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Crown moisture and prediction of plant mortality in drought-stressed alfalfa

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Abstract Withholding alfalfa (*Medicago sativa* L.) irrigations during the summer, a practice referred to as summer irrigation termination (SIT), can conserve substantial amounts of water in long-season desert environments; however, plant mortality associated with SIT may be substantial. Proper timing of re-irrigation is critical for minimizing mortality and yield reductions following SIT. Procedures that would permit probable mortality prediction during drought stress would improve management efficiency with SIT. This study was conducted to determine (1) whether plant mortality occurs once the moisture content of the plant woody stem portions (crown) falls below some critical threshold and (2) if such a threshold could be used to predict the likelihood of plant mortality during SIT. Crown samples were taken from single, spaced, field-grown plants in Tucson, Arizona, at the end of a 84-day SIT period in 1994. A crown moisture content of about 42% was identified as a likely threshold critical for crown tissue SIT survival. This value was then used to predict whole-plant mortality of alfalfa grown in solid-seeded plots comparable to commercial fields. Crown samples were taken at five locations within the field along a solid gradient that was related to plant mortality. At each sampling location, the proportion of samples with less than 42% crown moisture was used to predict plant mortality. Predicted mortality slightly overestimated actual mortality but differences between predicted and observed mortality were significant for only one of five sampling locations. Alfalfa growers may be able to use this simple method of crown moisture determination to prevent permanent yield reductions by initiating irrigation before substantial portions of crowns fall below the threshold moisture content of 42%.

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Introduction

The annual water consumption of the perennial forage crop alfalfa (*Medicago sativa* L.) can be extremely high in long-season desert environments where nearly continuous year-round growth is possible. For example, irrigation application can exceed 200 cm per year in the southwestern United States (Robinson and Teuber 1992). Rising irrigation costs and water demand from municipalities have forced alfalfa growers in this region to use water more efficiently (Frate et al. 1991). During the period from July to September, peak water demand from municipalities and other crops coincides with low yields and low water use efficiency in alfalfa (Kipnis et al. 1989). In addition, hay quality and prices in the southwestern United States are typically lowest during this period (Hood 1992). This combination of factors drastically reduces the profitability of alfalfa production from July to September, and in extreme cases can lead to net losses for growers (Husman 1992). Discontinuation of irrigation during this period, forcing plants into semidormancy, could increase overall water use efficiency in alfalfa and make up to 800 mm of irrigation water available for other, more economical uses with minimal effects on farm income (Ottman et al. 1996; Robinson and Teuber 1992).

In addition to water conservation efforts, summer irrigation termination (SIT) has been considered in areas where heavy insect infestations severely affect the production of fall vegetable crops (Wrona 1992). Withholding alfalfa irrigation from mid-summer to fall could interrupt the nearly continuous year-round cropping pattern in long-season desert environments. Alfalfa can be the main host for insects such as the sweetpotato whitefly (*Bemisia tabaci*) between summer crop harvests and fall crop plantings (Natwick et al. 1992). Depriving whiteflies of a preferred host in early fall may reduce whitefly populations to a level where biological control agents or insecticides will be an effective control in fall crops.

In order to use SIT successfully, plant mortality must be limited to levels that do not lead to a permanent reduction in forage yield. Studies conducted to evaluate the effect of SIT on the productivity of alfalfa stands have produced mixed results. In Cyprus (Metochis and Orphanos 1981), central California (Frate et al. 1991), and central Arizona (Ottman et al. 1996), 56–63 days of SIT were associated with reduced forage yields only in the first post-SIT harvest. However, in the Imperial Valley of California (Wrona 1992) and in western Arizona (Ottman et al. 1996), stand density and post-SIT yield were significantly reduced on soils with a low water-holding capacity or if SIT was prolonged to October (>110 days) (Ottman et al. 1996; Wrona 1992).

