

PHYSIOLOGICAL FACTORS RELATED TO WATER-USE EFFICIENCY
OF ALFALFA (MEDICAGO SATIVA L.) GENOTYPES

by

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I hereby recommend that this dissertation prepared under my direction by Ghazi Majeed Al-Kawaz entitled Physiological Factors Related to Water-Use Efficiency of Alfalfa (Medicago sativa L.) Genotypes be accepted as fulfilling the dissertation requirement of the degree of Doctor of Philosophy

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ABSTRACT

A study was conducted at Tucson, Arizona to determine the variation in water-use efficiency among 150 genotypes of Medicago sativa L. cultivar 'Mesa-Sirsa' grown under three soil moisture regimes and to determine if there were physiological and anatomical parameters related to efficiency of water use and growth components of alfalfa genotypes at three different growth stages. Information was obtained that would contribute to the development of techniques in the selection of alfalfa genotypes efficient in water use.

Mesa-Sirsa genotypes varied in their water requirement. Some plants required three times more water to produce one gram of dry matter than other plants.

Soil-moisture content significantly affected the water-use efficiency in the seedling stage but not in the mature stage of growth. The amount of available soil moisture significantly influenced the production of dry forage, stems, and leaves at both seedling and mature stages of growth.

The relationship between water-use efficiency and growth components studied was significantly influenced by the amount of available soil moisture at the seedling stage but not at the mature stage of growth.

The water-use efficiency of Mesa-Sirsa seedlings grown under the medium soil moisture regime increased when dry matter production from forage, stems, and leaves increased. There was also a significant relationship between the total transpired water per plant and dry matter produced, number of stems and leaflets. Total water transpired was not significantly associated with the water-use efficiency of alfalfa seedlings.

At the mature stage of growth, there was a significant correlation between the water-use efficiency and each of the following factors: dry weight of forage and stems, number of leaflets, and stem-leaf ratio. The amount of water transpired per plant was significantly and positively correlated to dry matter production from forage, stems, and leaves, and to number of stems and leaflets per plant. There was no association between the total water transpired from a plant and the water requirement.

The relationship between the water requirement and transpiration rate significantly varied with the three growth stages. The most efficient alfalfa genotypes at floral bud or flowering stage transpired significantly less water per unit leaf area per unit time compared to the less efficient genotypes.

The rates of net photosynthesis and respiration varied among alfalfa genotypes and with growth stages. The rates of both decreased as plants progressed toward

maturity. At the floral bud stage plants with the highest water requirement also had the highest rates of photosynthesis and transpiration. At the vegetative stage of growth, respiration was directly associated with leaf-stem ratio and transpiration rate.

The number of xylem vessels was significantly associated with the water-use efficiency of alfalfa genotypes at the vegetative stage. The most efficient alfalfa plants had significantly less xylem vessels in the leaf midvein when compared to the least efficient plants. The thickness of palisade layer was also significantly related to water requirement of alfalfa genotypes at the floral bud stage but not at other stages. Efficient alfalfa genotypes developed a thinner palisade layer.

INTRODUCTION

Research on water utilization by agronomic crops has become a vital concern to investigators of arid and semi-arid areas as well as to those of other areas having limited water resources. The significance of this type of research is the development of selected crops that can use minimum quantities of water to produce maximum yields.

Researchers have already recognized the variation in water-use efficiency among plant species as well as among cultivars within species. The criteria in selecting water-efficient genotypes should not be based solely on units of water consumed per unit of plant dry matter produced, but also on their growth habits, on the efficiency of some physiological processes, and on anatomical features that may help to explain efficient use of water.

The objectives of this study were:

1. To determine if alfalfa genotypes of the Mesa-Sirsa cultivar vary in their water-use efficiency under greenhouse conditions.
2. To determine the effects of three soil-moisture regimes on water-use efficiency of alfalfa.
3. To measure the variation in the net photosynthetic, respiratory, and transpiration rates among Mesa-Sirsa genotypes at three different stages of growth.

4. To determine the association between water-use efficiency and net photosynthetic, respiratory, and transpiration rates.
5. To determine if anatomical characteristics of the leaves, petioles, and stems were associated with water-use efficiency of alfalfa genotypes.

LITERATURE REVIEW

Numerous investigators have shown that variation in the soil moisture status had a significant effect on growth and water requirement of agronomic crops.

Stern (57) reported that water was used more efficiently by cotton during wet season than during dry season. Scofield (52) stated that the water requirement was lower in spring and early summer and higher later in the summer and fall.

Early experiments by Briggs and Shantz (11) showed an increase in the water requirement when soil moisture content approached either extreme. In contrast to Briggs and Shantz's findings, Scofield (52) found that as the water stress conditions were increased the water requirement of alfalfa (Medicago sativa L.) remained the same. Data by Dobrenz et al. (19) showed that as soil-moisture stress increased, water-use efficiency and dry matter of production of blue panicgrass clones (Panicum antidotale Retz.) decreased. Wright (68) found that blue panicgrass exhibited a striking ability to produce forage under any of four soil-moisture-stress treatments. (Moisture-stress treatments were achieved by allowing the soil to dry to the wilting point at 15, 30, 45, and 60 cm deep between irrigations.) He also found that yield of this species was not

influenced by any of the water regimes applied. On the other hand, data presented by Burton, Prine, and Jackson (15) revealed that Coastal and Suwannee bermudagrasses (Cynodon dactylon [L.] Pers.) were more efficient during periods of drought than when water was more plentiful.

Smittle and Bradley (54) reported increased yield when available soil moisture was maintained above 50 per cent. Buckman and Brady (14) stated that for optimum plant growth, water should be applied when 50 to 85 per cent of the available soil moisture was used. Leman et al. (39) agreed with this concept of an optimum soil moisture range. They found that alfalfa forage yield increased by frequent short irrigation. Alfalfa was irrigated when 30 cm tensiometers read 30 to 35 centibars, and water was held on plots for three hours. Maurer, Omrod, and Fletcher (42), however, were pessimistic about the concept of the optimum range of soil moisture applied during the entire growth period of a plant. They suggested that optimum soil moisture varied according to plant growth stages.

Other factors that may influence water requirement of plants are growth habit, morphology, anatomy, and physiology. Pendleton (49) stated that the particular characteristics contributing to water-use efficiency among and within species are not precisely known. He cited, however, the following plant features as possible contributing factors to differences in water-use efficiency:

maturity, extensiveness of the root system, leaf area and angle of inclination, leaf rolling, number, distribution and size of stomata, presence of waxy or corky epidermal cells, and the ability to go temporarily dormant. Using one or more of the aforementioned plant characteristics which may be associated with the most efficient genotypes among species, cultivars, and individual plants of the same cultivar may lead to the development of a new selection tool in plant breeding programs under both greenhouse and field conditions. In fact, Keller (36) suggested the use of water requirement as a selection tool for orchardgrass (Dactylis glomerata L.) breeding under both field and greenhouse conditions. This suggestion was confirmed by Cole (16) who obtained a significant variation in water-use efficiency among alfalfa genotypes within cultivars. He concluded that such findings would warrant additional investigation on water-use efficiency within cultivars.

Growth habits of plants could be used as an indication of the amount of water used by crops. Hanks, Gardner, and Florian (31) found that under dryland conditions in the Central Great Plains, yields of several crops and transpiration were directly related. They also concluded that the relation between transpiration and total dry weight was similar under greenhouse and field conditions. A similar statement was also made by Arkley (2) concerning the relationship of plant growth and transpiration.

In an experiment conducted by Keller (35) to determine the water requirement of 16 orchardgrass genotypes he revealed that genotypes high in herbage yield were low in water requirement. Baker and Hunt (3) found significant clonal differences in the number of tillers produced and in the efficiency of water use of grasses. In another experiment conducted on corn (Zea mays L.), it was found that water-use efficiency increased as corn yield increased (58).

As a plant reaches maturity, water consumption decreases. Slatyer and Bierhuizen (53) found that the rate of transpiration of a fully expanded cotton leaf after a 25-day period was half of the maximum value.

Stomata size and number have not been accepted yet as a definite feature indicating the differences in water-use efficiency among species and cultivars (44). However, Dobrenz et al. (20) found that stomata density and water-use efficiency of drought tolerant blue panicgrass clones were positively associated. Clones which had the highest stomata density were most drought tolerant. They also found in another experiment that clones most efficient in water-use had more vascular bundles in the leaves when compared to the least efficient clones (19).

Photosynthesis and Respiration

The rate of growth and development of field crops is governed mainly by two factors: the genetic constitution of that particular crop, and the environmental conditions under which the crop is grown. The environmental conditions exert a profound influence upon the expression of genotype. Thus, growth of a crop can be strongly affected by light, temperature, carbon dioxide, moisture conditions, and nutrient availability.

Photosynthesis and respiration are two important plant processes that ultimately determine the rate of crop growth and dry matter production.

Field crops accumulate dry matter at a rate determined by the intensity of solar radiation, the assimilation rate of individual leaves and other organs capable of photosynthesis, the size of photosynthetic system, and the loss of carbohydrates by respiration (43).

In the early stage of plant growth, dry matter is produced at a rate almost proportional to leaf area. When leaf shading increases with the progress of plant development, the rate of photosynthesis increases less rapidly than leaf area. A maximum leaf area index (the ratio of plant leaf area to the soil surface area) is reached when there are enough leaves to intercept all the radiation falling on the canopy. The change in dry matter production with age was attributed by Watson et al. (63) to a change

in photosynthetic rate. Pearce, Brown, and Blaser (48) measured net photosynthesis on alfalfa leaves of different ages from a growth chamber and from normal and thinned field plots. They found that net photosynthesis declined with leaf age after full expansion. The mean net photosynthetic rate for young leaves was $52 \text{ mg CO}_2/\text{dm}^2/\text{hr}$ when grown in the growth chamber. Similarly, Fuess and Tesar (25) reported that when they used 2- and 3-cut regimes on alfalfa stand, they obtained 17% more hay from alfalfa cut three times than from alfalfa cut two times. They attributed the difference in total yield to higher rates of net photosynthesis in the younger leaves of the 3-cut as compared with the 2-cut schedule. Furthermore, they found that leaves more than three weeks old were less than one-seventh as active photosynthetically as 5-day old leaves.

Monteith (43) stated that as the total leaf area increased, leaves low in the canopy received less and less light and their gross rate of photosynthesis decreased. The total rate of dry matter accumulation by the foliage continued to increase with leaf area until photosynthesis by the lowest leaves was just enough to compensate for their loss of carbohydrate by respiration.

