WATER-USE EFFICIENCY, PHOTOSYNTHESIS, AND GROWTH

COMPONENTS OF ALFALFA (MEDICAGO SATIVA L.)

MEASURED AT SEVERAL STAGES OF GROWTH

by

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF ILLUSTRATIONS

ABSTRACT

Experiments were conducted at Tucson, Arizona, to determine the relationship between water-use efficiency and six growth components at several stages of plant development. Two alfalfa (Medicago sativa L.) cultivars, Mesa-Sirsa and Sonora, and two clones of Mesa-Sirsa were utilized in this study.

Photosynthetic rate, total transpired water, leaf area, dry forage production, specific leaf weight, and transpiration rate were determined. The data were all statistically analyzed to determine those characteristics which could be used by plant breeders to select individual genotypes more efficient in water use.

When an entire harvest was utilized, correlation coefficients for older plants grown in the greenhouse and clones grown in the growth chamber showed most factors to be correlated with the amount of water utilized. Seedlings and clones grown in the greenhouse showed significant correlations among specific leaf weight, yield, and transpiration rate. When individual growth stages were compared, however, photosynthesis, yield, and specific leaf weight were not correlated with water-use efficiency except in the seedling experiment.

Water transpired per square decimeter was signifi-
cant in all of the entire harvest correlations and most of
the growth stage correlations.

INTRODUCTION

Alfalfa is Arizona's most important forage crop.
A shortage of water in the Southwest has put stress on
farmers to more carefully utilize water. Plant physiol-
ogists are now attempting to find more efficient techniques
to use for selecting plants that appear more efficient in
experimental studies.

Attempts have been made to relate plant charater-
istics to water-use efficiency. Stomate density was not
correlated with water-use efficiency (22). However, the
number of vascular bundles, dry weight, fresh weight,
proteins, and specific amino acids were all positively
correlated with water-use efficiency (23).

The objectives of this research were: (1) to deter-
mine the photosynthetic rate, water-use efficiency, specific
leaf weight, and other growth components of alfalfa at
different stages of plant development; and (2) to evaluate
the relationship between water-use efficiency and these
plant characteristics in order to establish a possible
relationship between water-use efficiency and other factors
which could be utilized in a breeding program.

1

REVIEW OF LITERATURE

Water-Use Efficiency

Consumptive use of water is defined by Erie, French, and Harris (28) as "the units of water used on a given area in transpiration, building of plant tissues, and evaporation from adjacent soil." Water requirement according to Briggs and Shantz (10) is the weight of water absorbed divided by the weight of dry matter produced exclusive of the root. Dobrenz et al. (22) calculated water-use efficiency by dividing the grams of water transpired by the grams of dry matter produced.

Kelley (48) in his research on 'Grimm' alfalfa (Medicago sativa L.) noted that the higher the available water content in the soil, the lower the water-use efficiency of the plant. There was no increase in plant yield just a decrease in is efficiency of water use. Joy (47) found that alfalfa which was watered when 30% of the available water was used had a reduced yield when compared with alfalfa plots that were watered when 60 and 90% of the available water was depeleted. Alfalfa, grown under different water table levels, was less efficient when the water table was high (6.1 dcm)(67). Tovey (67) also found that less dry matter was produced with a high water table, and less water was needed per unit of plant matter with

lower water tables. No change in growing cycle was noted
over various water table levels. Al-Kawaz (1) used several
'Mesa-Sirsa' plants to test water-use efficiency and
photosynthetic rates on single stems. He found such a wide
range in water-use efficiency and photosynthesis that his
correlations were non-significant.

The efficiency of grain sorghum (Sorghum vulgare
Pers.) has been investigated. The major factors which in-
fluenced the efficient use of water were available water
and the depth of water penetration (12). The efficiency
of sorghum grain ranged from zero to 6.3 metric tons per ha
per 2.54 cm (acre inch) of water on field plots. Safflower
(Carthamus tinctorius L. cultivar 'Frio') yielded an
average of 26.8 to 37 kg per ha per cm of water (27).
Wheat cultivars (Triticum vulgare 'Turkey' and 'Bluestem')
were compared by Briggs and Shantz (10). They showed that
between 995 (Turkey) and 1575 (Bluestem) g of water per g
of grain was necessary. Black (9) showed that crested
wheat grass (Agropyron desertorum Fisch., Schult,
'Standard') yielded 53 to 193 kg of forage per ha per cm
of water. The native grass yielded 43 to 171 kg of forage
per ha per cm of water used.

Briggs and Shantz (10) have shown that alfalfa
(cultivars 'Grimm' and 'Peruvian') used 657 to 659 g of
water per g of dry matter produced and 'Hairy Peruvian'
used 78% of that amount. Of the 17 native plants tested

ten were more efficient than Peruvian and 14 more efficient than Grimm. Tovey (67) learned that peak water used occurred at one tenth bloom and during high temperature periods. Cole (16) found that Mesa-Sirsa was more efficient at early stages of growth than Sonora, however, Sonora was more efficient when averaged over the entire growth cycle. Cole (16) and Baker and Hunt (6) have shown differences in water-use efficiency among clones of alfalfa.

Factors Which Influence Photosynthesis

Photosynthesis is affected by many growth and environmental factors. Jewiss and Woledge (45) showed that older leaves had a lower carbon dioxide assimilation rate in tall fescue (Festuca arundinacae Schreb.). Wilson and Cooper (71) found that in Lolium genotypes the maximum photosynthetic rate was found in the newly-expanded leaves. Fuess and Tesar (31) noted that frequent cutting raised the photosynthetic rate and that the younger leaves had a higher rate than the older leaves.

Egli, Pendleton, and Peters (24) found that the narrow leaf was more photosynthetically active than wide leaflets in contrasting soybean (Glycine max L.) cultivars. Kosonen (50) stated he was unable to measure the photosynthetic rates of plant tops and get maximum photosynthetic rates unless the root system was intact. In fact, he found a linear correlation between photosynthetic rate and total

production of the plants when roots 15 cm below the soil surface were included. Bailey (4) also stated that source for error in yield measurements, using photosynthesis as a basis for estimation, was failure to use root weights. Humphries and French (41) also found adequate root growth necessary to increase the photosynthetic rate of sugar beets (Beta vulgaris L.). Hofstra and Nelson (39) compared leaves of corn (Zea mays L.), sugar cane (Saccharum officinarum L.), sugar beets, soybeans, tobacco (Nicotiana tobacum L.), and pine (Pinus spp.) and suggested that the actual factor regulating photosynthetic rate was the translocation of photosynthate from the leaf to storage tissues. Hesketh (34) compared several species and showed that stomate number and chlorophyll content did not account for differences in photosynthesis and proposed that mesophyll resistance and dark reaction kinetics might be regulating factors.

Curtis, Ogren, and Hageman (18) showed that the differences in photosynthetic rate between two soybean cultivars ('Patterson' and 'Richland') were one hundred per cent; the Patterson variety had a photosynthetic rate of twelve mg CO_2 $dm^{-2}hr^{-1}$ compared with twenty-four for the Richland cultivar. Heichel and Musgrave (33) found that individual corn cultivars differed in photosynthetic rate by as much as 200%. Hybridization of corn cultivars failed to appreciably change the photosynthetic rate. It usually

fell at a level approximately half way between those of the parents (33).

Water

One environmental factor that affects photosynthesis is water. Relative humidity and soil water content both affect photosynthesis. Idso (42) showed that for every 6% decrease in relative humidity, there was a 1% drop in photosynthetic rate. In contrast, Nevins and Loomis (59) found that humidity had no effect on photosynthetic rate.

Lemon (52) proposed that a high transpiration rate was associated with a high photosynthetic rate. Denmead, Fritschen, and Shaw (20) found that photosynthesis stopped in corn if only the top leaves were wilted. Murata and Iyama (56) stated that even after eight days without water the photosynthetic rate of alfalfa was still 60% of normal. Moss, Musgrave, and Lemon (55) have shown that the photosynthetic rate of corn decreased appreciably even before plants reached the wilting stage. El-Sharkawy and Hesketh (25) suggested that water deficits in the soil lowered photosynthetic rate; however, the leaves of some range plants actually wilted but maintained high photosynthetic activity.

