following the scheduled deficit durations, essentially rehydrating the vines, which were more attractive to adult leafhoppers. Although we did not test this, a possibility is that once the deficit treatments ceased and the previously deficit treated plots received more

water, these vines went through a period of new shoot growth, providing more foliage for leafhoppers to feed and lay eggs on. This hypothesis is supported by the results of egg density from the 50% deficit treatment, which increased relative to the control from the second to the third generation (Figs. 13 and 14). However, the 25% deficit did not exhibit this pattern, suggesting that the irrigation deficit was too severe to allow recovery of vine growth. Other studies have found that oviposition at least partially explains a reduction in leafhopper nymphs to water stress. In a study on alfalfa, Hoffman and Hogg (1992), found that oviposition of potato leafhopper, *Empoasca fabae*, was lower on water stressed alfalfa plants. Daane and Williams (2003) found a 50% reduction in leafhopper oviposition on vines watered season-wide at 1.2 ET_c compared to vines watered at 0.6 ET_c. Our study, too, showed evidence that lower oviposition can be accomplished by imposing RDI. While an effect on leafhopper eggs was more pronounced under the 25% vs. 50% deficit, there was not a significant difference between the 3 week and 6 week durations. Interestingly, the effect on eggs remained through the third generation for the 25% intensity treatment, regardless of duration, suggesting that a severe deficit imposed in a shorter time frame may be just as effective as deficits imposed for longer durations, thereby, allowing viticulturists and winemakers the opportunity to simultaneously and harmoniously enjoy the benefits of RDI on fruit quality without causing unnecessary long term vine damage.

Costello (2008) noted that variability among soil types more than likely plays a role in the efficacy of the irrigation deficit treatments on leafhopper nymphal density. Different soil types hold water at varying capacities; this combined with high winter rain amounts (or lack thereof) can influence the onset of leafhopper density and occurrence of

oviposition, and influence the degree to which the irrigation deficits are actually experienced by the vines. This may partly explain why our pressure bomb readings from the deficit plots were not significantly different from the control, and why the stomatal conductance readings did not show a consistent and strong difference among treatments.

Recently, Costello (2008) described an idea that may explain why oviposition and leafhopper nymphal density can be reduced through RDI. Costello suggests nymphal mortality appears to increase on water stressed vines because of a physiological response in the leaf. It is known that plants respond to water stress by thickening their cuticle in an attempt to save water (Hopkins 1995). It is thought that the thicker cuticle discourages leafhopper feeding and oviposition by creating a more challenging surface to penetrate. To date, studies of this theory do not exist or have never been published. This study also suggests the possibility that reduced nymphal densities may be closely related to egg mortality.

Another untested theory on why leafhoppers prefer well watered vines is the possibility that water stressed vines reduce leafhopper feeding quality. Connor (1988) showed that oak lace bugs, *Corythucha arcuata* (Say), avoided water stressed foliage of white oak and preferred foliage that was well hydrated. Connor contributes this preference to higher quality feeding found on well watered leaves is due in part to a reduced metabolic cost to the insect when maintaining water balance in its ingestion mechanism, and a reduction in foliage toughness.

Table 1. Estimated season-wide (April 30-October 1) water applied by treatment in millimeters

		Treatment				
	Estimated ET_c	Control	50% irrigation-3 weeks	25% irrigation -3 weeks	50% irrigation-6 weeks	75% irrigation-6 weeks
2002	340	314	262	234	231	191
2003	325	305	268	244	239	214