King and Evans (37) studied the relation between the leaf area index of alfalfa and rates of net photosynthesis and dark respiration. Their results indicated that net photosynthesis rose rapidly with increase in LAI to a value

of 3 to 4 and continued to rise slowly up to the highest LAI values attained, above 10. At a light intensity of 3300 f.c., the highest rate of net photosynthesis was 44.5 mg CO₂/dm²/hr. They also reported that leaf respiration rate fell progressively with depth in the canopy. The highest respiration rate was 21.5 mg CO₂/dm²/hr.

According to the review of previous articles, it appears that as crops progress in maturity, dry matter accumulation slows down. The photosynthetic system gets less efficient, and more carbohydrates are lost by respiration (40). Therefore, the rates of dry matter accumulation during the whole growing season are governed by changes in the balance of photosynthesis and respiration (43).

The photosynthetic rate can be measured either directly by estimating the CO₂ input or the O₂ output per unit leaf area and time, or indirectly by determining the dry weight increase. Gregory (29) introduced the concept of the "net assimilation rate" which was defined as the rate of increase in total plant dry weight per unit of assimilating material per unit time. Williams (65, 66) replaced leaf area by dry weight and later by leaf protein. Watson (61, 62) stressed the use of leaf area for sake of uniformity, which later has been substituted by the leaf area index. Bongers (8) emphasized that the employment of a given unit of measurement may greatly affect the results.

Numerous researchers have studied the influence of light intensity on net assimilation rate. Briggs, Kidd,

and West (10), Gregory (30), Goodall (28), Watson (60), and Blackman, Black, and Kemp (6) all showed that light had a significant and positive effect on the net assimilation rate.

Variation in net assimilation rates among and within cultivars has been demonstrated. Ludlow and Wilson (41) reported that two grass cultivars had higher growth rates due to a high net assimilation rate. They explained that the high net assimilation rate of the grass was due to a higher photosynthetic rate, despite a much greater respiration rate compared with legumes. Watson and Hayashi (64) indicated that young sugar beet plants had a greater net assimilation rate than barley plants because they had a greater rate of photosynthesis. Respiration rates of whole plants for the two species were identical.

Blackman and Matthael (5) considered the importance of other factors and concluded that when CO_2 concentration and temperature were not limiting, the photosynthetic rate was proportional to the intensity of the incident light.

Recently, Gaastra (26), working with certain field crops determined the light intensity curves of photosynthesis at different leaf temperatures under normal and increased CO_2 concentrations. When the CO_2 concentration was nonlimiting, the photosynthetic rate increased with light intensity till a temperature sensitive light saturation was reached.

Bierhuizen and Slatyer (4) found that at all light intensities ranging from 1000 to 6000 ft-c, there was an almost linear response to CO₂ concentration up to values of 600 to 800 ppm. Above this CO₂ concentration, light became limiting. When CO₂ was a limiting factor, an increase in light intensity increased photosynthesis. Two reasons were given by the authors for such a phenomenon; stomata resistance was decreased, which increased CO₂ diffusion through the stomata; and the liquid phase permeability of the mesophyll cell walls to CO₂ transport was increased.

Similar work was conducted on two cultivars of soybean (Glycine max [L.] Merr.) by Brun and Cooper (13). They found that at light intensities above 5380 lux, the photosynthetic rates of both cultivars were limited by the CO₂ concentration of the surrounding atmosphere within the range of 270 to 1670 ppm. At normal atmospheric CO₂ concentration, photosynthesis of both cultivars was light saturated at about 21,530 lux. At a CO₂ concentration of 1670 ppm, photosynthesis was not light saturated in either cultivar at the highest light intensity tested.

Alexander and McCloud (1) reported that CO₂ uptake and ultimately dry matter production of bermudagrass involved the summation of many diverse effects on determining the degree to which the total leaf volume approaches light saturation during the growth period.

Light saturation, compensation point, and maximum rate of photosynthesis differ with the plant species and the conditions under which they had previously grown. Bohning and Burnside (7) investigated the relationship between light intensity and apparent photosynthesis in leaves of sun and shade plants and stated that at a light intensity of 100 to 150 ft-c, no net photosynthesis occurred in the leaves of sun plants. Light saturation was reached in the range of 2000 to 2500 ft-c. In the shade plants apparent photosynthesis was not zero until a light intensity as low as 50 ft-c was reached. Light saturation also occurred at a relatively low intensity of 500 to 1000 ft-c. The compensation point of the sun leaves was 100 to 150 ft-c as compared to 50 ft-c for the shade leaves.

Similarly, Wassink, Richardson, and Pieters (59) have shown that photosynthesis of detached leaves of Acer pseudoplatanus which were grown at low light intensity showed light saturation at a lower light intensity than those grown at high light intensity.

Forrester, Krotkov, and Nelson (23, 24) studied the effect of O_2 on the CO_2 exchange of detached dicot and monocot leaves. The results indicated that dicotyledon species (soybean) responded differently to O_2 than monocotyledon (corn) species. They found that apparent photosynthesis of soybean was inhibited by O_2 while the steady rate of respiration after a few minutes in the dark was not

affected. Part of the inhibition of apparent photosynthesis was shown to be a result of increased photorespiration. This stimulation of photorespiration by O_2 was manifested by an increase in the compensation point. On the other hand, the authors reported that twelve different cultivars of corn did not produce CO_2 in the light at any O_2 concentration. This was demonstrated by the zero CO_2 compensation point and the absence of a CO_2 burst in the first minute of darkness. The rate of photosynthesis was inhibited by O_2 and the inhibition was not completely reversible. However, the steady rate of respiration after a few minutes in the dark was not affected by O_2 .

Poskuta (50) used detached spruce twigs to measure photosynthesis, photorespiration, and respiration as influenced by oxygen concentration and light intensity and also showed that photorespiration rate was directly related to oxygen concentrations. Hoefstra and Hesketh (32) studied the effects of temperature on gas exchange of leaves from several species in the light and dark with air, O_2 -free air, and CO_2 -free air. They found that respiration in the light had a different sensitivity to temperature compared with respiration in the dark. At the lower temperatures, respiration in the light was higher than respiration in the dark. At temperatures above 40 C the reverse was observed. For any one species the maximum rates of photosynthesis and photorespiration occur at about

the same temperature. The maximum rate for dark respiration generally was found at a temperature about 10 C higher.

Moss (45) used only CO₂-free air and revealed that the rate of CO₂ evolution from the leaves of five species decreased when they were first illuminated, passed through a minimum, then increased to a higher rate than the original dark rate. Upon darkening, the rate of CO₂ evolution rapidly increased to a rate of CO₂ higher than illumination plateau, then gradually decayed to the original steady state dark respiration rate. The author (45) concluded that light enhanced a reaction which led to additional CO₂ evolution above normal dark respiration for many species when the CO₂ concentration in the atmosphere was low.

Moss (45) and Hoefstra and Hesketh (32) were in agreement with the finding that corn had no light-stimulated CO₂ evolution.

Data from several experiments showed that photosynthetic and respiratory rates varied with plant species and cultivars. Duncan and Hesketh (21) found differences among 22 races of corn in their net photosynthetic rates. Zelitch (69) also reported considerable variation among species studied in their rate of photorespiration. El-Tabbakh (22) measured the net photosynthetic rates of several species of grasses and several cultivars of alfalfa grown under both greenhouse and field conditions. He found that at a given temperature, alfalfa cultivars

varied in their net photosynthetic rates under both laboratory and field conditions.

Holmgren, Jarvis, and Jarvis (33) attributed the differences among tree species in net photosynthetic rates to differences in both stomatal and mesophyll resistance but the variation in the stomatal resistances was considerably larger than the variation in the mesophyll resistance. They reported also that within the species the variation in the minimum stomatal resistance was quite large in some cases whereas the variation in the mesophyll resistances was generally small.

It may also be possible that species and cultivar variation in net photosynthetic rates is due to differences in the diffusive resistance of the cuticle (46).

Data are not available in the literature indicating whether alfalfa genotypes of the same cultivar would show variation in photosynthesis, respiration, and transpiration.

MATERIALS AND METHODS

Planting

One hundred and fifty plastic pots (4.2 liter size) were placed in a greenhouse with a temperature range of 20 to 40 C. They were arranged in a randomized complete block design. Each block consisted of 30 pots. Each pot was filled with 400 grams of pea gravel in the bottom and 2000 grams of air dried soil mixture made up of three parts of desert Mohave clay loam (Typic Haplagid) and one part peat moss with 100 grams organic fertilizer (milorganite), and three grams sulfur added per 20 kg soil mixture. A hole 1.5 cm in diameter was bored at the base of each pot to allow for drainage of excess water from the soil during germination and plant establishment.

Fifty alfalfa seeds (Medicago sativa, cultivar Mesa-Sirsa)¹ were sown in each pot, covered with one-fourth inch layer of soil, and watered adequately to insure germination. Excess water was allowed to drain overnight. One week after germination, seedlings were thinned to five

1. Mesa-Sirsa alfalfa was officially released by the Arizona Agricultural Experiment Station and the Crops Research Division of the U.S.D.A. in 1966. It was developed from 13 plants having a high level of resistance to the spotted alfalfa aphid (Therioaphis maculata Buckton). These parent plants were selected from Sirsa No. 9 (P.I 235 736), an introduction from India.

per pot. When seedlings were 10 cm high, they were thinned to one healthy plant per pot.

Three water treatments were used to maintain soil moisture at different levels. Plants were watered to field capacity when 30, 60, and 90% of the total moisture content at field capacity remained in the soil.

When plants were thinned to one per pot, 8 g of coarse ground styrofoam were placed over the soil surface of each container to minimize soil evaporation as suggested by Dobrenz, Cole, and Joy (18). Then the three water regimes were assigned randomly to pots within each block.

A gravimetric method (12) was used to measure the amount of water lost by evaporation and transpiration. Two check pots per block were used for computing the loss of water by soil evaporation.

Pots were weighed and rewatered to the field capacity whenever the specified moisture content reached weights corresponding to the aforementioned water regimes. The difference between the initial weight of a pot and its weight at a given interval constituted the total loss of water by evaporation and transpiration. When water lost by evaporation was subtracted from the total water lost by evapotranspiration, the net weight represented the amount of water lost only by transpiration.