Light

Light is an important factor in photosynthesis measurements. Lemon (52) cited Yocum, who noted that the

entire canopy of the plant did not absorb any more light than that absorbed by one leaf (as a percentage of that available). Yocum, Allen, and Lemon (75) stated that the efficiency of light utilization of corn was only .42 as efficient as that of the algae Chlorella. They cited transpiration as the factor causing lowered efficiency.

Photosynthesis was calculated by measuring the light absorbed by plants in a corn field. In the experiment conducted by Allen, Yocum, and Lemon (2), reflected light and transmitted light were measured. They considered the light not transmitted or reflected to have been used by the plant. Most of the light utilized was absorbed in the upper half of the canopy. Denmead et al. (20) also found that the majority of the light absorption took place in the top half of the canopy. They found 75% of the total radiation was reflected and 73% of that not reflected was absorbed in the upper half. On cloudy days, however, the lower portion of the canopy was able to utilize more energy than the upper portion of the corn plant. Moss (54) has shown that corn, sugar cane, sunflower (Helianthus annus L.) and tobacco leaves, if illuminated from both sides, absorbed less CO_2 than if illuminated from the top only.

Lemon (52) showed a correlation between photosynthetic rates and light intensity. Bula, Rhykerd, and Langston (14) proposed that plants grown under high light intensities had 3 to 5 times greater photosynthetic rates

than those grown at low intensities. The photosynthesis
rates at 3000 fc (32.3 klux) were higher than those at 750
fc (53.8 klux). Jeffers and Shibles (43) found similar
results with soybeans. Brun and Cooper (13) worked with
soybeans and found that 21.5 klux gave maximum photo-
synthesis at 300 ppm carbon dioxide. If the carbon
dioxide concentration was increased to 1600 ppm, however,
an increase in photosynthesis was noted above 75.4 klux.

Wilfong (69) found that alfalfa was still responsive
to light when tested in a canopy illuminated at 10,000 fc
(107.6 klux). Pearce, Brown, and Blaser (61) reported that
light intensity affected the age of alfalfa leaves. At
high light intensities the leaves lived longer than those
grown at lower intensities. In 1919 Smith (65) felt that
the factor which influenced photosynthesis most was light
intensity. He also stated that temperature and carbon
dioxide concentration were major factors.

Temperature

Smith (65) has shown temperature to be an important
factor regulating photosynthetic rate. Jensen, Massengale,
and Chilcote (44) found that alfalfa grown at 27 C yielded
forage of lower quality than those plants grown at 16 C.
Optimum temperature ranges for several crop plants have
been investigated. Murata and Iyama (57) have shown ladino
clover (Trifolium repens L.) to have an optimum temperature

of 15 C. Jeffers and Shibles (43) reported that the
optimum temperature for soybeans was 25 to 30 C. Nevins
and Loomis (59) found 19 to 26 C was the best for sugar
beets. Hofstra and Hesketh (38) tested plants in normal
and oxygen-free air. The optimum temperatures for
corn, soybeans, and sugar beets in oxygen free air were
50, 37, and 35 C, respectively. Under normal conditions,
however, the optimum temperatures were 37, 30, and 35 C,
respectively. Hesketh (35) and Forrester, Krotkov, and
Nelson (29) have partial evidence that the higher tempera-
ture is permitted by lack of photorespiration. Additional
research by Hesketh (35) showed a response to oxygen free
air in all plants except corn which has been shown to lack
photorespiration. Forrester et al. (29) also found sugar
cane to be non-photorespiratory.

Friend (30) showed that by increasing the tempera-
ture from 20 to 30 C he could decrease the photosynthetic
rate of wheat. El-Sharkawy and Hesketh (25) also found in
several different plants that high temperatures caused a
lower photosynthetic rate. Winter and Pendleton (73)
reported than an ambient temperature of 55 C in the field
caused death or heavy damage to corn leaves.

Winter and Pendleton (73) also noted that the leaf
temperatures were 5 to 10 C higher than atmospheric
temperatures. Wilson and Cooper (72) have shown that

Lolium genotypes grown at temperatures of 9 to 15 C had higher photosynthetic rates than those grown at 21 C.

Curtis et al. (18) reported that the photosynthetic rate of soybeans was not directly related to yield. Luption (53) learned that when he tried to estimate the yield of wheat, he continually over-estimated the actual yield when using the CO_2 absorption technique. In cotton (Gossypium spp.) El-Sharkawy, Hesketh, and Muramoto (26) were not able to show a correlation between yield and photosynthesis; however, the leaf area production and temperature tolerances seemed to be somewhat related. Watson and Hayashi (68) attributed the increase in yield of sugar beets over barley (Hordeum vulgare L.) to higher photosynthetic rates. Lemon (52) stated that the best way to increase yield was to increase carbon dioxide uptake and not reduce transpiration rate.

Apparatus for Photosynthetic Measurement

Photosynthesis experiments reported by Wolf et al. (74) were performed utilizing an infra-red analyzer (Beckman IR-215). Several other methods had been used previous to this time. Osterhout (60) used a whole plant in a glass bell jar for his experiments. He also used an alkaline colorimetric solution to obtain photosynthetic rates. Catsky and Zedenek (15) found that colorimetric methods of measurement were not as accurate as the

infra-red system. Thomas and Hill (66) utilized a salt bridge and NaOH to find CO_2 absorption differences. Austin and Longden (3) used $C^{14}O_2$ but found that the plant favored normal carbon dioxide and their rates were slightly under the actual rates. Koller and Samish (49) used plastic bags in the field and Musgrave and Moss (58) used mylar canopies. Brown and Rosenberg (11) found that both of these methods created problems because of leaks and because plastic bags allowed seepage of carbon dioxide.

Transpiration

Dobrenz et al. (22) and Cole (16) found that stomate density was not significantly associated with water-use efficiency. Lemon (52) stated that transpiration was essential for exchange of carbon dioxide with the atmosphere. He also stated that transpiration rates depended on two sources of heat: (1) that left over because of inefficient light use by plant pigments and (2) ambient temperature. Allen et al. (2) stated that the upper portion of the canopy should transpire more because of the increased radiation load. Denmead et al. (20) have shown that transpiration occurring in the summer was much higher than evaporation because of the temperature. They also found that the net loss per year was about equal because of high evaporation rates in cooler temperatures partially caused by small corn plants in the spring and drying forage

in the fall. Lemon (52) found that evaporation was not influenced as much by plant cover as was expected due to heating of soil by infra-red light transmitted by leaves. Yocum et al. (75) equated transpiration and evaporation with light transmission and reflection and attributed the loss of photosynthetic efficiency to increased transpiration. Idso (42) stated that humidity influenced 90% of the water lost in transpiration and the soil moisture influence was only 10% except when the soil moisture level was near wilting point. Hagen et al. (32) reported that transpiration of ladino clover was lowered when the available soil moisture was below 50%. Hagen et al. (32) found that the dry weight production of plants and photosynthesis were not affected until all of the soil in the container was near the permanent wilting point.

Transpiration rates in the field were considered low by Peck, Vittum, and Gibbs (62), when working with alfalfa as compared with other field crops. They found a maximum use of 3.8 mm water per day. In 1958 Peck, Vittum, and Miller (63) found that the evapotranspiration rates for alfalfa were higher in the spring and summer than in the fall. Erie et al. (28) found that the highest water-use rate in Arizona was .36 inch (.91 cm) per day which was over twice the water-use rate noted in New York State as reported by Peck et al (63). The seasonal use for alfalfa in Arizona was 74.3 in (188.7 cm) per year; June and July

were the months of greatest use. August had lower rates
because the higher temperature caused partial dormancy. It
should be noted that alfalfa used appreciably more water
than other crops grown. Because of warm weather, it grows
almost continually.

It should be noted that alfalfa, unlike pinapple
(<u>Ananas</u> <u>comosus</u> [L.] Marr.) transpires all day. Joshi,
Boyer, and Kramer (46) found that the pineapple transpired
in the evening and at night rather than during the day
thereby reducing water loss.

