PHYSIOLOGICAL, MORPHOLOGICAL, AND ANATOMICAL CHARACTERISTICS OF MULTIFOLIOLATE AND 'LEW' ALFALFA (*MEDICAGO SATIVA* L.)

by

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ABSTRACT

Trifoliolate 'Lew' and Multifoliolate cycle-2 syn-1 (MF) germplasm sources were examined with respect to physiological and morphological characteristics, dry matter production, and various anatomical features. The purpose of this study was to determine if differences existed between the germplasm sources with respect to these factors, and if various factors were related.

Significant differences between germplasm sources were found for dark respiration (mg CO_2 dm^{-2} hr^{-1}), specific leaf weight, and the ratio of vascular tissue cross-sectional area to petiole cross-sectional area. No significant differences in yield or in leaflet to stem-petiole ratio were observed. Sixty-five percent of the MF plants displayed the multifoliolate characteristic.

A significant relationship (r = 0.61**) was observed between specific leaf weight and dark respiration. Yield and percent dry matter were negatively related (r = -0.63**). Palisade mesophyll thickness and leaf thickness were also correlated (r = 0.86**).

INTRODUCTION

Alfalfa (*Medicago sativa* L.) is the most important irrigated forage crop grown in Arizona (45). Average yield figures in kg/ha/year (tons/acre/year) for 1976 ranged from 4281.6 (2.4) in Apache County to 13,915.2 (7.8) in Yuma, with a total of 84,987 ha. (210,000 acres) harvested (2).

The great variation in yield throughout the state reflects a wide range of elevations and climatic conditions. At Springerville in Apache County, elevation 6,964 feet, the average length of the growing season for the years 1911 through 1945 was 128 days, whereas at Yuma in Yuma County, elevation 141 feet, the average growing season for the years 1930 through 1954 lasted 280 days, with extremes ranging from 223 to 341 days (41). Variation in yield also arises from a wide range of soil, insect, and disease problems.

Because of variation in growing conditions within the state, breeders in Arizona have been concerned with developing cultivars of alfalfa which are resistant to such problems as root rots, stem nematodes, various aphids, drought conditions, and high soil salinity. Varieties which are better adapted to specific conditions tend to produce higher forage yields. Forage quality is also a factor in many breeding programs in the state.

The research presented here was designed to compare the yield, quality, and some anatomical and physiological features of 'Lew' and an experimental multifoliolate alfalfa. Lew is the newest cultivar released by Dr. M. H. Schonhorst at The University of Arizona and was developed for southern Arizona conditions[1]. The experimental Multifoliolate cycle-2 syn-1 line was developed from the initial crossing (6) of a multifoliolated 'Ladak-65' alfalfa clone with 'Mesa-Sirsa' which was also developed at The University of Arizona (39).

[1]Personal communication from the files of Dr. M. H. Schonhorst, University of Arizona, "Notice of Naming and Release of Lew Alfalfa".

LITERATURE REVIEW

The ultimate objectives of forage research are to increase yield (8, 16) and quality (8). Donald (16) indicated that breeding for insect resistance had been the most widely used method in past years. He suggested that such breeding was ineffective given an optimum environment. Plants bred for insect and disease resistances have only a greater potential for reaching their maximum inherent yields (26).

Selection of plants which show a greater efficiency of conversion of light and nutrients into yield is an approach which has received little attention by plant breeders (8). According to Pierce and Lee (33) the ultimate limitation to increasing crop production is the efficiency with which a crop absorbs and utilizes solar energy. Donald (16) indicated that yield is a direct outcome of the extent and duration of photosynthesis, and that plant breeders should therefore be concerned with the effectiveness of this process as it relates to yield.

Carlson et al. (8) stated that physiological traits, as well as morphological traits which can be closely associated with physiological traits, may be used as selection criteria for yield and quality increase. However, heritable differences in these traits must exist within a crop. The physiological or morphological trait must be easily measured, and these traits must be closely related to yield. These researchers also concluded that photosynthetic rate was variable enough among genotypes to be used as a characteristic in a selective breeding program. Foutz, Wilhelm and Dobrenz (22) found that morphological factors were superior to physiological factors as indicators of yield in alfalfa, and suggested that morphological factors be used in selection for yield.

Many researchers have found significant positive correlations between specific leaf weight (SLW) and apparent photosynthesis on a unit leaf area basis (8, 12, 13, 27, 32, 33, 49, 50). Delaney, Dobrenz, and Poole (14) found that SLW, as well as photosynthesis (PS), dark respiration (DR) and leaf area index (LAI), is reduced during periods of high temperatures. Ku and Hunt (30) reported a similar relationship between SLW and air temperature. These researchers also, however, stated that there is no significant relationship between SLW and net carbon exchange on a leaf area basis. Porter and Reynolds (34) found, however, that the SLW of their samples is higher on sampling dates preceeded by higher mean air temperatures. Bula (7) also reported higher SLW's at higher temperatures.

SLW and net PS in alfalfa are dependent on light intensity (33). Chatterton, Lee, and Hungerford (10) found diurnal changes in SLW of alfalfa associated with changes in incident solar light intensity.

Hesketh and Baker (27) concluded that leaf thickness may directly affect PS. Lewis (31) stated that the resistance of the leaf mesophyll to the diffusion of CO_2 is inversely proportional to the thickness of the leaf, suggesting that thicker leaves may have the capacity for more rapid CO_2 exchange rates at light saturation. Lewis (31) also stated that variation in leaf thickness was related to the number of palisade cell layers and the length of these cells. Delaney and Dobrenz (13) further demonstrated that SLW is positively associated with leaf thickness, and that PS is significantly dependent upon the thickness of the palisade layer.

Chatterton (9) studied diurnal changes in SLW, PS on a unit leaf area basis, and translocation of assimilates in alfalfa leaves. He found that on a diurnal basis PS and SLW are significantly negatively correlated, and he associated this with feedback inhibition of PS due to the buildup of assimilates in the leaf. As assimilates build up, SLW increases and PS decreases. He also found differences in the magnitude of PS associated with clones displaying different inherent SLW's, but no data were presented.

Sato (38) found that alfalfa plants grown in lower temperature regimes have greater leaf thickness, lower palisade cell density, larger and longer palisade cells, lower stomatal densities, and larger intercellular spaces than those grown under higher temperatures. Bula (7) found that plants grown at higher temperatures have less leaf tissue, less leaf area, and smaller, more densely packed leaf cells. He suggested that these temperature anatomy relationships partially explain lower alfalfa yields in the warmer summer months. Ku and Hunt (30) reported findings similar to those of Bula (7) and Sato (38), with the exception that they found greater leaf densities at lower temperatures. Ku and Hunt (30) also found that net carbon dioxide exchange rates were greater at lower temperatures for non-saturating light conditions.

Light absorbtion by leaves has been related to leaf thickness and density. Gausman and Allen (24) found that thicker leaves with more complex mesophylls absorb more light per unit leaf area than thinner, less complex leaves.

Selection of higher yielding alfalfa on the basis of higher apparent PS/unit leaf area, however, does not seem feasible (37). Although Carlson et al. (8) found relationships between SLW and PS/unit leaf area, they reported no significant association between PS/unit leaf area and yield on a total plant basis. Other researchers have lent further support to this conclusion (12, 22, 34, 35). Foutz, Wilhelm, and Dobrenz (22) also found no correlation between SLW and yield per plant, and although Song and Walton (42) found that SLW is a heritable trait in alfalfa, they drew the same conclusion.

A significant and positive correlation does exist, however, between PS on a total plant basis and yield of alfalfa (12, 13, 22, 35). Yield has also been related to leaf area per plant (12, 13, 22), LAI (14), DR per plant (12, 22), and post-illumination burst (PIB) per plant (22).