Water-Use Efficiency

Water-use efficiency of plants was determined at the seedling and mature stages of plant growth. At the seedling stage, alfalfa plants were harvested when they reached the first bloom. Plant tops were harvested 7 cm above the soil surface. They were oven dried at 80 C for 24 hours, and weighed on an electric Mettler balance that was accurate to 0.001 gram. The water-use efficiency was then determined by dividing the quantity of water transpired by a plant during the period of growth by the dry matter of tops produced during the same period of growth.

Mature alfalfa plants were harvested when they were near the second flowering (flowering after first clipping). Harvesting, weighing, drying, and computation of water-use efficiency were determined the same way as in the seedling stage.

Additional measurements were made on each seedling and mature alfalfa plant which included the number of leaflets, number of stems, dry weight of leaflets, dry weight of stems, and stem-leaf ratio.

Statistical analyses and correlation coefficients of the above parameters were processed by Numerical Analysis Laboratory, University of Arizona.

Measurement of Photosynthesis and Respiration

Mesa-Sirsa alfalfa seeds were planted in 45 plastic pots. Planting procedures were similar to those used previously. Pots were divided into three equal sets in order to obtain plants of three different growth stages-- plants before floral-bud development (8-week old), plants with floral buds (10-week old), and plants at flowering stage (12-week old). Pots were rewatered to field capacity when 60% of the total moisture content remained in the soil. Plants were thinned to one per pot when seedlings were 10 cm high.

Eight grams of coarse ground styrofoam were placed on the soil surface of each pot. One pot without a plant was used as a check to account for the amount of water lost by evaporation in the treated pots (with plants) at each growth stage.

Photosynthetic and respiration rates were measured for ten plants selected at random from the specified growth stages. An air-sealed leaf chamber technique as described by Wolf et al. (67) was used for leaf photosynthesis and respiration determinations. A Beckman infrared CO₂ analyzer (Beckman model IR215A, Beckman Instruments, Inc., Fullerton, California, U.S.A.) was calibrated with standard air-CO₂ mixture prepared by the Matheson Co., Inc., East Rutherford, New Jersey.

The air-sealed chamber consisted of three chambers, mixing, reference, and leaf chamber. The leaf chamber opened from one end to facilitate the insertion of alfalfa branches (Figure 1). Air from outside the laboratory room was mixed thoroughly, forced into the mixing chamber, and pulled through the leaf chamber by a suction pump at a rate of 1.79 liters per minute. Air was regulated by a flow meter manufactured by Manostat Corporation, New York.

The air sample passing through the analyzer was dried by passing through a column of anhydrous calcium sulfate. The output of the analyzer was fed to a continuous model H Azar recorder (Leads and Northrup, Philadelphia, Pennsylvania) where the signal was amplified.

By differentially sampling the air for CO₂ content as it passed through the reference chamber and through the leaf chamber, CO₂ uptake and output could be recorded. Thus net photosynthetic and respiration rates were computed.

Respiratory and net photosynthetic rates were measured when leaf chamber temperatures were 25 and 33 C, respectively. The net photosynthetic rate was also measured under light of 6000 ft-c; an intensity obtained from seven 300-watt incandescent lights.

Net photosynthetic and respiration rates obtained were expressed as mg CO₂ per square decimeter of leaf area per hour according to the following formula:

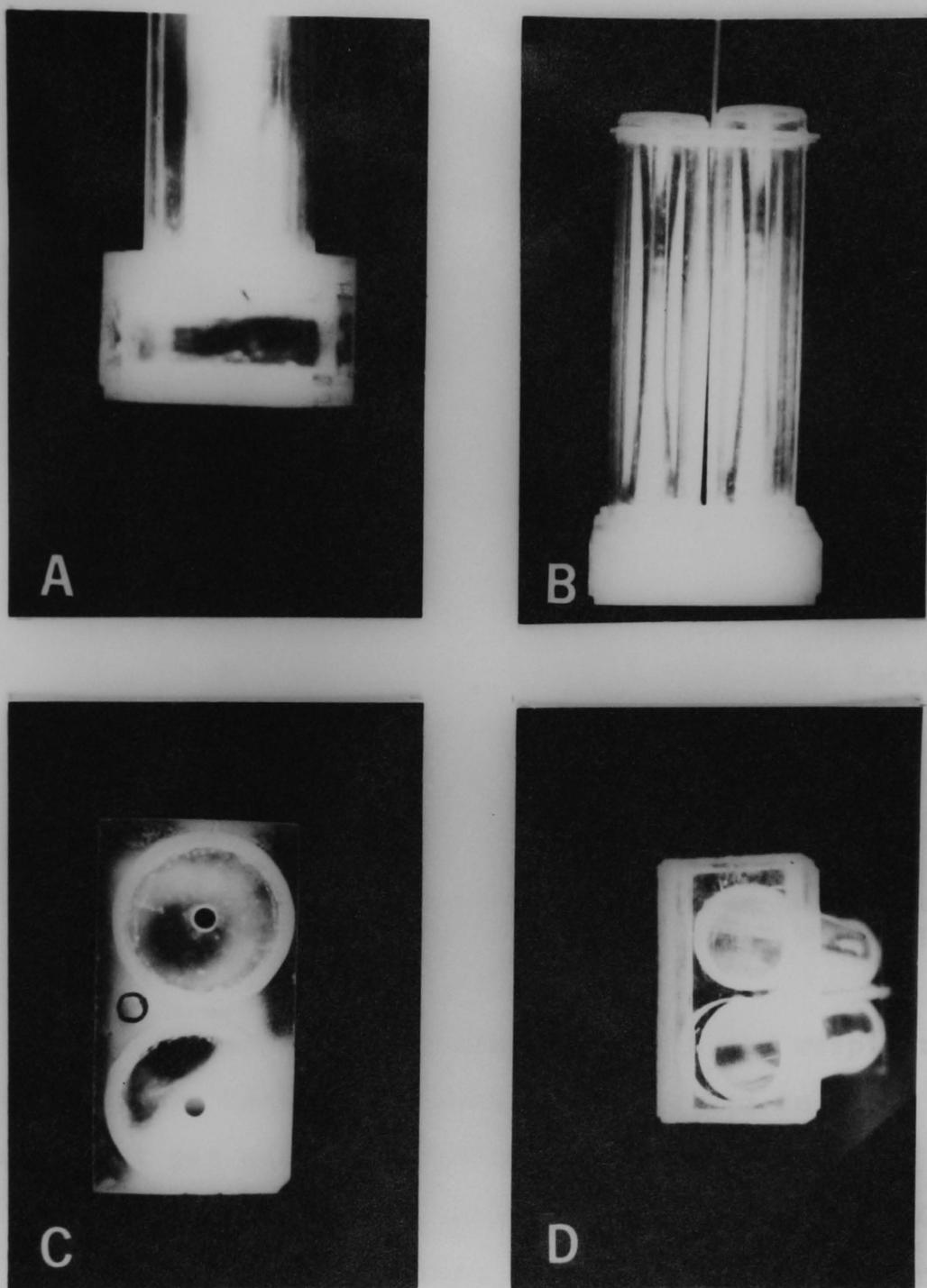


Fig. 1. The air-sealed leaf chamber used for measuring the net photosynthetic and respiratory rates -- A. Side view of mixing chamber. B. The general view of air-sealed chamber showing the mixing, reference, and leaf chambers. C. The inlet of air into the mixing chamber placed between the reference and leaf chamber outlets. D. The open end of leaf chamber where an alfalfa branch could be inserted.

$$R \text{ or } P = K \cdot F \cdot \Delta\text{CO}_2/A$$

where: R = respiration rate mg CO₂/dm²/hr

P = net photosynthetic rate mg CO₂/dm²/hr

F = The air flow through the chamber in liters per minute. This reading was from the flow meter and its calibration curve, and was converted to liters/hr.

K = The constant of conversion of volume of CO₂ to milligrams of CO₂ where 22.4 liters of CO₂ at 0 C and one atmosphere pressure equal to 44000 mg.

$$\frac{44000}{22.4} \left(\frac{273}{273+T} \right) \left(\frac{697}{760} \right) = \text{mg CO}_2/\text{liter}$$

is an approximate empirical conversion factor from liters of CO₂ to mg CO₂ for the location of Tucson, Arizona.

ΔCO_2 = calibrated value (ppm) per division on the recorder X Δ readings on the recorder for photosynthesis or respiration.

A = The leaf area in square decimeters determined by tracing leaves on paper of known weight per unit area.

After measurement of photosynthesis and respiration, plant tops were harvested. Leaves were traced on paper of known weight per unit area for leaf area determination. The leaves and stems were separated and oven dried at 80 C for 24 hours. Total dry matter was used for computation of water-use efficiency as was done previously.

Correlation coefficients of photosynthetic and respiratory rates with water-use efficiency, transpired water, transpiration rate, total dry matter, dry matter of

leaflets, dry matter of stems, stem-leaf ratio, and leaf area were determined.

Determination of Transpiration Rate

Transpiration rate of alfalfa plants at three growth stages was measured by a gravimetric method. Plants that were grown for photosynthetic and respiration rate determinations were also used for transpiration rate measurements. When plants reached the specified growth stage, soil moisture content in pots was returned to field capacity. Two days later, pots were weighed and amount of water transpired was computed. Total leaf area per plant was calculated according to the method used previously. The quantity of water transpired per square decimeter of leaf area per hour was computed.

Anatomy

Fully expanded leaflet, petiole, and stem samples were obtained from the uppermost internode of an alfalfa branch of each plant. The samples were collected from plants that were grown for photosynthesis and respiration measurements.

A section of the middle leaflet, petiole, and stem was cut, killed in FAA, and prepared for sectioning according to techniques described by Johansen (34).

Leaflets, petioles, and stems were microtomed to 10 μ thick, mounted, and stained with safranin and fast

green. The mean palisade and spongy layer thickness of leaf cross section was measured from five locations on the leaf under the microscope field of 0.22 mm (high dry). The number of xylem vessels of petiole, leaf midvein, and stem cross section of each of the ten plants was counted under the microscope. The number of xylem vessels was obtained from the mean of two counts.

RESULTS AND DISCUSSION

Effects of Three Water Regimes on Water-Use Efficiency and Several Other Characteristics of Mesa-Sirsa Genotypes

Seedling Stage

The amount of soil moisture available for growth and development of alfalfa significantly influenced water-use efficiency in the seedling stage (Table 1).