Specific Leaf Weight

Specific leaf weight has been defined by Barnes
et al. (7) as the leaf dry weight per unit of leaf area.
They found that low intensity or poor spectral quality in
light caused a lowering of the specific leaf weight.
Specific leaf weight could be a good breeding tool for
selection of alfalfa plants with higher photosynthetic
rate as long as the plants are grown under the same condi-
tions as those to be grown in actual practice. Cooper and
Qualls (17) found that when alfalfa leaves were shaded, the
leaf area per unit weight ratio increases over 50%. Pearce
et al. (61) found that the specific leaf weight of alfalfa
increased with age in the growth chamber and decreased with
age in the field. Wilson and Cooper (70) found that the
smaller, thinner leaves of <u>Lolium</u> had the highest

photosynthetic rate. Wilson and Cooper (71) however, found no correlation between photosynthesis and specific leaf weight. Jewiss and Woledge (45) stated that leaf weight per area in tall fescue (Festuca arundinacea Schreb.) remained constant irregardless of age. Barnes et al. (7) found specific leaf weight in alfalfa closely correlated to yield.

METHODS AND MATERIALS

Plants of Sonora and Mesa-Sirsa alfalfa were grown under greenhouse and growth chamber conditions and used to evaluate the relationship between photosynthesis, efficient use of water, specific leaf weight, and other components of growth. This research was conducted in four separate experiments. Pot culture techniques and environmental conditions will be described for each aspect of the study.

Apparatus

The apparatus used to measure photosynthetic rates was similar for all four experiments. The plant and pot were placed under a cylindrical plexiglass tube closed at one end. This chamber had an outside diameter of 15.2 cm and a length of 45.6 cm (Figure 1). The chamber had two inlets at the base that were covered by a baffle plate containing 6 to 8 holes each for distribution of air (Experiments 1 and 2). Austin and Longden (3) showed numerous inlets to be more accurate at distributing the air than just a single jet inlet. The light source was a bank of 500-watt flood lights which were submerged in water to control the heat. This was contained in a plexiglass housing which gave 134.55 klux directly below the housing. The plexiglass chamber (Figure 2) was connected to the

Figure 1.   Plexiglass chamber used to measure photosynthesis
            in Experiments 1 and 2.
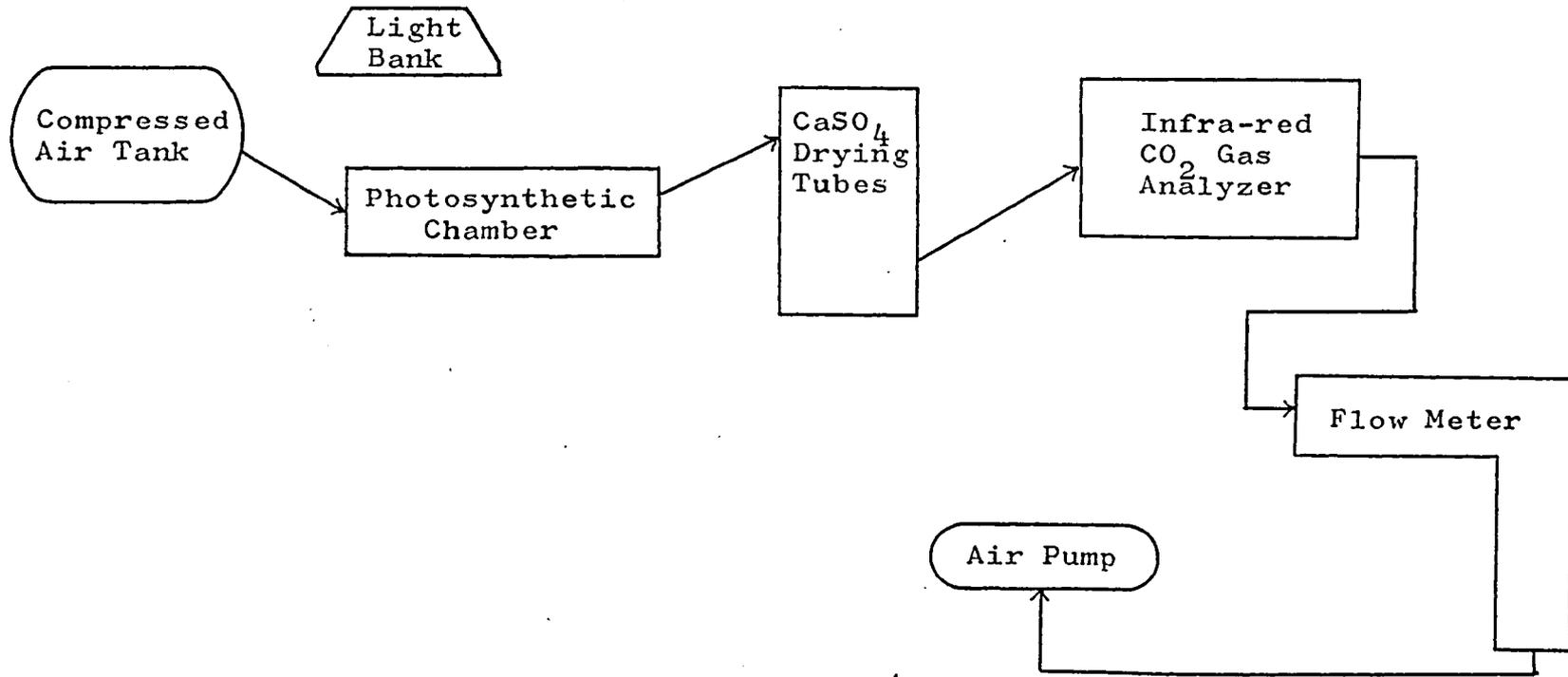
Figure 2. Flow diagram of photosynthetic apparatus used in these experiments.

analyzer by tygon tubing cited as the type that absorbed
the least CO_2 (11). They also supported the use of an
indicating $CaSO_2$ column to remove transpired water from the
air rather than a silica gel column. A micronite filter
was used to remove dust from the air being drawn through
the system. A Gibson flow meter was used to measure flow
rate. Air was pulled through the chamber and analyzer by
a pump at the end of the system; air was not recycled. Brown
and Rosenberg (11) proposed that air should be pressurized
and forced through the system. Decker (19), Hesketh (36),
and Zelitch (76), however, emphasized the need for high
flow rates, as high as 60 liters (l) per minute. Due to
filter sizes and an overheating problem in the $CaSO_4$ filter
system, flow rates of 2.1 to 6.1 l per min were used. The
Beckman Infra-Red Analyzer (IRA-215) was calibrated by
using standardized gases from Matheson Company. At the
initiation and termination of a run the instrument was
calibrated with standard gases. This figure was used to
calculate the total CO_2 removed from the atmosphere.
Brown and Rosenberg (11) have shown that carbon dioxide
concentration may change after one-third of the tank is
used; however, if both tanks change together the error in-
volved should be negligible. Bate, D'Aoust, and Canvin
(8) found some gas to be slightly off in analysis; there-
fore, ours were tested before delivery for actual analysis

and this reading was used. Photosynthetic rate was calculated using the formula proposed by Hesketh and Moss (37).

Experiment 1

In the first experiment, the pots used for alfalfa plants were 15 cm in circumference and 17.5 cm high with a volume of 4.2 liters. A hole 1.25 cm in diameter was drilled at the base of the pot and a rubber stopper (number 0) was placed in the hole. The pot and stopper were placed on a top-loading balance and the weight was brought up to 500 grams with pea gravel. Two thousand grams of a soil mixture (Mohave clay loam [typic haplargid], peat moss in a 3:1 ratio) was added to the pot to bring the weight to 2500 grams.