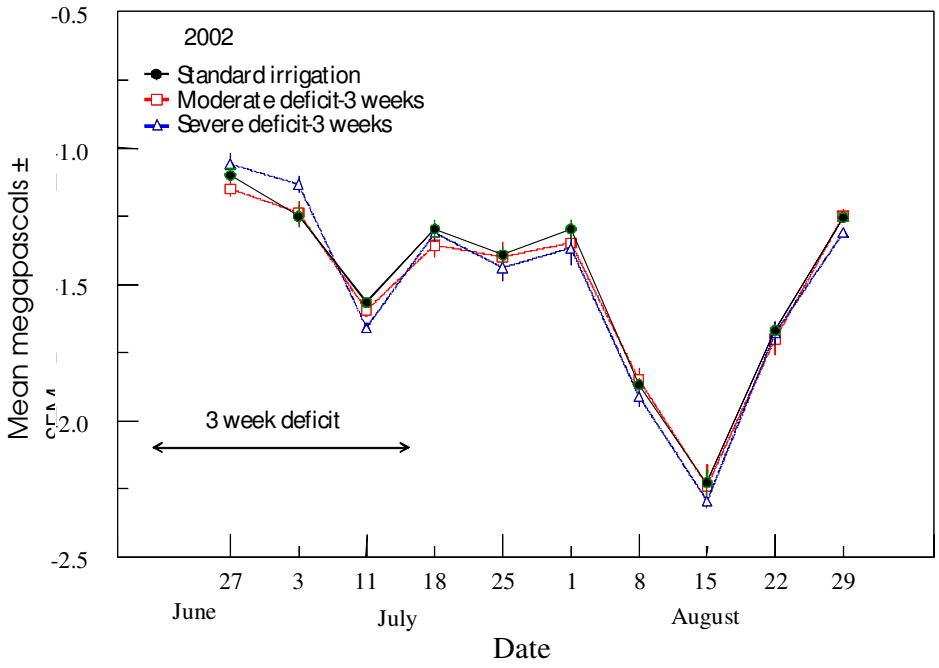

Figure 1. Leaf water potential (mean megapascals ± standard error) over time, 2002, 3 week deficit duration.

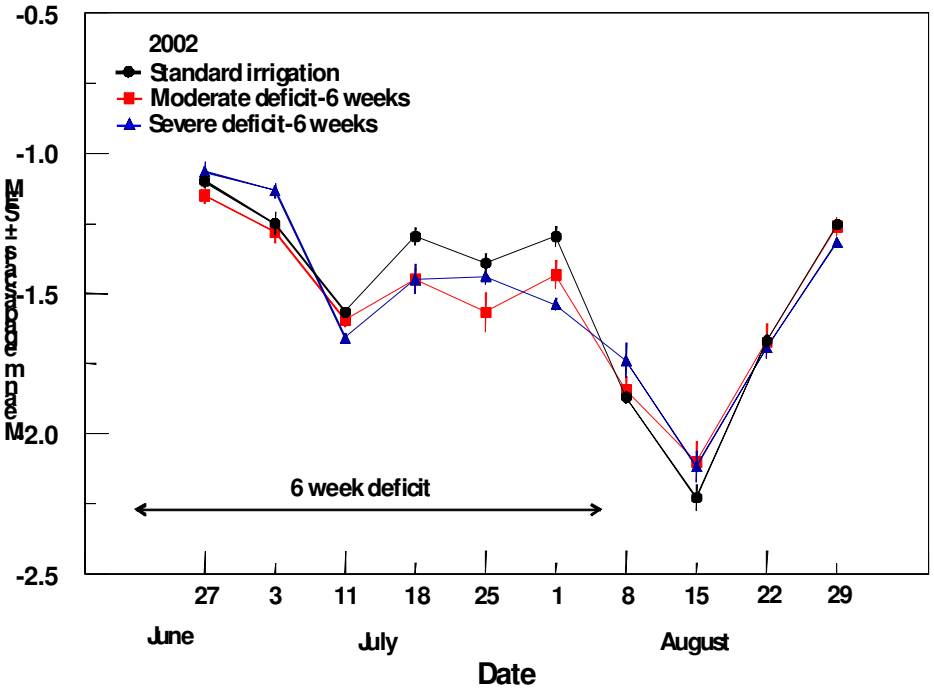

Figure 2. Leaf water potential (mean megapascals ± standard error) over time, 2002, 6 week deficit duration.