New herbaceous shoots originate from the perennial basal (woody) portion of the stem following SIT, an area referred to as the crown (Steward 1926). Even after all herbaceous shoots have died, plants can recover from drought stress if crown tissue is capable of producing new shoot growth from axillary buds within the woody crown tissue. The degree of mortality in crown tissue (100% crown mortality = whole-plant mortality) will be a major factor affecting post-SIT productivity of individual plants and whole alfalfa stands. Crown mortality during SIT is likely to depend on the amount of water lost from this tissue. Crown moisture content may therefore indicate whether or not a plant will survive SIT. Where soil conditions may lead to high plant mortality or under conditions that lead growers to extend the SIT period beyond 110 days, irrigation timing will be of utmost importance to minimize mortality. Reports of high mortality rates in commercial fields that were subjected to SIT in the Imperial Valley (Wrona 1992) indicate the need for an objective criterion that will allow growers to schedule irrigation based on plant mortality estimates.

The objective of this study was to test the hypothesis that crown mortality occurs when crown moisture content falls below some critical threshold level. Parts of crowns from field-grown plants were sampled to determine their moisture content and the remaining unsampled crown area was used to assess mortality during SIT. Data from these experiments were used to determine a crown moisture value at which death will occur. After this threshold value had been established, it was used to predict alfalfa plant mortality in a second field trial exposed to SIT.

Materials and methods

The experimental site was located at the Campus Agricultural Center, Tucson, Arizona. Plant material used to established a crown moisture content threshold were single, spaced plants of a composite population of very nondormant Arabian and North African ecotypes (AZ91-AC). Seed was produced in a bee cage on plants that represented bulked open-pollinated Syn-1 seed from the six highest-yielding entries (described in Smith et al. 1991) in a 2-year field trial of extremely nondormant Arabian and North African ecotypes at Tucson, Arizona. Individual plants of this composite were sown in two contiguous field plots, which were treated equally and considered replicates, on a soil classified as Pima clay loam (Fine-silty, mixed (calcareous), thermic Typic Torrifluvent) on 30 September 1993. Each plot contained 240 plants (ten rows, each with 24 plants), which were spaced 25 cm within and 50 cm between rows. The two outside rows and three plants at both ends of rows were treated as borders. Plots were sprinkle irrigated until seedlings were established and flood irrigated at approximately 120% of cumulative evapotranspiration thereafter (60 mm of irrigation every 5–10 days), except for the interval from 18 July to 10 October 1994, when irrigations were withheld for a 84-day SIT period. Cumulative evapotranspiration during the SIT period was 558 mm and precipitation 85 mm (data obtained from the Arizona Meteorological Network at the Campus Agricultural Center, Tucson, Arizona). The volumetric soil moisture content was measured with a neutron probe at depths of 15 and 105 cm on days 2, 42, and 84 of the SIT period.

Forage was harvested when most plants were at approximately 10% bloom five times prior to the SIT period (10 cm stubble). We considered the day of the last pre-SIT harvest to be day 1 of SIT because the next irrigation would have been scheduled for that day. On day 4 of SIT, the percentage of the crown area that produced new shoots was estimated visually. This procedure was repeated after the first post-SIT harvest in December 1994. From both estimates, crown mortality was calculated as a percentage of the productive pre-SIT crown area.

On days 5, 42, and 84 of the 1994 SIT period, one branch (approximately 3 cm in length and 2 g in weight) of the woody, lower part of the crown was sampled on 60 plants in both plots (three alternate rows per plot with 10 plants sampled per row). Crown samples were wrapped in aluminum foil, stored in plastic bags, and placed on ice for transportation to the laboratory. Prior to fresh weight determination, all nonwoody plant material was removed from crown samples. This included dry stubble and any herbaceous shoot tissue. Samples were shock-frozen in liquid nitrogen and stored at −80 °C. Dry weight was determined after lyophilization for 24 h. Crown moisture content was calculated as percent moisture in the fresh sample. Crown mortality in neighboring rows was used to determine whether sampling influenced survival and crown mortality. A one-sided *t*-test was conducted to compare mean crown mortality of sampled and unsampled rows.