Two alfalfa cultivars, Mesa-Sirsa and Sonora, were seeded into fifty pots at the rate of 10 to 15 seeds per pot. When the first trifoliate leaf had unfolded, they were thinned to three plants per pot. The three plants were allowed to grow until they were 10 to 12.5 cm tall then thinned to one plant per pot. The plants were fertilized with 327 cc of a fertilizer mixture (55 ml of Vigaro 10-10-10 in 3.79 liters of water). The plants were harvested four times before the actual tests were conducted. Four plants of each cultivar were assembled into ten replications in a randomized block design. One unplanted check pot was included with the eight planted pots. All

of these pots were covered with styrofoam pellets as
described by Dobrenz, Cole, and Joy (21). The pots were
brought up to 3040 g with water and rewatered when the
weight dropped below 2890 g (55% available water). The
check pots were also watered and the loss of water from
the check was deducted from the planted pots. The experi-
ment was started over on January 27, 1970, because the
soil drench Azodrin, 3(dimethoxyphosphinoxy)-M-methyl-
cis-crotonamide, added in an attempt to control spider
mites (Tetranychus spp.), defoliated the plants after they
were 30.5 cm in height. In the second harvest the plants
were harvested at four stages of growth. The greenhouse
temperature had an average high of 31 C and a low of 9 C.
When the majority of the plants were 15.2 cm high, two
randomly selected plants, one of each cultivar, were
harvested. The cultivars were weighed every two hours
for a 24-hour period to determine the daily diurnal
transpiration rates and then photosynthetic rates were
determined using a flow rate of 4.8 l per min. The plants
were then harvested and the leaf area determined. Using
an adaptation of Robison and Massengale (64) and Hughes,
Cocshull, and Heath (40), leaf disks were taken with a
Meith silk punch. The holes were .62 cm in diameter. The
disks were dried and weighed to calculate total leaf weight.

In this experiment, the plants were watered when
they utilized 55% of the available soil moisture and

brought back to field capacity using the gravimetric method described by Briggs and Shantz (10), LaRue (51), and Cole (16). Total transpired water was calculated by subtracting the water lost from the check pots from the total water lost from each pot containing an alfalfa plant. The weighing method was found to be more accurate than the Cobalt Chloride treatment used by Bailey, Rothacher, and Cummings (5).

Experiment 2

The second experiment was carried out on two Mesa-Sirsa clones. Sections of stems were cut and dipped in a rooting solution called Jiffy Grow (one part Jiffy Grow to 20 parts of water). They were placed in a special starting bed made of a sand medium with a water mist which sprayed for one second every thirty minutes. Cuttings were placed in pots as described in Experiment 1 and grown in a greenhouse. Plants of each cultivar were assembled into five replications in a randomized complete block design. The temperature ranged between 30.5 C and 22 C. The photosynthetic rate was measured using a flow rate of 4.7 l per min. The leaf area was determined as in Experiment 1. The total area was then calculated using specific leaf weight and leaf dry weight.

Experiment 3

Alfalfa seedlings were used in the third experiment. Smaller pots 10 cm in diameter and 12.5 cm in height were used. The pots had a volume of .95 liter. A hole 1.25 cm in diameter was drilled at the base of the pot and a rubber cork (number 0) was placed in the hole. The pot and cork were put on a scale and pea gravel was added until the total weight was 150 g. One thousand g of soil was then added to each pot. Fifty-five pots were planted with 3 seeds of Mesa-Sirsa and another 55 pots with 3 seeds of Sonora. The seedlings were thinned to one plant per pot when the first true leaves appeared.

The plants were allowed to grow to maturity and then harvested. The temperature ranged from an average high of 30.5 C to a low of 19 C. A smaller chamber (7.6 cm in diameter and 45.6 cm tall) was used to measure photosynthesis in Experiment 3 (Figure 3). The upper end of the chamber was closed with a rubber gasket sealed with silicon grease. The plants were placed in the chamber and more gas was pumped into a base inlet than they needed for measurement. This formed an air seal. A flow rate of 2 l per min was used to monitor photosynthetic rate. The plants were tested at only two stages of growth: (1) 5 to 6 inches, and (2) pre-bloom. The leaf area was taken as in Experiments 1 and 2.
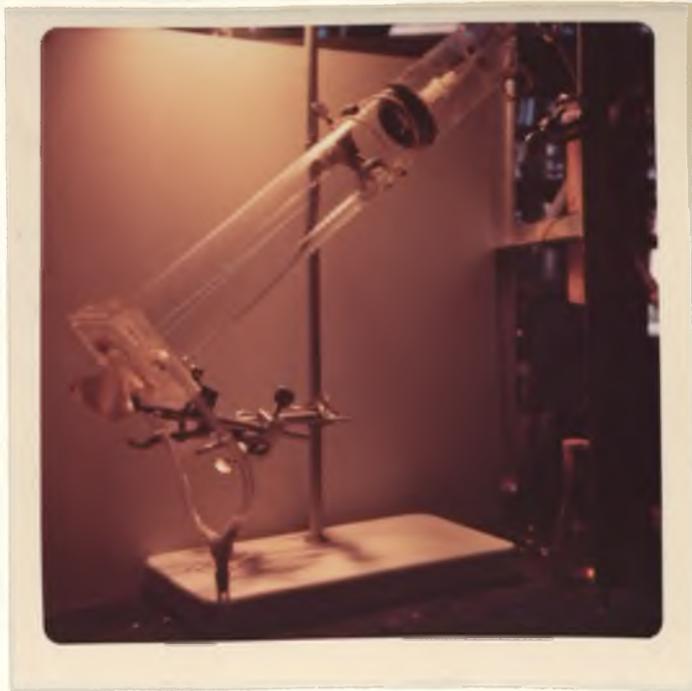
Figure 3. Plexiglass chamber used to measure photosynthesis
           in Experiments 3 and 4.

Experiment 4

This experiment, unlike the others, was carried out in the growth chamber. The two clones of Mesa-Sirsa were the same as those used before in Experiment 2. After planting, they were acclimatized to the chamber. They were harvested one time in the growth chamber before use in the experiment. The plants were harvested twice. Photosynthetic rate and total water transpired were measured at three stages of growth in the first harvest. The plants were then allowed to regrow and were all measured at the same stage of growth to determine the reason for photosynthetic differences. The run started on October 20, under a controlled temperature of 27 C during the day and 20 C at night. The photosynthetic rate of the plants was measured in the same chamber used in Experiment 3. The flow rate used for measurement was 2 l/min except for Harvest 3 which was run at 3 l/min. The leaf area was determined using an air flow planimeter.

RESULTS AND DISCUSSION

Photosynthesis

The photosynthetic rate of alfalfa showed considerable variation among experiments and between the different stages of plant development which were evaluated during each growth period (Table 1). Plants in Experiment 1 had significantly lower photosynthetic rates (18 mg dm^{-2} hr^{-1}) when stems were 30.5 cm compared to 25.6 mg dm^{-2} hr^{-1} for stems which were evaluated when they had reached a height of 15.3 cm. This decrease in photosynthetic rate with increasing plant maturity was also evident during the second growth period of these same plants. Alfalfa plants in Experiment 2 had an average photosynthetic rate of 46.6 mg $dm^{-2}hr^{-1}$ in the early stages of development and this rate dropped to 30.5 mg $dm^{-2}hr^{-1}$ at the one-tenth bloom stage of growth. Plants which were grown under low light intensity in the growth chamber (Experiment 4) did not display this decline in photosynthetic rates with maturity. Plants grown under the lower light intensity had a photosynthetic rate of 40.5 mg $dm^{-2}hr^{-1}$ compared with 25.0 mg $dm^{-2}hr^{-1}$ for the earlier stages of development.

Photosynthetic rates of Mesa-Sirsa and Sonora were not significantly different (Table 2). Plants of these two cultivars were used in Experiments 1 and 3 and although

25

Table 1. Mean photosynthetic rates, growth components, and water requirement of alfalfa measured at several stages of plant development in four experiments.