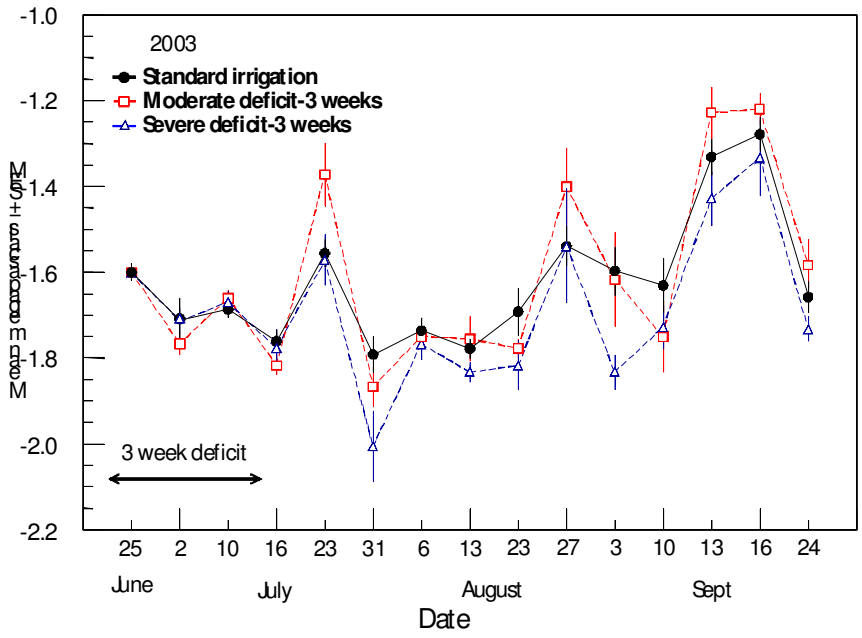

Figure 3. Leaf water potential (mean megapascals ± standard error) over time, 2003, 3 week deficit duration.

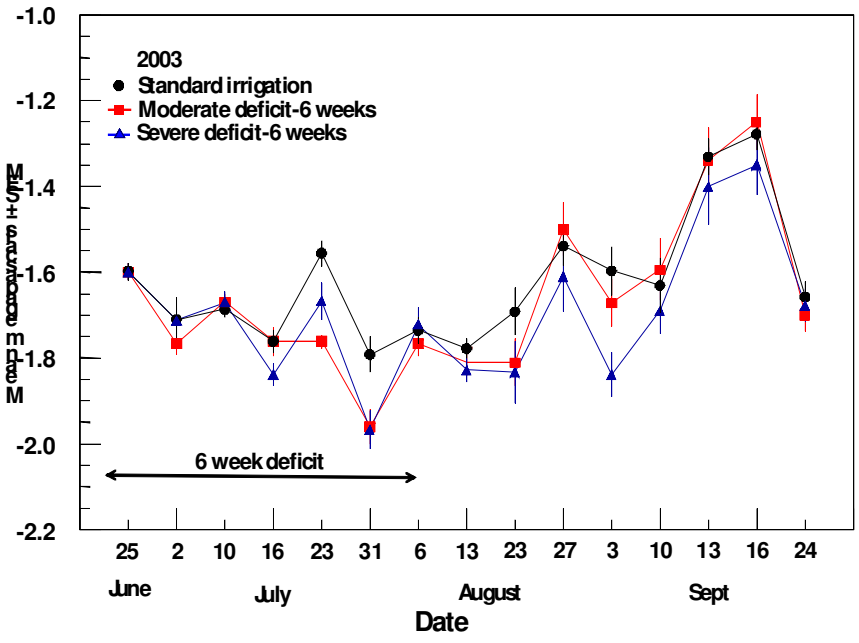

Figure 4. Leaf water potential (mean megapascals ± standard error) over time, 2003, 6 week deficit duration.

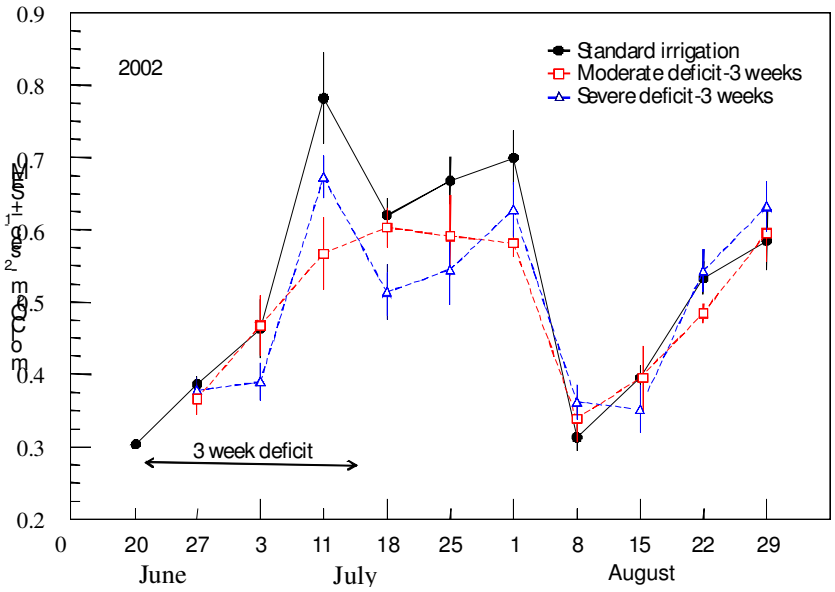

Figure 5. Stomatal conductance (mean mol CO_2 m^{-2} sec^{-1} ± standard error) over time, 2002, 3 week deficit duration.

