Walnut Marketing Board
Final Project Report
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Project Title: Irrigation Management and the Incidence of Kernel Mold in Walnut

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ABSTRACT
There is no doubt that kernel mold reduces edible yield and therefore price received for the crop. The increase in incidence of mold, especially by late varieties, seems to be real. The approach we took to evaluate the relationship between kernel mold and water deficits was sound. We withheld irrigation water while monitoring the tree water status with a pressure chamber until significant differences in water stress were achieved. Two closely controlled trials in 2001 and three in 2002 were conducted to access the effects of water deficits on the incidence of walnut kernel mold in mature Chandler orchards. In the 2001 Chico trial, a comparison was made between well watered and significant water stress conditions occurring at a time when it was hypothesized to have the most effect on the incidence of mold mid-late season. In 2002, the severity of water stress was increased and the time of water stress moved to earlier in the season. In this trial (Chico), no relationship between mold and water deficits was found in either 2001 or 2002. The Clements trial (conducted in 2001) achieved stress to a lesser differential but at two timings, mid and late plus midseason. The level of deficits had no significant effect on the presence of kernel mold. At the San Joaquin site, no significant differences in the incidence of kernel mold were found between treatments. At the Tehama site, significantly fewer molds occurred in the full water treatment when compared to the mild and moderate treatments trending towards a significant relationship between the molded nuts and seasonal MDSWP. Another hypothesis advanced is that the nuts that fall early onto the wet soil surface will have a higher incidence of mold. To address this issue, nuts were collected from trees prior to shaking that were on the soil surface still moist from micro-sprinkler irrigation. No significant difference in mold was found between windfall and shake nuts with or without water stress in 2001 or in 2002. Specifically, there is no strong relationship between the incidence of mold and water stress as imposed in 5 trials over two years.

PROBLEM AND ITS SIGNIFICANCE
Kernel mold is a serious and increasing problem facing walnut growers and handlers. Mold reduces yield, kernel quality and grower returns.

Numerous theories have been advanced as to the cause of kernel mold. Some see the increase in mold as related to the time windfall nuts are on the ground. Another hypothesis advanced is that the nuts that fall early onto the wet soil surface will have a higher incidence of mold. Observation of kernel mold problems has resulted in an emergence of a causal theory that mid/late season water stress is a major factor in kernel mold. This is supported by the fact that: (1) later harvest cultivars such as Chandler and Howard continue to use water that can effect the nut past the time of earlier harvest cultivars, and (2) many young, later harvest cultivar orchards are achieving full canopy coverage. This maximizes tree water use and increases the potential for orchard under-irrigation and tree water deficits.

This study seeks to determine the responses of walnut to the presence/absence of mid-late season water deficits and the affect of these deficits on the incidence of kernel mold. The incidence of mold in windfall nuts onto a moist soil surface will also be evaluated.

OBJECTIVE
Determine incidence of kernel mold in walnut under different irrigation regimes causing presence/absence of water deficits.

PLANS AND PROCEDURES:
Three orchards were chosen for this experiment. The plan was to create water deficits by withholding irrigation water, measure the intensity and duration of water deficits using a pressure chamber, and then measure the effects on the occurrence of kernel mold.

Chico Site. The first site is located in Butte County, slightly south of Chico, California. The site has a deep, high water holding capacity soil and is planted to the Chandler cultivar. The mature orchard has a history of increasing mold over time as evidenced by crop grade sheets. Irrigation is provided by a micro-sprinkler irrigation system with one sprinkler per tree. Ten plots were designated, each containing 12 trees. Each plot consisted of 3 rows and 4 trees in each row. The experimental design is a randomized complete block with two treatments and five replications. Deficits began in Treatment 2 in July and intensified through August. The deficit treatment was compared to a fully irrigated control (T1).