The following year (1995), crown moisture content was used to predict mortality in field plots that closely resembled conditions in commercial alfalfa fields in this region. The experiment was located at the Campus Agricultural Center, Tucson, Arizona, on a soil classified as a Pima-Agua Complex: Pima clay loam [Fine-silty, mixed (calcareous), thermic Typic Torrifluvent]; Agua sandy clay loam [Fine-loamy over sandy, mixed (calcareous), thermic Typic Torrifluvent]. Six experimental populations were sown in a latin square design on 30 March 1995 at a seeding rate of 6.7 kg ha^{-1}. Plots contained five 1-m-rows with 15-cm spacing between rows and 30 cm between plots. Initially, 60 seeds m^{-1} were sown; these were later thinned to 22 plants m^{-1}. A 7-row border seeded with the cultivar Lew (20 kg ha^{-1}) surrounded the experimental area. The field was flood irrigated at approximately 130% of cumulative evapotranspiration (70 mm per irrigation every 5–10 days) until 1 August 1995, when a 71-day SIT period was imposed. Cumulative evapotranspiration during the SIT period was 471 mm and precipitation 81 mm (data obtained from the Arizona Meteorological Network at the Campus Agricultural Center, Tucson, Arizona). Plots were harvested twice prior to the SIT period when most plants were at approximately 10% bloom (10 cm stubble). Live plants in each plot were counted on day 3 of SIT and following the first post-SIT forage harvest on 17 November 1995. An ANOVA was conducted and demonstrated no differences in plant mortality between experimental populations in the latin square.

Soil variability strongly affected the plant response during the SIT period along a gradient from southwest (all leaves desiccated) to northeast (the majority of leaves remained turgid) within the experimental field. This gradient allowed us to take crown samples from areas with the experimental field that had experienced different degrees of drought stress. Crown samples were taken from five locations along this gradient prior to initiating irrigations. These five locations represented the whole range of stress intensities observed in our field and were considered nonrandomly arranged treatments. We designated these treatments (locations) A–E (A = plants most stressed, E = least stressed).

Because plants that are grown in rows have smaller crowns than single, spaced plants, whole crowns instead of crown parts were sampled in this experiment. A total of 13–20 ($n=78$) crown samples were obtained per sampling site. Samples were taken from the innermost border row instead of the 1-m^2 plots because these plots were needed to estimate actual plant mortality. Crown moisture content was determined as before except that samples were dried in a microwave at low intensity (defrost). The time needed to dry samples was split into six runs of 75 s each with 30 s between runs to avoid burning the crown tissue. A microwave was used for drying crown samples in order to evaluate a simplified method that could easily and quickly be used by alfalfa growers or Extension personnel. Moisture content was calculated as a percentage of fresh weight. The proportion of samples with less than 42% crown moisture was used as an estimate of expected mortality.

Plant mortality was determined after counting live and dead plants in each 1-m^2 plot. The mean of two plots adjacent to each sampling site was used to estimate observed mortality. A regression analysis was conducted to determine the goodness of fit between predicted and observed values.

To investigate the cause of the differences in apparent stress intensities in our field, soil profiles were exposed at each sampling site by digging a trench (150 cm in depth) after the experiment had been terminated. This allowed us to measure the depth of the topsoil (A and C horizons in Pima clay loam, A horizon in Agua sandy clay loam). The differences in the degree of wilting observed for plants at different locations within the field corresponded to a gradient in texture and depth of the topsoil. In the southwestern part of the experimental field where treatments A, B, and C were located, the topsoil was classified as sandy clay loam and reached to a depth of 44, 48, and 65 cm for treatments A, B, and C, respectively. The topsoil in the northeastern part of the field was heavier (clay loam) and reached to depths of 105 cm for treatment D and 115 cm for treatment E. Gravelly or fine sand with an extremely low water-holding capacity was encountered at greater depths.