Alfalfa genotypes grown under the low, medium, and high water regimes (30, 60, and 90% of total soil moisture at field capacity) transpired 675, 706, and 1208 g water, respectively, for each gram of dry matter produced. Water-use efficiency was significantly higher in the low and medium soil moisture regimes than in the high. No significant differences were found between the low and medium water levels (Table 1).

The total water transpired per plant during the growth period varied significantly ($p = 0.01$) with the three water regimes. Alfalfa grown under the low, medium, and high water levels lost 1090, 1463, and 1103 g water per plant, respectively. Genotypes under the medium soil moisture content transpired significantly greater amounts of water compared with plants grown under the other regimes. There were no significant differences, however,

Table 1. Mean values of several characteristics of Mesa-Sirsa alfalfa seedlings grown under greenhouse conditions with three soil moisture levels.^{1/}

Characteristics	Moisture levels		
	90% f.c. ^{2/}	60% f.c.	30% f.c.
Water-use efficiency	1208 b ^{3/}	706 a	675 a
Transpired water (g)	1103 a	1463 b	1090 a
Dry matter of tops (g)	1.521 a	2.150 b	1.740 ac
Dry matter of stems (g)	0.728 a	0.946 a	0.772 a
Dry matter of leaves (g)	0.793 a	1.204 b	0.968 ac
Stem-leaf ratio	0.852 a	0.804 a	0.788 a
Number of stems	3.240 a	5.060 b	4.680 bc
Number of leaves	183 a	260 b	245 b

^{1/}The mean values were obtained from 50 plants for each moisture regime.

^{2/}f.c. refers to the soil moisture content at field capacity.

^{3/}Means followed by the same letter are not significantly different according to the Duncan Multiple Range test.

among genotypes grown under the low and high moisture levels (Table 1).

The soil moisture content significantly ($p = 0.01$) affected dry matter production of Mesa-Sirsa alfalfa. Genotypes grown under the medium water level produced significantly greater amounts of dry forage than did those under the other two levels. However, there were no significant differences in the dry matter production among genotypes grown under the low and high soil moisture regimes (Table 1).

Alfalfa genotypes produced significantly ($p = 0.01$) more dry matter of leaves under the medium range of soil moisture, and less under the low and high water regimes. However, leaf dry matter produced under the low water regime was significantly ($p = 0.05$) greater than that produced under the high soil moisture (Table 1).

The average number of stems produced on a plant differed significantly among the three soil moisture regimes. Alfalfa grown under the low, medium, and high moisture levels produced 4.7, 5.0, and 3.2 stems per plant, respectively. A significantly higher number of stems was produced on plants grown under the low and medium water levels as compared to the high (Table 1).

Soil moisture regime significantly ($p = 0.01$) influenced the number of leaflets produced on alfalfa genotypes. Plants grown under the low, medium, and high

moisture regimes developed 245, 260, and 183 leaflets, respectively. Plants grown under the low and medium soil moisture levels produced significantly more leaves per plant compared to plants grown under the high soil water regime (Table 1). The three soil moisture regimes did not significantly induce differences in the leaf-stem ratio and stem dry matter production of alfalfa.

Mature Stage

The soil moisture content apparently did not have a significant effect on the water-use efficiency of mature alfalfa genotypes (Table 2). However, it did have a significant effect on the total water transpired per plant. The amount of water transpired from alfalfa genotypes was significantly greater in the low and medium water regimes, and lower in the high. Mature alfalfa plants grown under the low, medium, and high soil moisture levels transpired 1280, 1452, and 894 g water per plant, respectively.

Dry matter of alfalfa tops was strongly influenced by changing the level of soil moisture content. Plants grown under the medium water regime produced significantly more dry matter of tops than they did under the low and high moisture levels (Table 2). Alfalfa that received low, medium, and high water regimes produced 1.317, 1.616, and 0.977 g dry matter per plant, respectively.

Table 2. Mean values of several characteristics of Mesa-Sirsa mature alfalfa plants grown under greenhouse conditions with three soil moisture levels.^{1/}

Characteristics	Moisture levels		
	90% f.c. ^{2/}	60% f.c.	30% f.c.
Water-use efficiency	1261 a ^{3/}	984 a	1115 a
Transpired water (g)	894 a	1452 b	1280 c
Dry matter of tops (g)	0.977 a	1.616 b	1.317 c
Dry matter of stems (g)	0.612 a	0.798 a	0.648 a
Dry matter of leaves (g)	0.595 a	0.798 a	0.668 a
Stem-leaf ratio	0.896 a	0.980 a	0.925 a
Number of stems	4.820 a	6.480 b	6.180 b
Number of leaves	167 a	231 b	220 b

^{1/}The mean values were obtained from 50 plants for each water regime.

^{2/}f.c. refers to soil moisture content at field capacity.

^{3/}Means followed by the same letter are not significantly different according to the Duncan Multiple Range test.

Alfalfa genotypes produced significantly more stems and leaves in the medium soil moisture regime than they did in the other two levels. Genotypes grown under the low, medium, and high moisture status had on the average 6.1, 6.5, and 4.8 stems per plant, respectively. These same plants produced 220, 231, and 167 leaflets per plant, respectively (Table 2).

Table 2 shows that varying the soil moisture content did not significantly change the water-use efficiency, stem dry matter, leaf dry matter, and stem-leaf ratio of mature alfalfa genotypes.

Variations in Water-Use Efficiency

The ten most and ten least efficient from each of seedlings and mature plants selected from 150 alfalfa genotypes revealed a considerable variation in their efficiency of water use. Table 3 shows that the most-efficient seedlings consumed on the average only one-half the amount of water used by the least efficient seedlings in the production of one gram of dry matter. The most efficient mature alfalfa plants transpired only one-third the amount of water transpired by the least efficient plants to produce the same amount of dry matter.

The differences in the water-use efficiency between the least and most efficient alfalfa genotypes were due in part to differences in the dry matter production. The most

Table 3. Water-use efficiency under greenhouse conditions of the ten most and the ten least efficient Mesa-Sirsa alfalfa genotypes for each of the high, medium, and low soil moisture regimes at the mature and seedling growth stages.

Plant rank no.	Seedling stage				Mature stage			
	Moisture level			Mean	Moisture level			Mean
	90% f.c. ^{1/}	60% f.c.	30% f.c.		90% f.c.	60% f.c.	30% f.c.	
	<u>Most efficient</u>							
1	510	440	363		629	300	596	
2	518	497	363		663	587	658	
3	519	419	437		695	636	669	
4	534	520	467		701	660	674	
5	544	521	483		711	676	675	
6	566	539	484		739	682	677	
7	582	547	489		748	706	694	
8	593	510	497		751	719	705	
9	628	572	519		753	759	706	
10	645	573	535		756	762	757	
Mean	564	514	464	519	715	649	681	681
	<u>Least efficient</u>							
1	2310	999	1885		2913	1692	2541	
2	1553	978	1313		2590	1603	2377	
3	1373	974	1007		2253	1491	2326	
4	1359	948	966		2250	1477	1708	
5	1328	940	926		2157	1431	1687	
6	1308	926	917		2089	1351	1494	
7	1237	885	826		1833	1292	1354	
8	1068	865	790		1672	1284	1353	
9	1034	857	775		1520	1219	1349	
10	1021	829	756		1378	1192	1332	
Mean	1359	920	1016	1098	2065	1403	1752	1740

^{1/}f.c. refers to soil moisture content at field capacity.

efficient seedlings produced 93% more dry matter than the least efficient. Similarly, the most efficient mature alfalfa plant produced 252% more dry matter than the least efficient one. Generally, the most efficient seedlings and mature alfalfa genotypes produced 140% more dry matter than the least efficient seedlings and mature plants (Table 4).

Another factor that contributed to the differences in the water-use efficiency between the most and least efficient alfalfa genotypes was the total water transpired per plant during the experiment. The most efficient seedlings transpired 160 g more water than the least efficient, and the most efficient mature alfalfa plants transpired 300 g more than the least efficient. On the average, the most efficient seedlings and mature genotypes transpired 284 g more water per plant during the period of growth than the least efficient (Table 5).

Correlation Coefficients

The soil moisture content significantly influenced the relation between the water-use efficiency and several other plant parameters in the seedling stage. However, this relationship was more pronounced under the medium moisture regime when compared with the low and high. Water-use efficiency was significantly and negatively related ($r = 0.50$) to the dry matter production of tops, dry matter of stems, and dry matter of leaves (Table 6).

Table 4. Forage dry matter (g) production under greenhouse conditions of the ten most and the ten least efficient Mesa-Sirsa alfalfa genotypes for each of the high, medium, and low soil moisture regimes at the mature and seedling growth stages.

Plant rank no.	Seedling stage				Mature stage			
	Moisture level			Mean	Moisture level			Mean
	90% f.c. ^{1/}	60% f.c.	30% f.c.		90% f.c.	60% f.c.	30% f.c.	
	<u>Most efficient</u>							
1	5.897	3.265	5.357		2.442	3.533	2.954	
2	2.224	2.604	1.694		2.252	2.862	2.534	
3	5.077	1.794	0.551		2.172	2.594	2.354	
4	4.486	3.715	1.643		2.081	3.104	1.582	
5	2.544	3.174	1.032		1.080	2.492	1.863	
6	3.135	1.774	1.934		1.271	1.632	2.413	
7	2.134	2.764	1.823		1.752	2.381	2.584	
8	4.126	2.344	1.663		1.402	0.780	1.982	
9	3.526	3.215	1.593		0.291	1.261	1.542	
10	0.982	1.874	1.373		2.452	1.532	2.164	
Mean	3.413	2.652	1.866	2.666	1.720	2.217	2.197	2.067
	<u>Least efficient</u>							
1	0.171	2.505	1.432		0.080	0.851	0.390	
2	0.150	0.901	0.622		0.217	0.992	0.300	
3	1.284	2.855	1.534		0.091	0.750	0.430	
4	0.382	2.103	1.572		0.100	0.810	0.411	
5	1.003	1.374	2.485		0.051	0.680	0.600	
6	0.221	2.715	2.225		0.180	0.741	0.470	
7	0.262	0.812	2.005		0.210	1.362	0.871	
8	0.782	2.255	2.064		0.131	1.012	0.490	
9	0.594	1.423	1.592		0.350	1.481	1.000	
10	0.482	1.883	1.744		0.611	1.250	0.702	
Mean	0.533	1.882	1.728	1.380	0.202	0.993	0.566	0.587

^{1/}f.c. refers to soil moisture content at field capacity.