Sprinklers in the deficit treatment were changed in size to emit half the amount of the non-deficit treatment on 6/10/02. Additionally, the sprinklers were plugged on that date in the deficit treatment and left so until significant deficits were measured. On 8/22/02, the sprinklers within each stressed plot (T2) were unplugged. At that time of year, the orchard was irrigated twice a week (Tuesday and Friday) for 18 hours each time. Sprinklers in the deficit treatment emit 16.4 gallons/hour compared to the control, emitting 32.8 gallons/hour. The water applied significantly reduced the water deficits within a short time period. The sprinklers were again plugged and remained so through irrigation cutoff for the entire field (9/30/02). Stem water potential measurements were collected on Thursday (the day before the next irrigation) of each week in order to measure when the highest level of water stress that irrigation cycle. The water

status of the two center trees of each plot was monitored by weekly measurements of midday stem water potential (MDSWP) using a pressure chamber. Leaves in low, shaded positions near the base of the tree were bagged at least 15 minutes before sampling and placed immediately in the pressure chamber (still enclosed in the bag). Nut samples were collected from the same trees that were monitored for MDSWP.

San Joaquin County Site. The orchard is a mature Chandler orchard planted on Paradox rootstock at a 32' by 32' equilateral triangle arrangement (49 trees/acre). The soil is a Cogna loam, which is a deep well drained alluvial derived soil. The orchard is irrigated with Nelson R10 sprinklers at one per tree. Variation in irrigation treatments was achieved using different size nozzles with the high, medium and low irrigation treatments applying 0.066, 0.056, 0.047 inches per hour. There are four replications of each irrigation treatment with 3 rows per replication. A replication consists of 18 trees receiving the same irrigation treatment. The middle four trees in each plot were used for detailed water potential, nut and shoot growth measurements. Midday stem water potential was measured approximately every 10-14 days (generally near the end of an irrigation cycle) on 4 trees per plot using methods similar to those described above for the Chico site.

Tehama County Site. The orchard was planted in 1994 on a 30' by 18' spacing (81 trees/acre). The soil is a Maywood sandy loam. The variety is Chandler alternating on Northern California Black and Paradox rootstocks. The orchard is irrigated with one Nelson R-5 micro-sprinkler per tree. Variation in irrigation treatments was achieved using different size nozzles with the high, medium and low irrigation treatments applying 0.060, 0.049, 0.039 inches per hour. In order to allow fine tuning of the irrigation treatments, manual shutoff valves were placed on each irrigation line in order to allow turning the water on and off to these plots as needed. There are four replications of each of the three irrigation treatments with 3 rows per replication and 12-13 trees per row as well as guard rows between plots. Midday stem water potential was measured approximately every 7-10 days on 6 trees per plot (total of 24 trees per treatment) using methods similar to those described above for the Chico site. Although rootstocks originally alternated between Northern California Black and Paradox rootstocks, replanting was done with Paradox. This study ended up with a total of 14 Paradox and 10 Northern California Black rooted trees being monitored for midday stem water potential in each treatment.

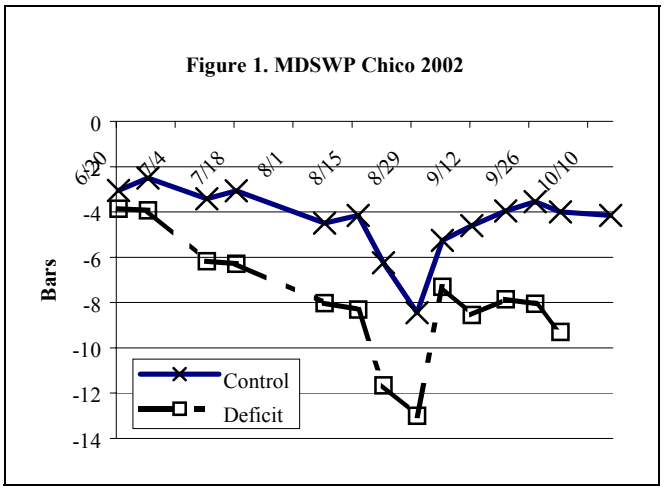
Yields were monitored by harvesting the individual monitored trees at both the San Joaquin and Tehama sites. After each individual tree was shaken, the nuts were swept into windrows and harvested with a small manually pulled cup-type harvester at the Tehama County site and the grower's harvester at the San Joaquin County site. Sub-samples were taken for drying, size and quality analysis.