Results and discussion

Determination of a threshold crown moisture content

The comparison of mean whole-plant mortality in rows that were sampled and not sampled showed that removing crown parts for moisture determination did not affect mortality. Whole-plant mortality in rows of sampled plants was 32.3 ± 4.6% (mean ± SE) compared to 28.7 ± 4.4% in neighboring unsampled rows. On individual plants, mean crown mortality was 41.1 ± 6.7% in sampled rows and 35.1 ± 6.7% in unsampled rows. Neither whole-plant or crown mortality was significantly different between rows. In comparison, crown mortality in a normally irrigated control plot was 11.8 ± 7.6%. The volumetric soil moisture content measured at a depth of 15 cm decreased from 22.8% at day 2 of SIT to 18.5% at day 42 and 15.7% at day 84. The respective values for measurements taken at a depth of 105 cm were 17.7, 12.4, and 7.8%.

Mean crown moisture content of single, spaced plants decreased from 66.7 ± 0.4% on day 5 of SIT to 49.7 ± 0.5% on day 42. Crown moisture content was not significantly correlated with final crown mortality for either sampling date. Crown moisture content had further decreased to 6.6 ± 1.2% with 100% crown mortality on day 84 of SIT. The majority of plants with less than 25% crown mortality were found in a cluster between 41 and 48% moisture content (Fig. 1). Most plants with a crown mortality of 100% were in a cluster below 10% moisture. Only a few plants were found between the two clusters. We attribute this lack of intermediate values to rapid water loss in tissue that has died and lost its water retention capacity. Crown moisture content apparently approaches a threshold level that is critical for survival towards the end of the SIT period. Judging from the distribution of data in Fig. 1, a moisture content of approximately 42%, as measured in this experiment, may be such a threshold level. Only 1 of 32 plants at or above 42% did not survive SIT, but 22 of 27 plants below 42% had crown mortality greater than 50%. At a crown moisture content between 42 and 43%, 7 of 8 plants had less than 25% crown mortality. During the transition phase between the extremes of complete survival (0% crown mortality) and complete plant death (100% crown mortality), dead tissue could have been sampled from partly living plants. This may explain why several of the plants with a crown moisture content below 42% were still alive, and in 3 cases had less than 25% crown mortality.

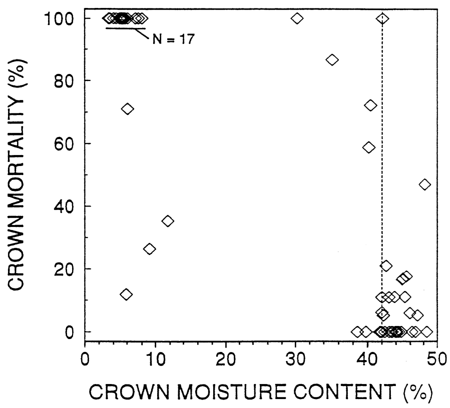

Fig. 1 Crown mortality after 84 days of summer irrigation termination as influenced by crown moisture content. Crown moisture content was determined as g/g fresh weight × 100%. The *vertical line* at 42% crown moisture represents an approximate threshold moisture content necessary for crown survival

Prediction of plant mortality in field plots

If a crown moisture content of at least 42% is of some physiological relevance for survival of crown tissue during SIT, it may be possible to use that threshold to predict plant mortality in alfalfa fields under SIT and to resume irrigation before the majority of plants approach that threshold.