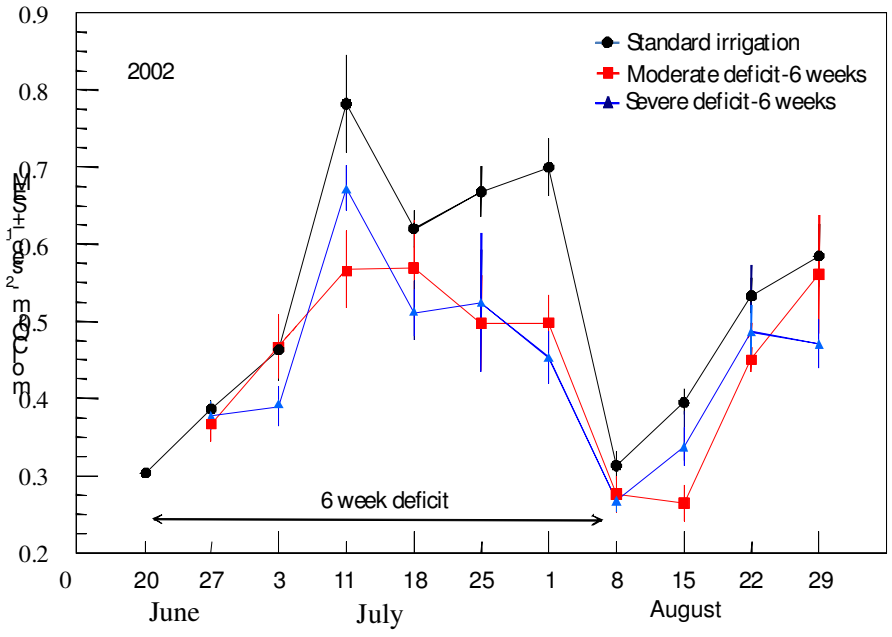

Figure 6. Stomatal conductance (mean mol CO_2 m^{-2} sec^{-1} ± standard error) over time, 2002, 6 week deficit duration.

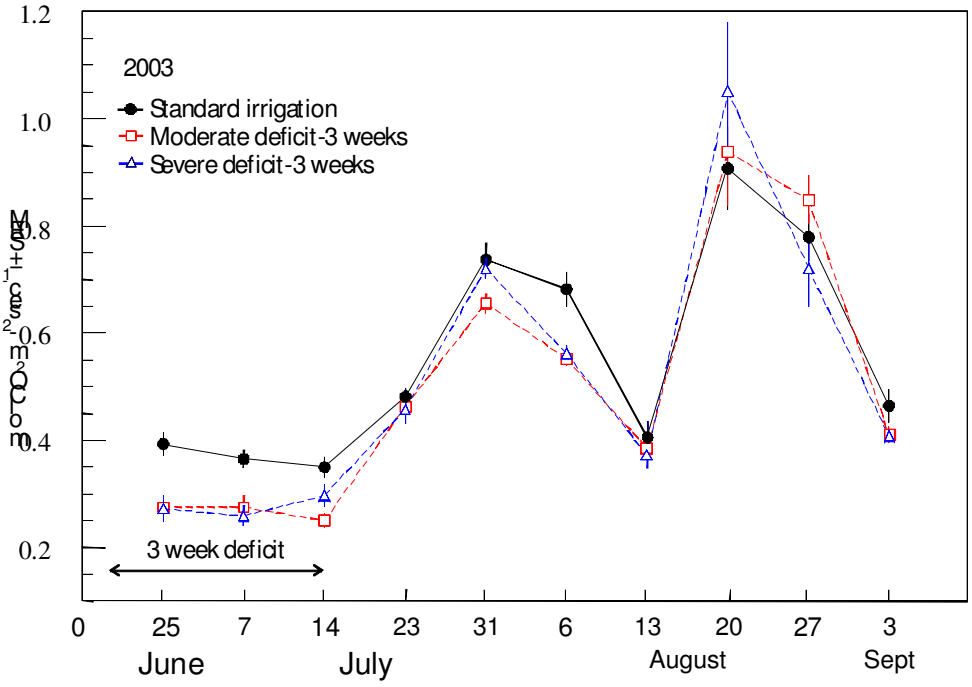

Figure 7. Stomatal conductance (mean mol CO_2 m^{-2} sec^{-1} ± standard error) over time, 2003, 3 week deficit duration.