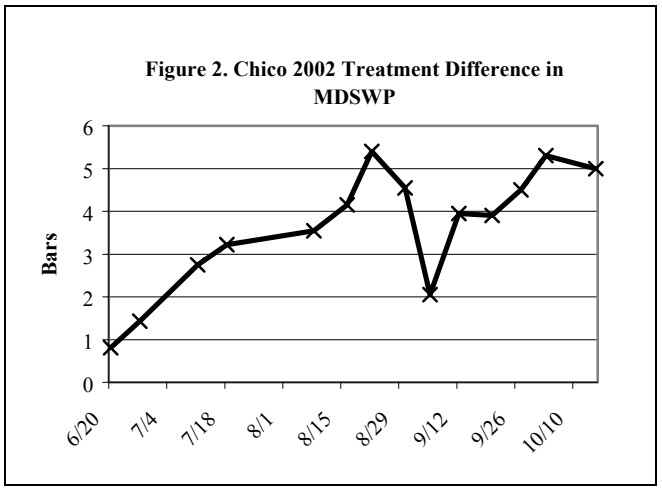
Nuts found to contain mold at grading (Diamond Walnut) were individually stored for identification by microscopic examination. Molds are currently being isolated into pure culture and identified from culture. Identification is based on standard taxonomic works and comparison with reference cultures as needed. Mold identification is currently under way. Results in part due to the lateness of the season and the time necessary to complete the work will not be reported in this document.

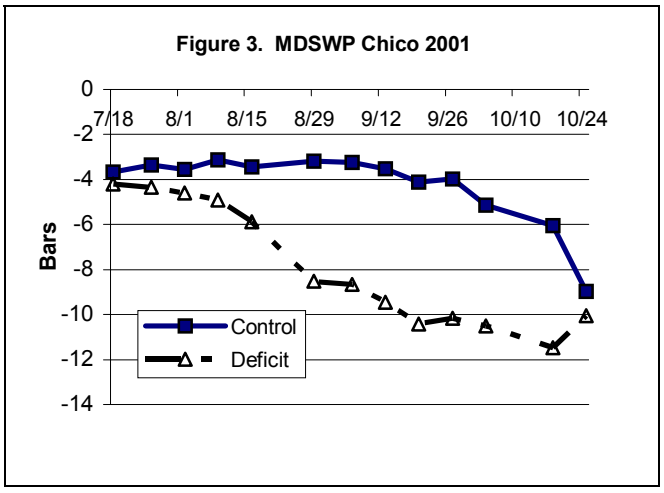
RESULTS

Chico Results.

Midday stem water potential (MDSWP) was measured from 6/20/02 through 10/15/01. The goal was to impose water deficits earlier than last season (2001). Significant differences in MDSWP were noted between treatments as a result of the plugged sprinklers (Figures 1 and 2). By 8/22/02, MDSWP levels in the deficit treatment had reached -13 bars. At this time sprinklers were unplugged and allowed to apply about one half the volumes as in the control treatment. After a few irrigations, MDSWP had recovered to near the control treatment at which time the sprinklers were again plugged until harvest. The differential between the control and deficit treatment reached a maximum of -5.3 bars by harvest (Figure 2). T1 always exhibited a better water status than T2. The control (T1) was irrigated at a near baseline (well irrigated) condition until 8/29/02 when irrigation volumes were reduced. Upon irrigation using the original schedule, water deficits were relieved to near baseline through harvest. The maximum water deficits were measured on 8/30/02 at –13 bars (Table 1). This compares to a MDSWP maximum at –11.5 bars on 10/18/01(Figures 3 and 4). Essentially, the deficits caused by withholding irrigation occurred earlier and to at a more negative level than 2001.