Delaney, Dobrenz, and Poole (14) found that LAI was closely related to yield in alfalfa. Total leaf area per plant is also related to yield (11, 13). Song and Walton (42) found that although leaflet size in alfalfa is a heritable trait and is responsive to selection, leaflet size on a per leaf basis as opposed to leaflet area per plant is not related to yield. Potter and Jones (35) indicated that the rate of leaf area expansion is highly correlated with grow rate of a plant, and that partitioning of photosynthate into leaf area expansion is very important. Yield has been related to leaf area on a per plant basis as the product of leaf area/plant and PS/unit leaf area (22, 35). This further defines the relationship between PS/plant and yield/plant, as PS/unit leaf area x leaf area/plant = PS/plant.

SLW and LAI have been significantly and negatively correlated (36), and SLW and area per leaflet of alfalfa were negatively related (12). Selection for large and thick leaves would, however, increase PS/unit leaf area while maintaining total leaf area/plant. This morphological combination could potentially increase yield.

Gallagher, Ashley, and Brown (23) suggested that the capacity of the phloem tissues in leaf petioles may limit the rate of translocation from leaves. Stephenson, Brown, and Ashley (44) further demonstrated a relationship between net PS and translocation, but concluded, due to the low value correlation coefficient ($r = 0.59$) that other factors are involved which affect the PS -- translocation relationship. Chatterton, Lee, and Hungerford (10) showed that on a diurnal basis an increase in SLW is accompanied by an increase in total amount of nonstructural carbohydrates in a leaf, and that these two variables are significantly and positively correlated. They (10) suggested that plants should be selected for efficient translocation of photosynthates if translocation influences the rate of PS or crop yield. In subsequent research, Chatterton (9) concluded that PS may be feedback inhibited by an accumulation of photosynthate in leaves due to slow translocation.

Bingham (3) found that vascular bundle size in petioles of leaves from multifoliolate (MF) plants was twice that found in petioles of leaves from trifoliolate (TF) plants, and suggested that, based on this characteristic, MF plants are more adequately equipped to carry on photosynthesis. However, Bingham and Murphy (4) found no association between the MF characteristic and an increase in mean percent of leaf dry weight per plant. As the number of leaflets per leaf increased the leaflet weight decreased. These authors did not state whether this inverse relationship was associated with leaflet area, leaflet thickness, or both.

Brick, Dobrenz, and Schonhorst (5) found that the (MF) characteristic in alfalfa is easily transmittable and heritable. They also observed an increased leaf percentage and a higher SLW displayed by MF progeny as opposed to TF plants. Ferguson and Murphy (18) also found a higher leaf percentage in MF plants when compared to TF plants from several cultivars. These data suggest that MF plants may have a higher photosynthetic capacity and the potential to produce more dry matter of higher quality.

Many researchers have associated leafiness with forage quality in alfalfa (1, 7, 15, 17, 19, 20, 25, 29, 36, 40, 46, 48). Smith and Struckmeyer (40) found that alfalfa leaves were more important than stems as a source of carbohydrates, proteins, and minerals. Dobrenz, Schonhorst and Thompson (15) found crude protein percentage in leaves of 5 alfalfa cultivars to range from 27.5 to 26.5 and in stems of the same cultivars to range from 11.6 to 10.5. Anderson (1) reported that leaves of alfalfa contain about twice as much protein and are about twice as high in energy value as the stems. Stahmann (43) found that among crops currently grown, alfalfa has the greatest potential for protein production.

The nutritive value of alfalfa forage tends to decline as the forage matures (19), but the nutritive value deteriorates much more slowly in leaves than in stems (20, 29). Vorachek (47) cited considerable research which showed that as alfalfa matures, there is an increase in lignin and fiber content with an accompanying decrease in soluble cell contents. These biochemical changes during maturation cause a decrease in forage quality and palatability. Vorochek (47) found in his study that increased fiber content tended to lower the digestibility of alfalfa. Hanna, Monson, and Burton (25) suggested that because the reduction in quality of leaves of forage plants with advancing maturity is much slower than in the stem, the use of dwarf phenotypes to increase percent leaves might be a way to improve overall forage quality. Varga et al. (46) also inferred that smaller alfalfa plants with a greater percent leaves might improve forage quality.

Effects of temperature and daylength on the quality of alfalfa have also been studied. Smith and Struckmeyer (40) reported that alfalfa leaves grown in cooler temperatures have a higher nonstructural carbohydrate content than leaves grown at higher temperatures. Sato (38) showed that under lower temperatures ($10^{\circ}C$ to $15^{\circ}C$) and shorter days, the leaf to stem ratio and the nitrogen content of the leaves of alfalfa are higher than under high temperatures (25°C to 30°C) and longer daylengths. Faix (17), however, found that although percent leaves increases with shorter days, temperatures above 22°C also cause an increase in leaf percent. Dobrenz, Schonhorst, and Thompson (15) showed that the leaf to stem ratio in alfalfa is lower during the months of May, June, and July, generally the hottest months of the year in southern Arizona (monthly temperatures average 25°C to 35°C), than during the rest of the growing season.

MATERIALS AND METHODS

In October of 1976, the alfalfa (*Medicago sativa*, L.) cultivar Lew and the experimental Multifoliolate Cycle-2 syn-1 (MF) were planted side-by-side in an 8.23 m (27 ft) x 129.54 m (425 ft) border (Figure 1) located at the USDA-SCS Plant Materials Center in Tucson, Arizona. A Brillion Cultipacker Seeder was used to plant the seed at a rate of 20 kg/ha (17.84 lb/A). Four 4.12 m x 7.62 m (13.5 ft x 25 ft) replicated plots of each germplasm source were located near the north end of the border as shown in Figure 1.

An initial forage harvest was made in April, 1977, to eliminate the winter overgrowth. Data were collected at subsequent harvests made at approximately 1-month intervals, corresponding to the 10% bloom stage of plant development. The harvest schedule was May 5, June 17, July 15, and August 19 for harvests 1, 2, 3, and 4 respectively.

Carbon dioxide exchange was measured on plants of the cultivar Lew, Multifoliolate Cycle-2 syn-1 plants which displayed the multifoliolate characteristic (MF-TF). On each of 3 days immediately preceeding each harvest date, two 33 cm (13-inch) stems of each plant type were gathered from each replication, placed in vials of distilled water, and allowed to equilibrate overnight in a controlled environment at 25^{o}C in darkness. CO_2 exchange measurements were made in a closed system similar to that described by Foutz (21) (Figure 2) using a Beckman model 215-A infared gas analyzer. Illumination