Stage of Growth	Photosynthetic Rate (mg CO_2 $dm^{-2}hr^{-1}$)	Specific Leaf Weight (g dm^{-2})	Water Transpired (g)	Yield (g)	Leaf Area (dm^{-2})	Water-Use Efficiency (water g dry wt g^{-1})	Transpiration Rate (g water dm^{-2})
			Experiment 1-Harvest 1				
15.3 cm	25.6 a	.287 a	533 a	1.20 a	2.33 a	397.7 a	232.4 a
30.5 cm	18.0 b	.308 a	1156 b	2.45 b	3.91 b	487.9 b	302.0 b
			Experiment 1-Harvest 2				
15.3 cm	34.1 a	.273 a	518.2 a	1.48 a	2.73 a	346.6 a	188.6 a
30.5 cm	31.3 a	.295 a	984.6 a	2.22 b	3.77 b	466.4 ab	263.9 ab
Pre-bud	31.2 a	.308 a	1544.6 b	3.13 c	4.61 bc	494.6 ab	336.5 b
1/10 bloom	28.6 a	.356 b	2214.9 c	3.66 c	4.72 c	587.3 b	457.7 c
			Experiment 2				
15.3 cm	46.6 b	.248 a	621.5 a	.42 a	.79 a	766.0 a	415.8 a
30.5 cm	19.2 a	.362 b	817.3 b	.90 b	1.23 ab	692.9 a	517.8 a
Pre-bud	35.6 ab	.455 c	977.0 c	1.43 c	1.49 b	694.5 a	701.1 b
1/10 bloom	30.5 ab	.459 c	1079.6 c	1.46 c	1.51 b	744.5 a	745.5 b
			Experiment 3				
15.3 cm	18.0 a	.303 a	126.3 a	.19 a	.34 a	1002.6 a	437.4 a
Pre-bloom	49.7 b	.374 a	345.4 b	.58 b	.74 b	629.8 a	481.2 a
			Experiment 4-Harvest 1				
15.3 cm	25.0 a	.342 a	297.6 a	.57 a	.73 a	519.4 a	408.2 a
Pre-bud	27.0 a	.348 a	536.6 b	.83 a	.99 ab	648.4 b	540.2 a
1/10 bloom	40.5 b	.452 b	1201.7 c	1.42 b	1.24 b	850.6 c	796.7 b
			Experiment 4-Harvest 2				
1/10 bloom	20.4 a	.361 a	1289.2 a	1.19 a	1.25 a	1118.6 a	1054.9 a
1/10 bloom	32.2 a	.384 a	1465.2 a	1.31 a	1.29 a	1282.5 a	1250.5 a
1/10 bloom	25.6 a	.383 a	1947.0 a	2.06 a	1.71 a	950.0 a	1180.7 a

Means followed by the same letter are not significantly different at the .01 level according to Duncan's Multiple Range Test. Harvests were statistically analyzed individually.

Table 2. Mean photosynthetic rate, growth components, and water requirement of two cultivars and two clones of alfalfa.

Plant Material	Photosynthetic Rate $(mg\ CO_2\ dm^{-2}hr^{-1})$	Specific Leaf Weight $(g\ dm^{-2})$	Total Water Transpired (g)	Yield (g)	Leaf Area (dm^2)	Water-Use Efficiency (water g dry wt g^{-1})	Transpiration Rate $(g\ water\ dm^{-2})$
Experiment 1-Harvest 1							
Mesa-Sirsa	22.6	.308	869	1.89	3.10	445.9	274.7
Sonora	21.0	.287	280	1.77	3.13	439.7	259.7
Experiment 1-Harvest 2							
Mesa-Sirsa	30.2	.340	1251	2.60	3.87	454.3	307.5
Sonora	32.4	.313	1380	2.64	4.04	483.1	315.8
Experiment 2							
Clone 5	30.8	.396	612	.93	1.02 a	698.3	617.6
Clone 11	35.2	.366	885	1.18	1.49 b	750.6	572.5
Experiment 3							
Mesa-Sirsa	33.2	.349	251	.42	.58	642.7	464.8
Sonora	35.4	.328	221	.35	.50	989.7	453.7
Experiment 4-Harvest 1							
Clone 5	31.3	.399	750	1.04	1.09	681.2	653.4
Clone 11	30.4	.362	607	.84	.89	664.4	630.1
Experiment 4-Harvest 2							
Clone 5	26.4	.372	1800	1.67	1.69	1122.7	1094.7
Clone 11	25.8	.380	1335	1.38	1.15	1112.1	1229.3

No factors significantly different at the .01 level of Duncan's Multiple Range Test except leaf area in Experiment 2.

photosynthetic rates were lower during the first harvest of
Experiment 1, the two individual cultivar rates were not
significantly different. Mesa-Sirsa averaged 30.2 and 33.2
mg dm^{-2}hr^{-1} for the second harvest of Experiment 1 and for
Experiment 3, respectively. The photosynthetic rates for
Sonora plants in these same experiments were 32.4 and 35.4
mg dm^{-2}hr^{-1}, respectively.

Two clones of Mesa-Sirsa used in Experiments 2 and
4 had similar photosynthetic rates (Table 2). Clone 5 had
an average rate of 30.8, 31.3, and 26.4 mg dm^{-2}hr^{-1} for
Experiment 2 and the first and second harvest of Experiment
4, respectively. Clone 11 had an average carbon dioxide
incorporation rate of 35.2, 30.4, and 25.8 mg dm^{-2}hr^{-1}
respectively for these same experiments.

The variability in alfalfa photosynthetic rates
between experiments may have been due to the wide range in
environmental conditions which existed in the greenhouse
during different periods of the growing season.

Specific Leaf Weight

In every experiment and harvest the specific leaf
weight (SLW) increased as the plant aged (Table 1). These
results are in agreement with those reported by Cooper and
Qualls (17) who found this same relationship. Pearce et al.
(61) also found this relationship, however, they showed
that the effect was opposite that found in field experiments.

Alfalfa leaves produced during the first harvest of Experiment 1 had a low SLW of .287 g dm^{-2} when stems were harvested at a height of 15.3 cm. The SLW was .308 for the same experiment when stems reached a height of 30.5 cm. In all experiments where three or more stages of growth were tested, the SLW changes were significant at the .01 level. The SLW showed a tendency to increase as the temperature and day length increased. The SLW was .248 mg dm^{-2} in the spring. On plants tested in the greenhouse it had increased to .303 in the fall. Lower light intensity in the growth chamber caused the SLW to increase to .342 when stems had reached a height of 15.3 cm. The stage was similar to the 1/10 bloom growth stage in Experiment 1.

The specific leaf weight of Mesa-Sirsa and Sonora was not significantly different (Table 2). Cultivars and clones were both consistent when they were compared, i.e., Mesa-Sirsa always had a higher SLW than Sonora and Clone #5 always had a higher SLW than Clone #11 except at maturity. Clone #5 was the genotype with the highest SLW when growth was initiated, however SLW of Clone #11 increased faster and was higher in the 1/10th bloom growth stage. This last factor was possibly due to leaflet shape. Clone #5 had broad leaflets while Clone #11 had long thin leaflets.

Transpiration

The total water transpired per plant increased with each successive growth stage (Table 1). The quantity of water transpired also increased as the maximum and minimum temperatures increased. The total transpired water per pot in Experiment 1 was 533 g of water when plant stems were 15.3 cm in height at the first harvest. In Experiment 2 the total water transpired was 621.5 g on a plant that was approximately 1/3 the size of the plant in Experiment 1.

In Experiment 4, harvest 2, the plants harvested at the younger stages of growth in the previous harvest had a lower water requirement than those that had been harvested at maturity. The plants used over 600 g more water when harvested at 1/10 bloom in the previous experiment than those plants harvested at the 15.3 cm height.

The cultivars were inconsistent in the total amount of water transpired. Plants of Mesa-Sirsa transpired more water than those of Sonora during the first growth period of Experiment 1. Sonora transpired more water than Mesa-Sirsa during the second growth period of this same experiment (Table 2). Mesa-Sirsa used more water in the early stages of growth; however, Sonora utilized more water during the maturation stage. The clones were also inconsistent, possibly a result of leaflet shape.

In every experiment the transpiration rate (g water dm^{-2}) increased as the plant aged (Table 1). Water-use

efficiency increased at each stage of growth through most

experiments, but was not consistent. Transpiration rate

per dm^2 increased as the plants developed within harvests.

The quantity of water transpired increased as the tempera-

ture increased and as the plant aged, illustrating the

effect of temperature and leaf age on transpiration rates.

The water transpired when compared by cultivar or clone

was not consistent.

A record was made of the transpiration pattern

(Experiment 1, Harvest 1) over a 24-hour period (Table 3).

The total water lost over one day at the 15.3 cm stage was

81 g compared with a 154.5 g loss at the 30.5 cm height.