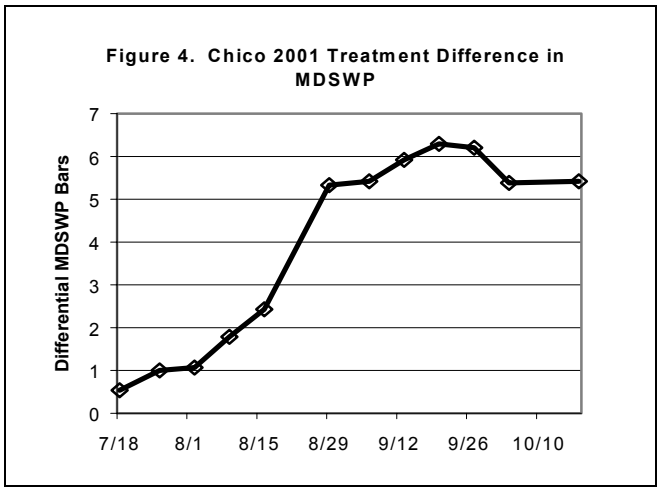


Table 1. Midday Stem Water Potential, Chico 2002

Treatment	Average		Maximum	
1	-4.42	b	-8.45	b
2	-8.00	a	-13.00	a
P =	0.0014		0.0035	

Common letters among means within columns denote no significant difference at P ≤ 5% using Duncan's mean separation.

The incidence of kernel mold and other grading parameters was measured in each plot by first collecting 200 nuts of the windfall nuts, followed by sweeping and removing all windfall nuts from the tree area, then collecting 200 nuts of the shake nuts. Diamond Walnut, using their standard grading system, evaluated each sample separately. Table 2 shows the harvest nut grading for the number of baby large nuts, mold, insects, shrivel, adhering hull, reflected light (RLI 1), stain and edible kernel weight. No significant differences were found in any parameter in windfall or shake nuts between treatments. However, differences between the windfall and shake nuts are illuminating. Windfall nuts were observed to have significantly more percentage insects and adhering hulls. Edible kernels percent were significantly higher in the shake nuts. It is important to remember the windfall nuts are a small component of the total harvest.

Table 2. Chico 2002 Walnut Harvest

Treatment	% Large	% Mold	% Insect	% Shrivel	% Adhering Hull	RLI	% Stain	% Edible Kernel
Windfall								
1	93.6	3.96	1.94	5.87	9.67	49.6	0.84	44.2
2	92.2	2.96	3.43	3.82	8.22	50.4	2.91	43.7
P =	0.6686	0.3439	0.3085	0.2348	0.5305	0.728	0.1782	0.6418
Shake								
1	86.1	2.45	0	0.25	1.14	50.6	0.66	47.9
2	89.8	1.89	0	1.89	2.84	50.6	0	46.4
P =	0.4384	0.6385		0.1374	0.2070	0.1056	0.2105	0.0698

Common letters among means within columns denote no significant difference at P ≤ 5% using Duncan's mean separation.

San Joaquin Results

Midday stem water potential. There was some difficulty in maintaining the target midday stem water potentials (MDSWP) at both the San Joaquin and Tehama sites in 2002. In interpreting these data, it is important to realize that the MDSWP measurements at the San Joaquin site were generally done near the end of the 14-day irrigation cycle so they represent the most stressed conditions that the trees experienced. The treatment average MDSWP would have been somewhat less negative. The MDSWP for the treatments tended to bounce above and below the target levels (Figure 5) during May-June, then tended to be below target values during July until the middle of August followed by a recovery above target levels from mid-August through mid-September (Figure 6).