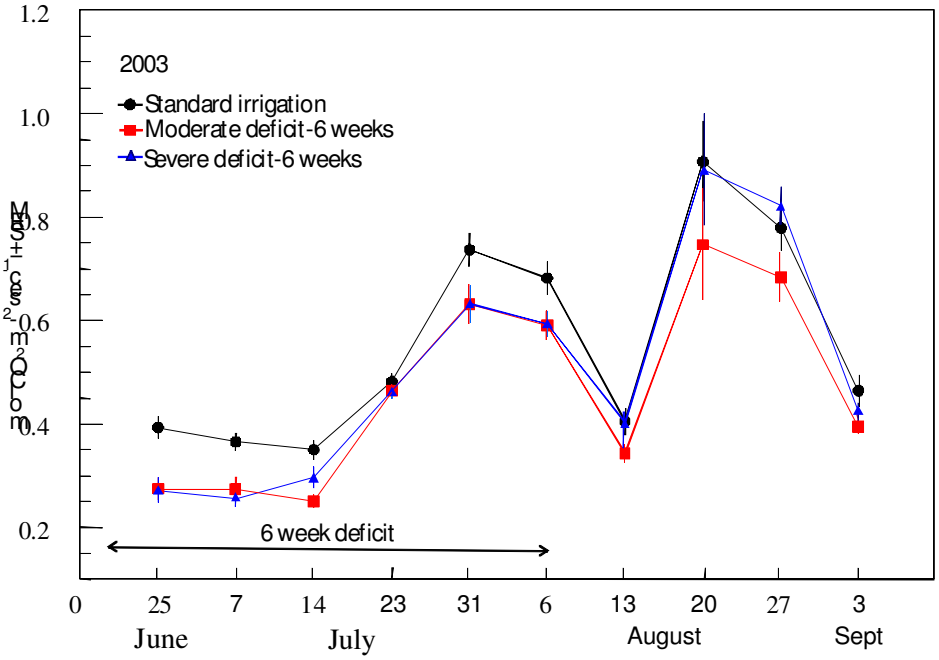

Figure 8. Stomatal conductance (mean mol CO_2 m^{-2} sec^{-1} ± standard error) over time, 2003, 6 week deficit duration.

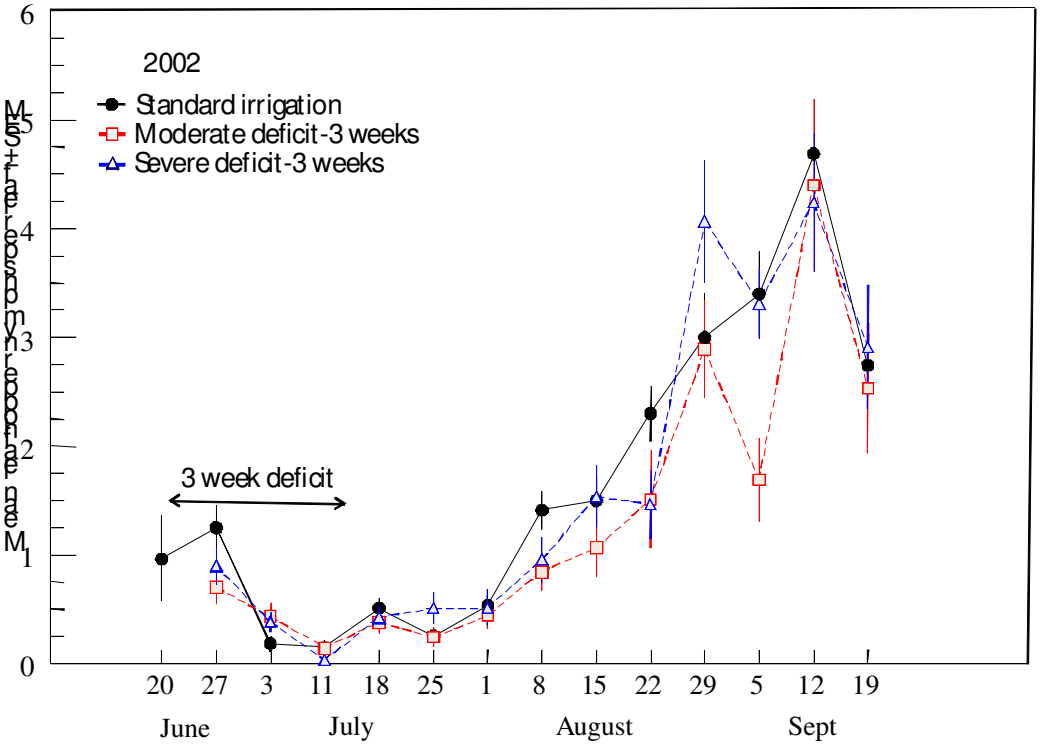

Figure 9. Mean leafhopper nymphs per leaf (± standard error) over time, 2002, 3 week deficit duration.