Table 5. Total transpired water (g) under greenhouse conditions of the ten most and the ten least efficient Mesa-Sirsa alfalfa genotypes for each of the high, medium, and low soil moisture regimes at the mature and seedling growth stages.

Plant rank no.	Seedling stage				Mature stage			
	Moisture level			Mean	Moisture level			Mean
	90% f.c. ^{1/}	60% f.c.	30% f.c.		90% f.c.	60% f.c.	30% f.c.	
	<u>Most efficient</u>							
1	3009	1437	1943		1536	1059		
2	1152	1294	517		1493	1679	1762	
3	2634	931	241		1509	1651	1667	
4	2394	1930	768		1458	2050	1574	
5	1363	1654	499		768	1684	1066	
6	1775	957	937		939	1113	1257	
7	1243	1513	892		1310	1680	1634	
8	2448	1289	826		1053	561	1793	
9	2213	1839	827		219	957	1398	
10	634	1074	735		1854	1168	1089	
Mean	1886	1392	818	1365	1214	1360	1324	1299
	<u>Least efficient</u>							
1	395	2503	270		233	1440	991	
2	233	881	817		562	1590	713	
3	1764	683	1545		205	1118	1090	
4	519	1994	1520		225	1196	702	
5	1332	1292	2302		110	973	1012	
6	289	2514	2042		376	1001	702	
7	324	719	1656		385	1760	1179	
8	835	1951	1630		219	1299	663	
9	614	1220	1234		532	1805	1349	
10	492	1561	1004		842	1490	935	
Mean	680	1532	1402	1207	369	1367	934	890

^{1/}f.c. refers to soil moisture content at field capacity.

Table 6. Correlation coefficients of water-use efficiency with several plant characteristics of Mesa-Sirsa seedlings grown under greenhouse conditions with three soil moisture levels.^{1/}

Characteristics	Moisture level		
	90% f.c. ^{2/}	60% f.c.	30% f.c.
Transpired water	-0.29	+0.05	+0.06
Dry matter of tops	-0.33	-0.47**	-0.19
Number of leaves	-0.32	-0.11	-0.12
Number of stems	-0.33	-0.17	-0.15
Stem-leaf ratio	-0.32	+0.008	-0.03
Dry matter of stems	-0.31	-0.46**	-0.19
Dry matter of leaves	-0.33	-0.45**	-0.17

^{1/}Correlation coefficients were determined from 50 alfalfa plants.

^{2/}f.c. refers to soil moisture content at field capacity.

As the amount of dry matter of forage, stems, and leaves increased, the quantity of water required to produce one gram dry matter decreased.

The total water transpired per plant was directly and significantly associated with dry matter production, stems and leaves, and number of stems and leaflets (Table 7). Plants which produced the most dry matter, the highest number of stems and leaflets, also transpired the greatest amount of water.

The levels of soil moisture did not change the relation between water-use efficiency and other plant characteristics in the mature stage of growth for the alfalfa genotypes (Table 8). The water-use efficiency was inversely related to dry matter of forage, dry matter of stems and leaves, number of leaflets, and stem-leaf ratio. As dry matter, number of leaflets, and stem-leaf ratio increased, less water was needed to produce one gram of dry matter.

Total transpired water was proportionally increased as the dry matter of forage, leaves, and stems increased (Table 9). This relationship was similar to that in the seedling stage.

Alfalfa seedlings were more sensitive to the high soil moisture content than the mature plants. The roots of alfalfa seedlings were not sufficiently extensive to absorb a large amount of water, and the total quantity of

Table 7. Correlation matrices of several characteristics of Mesa-Sirsa alfalfa seedlings grown under greenhouse conditions with three soil moisture levels.^{1/}

Characteristics	Water-use efficiency	Transpired water (g)	Dry matter of tops (g)	Number of leaves	Number of stems	Stem-leaf ratio	Dry matter of stems (g)	Dry matter of leaves (g)
Water-use efficiency	1.00							
Transpired water	-0.22	1.00						
Dry matter of tops	-0.32	0.88**	1.00					
Number of leaves	-0.28	0.74**	0.76**	1.00				
Number of stems	-0.28	0.53**	0.52**	0.61**	1.00			
Stem-leaf ratio	-0.20	-0.28	0.28	0.10	-0.06	1.00		
Dry matter of stems	-0.29	0.84**	0.96**	0.69**	0.42	0.48	1.00	
Dry matter of leaves	-0.32	0.86**	0.96**	0.78**	0.58**	0.07	0.86**	1.00

^{1/}Correlation coefficients were determined from 150 alfalfa plants.

Table 8. Correlation coefficients of water-use efficiency with several characteristics of mature Mesa-Sirsa alfalfa plants grown under greenhouse conditions with three soil moisture levels.

Characteristics	Correlation _{1/} coefficients (r)	Correlation _{2/} coefficients (r)
Transpired water	-0.40 ^{3/}	-0.34
Dry matter of tops	-0.58**	-0.54**
Number of leaflets	-0.52**	-0.47**
Number of stems	-0.35	-0.32
Stem-leaf ratio	-0.54**	-0.52**
Dry matter of stems	-0.49**	-0.49**
Dry matter of leaves	-0.48**	-0.48**

^{1/} Correlation coefficients were determined without accounting for the error term due to moisture levels and replications.

^{2/} Correlation coefficients were determined with accounting for the error term due to moisture levels and replications.

^{3/} Correlation coefficients were determined from 150 alfalfa plants.

Table 9. Correlation matrices of several characteristics of mature Mesa-Sirsa alfalfa plants grown under greenhouse conditions with three soil moisture levels.^{1/}

Characteristics	Water-use efficiency	Transpired water (g)	Dry matter of tops (g)	Number of leaves	Number of stems	Stem-leaf ratio	Dry matter of stems (g)
Water-use efficiency	1.00						
Transpired water	-0.34	1.00					
Dry matter of tops	-0.54**	0.83**	1.00				
Number of leaves	-0.47**	0.72**	0.79**	1.00			
Number of stems	-0.32	0.58**	0.51**	0.57**	1.00		
Stem-leaf ratio	-0.52**	0.41	0.56**	0.43	0.22	1.00	
Dry matter of stems	-0.49**	0.68**	0.87**	0.67**	0.38	0.72**	1.00
Dry matter of leaves	-0.48**	0.77**	0.85**	0.73**	0.49**	0.28	0.91**

^{1/}Correlation coefficients were determined from 150 alfalfa plants.

transpired water was small compared to the mature alfalfa plants. Thus, more water remained in the soil and probably caused poor soil aeration. The poor soil aeration might have retarded the normal physiological processes.

Seedlings, therefore, were more efficient in the water use when they were grown in soils treated with the low and medium water regimes. This higher water-use efficiency of alfalfa seedlings was attributed to the significant increase in dry forage, number of stems, and number of leaflets per plant. Similar results were reported on the effect of soil moisture levels on the efficiency of water-use by crops (9, 11, 15, 19, 27, 38, 47, 51, 52, 55, 56). However, seedlings which were grown under the medium soil moisture range produced significantly more dry forage, more dry matter of leaves, and greater number of stems and leaflets. These data indicate that plants grown under the medium soil moisture level had higher net assimilation rates.

The Relation Between Transpiration Rate and Water-Use Efficiency

Mesa-Sirsa alfalfa genotypes exhibited wide differences in the amount of water transpired per unit dry matter of tops produced (Table 10). They also showed variation in their transpiration rates. The transpiration rates were higher on alfalfa plants in the vegetative stage, and lower when they were at the floral bud and flowering stages,

respectively. Slatyer and Bierhuizen (53) reported that the transpiration rate was reduced to one-half on cotton leaves 25 days after full expansion.

The relationship between transpiration rate and water-use efficiency became stronger as plants progressed toward maturity. The transpiration rate was significantly associated ($r = .698$) with the water-use efficiency when alfalfa plants reached floral bud development, and this relationship became significantly stronger when alfalfa genotypes reached the flowering stage ($r = 0.82$). These data suggest that the stage of growth must be considered in the selection of water-efficient genotypes. The most efficient alfalfa genotypes transpired less water per unit leaf area per unit time (Table 10).

The Relation of Net Photosynthetic and Respiratory Rates with Several Alfalfa Characteristics

Alfalfa genotypes of all growth stages showed differences in net photosynthetic rates. However, the NAR was higher for genotypes that reached the vegetative and floral bud stages compared to that for genotypes at the flowering stage (Tables 12, 13, 14). Net photosynthetic rates ranged from 7.0 to 18.1, 4.0 to 17.3, and 2.2 to 9.4 mg CO₂/dm²/hr for alfalfa genotypes at the vegetative, floral bud, and flowering stages, respectively.

The relationship between the net photosynthetic rate and each of the factors of water-use efficiency, total

Table 10. Water-use efficiency and transpiration rates of ten Mesa-Sirsa alfalfa genotypes at three different growth stages grown under greenhouse conditions.

Plant no.	Plants at vegetative stage		Plants at floral bud stage		Plants at flowering stage	
	Water-use efficiency	Transpiration rate (g H ₂ O/dm ² /hr)	Water-use efficiency	Transpiration rate (g H ₂ O/dm ² /hr)	Water-use efficiency	Transpiration rate (g H ₂ O/dm ² /hr)
1	620	2.36	646	1.95	609	1.43
2	627	2.49	684	2.27	660	1.07
3	655	2.65	691	1.95	806	1.42
4	738	2.23	696	1.83	851	1.00
5	770	2.94	799	2.44	882	1.33
6	794	2.92	805	2.09	942	1.52
7	903	3.33	810	1.96	1110	1.88
8	1350	2.02	833	1.71	1194	1.80
9	1415	2.28	962	2.02	1395	2.01
10	1660	4.14	1246	2.98	--	--
Mean	953	2.74	817	2.12	845	1.35
Correlation coefficient	0.345		0.698*		0.820**	

transpired water, transpiration rate, forage dry matter, stem dry matter, leaf-stem ratio, and total leaf area per plant was not consistent with the growth stages. Net photosynthetic rate was significantly related to transpiration rate, and water-use efficiency ($r = 0.669$ and 0.770 , respectively), when plants were at the floral bud stage. None of the aforementioned characteristics were significantly related to net photosynthetic rates when alfalfa plants were at the vegetative and flowering growth stages (Table 11).