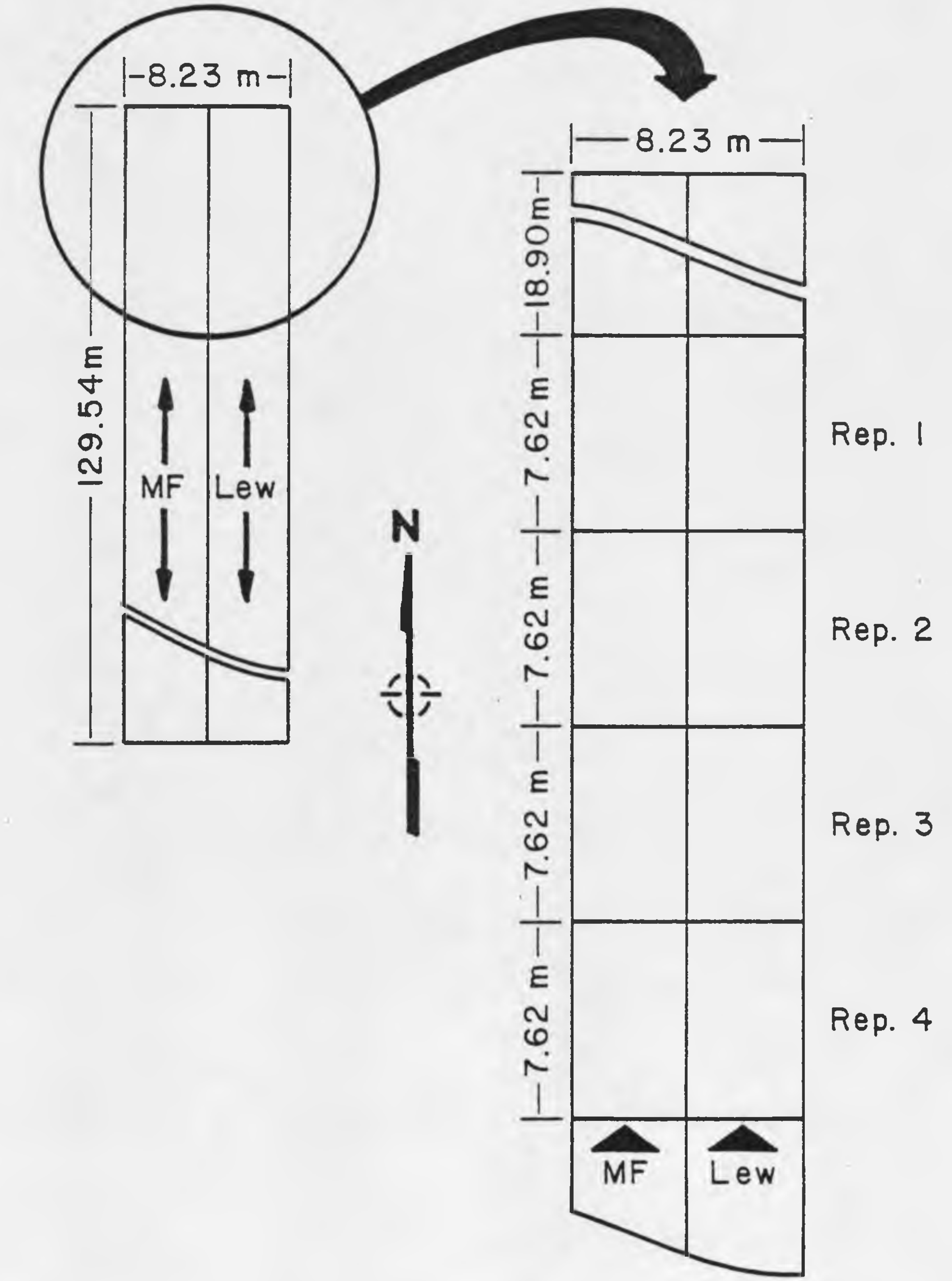

Figure 1. Plot design for comparative study of the MF and Lew germplasm sources.

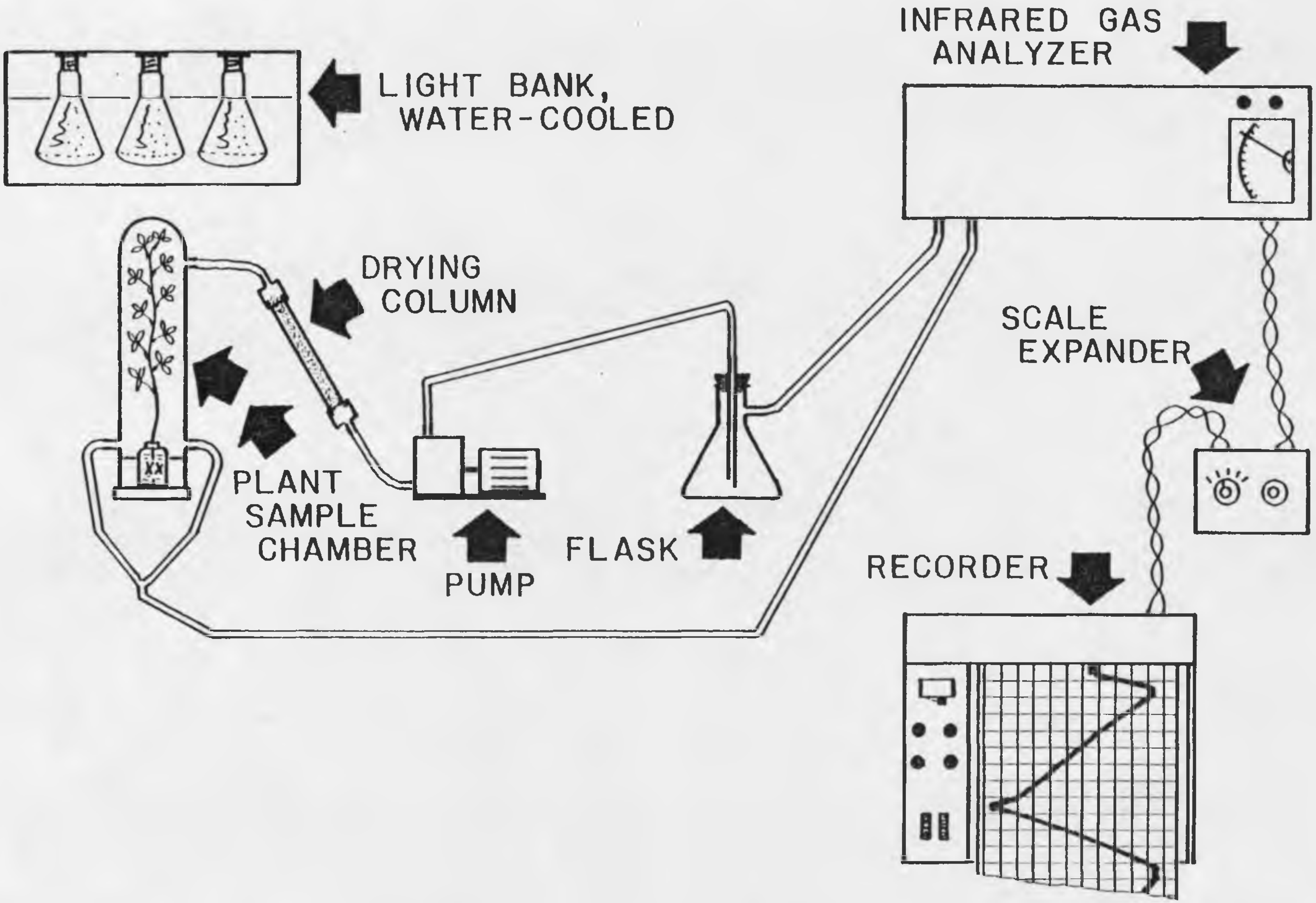

Figure 2. Diagram of the closed system used for physiological measurements, adapted from Foutz (21).

(2,000μ Einsteins) was provided by seven 500-watt flood lamps suspended in a circulating water bath to reduce heat immediately above the cylindrical glass CO_2 exchange chamber. Leaflet areas were measured using a Hayashi-Denko model AAM-5 automatic area meter. Rates of apparent photosynthesis (PS), post-illumination burst (PIB), and dark respiration (DR) were obtained in ppm CO_2/minute and were converted to mg $CO_2/dm^2/hr$ by Equation 1.

EQUATION 1:

$$\text{mg } CO_2 \text{ dm}^{-2} \text{ hr}^{-1} = \frac{(\Delta CO_2)(44{,}000 \text{ mg}CO_2/M)(V_S)(60 \text{ min/hr})(P/760)(273/T)}{(22.4 \text{ l/M})\ (LA)\ (10^6 \text{ ppm})}$$

where: CO_2 = rate of gas exchange in ppm

V_S = volume of system in liters

P = atmospheric pressure in mm Hg

T = system temperature in ^oK

LA = leaf area of sample in dm^2

Specific leaf weights for sample material were expressed as mg dry weight/cm^2 leaf area. Material was dried for 24 hrs in a forced air oven at 80^oC before weighing.

At each harvest, a Jari forage mower was used to cut a .81 meter (32 inch) harvest strip through the length of each replication. Cut material was weighed, and a sample was extracted, weighed, dried as described above, and reweighed. Percent dry matter was determined and yield in kg/harvest strip dry weight was calculated according to the following method (Equation 2).