The peak in water use was found at the 12 to 2 and 2 to 4

p.m. period. The rate for the 2 to 4 p.m. period was

actually higher in the taller plants. Transpiration rates

are shown in Table 4. The shorter plants transpired more

water during the night, plants at the 15.3 cm growth stage

used 4.2 g dm^{-2} versus 2.7 g dm^{-2} for the 30.5 cm growth

stage. The taller plants transpired more water during the

day. They used from .6 g water dm^{-2} to 2.4 g water dm^{-2}

more than the plants at the 15.3 cm growth stage.

Yield and Leaf Area

Generally, the yield means followed the same trend

as the SLW (Table 1). Dry forage production increased very

Table 3. Water transpired utilizing two cultivars of alfalfa and two stages of growth measured at two-hour intervals.

	Water Lost Overnight	Water Used (g)				
		8-10 a.m.	10-12 a.m.	12-2 p.m.	2-4 p.m.	Total
Cultivars						
Mesa-Sirsa	10.2 a	10.1 a	27.8 a	37.0 a	36.0 a	121.5 a
Sonora	11.2 a	9.1 a	26.2 a	35.0 a	33.4 a	114.0 a
Stages of Growth						
15.3 cm	9.9 a	3.8 a	18.8 a	26.0 a	22.4 a	81.0 a
30.5 cm	11.5 a	15.4 a	35.2 b	46.1 b	46.9 b	154.5 b

Numbers in columns followed by the same letter are significantly different at the .01 level according to Duncan's Multiple Range Test.

Cultivars and growth stages analyzed separately.

Table 4. Transpiration rate of two alfalfa cultivars at two stages of growth measured at two-hour intervals.

	Water Lost Overnight	Water (g) dcm^{-2}				
		8-10 a.m.	10-12 a.m.	12-2 p.m.	2-4 p.m.	Total
Cultivars						
Mesa-Sirsa	3.3	3.3	9.0	11.9	11.6	39.2
Sonora	3.6	2.9	8.4	11.2	10.7	36.4
Stages of Growth						
15.3 cm	4.2	1.6	8.1	11.2	9.6	34.8
30.5 cm	2.9	3.9	9.0	11.8	12.0	39.5

quickly in the initial growth stages then increased more slowly as the plants matured. The leaf area, however, increased very rapidly up to the 30.5 cm stage, then leaf formation almost ceased as the buds and floral parts developed. The wide variance in yield and leaf area between experiments was due to differences in plant age. The plants in Experiment 1 were quite old and had numerous stems while those in Experiment 3 were seedlings that averaged two stems per plant.

Cultivar and clonal differences were not consistent except within experiments. Since the same plants were used for two harvests in both Experiments 1 and 4, similar results were obtained. The second experiment was the only experiment where transpiration rate was significant. This indicated that the change in yield and in leaf area were factors of both growth and aging.

Water-Use Efficiency

As plants aged in each growth period, they required more water per unit of dry matter produced. The most efficient plants were found in the 15.3 cm growth stage except seedlings and clones tested in the greenhouse. In Harvest 2 of Experiment 1, for example, the water requirement increased from 346.6 when stems had reached a height of 15.3 cm to 587.3 at 1/10 bloom. In general, increase in water requirement from seedlings to plants 6 to 8 months

old was 629.8 at 30.5 cm growth stage in seedlings to 446.4

in the second harvest of Experiment 1 at the same height.

The use of averages caused discrepancies in the tables due

to increase in water-use efficiency (WUE), i.e., the

seedling experiment had one plant that used 4700 g of water

to produce one gram of dry weight. This caused the in-

flated water-use efficiency figure at the 15.3 cm growth

state of 1002.6 g water per g dry weight. The same problem

is illustrated in Table 2 where the same plant raised the

WUE of the cultivar, Sonora. If that one plant had not

been included in the average, the mean would have been

653.5 g water per g dry weight.

Temperature increased in the greenhouse and growth

chamber over the entire growing season. This caused an

increase in water transpired from 445.9 grams of water per

gram of dry weight at 15.3 cm plant height in Experiment 1

to 681.2 in Experiment 4 at the same height. The second

experiment showed a decrease in water-use efficiency caused

by the utilization of young plants and high night tempera-

tures. This was also shown in Experiment 4 by the quantity

of water utilized per gram of dry matter produced in the

growth chamber where the temperature was kept above 20 C

at all times.

WUE is difficult and time consuming to measure.

This thesis originated as an attempt to find a tool to

expedite selection of efficient genotypes. The relationship

of water-use efficiency to photosynthetic rate, SLW, total transpired water, yield, and water transpired dm^{-2} was determined on all plants within each harvest (Table 5) and within each growth stage (Table 6).

Photosynthetic rate was positively correlated with WUE only on plants grown in the growth chamber when all plants within the harvest were used (Table 5). It was not significant at any stage of growth in any experiment. Specific leaf weight was significantly related to WUE in older plants grown in the greenhouse and clones grown in the growth chamber when all plants within a harvest were analyzed. Seedlings showed a correlation coefficient of -.61 between SLW and WUE when all plants within a harvest were analyzed; however, only the 15.3 cm growth stage of the seedling experiment was significantly correlated with WUE when individual growth stages were tested. Total water transpired was positively correlated in most experiments when the correlations were made on all plants within harvests. The total amount of transpired water was positively correlated with WUE with plants at the 15.3 cm, 30.5 cm, and pre-bud growth stages of Experiment 1, Harvest 2, and the 15.3 cm growth stage in the seedling experiment. Although significant correlation coefficients were found in most harvests, individual stages of growth were significant only when WUE was compared with transpiration rates.

Table 5. Correlation coefficients for water-use efficiency versus photosynthetic rate and other growth components across all stages of growth within each harvest.

	Water-Use Efficiency Versus				
	Photo-synthetic Rate	Specific Leaf Weight	Transpired Water	Yield	Water Transpired (dm^{-2})
Experiment 1-Harvest 1 Cultivars	-.14	omitted	.51**	.25	.80**
Experiment 1-Harvest 2 Cultivars	-.18	.63**	.87**	.65**	.92**
Experiment 2 Clones	.02	.11	.06	.22	.32*
Experiment 3 Cultivars	-.27	-.61**	-.31	-.41*	.50*
Experiment 4-Harvest 1 Clones	.74**	.72**	.91**	.82**	.94**
Experiment 4-Harvest 2 Clones	.06	-.10	.52**	.65**	.60**

*Significant at the .05 level.

**Significant at the .01 level.

Table 6. Correlation coefficients for water-use efficiency versus several growth components and photosynthesis of alfalfa.

Stage of Growth	Water-Use Efficiency Versus				
	Photo-synthesis	Total Water Transpired	Yield	Specific Leaf Weight	Water Transpired dm^{-2} Leaf Area
Experiment 1-Harvest 1					
15.3 cm	.38	.17	-.33	omitted	.85*
30.5 cm	.22	.13	-.27	omitted	.63
Experiment 1-Harvest 2					
15.3 cm	.38	.88**	.31	.24	.87*
30.5 cm	-.38	.94**	-.37	.55	.97**
Pre-bud	.03	.67	-.04	-.28	.85*
1/10 bloom	.59	.72	-.54	.41	.65
Experiment 2					
15.3 cm	-.08	.35	-.32	.41	.99**
30.5 cm	-.35	.10	-.42	-.42	.35
Pre-bud	-.03	.11	-.41	.13	.79*
1/10 bloom	-.30	.53	-.15	-.25	.31
Experiment 3					
15.3 cm	-.16	-.58	-.75*	-.61*	.56
30.5 cm	-.32	-.18	-.79**	-.20	.79**
Experiment 4-Harvest 1					
15.3 cm	.54	.39	.22	.48	.85*
30.5 cm	-.08	.43	.11	.35	.83*
1/10 bloom	.57	-.32	-.68	-.27	.30

Table 6.--<u>Continued</u>

			Experiment 4-Harvest 2		
1/10 bloom	.61	-.59	-.81*	.41	.53
1/10 bloom	-.14	-.52	-.61	-.11	.90**
1/10 bloom	.02	.51	-.56	.03	-.18

*Significant at the .05 level.