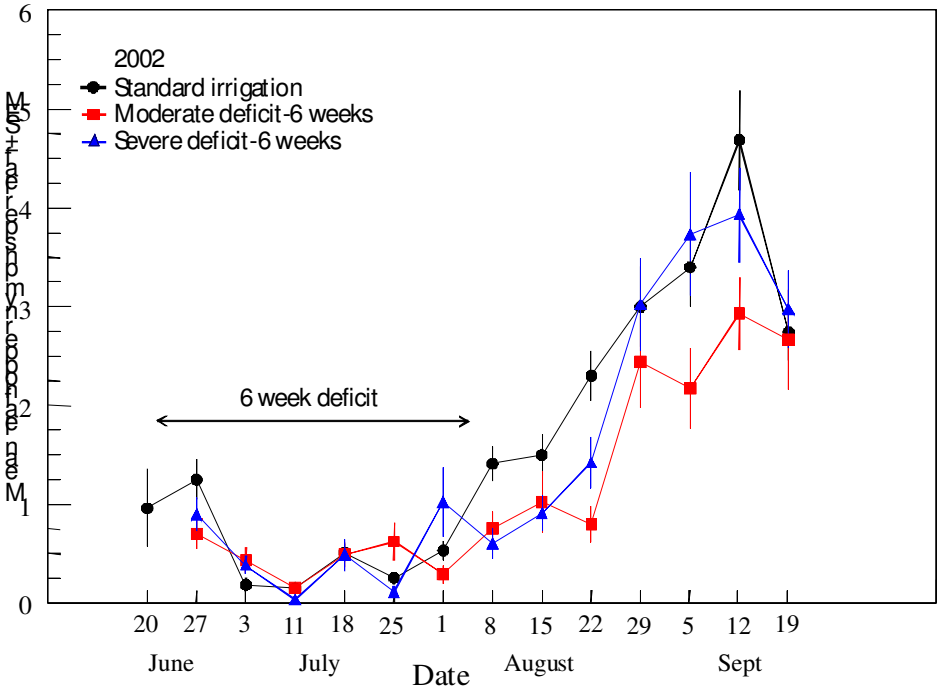

Figure 10. Mean leafhopper nymphs per leaf (± standard error) over time, 2002, 6 week deficit duration.