EQUATION 2:

$$\text{Yield (kg/harvest strip)} = W_H\,(D_S/W_S)$$

where: W_H = Fresh Weight of Harvest strip in kg

D_S = Dry Weight of Subsample in grams

W_S = Fresh Weight of Subsample in grams

Leaflet to stem-petiole ratios were determined from a subsample of 6 stems chosen from the harvest material in each replication.

Percentage stems which displayed the multifoliolate characteristic was estimated from a subsample of 30 stems drawn at random from the harvest material in the Multifoliolate Cycle-2 syn-1 germplasm.

Leaflet and petiole samples from the sixth node of 10 MF-MF, 10 MF-TF and 10 Lew plants were collected for anatomical study in September, 1977. Material was fixed, dehydrated, sectioned, and stained according to procedures outlined by Johansen (28). Photomicrographs were taken of the various sections of each sample, and a stage micrometer was photographed at each of the magnifications used. Scales were made from projected images of the micrometer. Exact anatomical measurements were then obtained from projected images of the sample sections. Cross-sectional areas of tissues within the petiole were calculated from the areas of paper models, drawn from the projected image which were determined using a Hayashi-Denko model AAM-5 automatic area meter. Leaf thickness, palisade mesophyll thickness, spongy mesophyll thickness, and number of palisade cell layers were determined from 10-micron leaf-tissue cross-sections. Xylem, phloem, vascular bundle, and petiole cross-sectional areas were calculated from measurements made on 10-micron petiole cross-sections.

RESULTS AND DISCUSSION

Physiological Characteristics

Apparent photosynthetic (PS) rates averaged over the four harvests were 11, 10, and 10 mg CO_2 dm^{-2} hr^{-1} for the MF-MF, MF-TF, and Lew germplasm sources, respectively (Table 1). Differences in PS among the germplasm sources were not significant when averaged over the four harvests or at each individual harvest date (Table 2). The harvest period means for PS were slightly lower than those observed for alfalfa by Retzinger (37) and Foutz (21). PS rates declined at each harvest for all three germplasm sources (Figure 3). An analysis of variance by harvest showed that differences in PS between harvest dates were significant at the 1% level (Table 3).

Mean post-illumination burst (PIB) rates for the three germplasm sources over all harvests were 16, 15, and 15 mg CO_2 dm^{-2} hr^{-1} for MF-MF, MF-TF, and Lew, respectively (Table 1). These values fall slightly higher than the maximums observed by Retzinger (37), but are in the low end of the range observed among germplasm sources for the entire growing period or at individual harvest dates (Table 2). The trend in PIB over the harvest period (Figure 4) did not show a linear decline, in fact, a peak was noted at the July 15 harvest. Significant (5% level) differences in PIB occurred between harvest dates (Table 3).

Dark respiration (DR) rates were 4, 3, and 3 mg CO_2 dm^{-2} hr^{-1} for the MF-MF, MF-TF, and Lew germplasm sources, respectively, when

Table 1. Mean values ($\bar{x}$) and ranges (r) of apparent photosynthesis, post-illumination burst, dark respiration, and specific leaf weight for the MF-MF, MF-TF, and Lew germplasm sources over all harvests.

		Germplasm Source		
		MF-MF	MF-TF	Lew
Apparent Photosynthesis	$\bar{x}$	11	10	10
($mg\ CO_2\ dm^{-2}\ hr^{-1}$)	r	3-26	3-24	2-24
Post-Illumination Burst	$\bar{x}$	16	15	15
($mg\ CO_2\ dm^{-2}\ hr^{-1}$)	r	8-32	6-28	5-28
Dark Respiration	$\bar{x}$	4	3	3
($mg\ CO_2\ dm^{-2}\ hr^{-1}$)	r	1-9	2-8	2-6
Specific Leaf Weight	$\bar{x}$	4.41	4.22	3.74
($mg\ dm^{-2}$)	r	6.78-3.13	7.95-2.86	5.10-2.71

Table 2. Summary of analysis of variance among the MF-MF, MF-TF, and Lew germplasm sources for apparent photosynthesis, post-illumination burst, dark respiration and specific leaf weight by individual harvest date and over all harvests.

	Harvest Data				Four
	May 5	Jun. 17	Jul. 15	Aug. 19	Harvests
Apparent Photosynthesis	NS	NS	NS	NS	NS
Post-Illumination Burst	NS	NS	NS	NS	NS
Dark Respiration	*	NS	NS	NS	*
Specific Leaf Weight	*	NS	**	NS	*

** Significant to 0.01 level

* Significant to 0.05 level

NS Non-significant

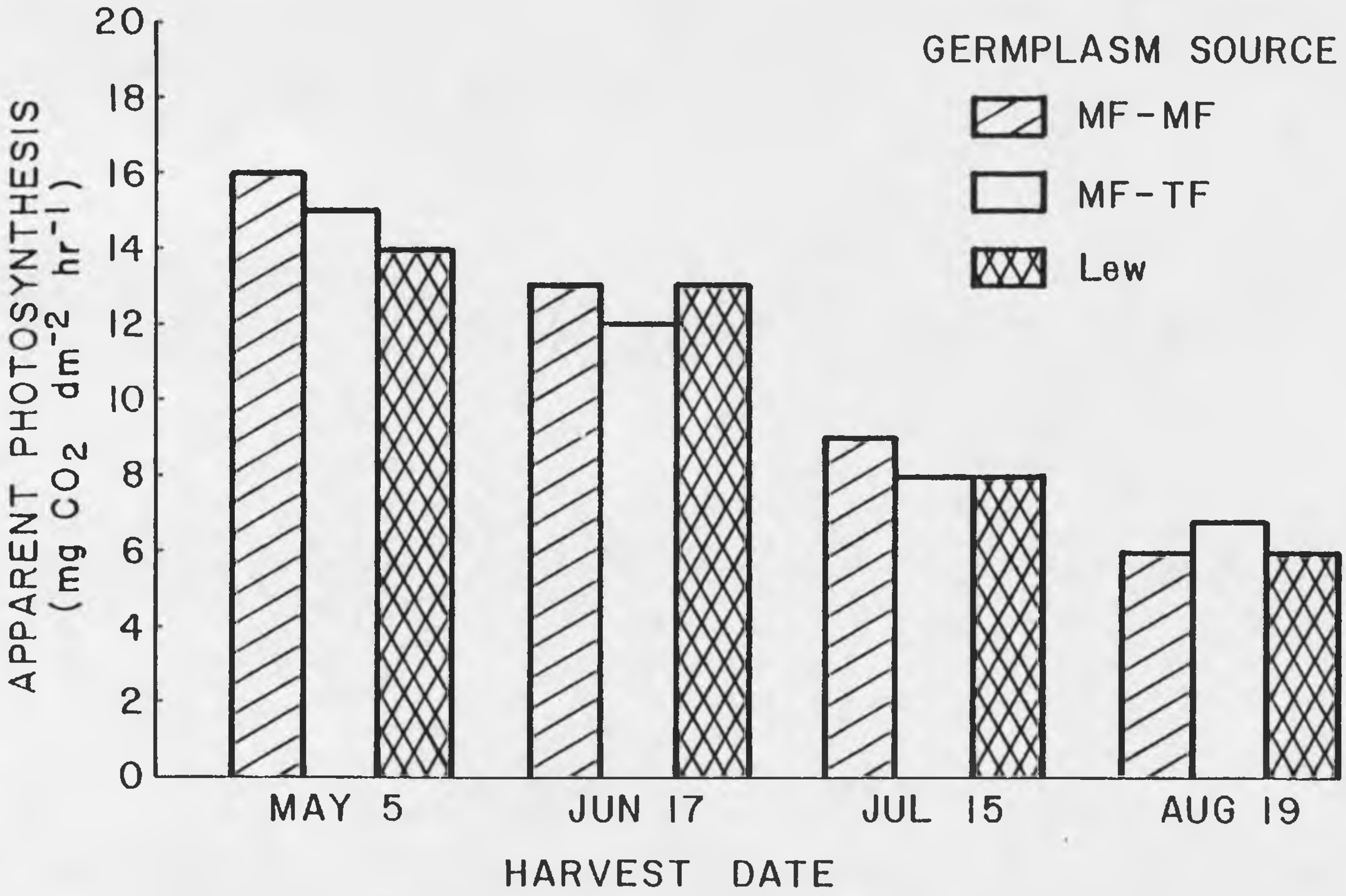

Figure 3. Mean apparent photosynthetic rates of the MF-MF, MF-TF, and Lew germplasm sources as measured at four harvest dates in 1977.