**Significant at the .01 level.

Water transpired per unit of leaf area was positively, significantly, correlated with WUE in every experiment when all growth stages were used. These two factors were usually significantly correlated at the 15.3 cm and/or 30.5 cm growth stage within each harvest. The only exception was Experiment 2, where a problem with salt accumulation partly affected all results.

SUMMARY

Four experiments were conducted in greenhouse and growth chamber environments at Tucson, Arizona, to determine the relationship between water-use efficiency and photosynthetic rate, transpiration rate, total transpired water, dry forage production, and specific leaf weight of alfalfa.

Mesa-Sirsa and Sonora cultivars were seeded in pots that contained a specified amount of soil. Cuttings from two clones of Mesa-Sirsa were established and utilized to determine the variation within cultivars that was associated with the physiological and growth component factors and how these components were related to water-use efficiency when genetic variation was reduced.

Photosynthetic rates were evaluated with an infra-red gas analyzer connected with plant chambers which accommodated the entire alfalfa plant.

The photosynthetic rates of Mesa-Sirsa and Sonora cultivars were not significantly different. Considerable variation in CO_2 uptake was found from one experiment to another; however, environmental conditions and plant age would account for the differences among experiments. Photosynthetic rates were higher in plants that were measured early in the growth cycle. Rates declined as the plants matured toward the 1/10 bloom stage except for

alfalfa seedlings grown in the greenhouse and clones grown under low light intensity. Photosynthetic rates were not related to the efficient use of water except for plants grown under low light intensity.

The transpiration rate of alfalfa was highest during the four-hour period from 10 a.m. to 2 p.m. The amount of water lost per unit of leaf area did not differ significantly between cultivars or clones within a cultivar; however, a significant relationship existed between WUE and transpiration when the rates were calculated by dividing total transpired water by leaf area.

Specific leaf weight was not different for Mesa-Sirsa or Sonora cultivars or between clones within Mesa-Sirsa. The weight per unit of leaf area was lower during the early stages of a growth cycle and continued to increase as plants matured. Specific leaf weight increased during periods of relatively high maximum and minimum temperatures.

Alfalfa plants which were harvested at the early stages of the growth cycle were more efficient in the use of water than mature plants. The water requirement was lowest during the period of most rapid dry matter accumulation which occurred during the first growth stage following forage removal. Subsequent growth occurred much more slowly, thus, existing transpiring surfaces raised the water requirement at maturity. The difference in the water-use efficiency among experiments was a result of

inadequate control of temperature and humidity. As maximum and especially minimum temperatures throughout the season increased, additional units of water were required to produce a unit of dry matter.

Water-use efficiency was compared with photosynthetic rate, specific leaf weight, total transpired water, yield, and transpiration per decimeter squared. Correlation coefficients were calculated for all plants within harvests and within each separate growth stage. Several factors were statistically significant when all plants within a harvest were used to calculate correlation coefficients. Few factors were significantly related within individual growth stages. The transpiration rate, determined by dividing total water lost by leaf area, was the only factor that was consistently related with WUE.

REFERENCES CITED

1. Al-Kawaz, G. M. 1970. Physiological factors related to water-use efficiency of alfalfa (Medicago sativa L.) genotypes. Ph. D. Dissertation. University of Arizona, Tucson.

2. Allen, L. H., C. S. Yocum, and E. R. Lemon. 1964. Photosynthesis under field conditions. VII. Radiant energy exchanges with a corn crop canopy and implications in water-use efficiency. Agron. J. 56:253-259.

3. Austin, R. B., and P. C. Longden. 1967. Rapid method for the measurement of rates of photosynthesis using $C^{14}O_2$. Ann. of Bot. 31:245-253.

4. Bailey, L. F. 1940. Some water relations of three Western grasses. I. The transpiration ratio. Amer. J. Bot. 27:122-128.

5. Bailey, L. F., J. S. Rothacher, and W. H. Cummings. 1952. A critical study of the cobalt chloride method of measuring transpiration. Plant Physiol. 27:563-574.

6. Baker, J. N., and O. J. Hunt. 1961. Effects of clipping treatments and clonal differences on water requirement of grasses. Range Manage. 14:216-219.

7. Barnes, D. K., R. B. Pearce, G. E. Carlson, R. H. Hart, and C. H. Hanson. 1969. Specific leaf weight differences in alfalfa associated with variety and plant age. Crop Sci. 9:421-423.

8. Bate, G. C., A. D'Aoust, and D. T. Canvin. 1969. Calibration of infra-red CO_2 gas analyzers. Plant Physiol. 44:1122-1126.

9. Black, A. L. 1962. Nitrogen and phosphorus fertilization for production of crested wheatgrass and native grass in northeastern Montana. Agron. J. 54:316.

10. Briggs, L. J., and H. L. Shantz. 1914. Relative water requirements of plants. J. Agr. Res. 3:1-64.

11. Brown, K. W., and N. J. Rosenberg. 1968. Errors in sampling and infra-red analysis of CO_2 in air and their influences in determination of net photosynthetic rate. Agron. J. 60:309-311.

12. Brown, P. L., and W. D. Shrader. 1959. Grain yields, evapotranspiration, and water-use efficiency of grain sorghum under different cultural practices. Agron. J. 51:339-343.

13. Brun, W. A., and R. L. Cooper. 1967. Effects of light intensity and carbon dioxide concentration on photosynthetic rate of soybeans. Crop Sci. 7: 451-454.

14. Bula, R. J., C. L. Rhykerd, and R. G. Langston. 1959. Growth responses of alfalfa seedlings under various light regimes. Agron. J. 51:84-86.

15. Catsky, J., and S. Zedenek. 1966. Suitable indicators and an altered empirical equation for calculating the CO_2 concentration in colorimetric determinations of photosynthetic rate. Biol. Plant. 8:60-74.

16. Cole, D. F. 1969. Water-use efficiency of seven cultivars of alfalfa (Medicago sativa L.). M.S. Thesis, Department of Agronomy, University of Arizona, Tucson.

17. Cooper, C. S., and M. Qualls. 1967. Morphology and chlorophyll content of shade and sun leaves of two legumes. Crop Sci. 7:672-673.

18. Curtis, P. E., W. L. Ogren, and R. H. Hageman. 1969. Varietal effects in soybean photosynthesis and photorespiration. Crop Sci. 9:323-327.

19. Decker, J. P. 1947. The effect of air supply on apparent photosynthesis. Plant Physiol. 22:561-571.

20. Denmead, O. T., L. J. Fritschen, and R. H. Shaw. 1962. Spatial distribution of net radiation in a corn field. Agron. J. 54:505-510.

21. 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.

22. 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.

23. Dobrenz, A. K., L. N. Wright, M. A. Massengale, and W. A. Kneebone. 1969. Water-use efficiency and its association with several characteristics of blue panicgrass (_Panicum antidotale_ Retz.) clones. Crop Sci. 9:213-216.

24. Egli, D. B., J. W. Pendleton, and D. B. Peters. 1970. Photosynthetic rate of three soybean communities as related to carbon dioxide levels and solar radiation. Agron. J. 62:411-414.

25. El-Sharkawy, M., and J. D. Hesketh. 1964. Effect of temperature and water deficit on leaf photosynthetic rates of different species. Crop Sci. 4:514-518.

26. El-Sharkawy, M., J. Hesketh, and H. Muramoto. 1965. Leaf photosynthesis and other growth characteristics among twenty-six species of _Gossypium_. Crop Sci. 5:173-175.

27. Erie, L. J., and O. F. French. 1969. Components of safflower as affected by irrigation regimes. Agron. J. 61:111-113.

28. Erie, L. J., O. F. French, and K. Harris. 1965. Consumptive use of water by crops in Arizona. Arizona Agr. Expt. Sta. Tech. Bul. 169.

29. 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. 41:1781-1787.

30. Friend, D. J. C. 1969. Net assimilation rate of wheat as affected by light intensity and temperature. Can. J. Bot. 47:1781-1787.

31. Fuess, F. W., and M. B. Tesar. 1968. Photosynthetic efficiency, yields, and leaf loss in alfalfa. Crop Sci. 8:159-163.

32. Hagen, R. M., M. L. Peterson, R. D. Upchurch, and L. G. Jones. 1957. Relationships of soil moisture stress to different aspects of growth in Ladino clover. Proc. Soil Sci. Soc. Amer. 21:360-365.