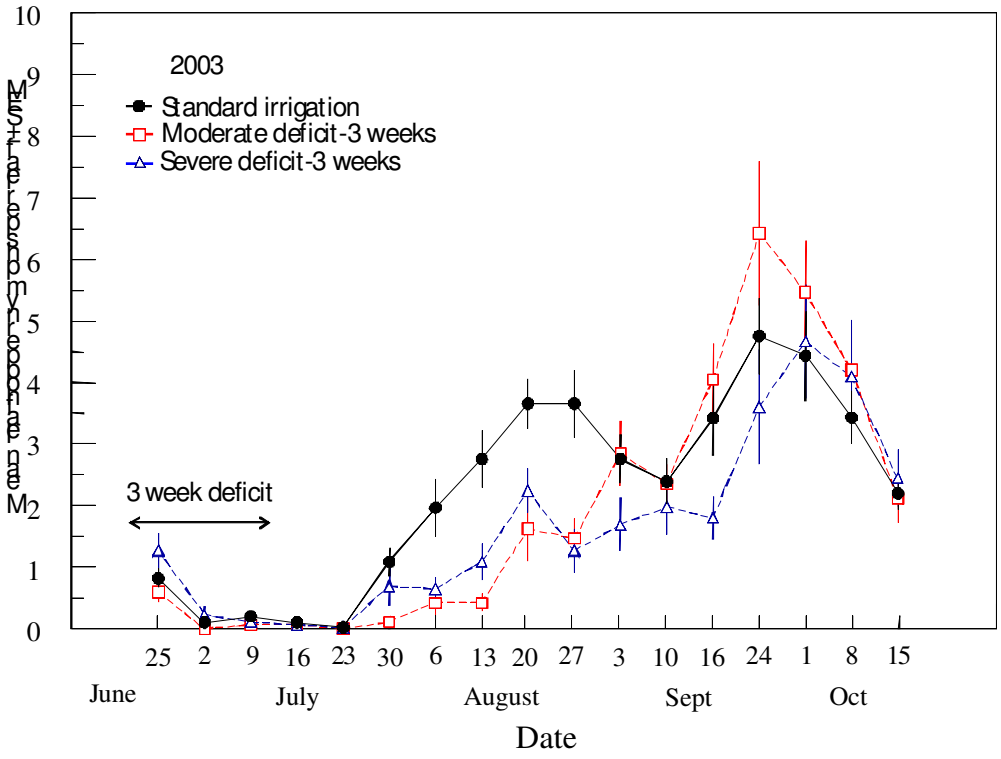

Figure 11. Mean leafhopper nymphs per leaf (± standard error) over time, 2003, 3 week deficit duration.

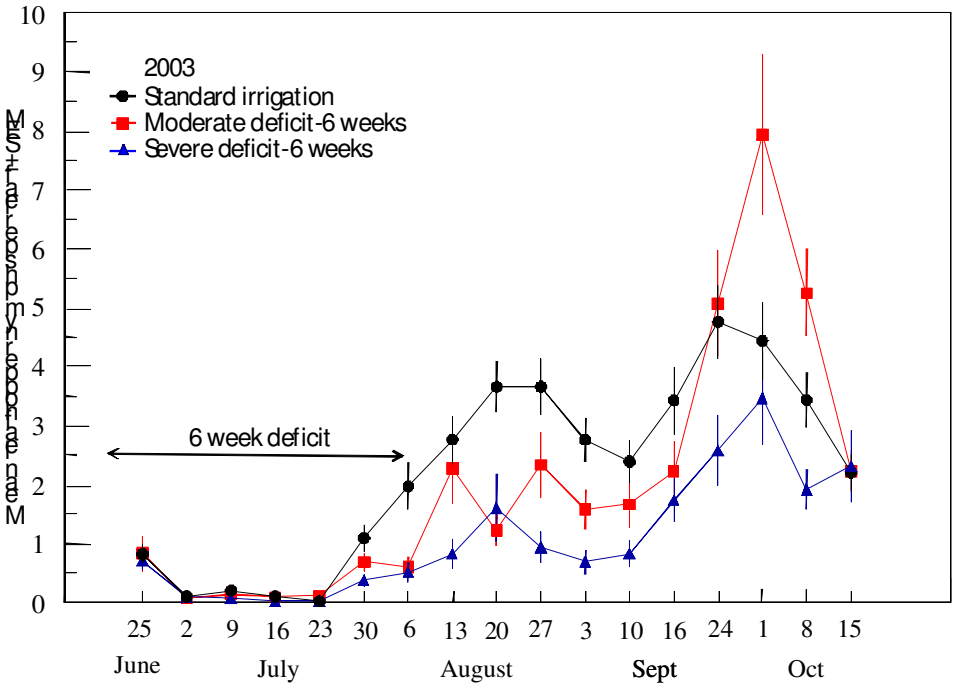

Figure 12. Mean leafhopper nymphs per leaf (± standard error) over time, 2003, 3 week deficit duration.