Table 3. Summary of analysis of variance among harvest dates for apparent photosynthesis, post-illumination burst, dark respiration, and specific leaf weight, including harvest date x germplasm source interaction.

	Harvest date	Harvest date x germplasm source
Apparent Photosynthesis	**	NS
Post-Illumination Burst	*	NS
Dark Respiration	**	NS
Specific Leaf Weight	**	NS

** Significant to 0.01 level

* Significant to 0.05 level

NS Non-significant

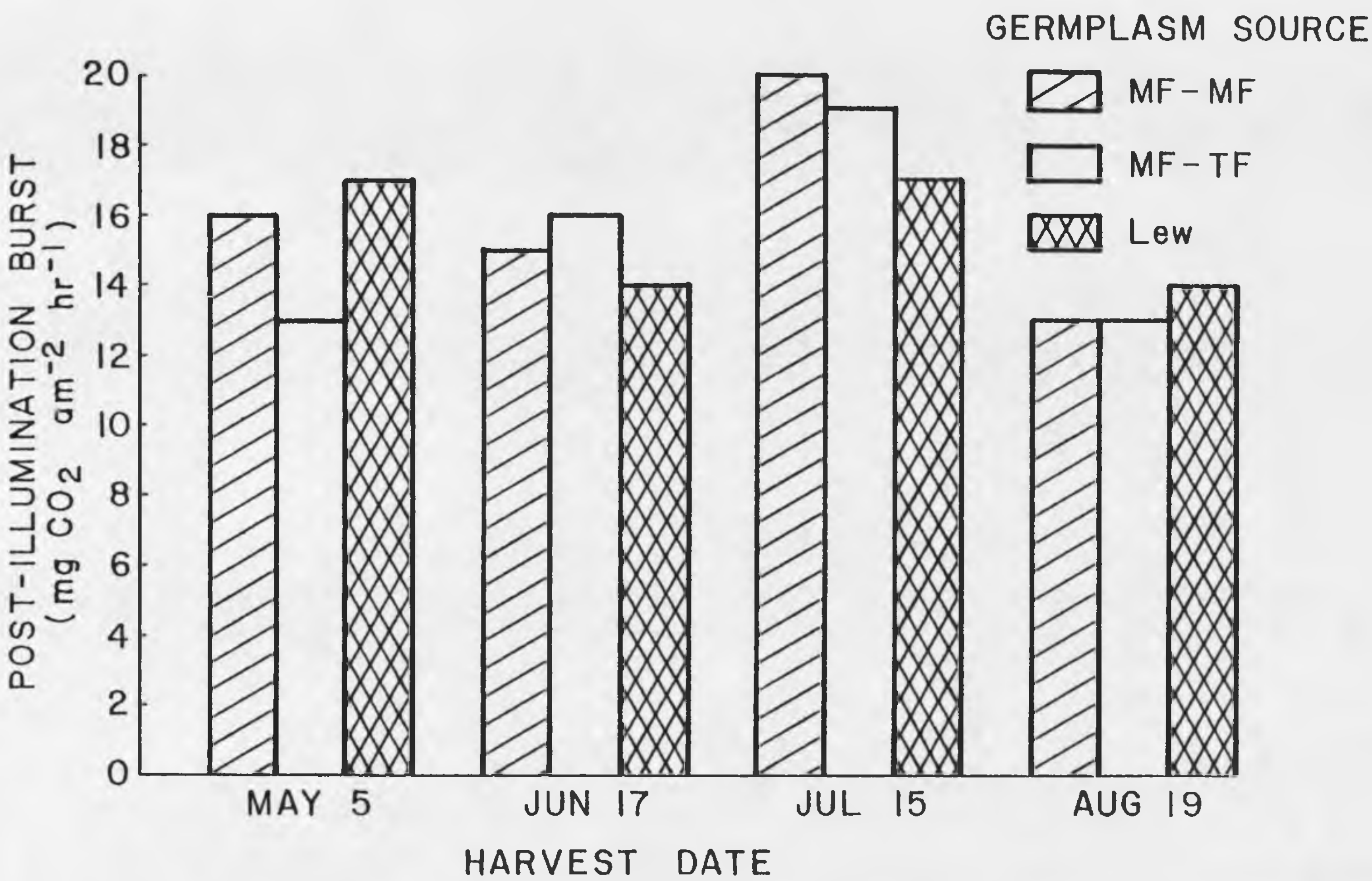

Figure 4. Mean post-illumination burst rates of the MF-MF, MF-TF, and Lew germplasm sources as measured at four harvest dates in 1977.

Dark respiration (DR) rates were 4, 3, and 3 mg CO_2 dm^{-2} hr^{-1} for the MF-MF, MF-TF, and Lew germplasm sources, respectively, when averaged over the four harvests in 1977 (Table 1). The value observed for the MF-MF germplasm source was similar to date obtained by Retzinger (37) for the MF cycle-1 syn-1 alfalfa. Variability for DR was one order of magnitude less than for either PS or PIB. A significant difference (5% level) in DR was observed among the three germplasm sources over the four harvests (Table 2); however, the differences in DR were only significant at the May 5 harvest when the analysis of variance (ANOVA) was conducted by harvest date. The significance over the four harvests may be partially explained by the fact that a larger number of cases was examined in the ANOVA for the four harvests than in the ANOVA at each individual harvest (144 observations vs. 36 observations, respectively). In general, the MF populations showed higher DR values. A trend of declining DR rates was observed at each harvest (Figure 5) similar to the trend for PS (Figure 3). This trend was also noted by Delaney, Dobrenz, and Poole (14). The differences in DR by harvest date were significant at the 1% level (Table 3).

Specific Leaf Weight

Germplasm sources showed significant differences with respect to specific leaf weight (SLW) at the May 5 and July 15 harvests. Differences among germplasm sources over all harvests were also significant (5% level) (Table 2). Mean SLW's for the growing season were