Alfalfa genotypes also showed differences in respiratory rates. Plants at the vegetative growth stage had higher respiratory rates than plants at the floral bud and flowering stages (Tables 12, 13, 14). The rates ranged from 2.5 to 10.8, 0.5 to 2.2, and 0.8 to 2.9 mg $\text{CO}_2/\text{dm}^2/\text{hr}$ for alfalfa genotypes at the vegetative, floral bud, and flowering stages, respectively. The rate of respiration was significantly and positively correlated with transpiration rate and leaf-stem ratio of plants ($r = 0.906$ and 0.915 , respectively) that were in the vegetative stage only. None of the eight characteristics was significantly related to respiration when alfalfa plants progressed toward floral bud and flowering growth stages.

Data by other researchers (17, 21, 22, 41, 69) indicated that species and cultivars within species differed in the net photosynthesis and respiration. However, no

Table 11. Correlation coefficients of net photosynthetic and respiratory rates with several characteristics of ten Mesa-Sirsa genotypes at three different growth stages grown under greenhouse conditions.

Plant characteristics	Plants at vegetative stage		Plants at floral bud stage		Plants at flowering stage	
	NPR ^{1/} (r)	RR ^{2/} (r)	NPR (r)	RR (r)	NPR (r)	RR ^{3/} (r)
Water-use efficiency	0.048	0.462	0.770**	0.222	-0.089	0.372
Transpired water	0.344	-0.616	-0.440	-0.475	-0.500	0.086
Dry matter of tops	0.190	-0.556	-0.597	-0.437	-0.344	-0.205
Leaf area	0.040	-0.540	-0.610	-0.589	-0.388	-0.594
Leaf-stem ratio	-0.190	0.915**	0.545	0.006	-0.127	-0.391
Transpiration rate	0.110	0.906**	0.669*	0.101	-0.113	0.589
Dry matter of stems	0.203	-0.571	-0.608	-0.405	-0.305	-0.129
Dry matter of leaves	0.176	-0.535	-0.563	-0.468	-0.308	-0.275

^{1/}NPR refers to net photosynthetic rate (mg CO₂/dm²/hr).

^{2/}RR refers to respiratory rate (mg CO₂/dm²/hr).

^{3/}Only nine plants used for determination of correlation coefficients.

Table 12. Physiological and growth component characteristics of Mesa-Sirsa alfalfa genotypes at the vegetative growth stage (8-week old) when grown under greenhouse conditions -- Plants were selected at random within each growth stage.

Plant no.	Net photo-synthetic rate (mg CO ₂ /dm ² /hr)	Respiration rate (mg CO ₂ /dm ² /hr)	Water-use efficiency	Transpired water (g)	Transpiration rate (g H ₂ O/dm ² /hr)	Forage dry matter (g)	Leaf dry matter (g)	Stem dry matter (g)	Leaf-stem ratio	Total leaf area (cm ²)
1	7.4	3.2	1350	378	2.02	0.28	0.16	0.12	1.33	40
2	7.0	2.9	738	310	2.23	0.42	0.26	0.16	1.62	75
3	11.2	3.4	620	632	2.36	1.02	0.54	0.48	1.12	152
4	12.3	3.8	794	143	2.92	0.18	0.11	0.07	1.57	27
5	12.8	2.6	627	514	2.49	0.82	0.46	0.36	1.28	107
6	15.6	2.5	655	498	2.65	0.76	0.40	0.36	1.11	87
7	8.4	6.9	770	185	2.94	0.24	0.15	0.09	1.67	36
8	10.8	10.8	1660	166	4.14	0.10	0.07	0.03	2.33	14
9	18.1	3.0	1415	481	2.28	0.34	0.19	0.15	1.27	42
10	15.1	8.7	903	298	3.33	0.33	0.22	0.11	2.00	45

Table 13. Physiological and growth component characteristics of Mesa-Sirsa alfalfa genotypes at the floral bud stage (10-week old) when grown under greenhouse conditions -- Plants were selected at random within each growth stage.

Plant no.	Net photo-synthetic rate (mg CO ₂ /dm ² /hr)	Respiration rate (mg CO ₂ /dm ² /hr)	Water-use efficiency	Transpired water (g)	Transpiration rate (g H ₂ O/dm ² /hr)	Forage dry matter (g)	Leaf dry matter (g)	Stem dry matter (g)	Leaf-stem ratio	Total leaf area (cm ²)
1	7.4	0.5	696	870	1.83	1.25	0.53	0.72	0.74	205
2	8.7	1.2	805	725	2.09	0.90	0.44	0.46	0.96	126
3	11.8	2.2	799	791	2.44	0.99	0.44	0.55	0.80	99
4	10.3	1.1	810	1012	1.96	1.25	0.55	0.70	0.79	189
5	5.6	1.9	833	558	1.71	0.67	0.27	0.40	0.67	84
6	13.6	1.5	962	558	2.02	0.58	0.32	0.26	1.23	81
7	4.0	1.2	691	891	1.95	1.29	0.60	0.69	0.87	190
8	11.0	1.8	646	866	1.95	1.34	0.59	0.75	0.79	190
9	5.2	0.5	684	944	2.27	1.38	0.63	0.75	0.84	193
10	17.3	1.4	246	573	2.98	0.46	0.23	0.23	1.00	49

Table 14. Physiological and growth component characteristics of Mesa-Sirsa alfalfa genotypes at the flowering stage (12-week old) when grown under greenhouse conditions -- Plants were selected at random within each growth stage.

Plant no.	Net photo-synthetic rate (mg CO ₂ /dm ² /hr)	Respiration rate (mg CO ₂ /dm ² /hr)	Water-use efficiency	Transpired water (g)	Transpiration rate (g H ₂ O/dm ² /hr)	Forage dry matter (g)	Leaf dry matter (g)	Stem dry matter (g)	Leaf-stem ratio	Total leaf area (cm ²)
1	2.5	2.5	806	2806	1.42	3.48	1.49	1.99	0.75	322
2	8.9	2.7	1194	1874	1.80	1.57	0.54	1.03	0.52	139
3	2.7	0.8	851	1765	1.00	2.07	1.06	1.01	1.05	328
4	3.2	1.4	609	1499	1.43	2.46	0.99	1.47	0.67	229
5	5.6	1.0	942	2241	1.52	2.38	1.00	1.38	0.72	255
6	2.8	1.0	882	3020	1.33	3.42	1.08	2.34	0.46	313
7	9.4	1.8	660	1736	1.07	2.62	1.19	1.44	0.82	303
8	2.2	1.8	1395	2859	2.01	2.05	0.89	1.16	0.77	266
9	3.5	2.9	1110	2322	1.88	2.09	0.76	1.33	0.57	187

information was available regarding genotypic variation within alfalfa cultivars for these physiological processes. The most efficient alfalfa genotypes at the floral bud stage of growth displayed lower transpiration rates which could be attributed to the restriction of water conduction by xylem vessels in the leaf midvein. These efficient plants were found to have less xylem vessels in the leaf midvein compared to the least efficient plants. However, the most efficient genotypes exhibited a lower rate of photosynthesis,

The Relation Between Water-Use Efficiency
and Stem, Leaf, and Petiole Anatomy

Alfalfa genotypes exhibited differences in palisade and spongy mesophyll thickness, number of xylem vessels in the leaf midvein, number of stem vascular bundles, number of xylem vessels in the stem, and number of xylem vessels in the petiole. However, the differences in most of the anatomical parameters were inconsistent with the growth stages studied, with the exception of palisade and sponge leaf mesophyll thickness. The thickness of palisade and sponge mesophyll decreased as growth progressed toward plant maturity (Tables 16, 17, 18).

Water-use efficiency was significantly and positively correlated ($r = 0.658$) to the number of xylem vessels in the leaf midvein when alfalfa genotypes were in vegetative stage. Also, water-use efficiency was positively

related ($r = 0.682$) to the palisade thickness when plants developed floral buds (Figure 2). Those plants which had the highest number of xylem vessels in the leaf midvein and the thickest palisade layers were the least efficient in water use. At the flowering stage of alfalfa plants, none of the anatomical features examined were significantly related to efficiency of water use (Table 15).

At the vegetative stage of growth, the number of xylem vessels may be a factor in restricting water conduction through the leaf midvein. As plants continued to develop, the palisade thickness became important in reducing the amount of water loss. The thinner palisade layers resulted in less cell surface area for water evaporation.

Data of this study suggested that further investigation should be conducted to verify more explicitly the relationship between the water-use efficiency and the physiological and anatomical parameters studied.