Figure 5. Target MDSWP Levels for Both the San Joaquin and Tehama Sites

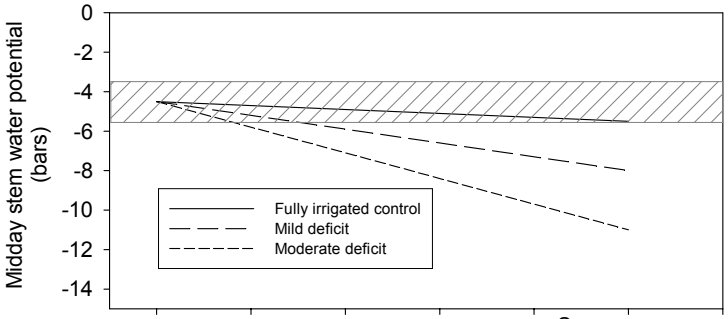
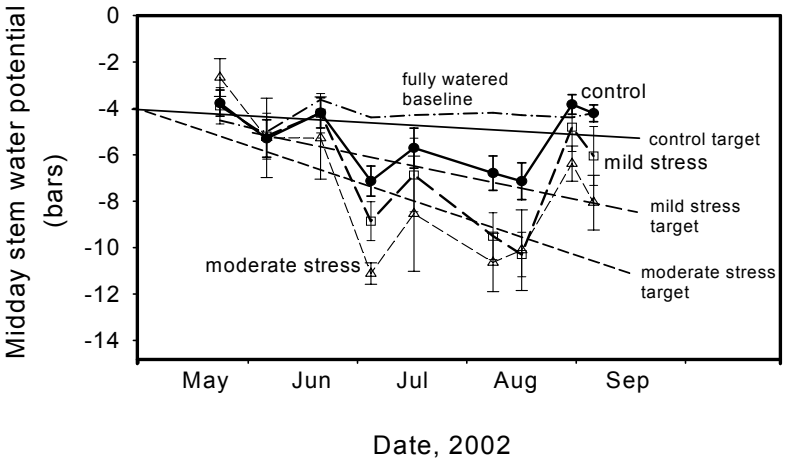


Figure 6. Midday stem water potential by irrigation treatment for San Joaquin County site for 2002 season. The control, mild and moderate stress are demarcated with solid circles, open squares and open triangles respectively. Error bars indicate plus or minus two standard errors.



San Joaquin County Site Harvest. There were no significant treatment impacts on overall yield for either fresh weight as measured at harvest or for weights after being adjusted to a constant dry weight (See Project Report: *Irrigation Management in Walnut Using Evapotranspiration, Soil and Plant Based Data).* There were significantly fewer large nuts in the moderate stress treatment compared to the control. There were no significant treatment impacts on mold, insect damage, shrivel, or adhering hulls at the San Joaquin site (Table 3). There was a significantly lower RLI in the moderate stress treatment compared to the control (Table 3).

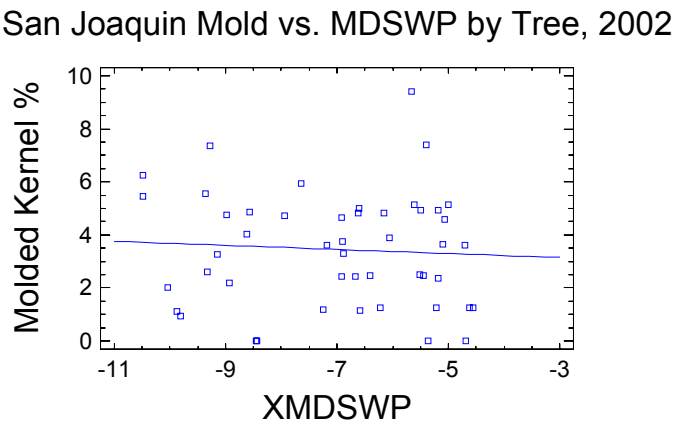
Table 3. Quality data for the San Joaquin County site from Diamond Walnut.
Harvest samples were obtained from each individual tree that was monitored for water potential.