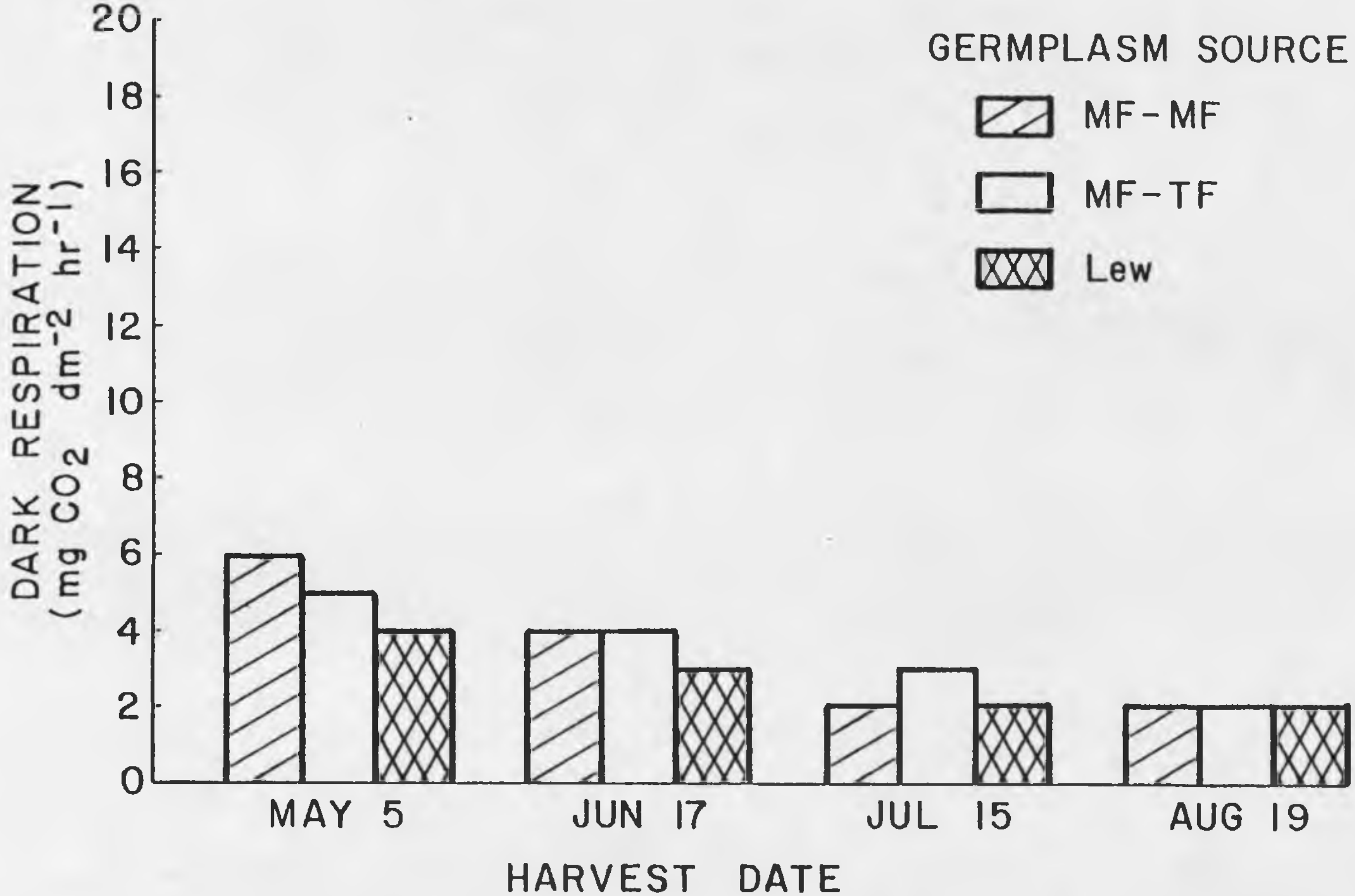

Figure 5. Mean dark respiration rates of the MF-MF, MF-TF, and Lew germplasm sources as measured at four harvest dates in 1977.

4.41, 4.22, and 3.74 mg dm^{-2} for MF-MF, MF-TF, and Lew, respectively (Table 1). These values are similar to those observed by Brick, Dobrenz, and Schonhorst (5); Foutz (21); Pearce et al. (32), and Retzinger (37). A significant difference was observed in the mean SLW at each harvest date (Table 3); no significant interaction between harvest date and germplasm source was noted. SLW showed a declining trend over the harvest period, but the SLW of the MF germplasm remained higher than Lew at each harvest date (Figure 6). Retzinger (37) also found that the MF cycle-1 syn-1 germplasm had higher SLW than Mesa-Sirsa germplasm.

Dry Matter Production

There was no significant difference in dry matter production between the MF and LEW germplasm sources over the harvest period (Table 4). There was, however, a significant difference (5% level) noted at the May 5 harvest, where MF outyielded Lew. A non-significant interaction occurred between the May 5 and June 17 harvest, and at all subsequent harvests Lew outyielded MF. It is possible that because the MF characteristic was obtained from Ladak-65 (6), a winter-hardy alfalfa, the MF is not as adapted to hot weather as Lew and suffered a greater "summer slump". Average yields were 0.34 and 0.36 kg m^{-2} for MF and Lew, respectively over the 1977 growth period (Table 5). These figures fall slightly under the averages (0.38 and 0.37 kg m^{-2} for MF syn-2 and Mesa Sirsa, respectively) observed by Retzinger (37). The lower yield can be partially explained by the infestation of an

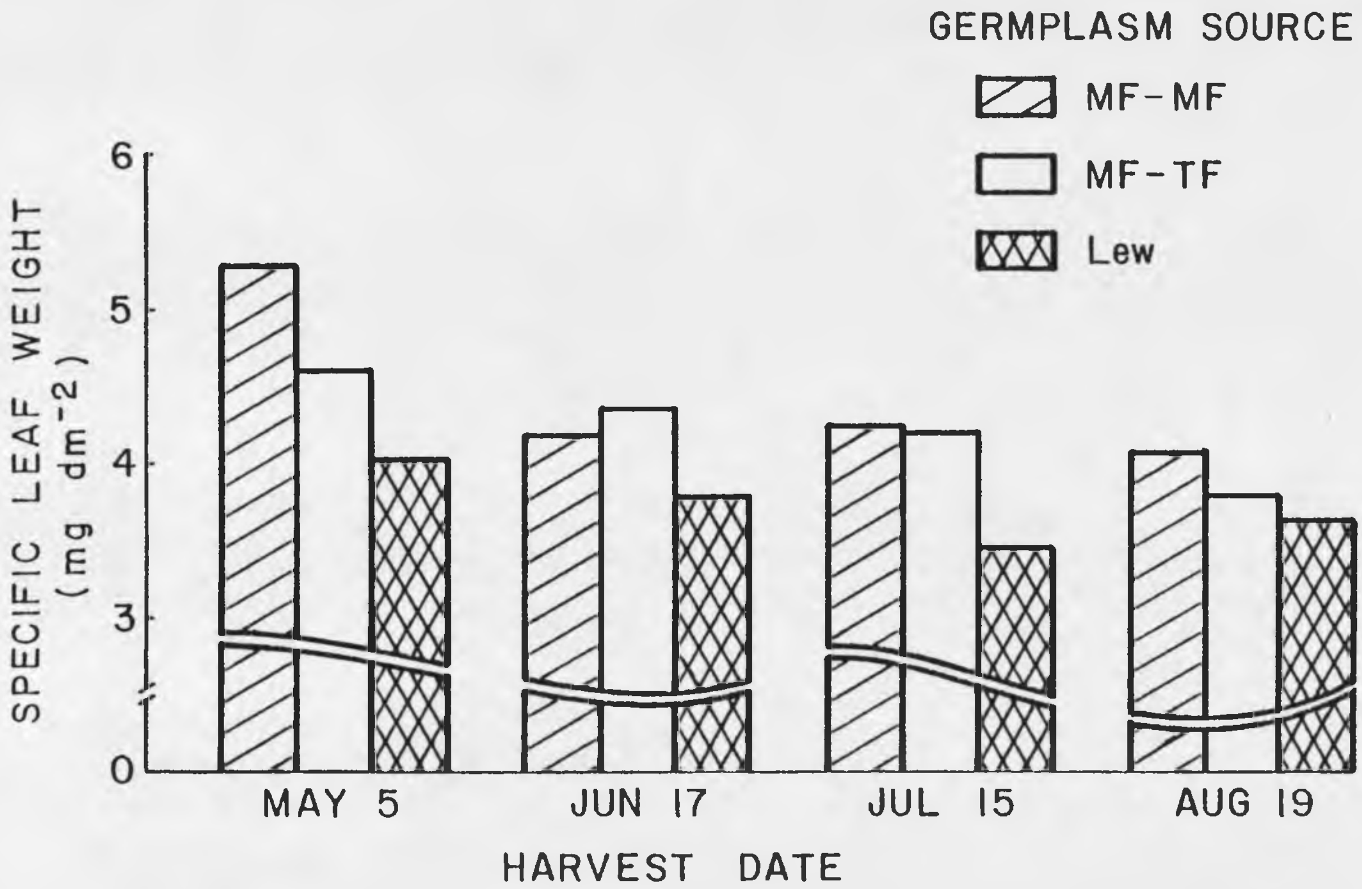

Figure 6. Mean specific leaf weights of the MF-MF, MF-TF, and Lew germplasm sources as measured at four harvest dates in 1977.