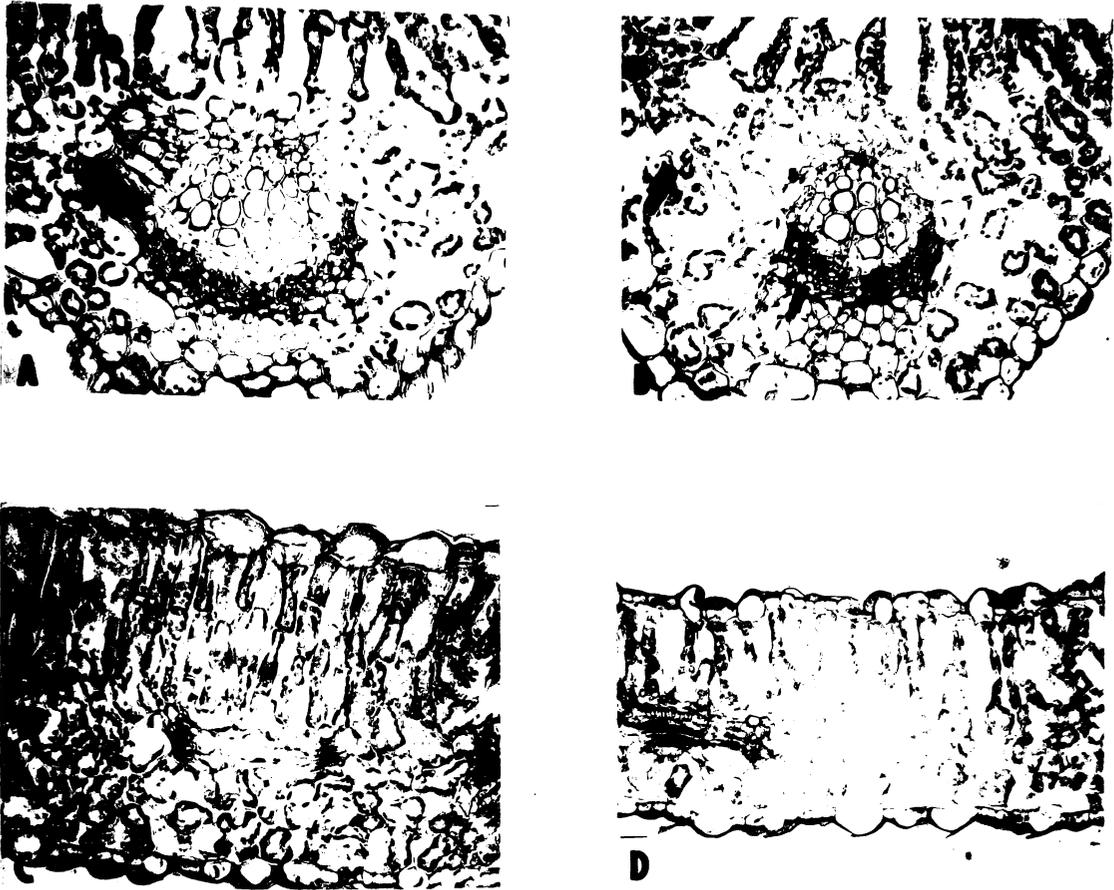


Fig. 2. The anatomy of Mesa-Sirsa alfalfa leaves obtained from plants at different growth stages -- A and B. Cross section of the leaf midvein at the vegetative stage showing the number of xylem vessels in the nonefficient and efficient genotypes, respectively (450x). C and D. Leaf cross section at the floral bud stage showing the thickness of palisade of the nonefficient and efficient alfalfa, respectively (450x).

Table 15. Correlation coefficients of water-use efficiency with several anatomical characteristics of leaves, stems, and petioles of ten Mesa-Sirsa alfalfa genotypes at three different growth stages grown under greenhouse conditions.

Anatomical characteristics	Plants at vegetative stage	Plants at floral bud stage	Plants at flowering stage ^{1/}
	(r)	(r)	(r)
Palisade thickness in leaf cross section	-0.131	0.682*	-0.050
Spongy layer thickness in leaf cross section	-0.284	0.567	-0.016
Number of vessels in leaf midvein	0.658*	0.278	-0.597
Number of vascular bundles in stem cross section	0.415	-0.365	0.543
Number of vessels in stem cross section	-0.209	0.602	-0.109
Number of vessels in petiole cross section	0.454	-0.051	-0.192

^{1/}Correlation coefficients were determined from nine alfalfa genotypes.

Table 16. Anatomical characteristics of Mesa-Sirsa alfalfa genotypes at the vegetative stage (8-week old) when grown under greenhouse conditions -- Plants were selected at random within each growth stage.

Plant no.	Palisade layer thickness (μ)	Spongy layer thickness (μ)	Number of vessels in leaf midvein	Number of vascular bundles in stem	Number of vessels in stem	Number of vessels in petiole
1	81.8	70.4	14	14	56	52
2	114.0	94.2	10	18	98	45
3	93.7	77.0	10	15	67	52
4	88.9	73.9	11	14	59	58
5	83.6	71.3	18	17	68	62
6	94.2	87.1	17	16	62	54
7	100.8	87.1	12	14	59	52
8	104.3	86.7	17	14	77	79
9	73.5	60.7	9	14	57	52
10	93.3	84.9	13	16	75	58

Table 17. Anatomical characteristics of Mesa-Sirsa alfalfa genotypes at the floral bud stage (10-week old) when grown under greenhouse conditions -- Plants were selected at random within each growth stage.

Plant no.	Palisade layer thickness (μ)	Spongy layer thickness (μ)	Number of vessels in leaf midvein	Number of vascular bundles in stem	Number of vessels in stem	Number of vessels in petiole
1	44.9	57.2	15	18	49	55
2	63.8	80.5	22	14	51	84
3	71.3	69.1	12	14	72	64
4	55.0	54.6	15	15	100	63
5	90.6	88.0	21	15	116	92
6	86.7	84.5	22	15	58	72
7	62.5	68.2	22	15	58	88
8	69.5	64.7	9	14	68	77
9	91.5	90.2	21	15	128	65
10	90.2	85.8	20	14	114	65

Table 18. Anatomical characteristics of Mesa-Sirsa alfalfa genotypes at the flowering stage (12-week old) when grown under greenhouse conditions -- Plants were selected at random within each growth stage.

Plant no.	Palisade layer thickness (μ)	Spongy layer thickness (μ)	Number of vessels in leaf midvein	Number of vascular bundles in stem	Number of vessels in stem	Number of vessels in petiole
1	81.0	84.7	23	13	78	82
2	60.8	51.0	17	10	85	71
3	80.3	68.0	14	14	71	48
4	70.4	66.8	15	14	53	65
5	66.1	67.6	15	15	78	42
6	75.0	68.1	19	13	91	105
7	90.2	78.9	14	13	92	59
8	79.3	66.4	12	14	51	43
9	60.3	65.7	15	15	78	70

SUMMARY

The water-use efficiency of 150 genotypes of Mesa-Sirsa alfalfa grown under three soil moisture regimes was determined under greenhouse conditions. Ten plants from each of the three different growth stages were used to measure the rates of net photosynthesis, respiration, and transpiration, and to examine the stem, leaf, and petiole anatomy. The objectives of this study were: to determine if Mesa-Sirsa alfalfa genotypes vary in their water-use efficiency, to investigate the effect of three soil moisture levels on water-use efficiency and other related characteristics for seedlings and mature alfalfa plants, and to determine plant parameters associated with the efficient water users within Mesa-Sirsa genotypes.

The results of this study indicated that:

1. Mesa-Sirsa genotypes exhibited variation in their water-use efficiency. Some plants required three times more water to produce a gram of dry matter than other plants in the same cultivar.
2. Soil moisture content had a highly significant effect on the water-use efficiency of alfalfa genotypes in the seedling stage but not in the mature stage.

The amount of available soil moisture significantly

influenced dry forage, stems, and leaves at both seedling and mature stages.

3. The soil moisture levels significantly changed the relationship between water-use efficiency and plant characteristics studied in the seedling stage but not in the mature stage of growth.
4. At the seedling stage, the water-use efficiency of Mesa-Sirsa genotypes grown under the medium soil moisture regime increased when dry matter production from forage, stems, and leaves increased. There was also a direct relation between the total transpired water per plant and dry matter produced, number of stems and leaflets. Total water transpired was not significantly associated with the water-use efficiency.
5. At the mature stage of growth, the amount of available soil moisture did not significantly change the relation between the water-use efficiency and other plant characteristics studied. At all water regimes, there was a direct association between the water-use efficiency and dry weight of forage and stems, number of leaflets and stem-leaf ratio. The amount of water transpired per plant was significantly and directly related to dry matter production from forage, stems, and leaves, and number of stems and leaflets per plant. No significant

association was found between the total water transpired per plant and the efficiency of genotypes in their water use.

6. The relation between the water-use efficiency and transpiration rate was significantly influenced by the growth stages. The efficient alfalfa genotypes at floral bud or flowering stage transpired significantly less water per unit leaf area per unit time compared to the more extravagant plants.
7. The rates of net photosynthesis and respiration varied among alfalfa genotypes and with growth stages. The rates decreased as plants progressed toward maturity. At the vegetative stage, respiration was directly associated to leaf-stem ratio, and transpiration rate. At floral bud stage, plants with the highest water requirement also had the highest rates of photosynthesis and transpiration.
8. The number of xylem vessels was significantly associated with the water-use efficiency of alfalfa genotypes at the vegetative stage of growth. Efficient alfalfa had significantly less xylem vessels in the leaf midvein compared to the non-efficient plants. The thickness of palisade layer was also directly related to water requirement of genotypes at the floral bud stage. Efficient

alfalfa genotypes developed a thinner palisade layer.

LITERATURE CITED

1. Alexander, C. W., and D. E. McCloud. 1962. CO₂ uptake (net photosynthesis) as influenced by light intensity of isolated bermuda grass leaves contrasted to that of swards under various clipping regimes. *Crop Sci.* 2:132-135.
2. Arkley, R. J. 1963. Relationships between plant growth and transpiration. *Hilgardia.* 34:559-583.
3. Baker, J. N., and O. J. Hunt. 1961. Effects of clipping treatments and clonal differences on water requirement of grasses. *J. Range Manage.* 14:216-219.
4. Bierhuizen, J. F., and R. O. Slatyer. 1964. Photosynthesis of cotton leaves under a range of environmental conditions in relation to internal and external diffusive resistances. *Aust. J. Bio. Sci.* 17:348-359.
5. Blackman, F. F., and G. L. C. Matthael. 1905. Optima and limiting factors. *Ann. Bot.* 19:281-295.
6. Blackman, G. E., J. N. Black, and A. W. Kemp. 1955. Physiological and ecological studies in the analysis of plant environment. An analysis of the effects of seasonal variation in daylight and temperature on the growth of *Helianthus annuus* in the vegetative phase. *Ann. Bot.* 19:527-548.
7. Bohning, R. H., and C. A. Burnside. 1956. The effect of light intensity on rate of apparent photosynthesis in leaves of sun and shade plants. *Amer. J. Bot.* 43:557-561.
8. Bongers, L. H. J. 1958. Changes in photosynthetic activity during algal growth and multiplication. *Meded Landb. hogeschool, Wageningen* 58:1-10.
9. Bourget, S. J., and R. B. Carson. 1962. Effect of soil moisture stress on yield, water-use efficiency, and mineral composition of oats and alfalfa grown at two fertility levels. *Can. J. Soil Sci.* 42:7-12.