Treatment	% Large	% Mold	% Insect	% Shrivel	% Adhering Hull	RLI
1	88.3 a	2.62 a	0.31 a	0.75 a	0.12 a	52.2 ab
2	88.8 a	3.37 a	0.12 a	0.94 a	0.25 a	50.6 b
3	77.3 b	2.87 a	0.50 a	1.12 a	0.37 a	52.9 a
LSD	6.8	1.35	0.44	0.72	0.40	1.87

Common letters among means within columns denote no significant difference at P $\leq$ 5% using Duncan's mean separation.

When regressed as the MDSWP against the kernel mold of each individual tree, a poor relationship exists (Figure 7). Since the P-value in the ANOVA table is only 0.5770, there is not a statistically significant relationship between the percent kernel mold and seasonal mean MDSWP. The R-Squared statistic indicates that the model as fitted explains 0.68% of the variability in percent mold. The correlation coefficient equals 0.0825, indicating a relatively weak relationship between the variables.

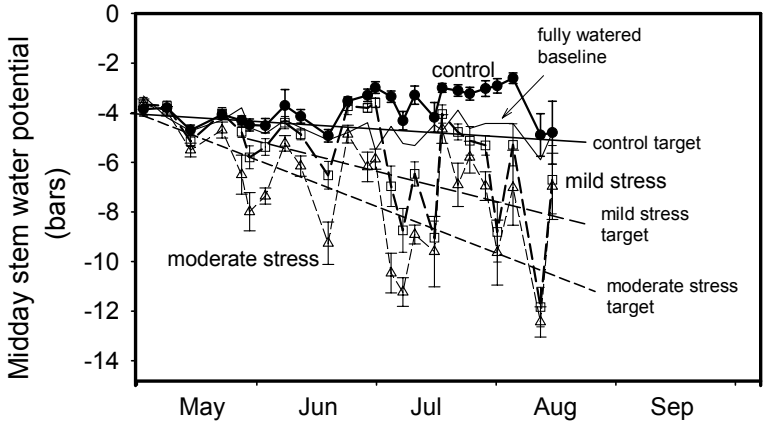
Figure 7. Relationship Between Kernel Mold and Seasonal Average MDSWP at the San Joaquin Site, 2002



Tehama Results

At the Tehama County site, there was also considerable variation of the MDSWP around the target values, but the fluctuations tended to be over a shorter time period (Figure 8). This may have been due to more fine adjustments in applications being possible due to a more frequent irrigation schedule at the Tehama site.

Figure 8. Midday stem water potential for Tehama County site for 2002 season. The control, mild and moderate stress are demarcated with solid circles, open squares and open triangles respectively. Error bars indicate plus or minus two standard errors.



Tehama County Site Harvest - The fresh weights were significantly higher for the control versus the moderate stress treatment (See Project Report: *Irrigation Management in Walnut Using Evapotranspiration, Soil and Plant Based Data)*. However, when the crop was dried to constant moisture content, there were no significant treatment effects on yield. There were significantly fewer large nuts in the moderate deficit treatment compared to the control. There was also significantly more mold and shrivel in both of the deficit treatments compared to the control although the levels for both were only about 1-2 percent higher than for the control. There were significantly more adhering hulls in the control treatment compared to the moderate stress treatment. There were no significant effects on nut color as measured by RLI (Table 4).

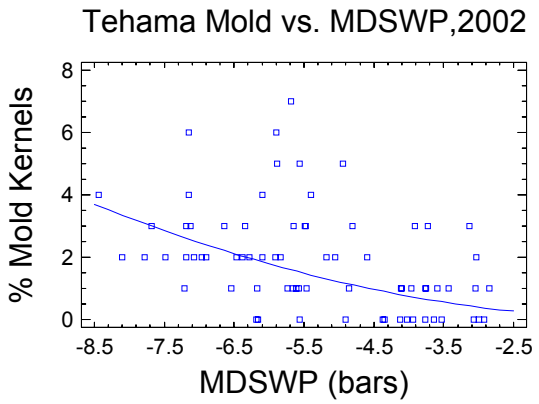
Table 4. Quality data for the Tehama County site from Diamond Walnut.
Harvest samples were obtained from each individual tree that was monitored for water potential.