Table 4. Summary of analysis of variance between the MF and Lew germplasm sources for dry matter production, leaflet to stem-petiole ratio, dry matter percent, and leaf weight by individual harvest date and over the four harvests.

	Harvest Date				Four
	May 5	Jun. 17	Jul. 15	Aug. 19	Harvests
Dry Matter Production	*	NS	NS	NS	NS
Leaflet to Stem-Petiole Ratio	NS	NS	NS	NS	NS
Dry Matter Percent	NS	NS	NS	NS	NS
Leaf Weight	NS	NS	NS	NS	NS

* Significant to 0.05 level

NS Non-significant

Table 5. Mean values of dry matter production, leaflet to stem-petiole ratio, dry matter percent, and leaf weight for the MF and Lew germplasm sources averaged over the four harvests.

	Germplasm Source	
	MF	Lew
Dry Matter Production ($kg\ m^{-2}$)	0.34	0.36
Leaflet to Stem-Petiole Ratio	0.70	0.69
Dry Matter Percent	21.6	21.8
Leaf Weight ($kg\ m^{-2}$)	0.14	0.15

unidentified problem which undoubtedly affected the yield of the four replications towards the final harvests. Yields by harvest were significantly different (1% level) (Table 6), and the trend (Figure 7) was similar to that noted by Retzinger (37).

Leaflet to Stem-Petiole Ratio

Mean leaflet to stem-petiole ratios (LSP) over the harvest period were 0.70 and 0.69 for the MF and Lew germplasm sources, respectively (Table 4). Brick, Dobrenz, and Schonhorst (5) observed LSP values of 0.88 and 0.72 for MF and Mesa-Sirsa germplasm sources, respectively. These figures also fall within the range of those observed by Foutz (21) and lie close to those observed by Retzinger (37). No significant differences in LSP were found between MF and Lew germplasm sources at any specific harvest or over the entire growth period (Table 5). Differences between harvest dates were also non-significant (Table 6). The trend of LSP by harvest (Figure 8) follows that observed by Delaney, Dobrenz, and Poole (14).

Dry Matter Percent

Dry matter percent (DMP) (100% minus percent moisture) averaged 21.6 and 21.8 for the MF and Lew germplasm sources, respectively, for the 1977 growing period (Table 4). Retzinger (37) obtained values of 80 to 85 percent moisture (15 to 20 DMP) for his harvest material throughout the growing season. No significant difference was noted between the two germplasm sources either over the harvest period or at

Table 6. Summary of analysis of variance among harvest dates for dry matter production, leaflet to stem-petiole ratio, dry matter percent, and leaf weight, including harvest date x germplasm source interaction.

	Harvest Date	Harvest Date x Germplasm Source
Dry Matter Production	**	NS
Leaflet to Stem-Petiole Ratio	NS	NS
Dry Matter Percent	**	NS
Leaf Weight	**	*

** Significant to 0.01 level

* Significant to 0.05 level

NS Non-significant

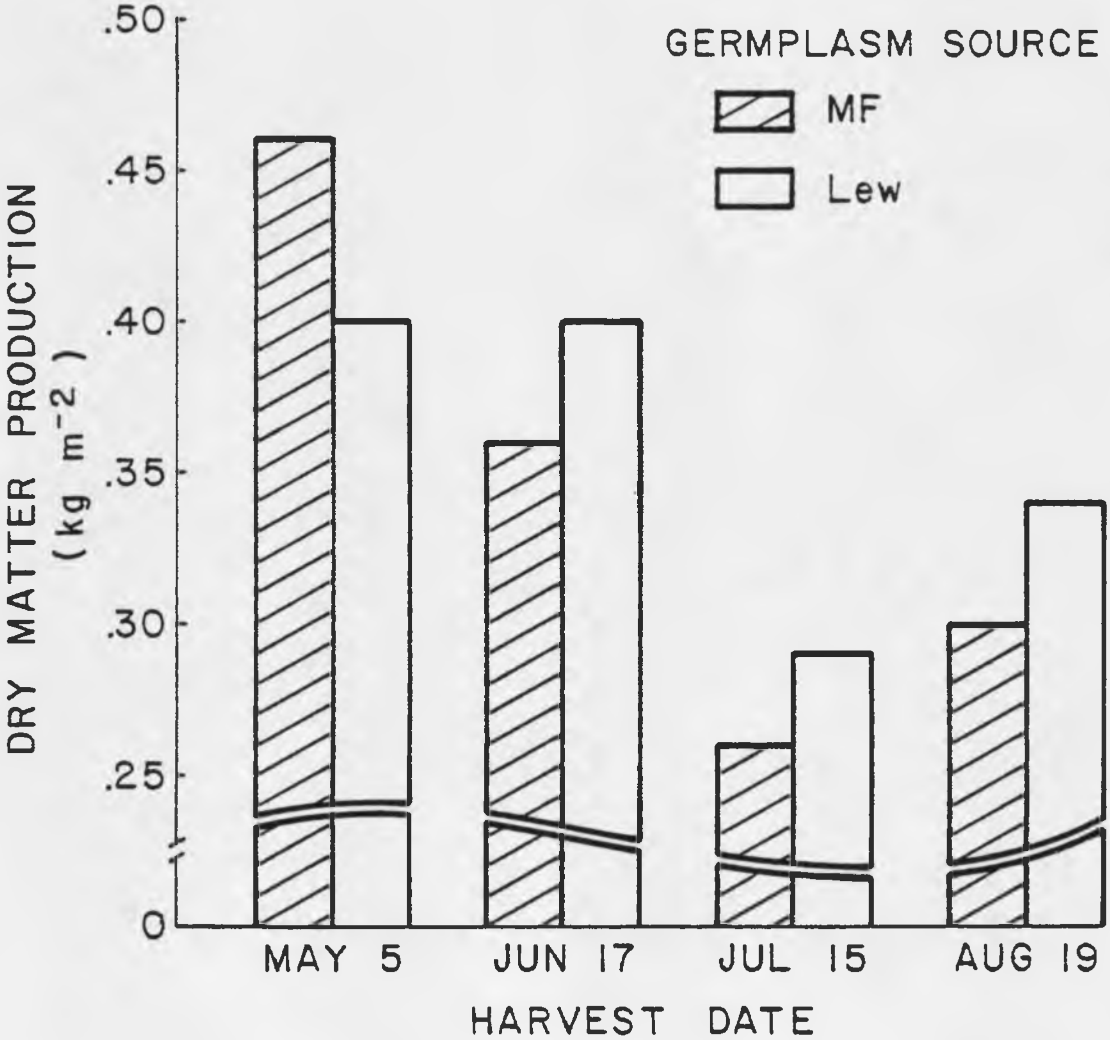

Figure 7. Dry matter production of the MF and Lew germplasm sources as measured at four harvest dates in 1977.