10. Briggs, G. E., F. Kidd, and C. West. 1920. A quantitative analysis of plant growth. *Ann. Appl. Biol.* 7:202.
11. Briggs, L. J., and H. L. Shantz. 1913. The water requirement of plants I. Investigation in the Great Plains in 1910 and 1911. *Bur. Plant. Ind., Bull.* No. 284.
12. Briggs, L. J., and H. L. Shantz. 1914. Relative water requirements of plants. *J. Agr. Res.* 3:1-64.
13. Brun, W. A., and R. L. Cooper. 1967. Effects of light intensity and carbon dioxide concentration on photosynthetic rate of soybean. *Crop Sci.* 7: 451-454.
14. Buckman, H. O., and N. C. Brady. 1960. The nature and properties of soil. The Macmillan Co., New York. Pp. 162-190.
15. Burton, G. W., G. M. Prine, and J. E. Jackson. 1957. Studies of drought tolerance and water-use of several southern grasses. *Agron. J.* 49:498-503.
16. Cole, D. F. 1969. Water-use efficiency of seven cultivars of alfalfa (Medicago sativa L.). M.S. Thesis. Agronomy Dept., University of Arizona.
17. Curtis, P. E., W. L. Ogren, and R. H. Hageman. 1969. Varietal effects in soybean photosynthesis and photorespiration. *Crop Sci.* 9:323-327.
18. Dobrenz, A. K., D. F. Cole, and R. J. Joy. 1968. Comparison of materials for reducing evaporation of soil moisture in water efficiency studies. *Agron. J.* 60:446.
19. Dobrenz, A. K., L. N. Wright, M. A. Massengale, and W. R. Kneebone. 1969. Water-use efficiency and its association with several characteristics of blue panicgrass (Panicum antidotale Retz.) clones. *Crop Sci.* 9:213-216.
20. Dobrenz, A. K., L. N. Wright, A. B. Humphrey, M. A. Massengale, and W. R. Kneebone. 1969. Stomate density and its relationship to water-use efficiency of Blue panicgrass (Panicum antidotale Retz.). *Crop Sci.* 9:354-357.

21. Duncan, W. G., and J. D. Hesketh. 1968. Net photosynthetic rates, relative leaf growth rates, and leaf numbers of 22 races of Maize grown at eight temperatures. *Crop Sci.* 8:670-674.
22. El-Tabbakh, A. E. 1967. The effect of leaf area development, leaf photosynthetic rates and temperature on growth and dry matter accumulation in certain species of forage crops. Ph.D. Dissertation Univ. Arizona (Libr. Congr. Card No. 68-3580) 118 p. Univ. Microfilms, Ann Arbor, Mich. (Diss. Abstr. 28:3560-B).
23. Forrester, M. L., G. Krotkov, and C. D. Nelson. 1966. Effect of oxygen on photosynthesis, photorespiration, and respiration in detached leaves. I. Soybean. *Plant Physiol.* 41:422-427.
24. Forrester, M. L., G. Krotkov, and C. D. Nelson. 1966. Effect of oxygen on photosynthesis, photorespiration, and respiration in detached leaves. II. Corn and other monocotyledons. *Plant Physiol.* 41:428-431.
25. Fuess, F. W., and M. B. Tesar. 1968. Photosynthetic efficiency, yields and leaf loss in alfalfa. *Crop Sci.* 8:159-163.
26. Gaastra, P. 1959. Photosynthesis of crop plants as influenced by light, carbon dioxide, temperature, and stomatal diffusion resistance. *Meded. Landb. hogeschool Wageningen* 59:1-68.
27. Gifford, R. O., and E. H. Jensen. 1967. Some effects of soil moisture regimes and bulk density of forage quality in the greenhouse. *Agron. J.* 59:75-77.
28. Goodall, D. W. 1945. The distribution of weight change in the young tomato plant. I. Dry weight changes of the various organs. *Ann. Bot.* 9:101-139.
29. Gregory, F. G. 1917. Physiological conditions in cucumber houses. *Rep. Exp. Res. Sta. Cheshunt.* 19 pp.
30. Gregory, F. G. 1926. The effect of climatic conditions on the growth of barley. *Ann. Bot.* 40:1-26.

31. Hanks, R. J., H. R. Gardner, and R. L. Florian. 1969. Plant growth--evapotranspiration relations for several crops in the Central Great Plains. *Agron. J.* 61:30-34.
32. Hoefstra, G., and J. D. Hesketh. 1969. Effects of temperature on the gas exchange of leaves in the light and dark. *Planta.* 85:228-237.
33. Holmgren, P., P. G. Jarvis, and M. S. Jarvis. 1965. Resistances to carbon dioxide transfer. *Physiol. Plant.* 18:557-573.
34. Johansen, D. A. 1940. *Plant microtechnique.* McGraw-Hill Book Co., Inc., New York. 523 p.
35. Keller, W. 1953. Water requirement of selected genotypes of orchardgrass, Dactylis glomerata L. *Agron. J.* 45:622-625.
36. Keller, W. 1954. Water requirement of Dactylis glomerata L. in the greenhouse as influenced by variations in technique and their interaction. *Agron. J.* 46:495-499.
37. King, R. W., and L. T. Evans. 1967. Photosynthesis in artificial communities of wheat, lucerne and subterranean Clover plants. *Aust. J. Biol. Sci.* 20:623-635.
38. Leather, W. J. 1911. Water requirements of crops, India I and India II. *Agric. Res. Inst. Chem. Services* 1:230-241.
39. Lehman, W. F., S. J. Richards, D. C. Erwin, and A. W. Marsh. 1968. Effect of irrigation treatments on alfalfa (Medicago sativa L.) production, persistence, and soil salinity in Southern California. *Hilgardia.* 39:277-295.
40. Loomis, R. S., and W. T. Williams. 1963. Maximum crop productivity: an estimate. *Crop Sci.* 3:67-72.
41. Ludlow, M. M., and G. L. Wilson. 1968. Studies on the productivity of tropical pasture plants. I. Growth analysis, photosynthesis, and respiration of Hamil grass and Sirato in a controlled environment. *Aust. J. Agric. Res.* 19:35-45.

42. Maurer, A. R., D. P. Omrod, and H. F. Fletcher. 1968. Response of peas to environment. IV. Effect of five soil water regimes on growth and development of peas. *Cand. J. Pl. Sci.* 48:129-137.
43. Monteith, J. L. 1965. Light and crop production. *Field Crop Abstr.* 18:213-219.
44. Montgomery, E. G. 1911. Methods of determining the water requirement of crops. *J. Amer. Soc. Agron.* 3:261-283.
45. Moss, D. N. 1966. Respiration of leaves in light and darkness. *Crop Sci.* 6:351-354.
46. Pallas, J. E., Jr., and A. R. Bertrand. 1966. Research in plant transpiration: 1963. USDA Prod. Res. Report.
47. Pallas, J. E., Jr., and A. R. Bertrand. 1967. Water use, stomatal action, and transpiration unaffected by atrazine. *Agron. J.* 59:139-142.
48. Pearce, R. B., R. H. Brown, and R. E. Blaser. 1968. Photosynthesis of alfalfa leaves as influenced by age and environment. *Crop Sci.* 8:677-680.
49. Pendleton, J. W. 1965. Increasing water-use efficiency by crop management. In: *Plant environment and efficient water use.* W. H. Pierre, D. Kirkham, J. Peseck, and R. Shaw (Eds.). Am. Soc. Agron. and Soil Sci. Soc. Madison, Wisconsin.
50. Poskuta, J. 1968. Photosynthesis, photorespiration and respiration of detached spruce twigs as influenced by oxygen concentration and light intensity. *Physiol. Plant.* 21:1129-1136.
51. Richards, L. A., and C. H. Wadleigh. 1952. Soil water and plant growth. In: *Soil physical conditions and plant growth.* B. T. Shaw (Ed.). Academic Press Inc., New York. 491 p.
52. Scofield, C. S. 1945. The water requirement of plants. USDA circular 735. 11 p.

53. Slatyer, R. O., and J. F. Bierhuizen. 1964. The influence of several transpiration suppressants on transpiration, photosynthesis, and water-use efficiency of cotton leaves. *Aust. J. Biol. Sci.* 17:131-146.
54. Smittle, D., and G. Bradley. 1966. The effects of irrigation, planting and harvest dates on yield and quality of peas. *Proc. Am. Soc. Hort. Sci.* 88: 441-446.
55. Stanhill, G. 1957. The effect of differences in soil-moisture status on plant growth: a review and analysis of soil moisture regime experiments. *Soil Sci.* 84:205-214.
56. Stephens, D. E., M. M. Overson, and G. A. Mitchell. 1943. Water requirement of wheat at the Sherman Branch Exp. Station. *Oregon Agric. Exp. Sta. Tech. Bull.* 1.
57. Stern, W. R. 1967. Seasonal evapotranspiration of irrigated cotton in a low latitude environment. *Aust. J. Agric. Res.* 18:259-269.
58. Timmons, D. R., R. F. Holt, and J. T. Moraghan. 1966. Effect of corn population on yield, evapotranspiration, and water-use efficiency in the Northwest corn Belt. *Agron. J.* 58:429-432.
59. Wassink, E. C., S. D. Richardson, and G. A. Pieters. 1956. Photosynthetic adaptation to light intensity in leaves of Acer pseudoplatanus. *Acta Bot.* 5: 247-256.
60. Watson, D. J. 1947. Comparative physiological studies on the growth of field crops. I. Variation in net assimilation rate and leaf area between species and varieties, and within and between years. *Ann. Bot.* 11:41-76.
61. Watson, D. J. 1952. The physiological bases of variation of yield. *Adv. in Agron.* 4:101-145.
62. Watson, D. J. 1958. The dependence of net assimilation rate on leaf area index. *Ann. Bot.* 22:37-54.

63. Watson, D. J., J. H. Wilson, M. A. Ford, and S. A. W. French. 1966. Changes with age in the photosynthetic and respiratory components of the net assimilation rates of sugar beet and wheat. *New Phytol.* 65:500-508.
64. Watson, D. J., and Ken-ichi Hayashi. 1965. Photosynthetic and respiratory components of the net assimilation rates of sugar beet and barley. *New Phytol.* 64:38-47.
65. Williams, R. F. 1936. Physiological ontogeny in plants and its relation to nutrition. II. The effect of phosphorus supply on the growth of the plant and its parts. *Aust. J. Exp. Biol. Sci.* 24:165-185.
66. Williams, R. F. 1939. Physiological ontogeny in plants and its relation to nutrition. VI. Analysis of the unit leaf rate. *Aust. J. Exp. Biol. Sci.* 27:123-132.
67. Wolf, D. D., R. B. Pearce, G. E. Carlson, and D. R. Lee. 1969. Measuring photosynthesis of attached leaves. *Crop Sci.* 9:24-27.
68. Wright, L. N. 1962. Effects of management practices on forage yield and per cent protein in blue panicgrass, Panicum antidotale Retz. *Agron. J.* 54:413-416.
69. Zelitch, I. 1966. Increased rate of net photosynthetic carbon dioxide uptake caused by the inhibition of Glycolate oxidase. *Plant Physiol.* 41:1623-1631.