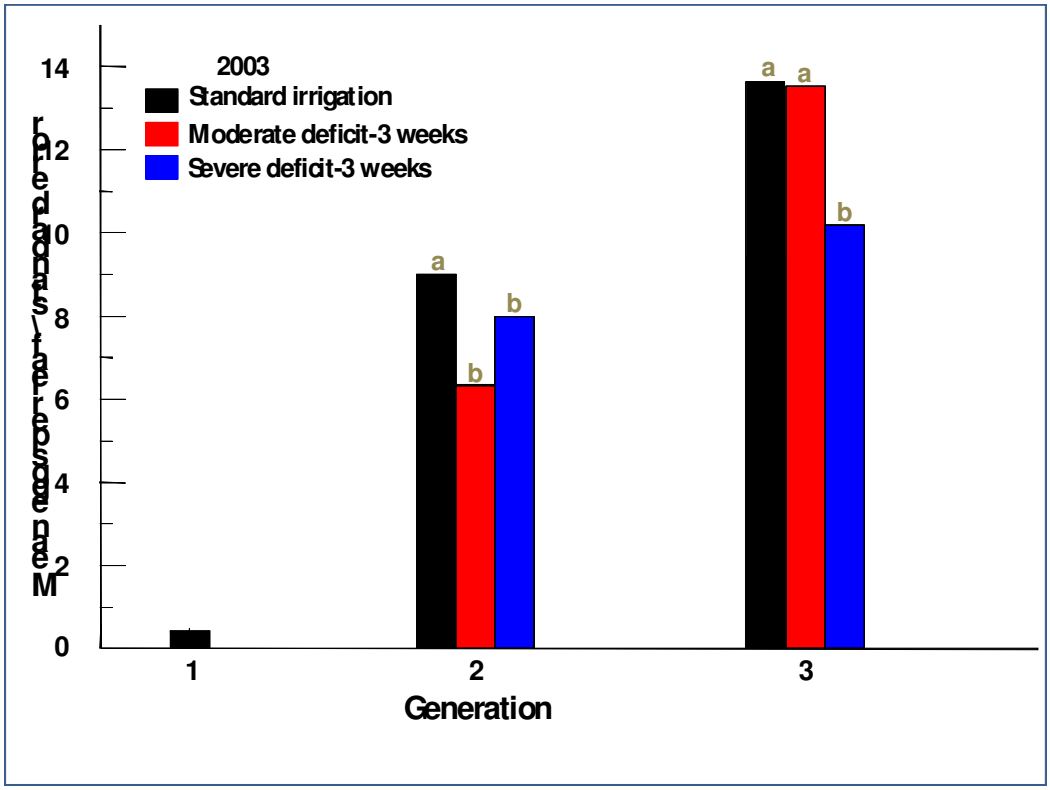

Figure 13. Mean leafhopper eggs per leaf (± standard error) 2003, 3 week deficit duration.

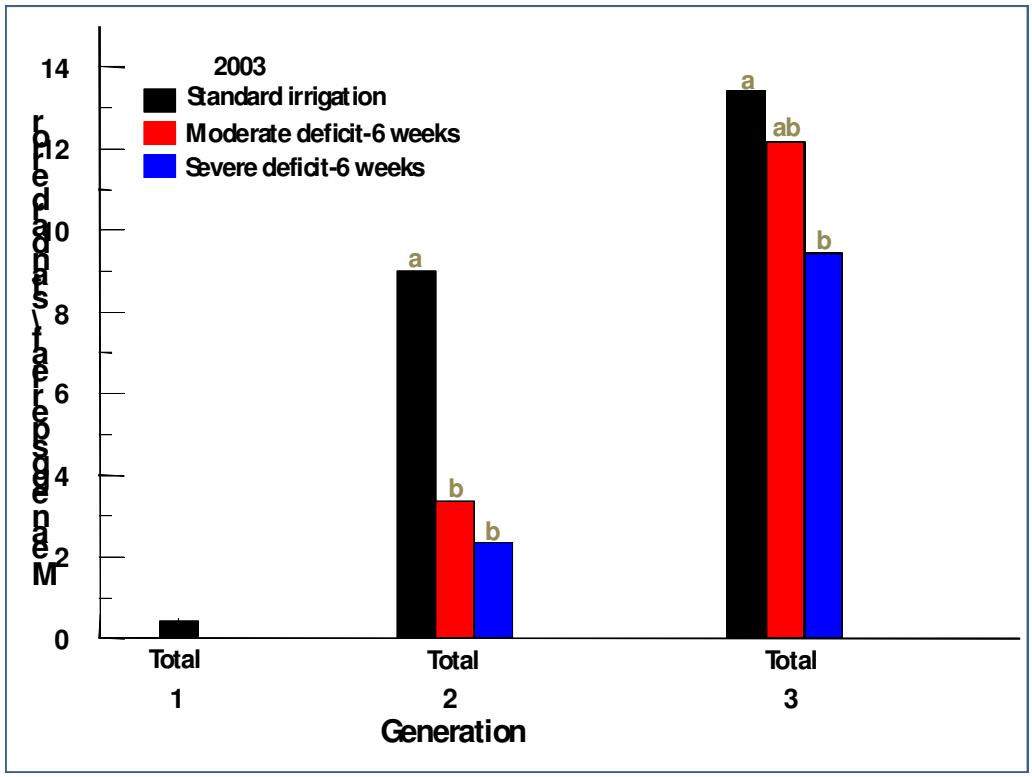

Figure 14. Mean leafhopper eggs per leaf (± standard error) 2003, 6 week deficit duration.

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