The soil gradient in our experimental field allowed us to test this hypothesis at different intensities of drought stress, conditions that would normally require the comparison of several field experiments with increasing duration of SIT. Because plants in densely seeded stands do not develop complex crowns during the first year, whole-plant mortality rather that partial crown mortality was used to

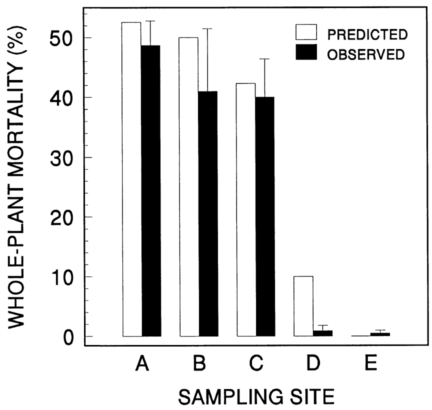

Fig. 2 Predicted and observed whole-plant mortality at five sites (A–E) along a soil gradient associated with differential plant response to summer irrigation termination. *Vertical bars* represent standard errors based on observed mortality from two plots in each site

estimate survival of SIT. Predicted mortality was compared to the mean observed mortality in the two plots next to the sampling site. These two plots contained different experimental cultivars; however, this should not have affected results presented in this study because whole-plant mortality was not significantly different between experimental populations.

The vast majority of plants in treatments D and E (topsoil exceeds 1 m in depth) did not approach the threshold value of 42% by the end of the SIT period, indicating that stress levels for these two treatments were not critical. This observation was in agreement with the fact that whole-plant mortality was less than 1% in plots of treatments D and E (Fig. 2). Where the topsoil depth was less than 50 cm (treatments A and B), whole-plant mortality increased to approximately 50% and we were able to closely predict this increase using 42% as the critical threshold level. The 95% confidence intervals for observed mortality overlapped with predicted mortality for four treatments. Only for treatment D did observed and predicted mortalities differ significantly. A regression analysis where the predicted values were treated as independent and observed values as dependent variables produced a highly significant ($r^2 = 0.97$) regression equation:

$$\text{observed mortality} = -4.2 + 1.01 \times \text{predicted mortality}.$$

On average, our prediction, based on the approximate moisiture threshold of 42%, overestimated actual mortality by 4.2%. Such a slight overestimation should be more acceptable to growers who would use predicted mortality to reschedule irrigation following SIT than an underestimation of mortality.

Certain assumptions were made in this study. Single, spaced plants that were able to develop complex crowns with several stems were used in the initial experiment to establish a critical crown moisture threshold for mortality. In using that threshold for predicting plant mortality in more densely seeded rows, we assumed that the threshold is the same for plants with large and small crowns. Results obtained in this study indicate that plants in a densely seeded alfalfa field may behave similarly to single, spaced plants. However, the critical moisture content could potentially decrease slightly as plants age and develop thicker bark (Teuber and Brick 1988).

Border rows that were used to obtain estimates for predicted mortality were seeded with the cultivar Lew but actual mortality was estimated on experimental populations derived from a composite of Arabian and North African ecotypes. Both Lew and the experimental populations are quite similar morphologically and developmentally inasmuch as both are classified as very nondormant (class 9), but we cannot exclude the possibility that they differed in their reaction to drought stress. Furthermore, border rows were seeded at a higher rate than field plots. Higher plant density may have increased whole-plant mortality in border rows due to increased inter-plant competition during drought stress. This could be a likely explanation for the tendency of our prediction to overestimate observed mortality.

Crown moisture content may increase even in dead crown tissue following precipitation. This may lead to an underestimation of the actual plant mortality rate if crown samples are taken immediately after such precipitation. Since dead plant tissue has lost its water retention capacity, a temporary increases in crown moisture of dead plants will be followed by a rapid decrease within a few days.

Despite such potential limitations, predicted whole-plant mortality closely agreed with observed whole-plant mortality in this study. In the absence of other suitable criteria, growers could use this simple and quick method of crown moisture determination as a guideline to re-schedule irrigation before a considerable portion of plants fall below the threshold of approximately 42% and plant density decreases to levels that permanently reduce forage yield following SIT. The exact number of plants needed to maintain acceptable yields following SIT will strongly depend on the age of the alfalfa stand undergoing SIT.

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