33. Heichel, G. H., and R. B. Musgrave. 1969. Varietal differences in net photosynthesis of Zea mays L. Crop Sci. 9:483-486.

34. Hesketh, J. D. 1963. Limitations to photosynthesis responsible for differences among species. Crop Sci. 3:493-496.

35. Hesketh, J. D. 1967. Enhancement of photosynthetic CO_2 assimilation in the absence of oxygen, as dependent upon species and temperature. Planta. 76:371-374.

36. Hesketh, J. D. 1968. Effects of light and temperature during plant growth on subsequent leaf CO_2 assimilation rates under standard conditions. Australian J. Biol. Sci. 21:235-241.

37. Hesketh, J. D., and D. Moss. 1963. Variation in the response of photosynthesis to light. Crop Sci. 3:107-110.

38. Hofstra, G., and J. D. Hesketh. 1969. Effects of temperature on the gas exchange of leaves in the light and dark. Planta. 85:228-237.

39. Hofstra, G., and C. D. Nelson. 1969. A comparative study of translocation of assimilated C^{14} from leaves of different species. Plant. 88:103-112.

40. Hughes, A. P., K. E. Cocshull, and O. V. S. Heath. 1970. Leaf area and absolute leaf water content. Ann. Bot. 34:259-265.

41. Humphries, E. C., and S. A. W. French. 1969. Photosynthesis in sugar beet depends on root growth. Planta. 88:87-90.

42. Idso, S. B. 1968. Atmospheric and soil-induced water stresses in plants and their effects on transpiration and photosynthesis. J. Theor. Biol. 21:1-12.

43. Jeffers, D. L., and R. M. Shibles. 1969. Some effects of leaf area, solar radiation, air temperature and variety on net photosynthesis in field grown soybeans. Crop Sci. 9:762-764.

44. Jensen, E. H., M. A. Massengale, and D. O. Chilcote. 1967. Environmental effects on growth and quality of alfalfa. Univ. of Nevada Agr. Exp. Sta. Tech. Bull. 7.

45. Jewiss, O. R., and J. Woledge. 1967. Effect of age on the rate of apparent photosynthesis in leaves of tall fescue (Festuca arundinacea Schreb.). Ann. Bot. 31:661-671.

46. Joshi, M. C., J. S. Boyer, and P. J. Kramer. 1965. Growth, carbon dioxide exchange, transpiration and transpiration ratio of pineapple. Bot. Gaz. 126: 174-179.

47. Joy, R. 1970. Consumptive water-use efficiency of alfalfa (Medicago sativa L.) grown under three irrigation regimes. M.S. Thesis, Department of Agronomy, University of Arizona, Tucson.

48. Kelley, O. J. 1954. Requirement and availability of soil water. Adv. Agron. 6:67-94.

49. Koller, D., and Y. Samish. 1964. A null point compensating system for simultaneous and continuous measure of net photosynthesis and transpiration by controlled gas stream analysis. Bot. Gaz. 125:81-88.

50. Kosonen, M. 1969. CO_2 production in relation to temperature and plant mass. Oikos 20:335-343.

51. LaRue, C. D. 1923. Simple weighing device for large scale transpiration experiments. Bot. Gaz. 75: 209-210.

52. Lemon, E. R. 1965. Energy conversion and water use efficiency in plants. Plant Environment and Efficient Water Use. Amer. Soc. of Agron. and Soil Sci. of Amer., Madison.

53. Luption, F. G. H. 1969. Estimation of yield in wheat from measurements of photosynthesis and translocation in the field. Ann. App. Bio. 64:363-374.

54. Moss, D. 1964. Optimum lighting of leaves. Crop Sci. 4:131-136.

55. Moss, D. N., R. B. Musgrave, and E. R. Lemon. 1961. Photosynthesis under field conditions. III. Some effects of light, CO_2, temperature and soil moisture on photosynthesis, respiration, and transpiration of corn. Crop Sci. 1:83-87.

56. Murata, Y., and J. Iyama. 1963. Studies on the photosynthesis of forage crops. I. Dirunal changes in the photosynthesis of several grasses and barley seedlings under constant temperature and light intensity. Crop Sci. Soc. Japan 31:311-315.

57. Murata, Y., and J. Iyama. 1963. Studies on the photosynthesis of forage crops. II. Influence of air temperature upon the photosynthesis of some forage and grain crops. Proc. Crop Sci. Soc. Japan 31:315-322.

58. Musgrave, R. B., and D. Moss. 1961. Photosynthesis under field conditions. I. A portable closed system for determining net assimilation and respiration of corn. Crop Sci. 1:37-41.

59. Nevins, O. J., and R. S. Loomis. 1970. A method for determining net photosynthesis and transpiration of plant leaves. Crop Sci. 10:3-6.

60. Osterhout, W. J. V. 1919. Apparatus for the study of photosynthesis and respiration. Bot. Gaz. 68:60-62.

61. 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.

62. Peck, N. H., M. T. Vittum, and G. H. Gibbs. 1968. Evapotranspiration rates for irrigated crops at Geneva, New York. Agron. J. 60:23-26.

63. Peck, N. H., M. T. Vittum, and R. D. Miller. 1958. Evapotranspiration rates for alfalfa and vegetable crops in New York State. Agron. J. 50:109-112.

64. Robison, G. D., and M. A. Massengale. 1967. Use of area weight relationship to estimate leaf area in alfalfa (Medicago sativa L. cultivar "Moapa"). Crop Sci. 7:394-395.

65. Smith, A. M. 1919. The temperature coefficient of photosynthesis: A reply to criticism. Ann. Bot. 33:517-536.

66. Thomas, M. D., and G. R. Hill. 1937. The continuous measurement of photosynthesis, respiration and transpiration of alfalfa and wheat growing under field conditions. Plant Physiol. 12:285-307.

67. Tovey, R. 1963. Consumptive use and yield of alfalfa grown in the presence of static water tables. Univ. of Nevada Agr. Exp. Sta. Tech. Bull. 232.

68. Watson, D. J., and K. Hayashi. 1965. Photosynthetic and respiratory components of the net assimilation rates of sugar beet and barley. New Phytol. 64: 38-47.

69. Wilfong, R. T. 1967. Relationships between leaf area index and apparent photosynthesis in alfalfa (Medicago sativa L.) and Ladino clover (Trifolium repens L.). Crop Sci. 7:27-30.

70. Wilson, D., and J. P. Cooper. 1969. Effect of light intensity and CO_2 on apparent photosynthesis and its relationship with leaf anatomy in genotypes of Lolium perenne L. New Phytol. 68:627-644.

71. Wilson, D., and J. P. Cooper. 1969. Apparent photosynthesis and leaf characters in relation to leaf position and age, among contrasting Lolium genotypes. New Phytol. 68:645-655.

72. Wilson, D., and J. P. Cooper. 1969. Effect of temperature during growth on leaf anatomy and subsequent light saturated photosynthesis among contrasting Lolium genotypes. New Phytol. 68: 1115-1123.

73. Winter, S. R., and J. W. Pendleton. 1970. Results of changing light and temperature regimes in a corn field and temperature effects on the apparent photosynthesis of individual leaves. Agron. J. 62:181-184.

74. Wolf, D. D., R. B. Pearce, G. E. Carlson, and D. R. Lee. 1969. Measuring photosynthesis of attached leaves with air sealed chambers. Crop Sci. 9:24-27.

75. Yocum, C. S., L. H. Allen, and E. R. Lemon. 1964. Photosynthesis under field conditions. VI. Solar radiation balance and photosynthetic efficiency. Agron. J. 56:249-253.

76. Zelitch, I. 1968. Investigations on photorespiration with a sensitive ^{14}C-assay. Plant Physiol. 43: 1829-1837.

74. Thomas, M. D., L. H. Hicton, and G. R. Hendricks. 1964.
Photosynthesis under field conditions. VI. Solar
radiation balance and photosynthetic efficiency.
Agron. J. 36:489-511.

75. Vartapetian, B. 1965. Investigations on photorespiration
with a sensitive ^{14}C-assay. Plant Physiol. 41:
1035-1037.