Treatment	% Large	% Mold	% Insect	% Shrivel	% Adhering Hull	RLI
1	96.0 a	0.83 b	0.0 a	1.04 b	1.25 a	51.5 a
2	94.5 a	2.62 a	0.12 a	2.29 a	0.83 ab	51.3 a
3	85.7 b	2.29 a	0.17 a	2.71 a	0.42 b	52.2 a
LSD	3.0	0.84	0.17	0.93	0.67	1.6

Common letters among means within columns denote no significant difference at $P \leq 5\%$ using Duncan's mean separation.

When regressed as the average seasonal MDSWP against the kernel mold of each individual tree, a weak relationship exists (Figure 9). This is the only trial of five conducted over two years that gives some indication that the incidence of mold is increased by increased water stress. The R-Squared statistic indicates that the model as fitted explains 19.3% of the variability in mold after transforming to a square root scale to linearize the model. The correlation coefficient equals -0.44, indicating a relatively weak relationship between the variables.

Figure 9. Relationship Between Kernel Mold and Seasonal Average MDSWP at the Tehama Site, 2002

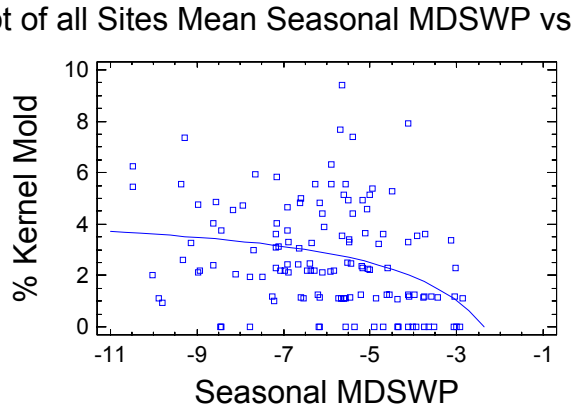


SUMMARY

There is no doubt that kernel mold reduces edible yield and therefore price received for the crop. The increase in incidence of mold, especially by late varieties, seems to be real. The approach we took was sound. We withheld irrigation water while monitoring the tree water status with a pressure chamber until significant differences in water stress were achieved. Two closely controlled trials in 2001 and three in 2002 were conducted to access the effects of water deficits on the incidence of walnut kernel mold in mature Chandler orchards. In the 2001 Chico trial, a comparison was made between well watered and significant water stress conditions occurring at a time when it was hypothesized to have the most effect on the incidence of mold mid-late season. In 2002, the severity of water stress was increased and the time of water stress moved to earlier in the season. In this trial (Chico), no relationship between mold and water deficits was found in either 2001 or 2002. The Clements trial (conducted in 2001) achieved stress to a lesser differential but at two timings, mid and late plus midseason. The level of deficits had no significant effect on the presence of kernel mold. At the San Joaquin site, no significant differences in the incidence of kernel mold were found between treatments. At the Tehama site, significantly fewer molds occurred in the full water treatment when compared to the mild and moderate treatments trending towards a significant relationship between the molded nuts and seasonal MDSWP.

When regressed as the MDSWP of all sites in 2002 against the kernel mold at all three sites, the relationship degrades over the Tehama site alone (Figure 10). The R-Squared statistic indicates that the model as fitted explains only 10.1% of the variability in mold after transforming to a square root scale to linearize the model. The correlation coefficient equals -0.32, indicating a relatively weak relationship between the variables.

Figure 10. San Joaquin, Tehama, and Chico Sites Combined Mean Seasonal MDSWP vs. % Kernel Mold, 2002



Another hypothesis advanced is that the nuts that fall early onto the wet soil surface will have a higher incidence of mold. To address this issue, nuts were collected from trees prior to shaking that were on the soil surface still moist from micro-sprinkler irrigation. No significant difference in mold was found between windfall and shake nuts with or without water stress in 2001 or in 2002.

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