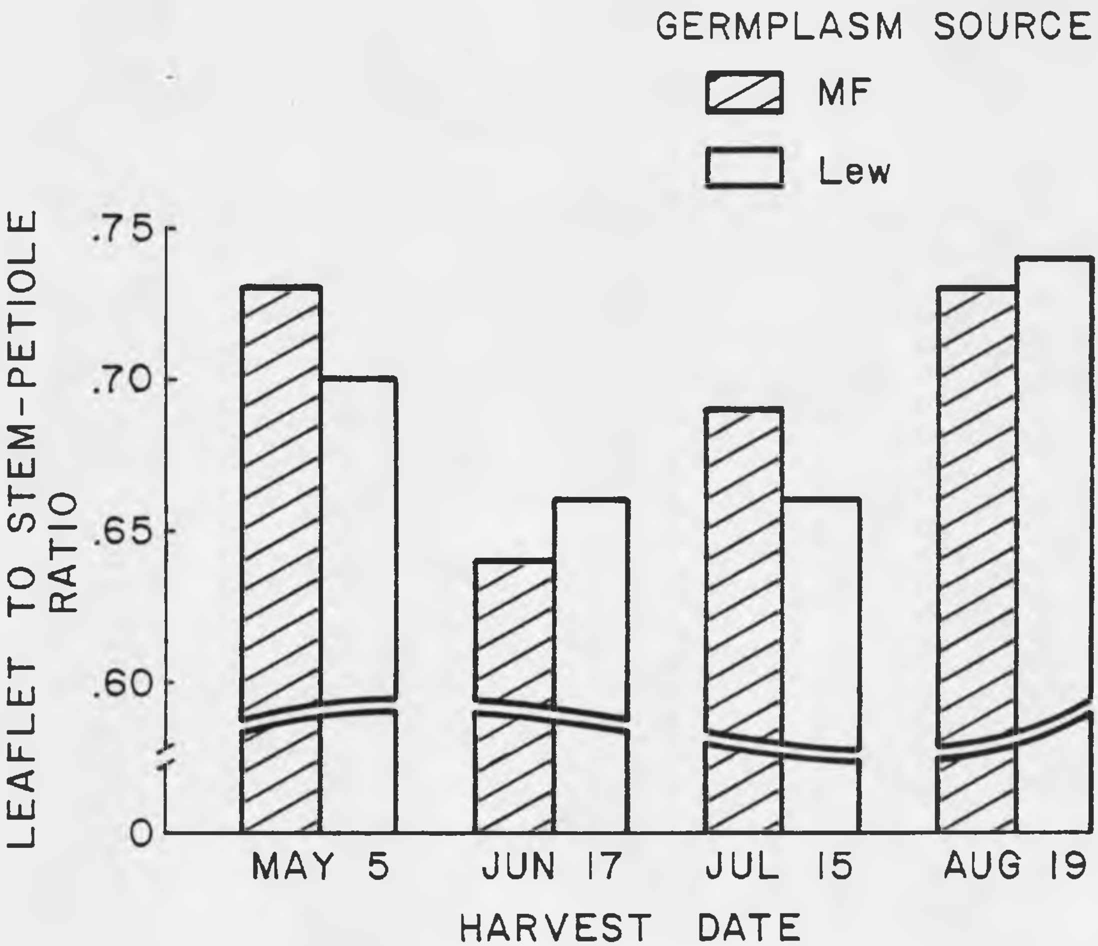

Figure 8. Leaflet to stem-petiole ratios of the MF and Lew germplasm sources at four harvest dates in 1977.

individual harvest dates (Table 5). DMP increased with subsequent harvest dates (Figure 9), and the differences between harvest date were shown to be significant at the 1% level (Table 6).

Leaf Weight

Leaf weight (LW) averaged 0.14 and 0.15 kg m^{-2} over the harvest period for MF and Lew germplasm sources, respectively (Table 4). Differences in mean LW between germplasm sources at individual harvest dates and over the harvest period were non-significant (Table 5). However, differences in LW between harvest dates were highly significant (1% level), and a significant interaction (5% level) with respect to LW occurred between harvest date and germplasm source (Table 6). An examination of the mean values for the two germplasm sources at each harvest date (Figure 10) shows that at the first harvest the LW of MF was higher than that of Lew, but at subsequent harvests the situation was reversed. A similar trend and interaction was shown to occur with dry matter yield (Figure 7).

Correlations

Pearson correlations for combined observations on MF-MF, MF-TF, and Lew germplasm sources over the four harvests (Table 7) showed that among the physiological factors, apparent photosynthesis (PS) was highly correlated with both dark respiration (DR) ($r = 0.65^{**}$) and post-illumination burst (PIB) ($r = 0.46^{**}$). Retzinger (37) and Delaney (11) also found significant correlations between these factors.

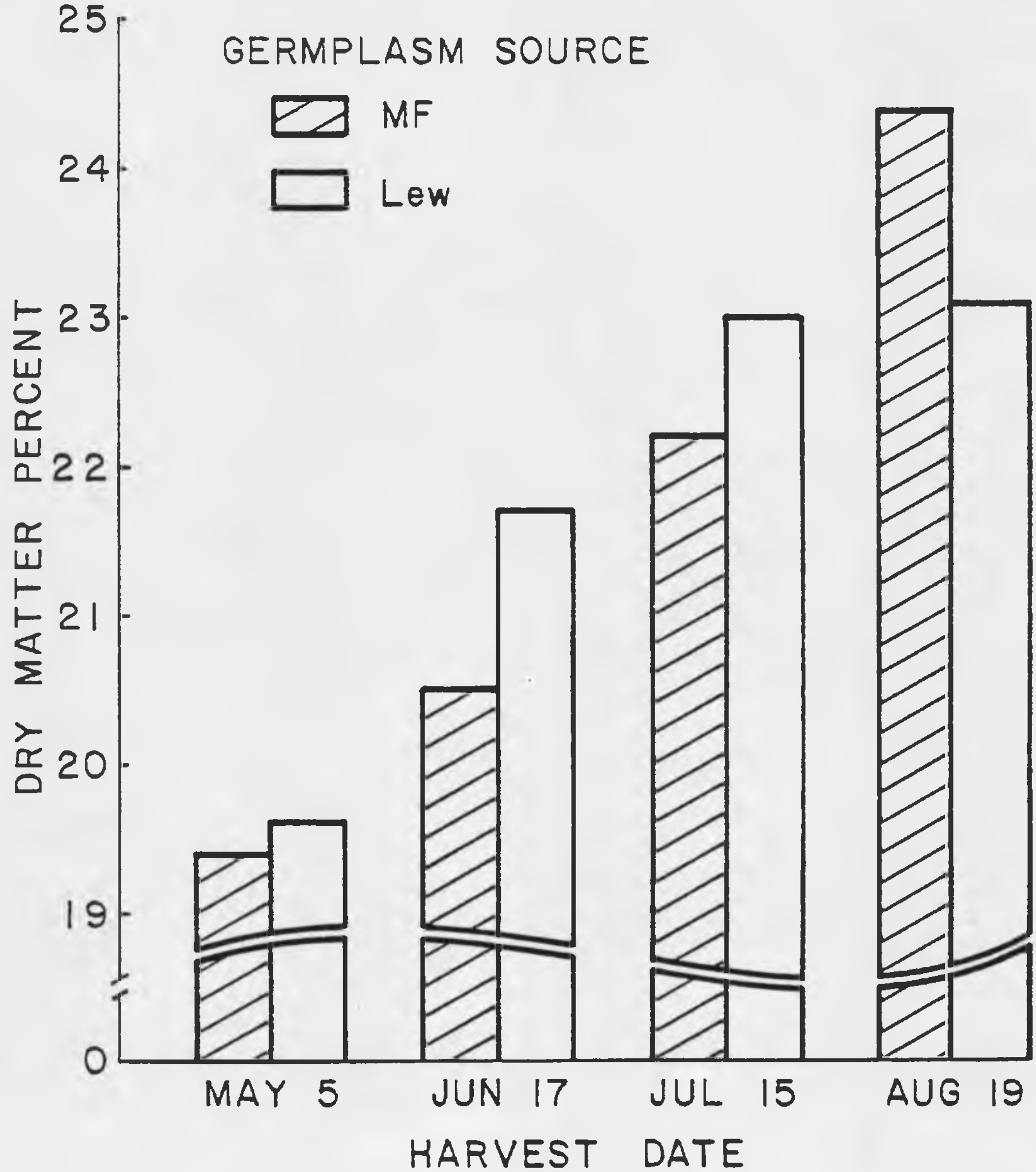

Figure 9. Dry matter percent of the MF and Lew germplasm sources at four harvest dates in 1977.

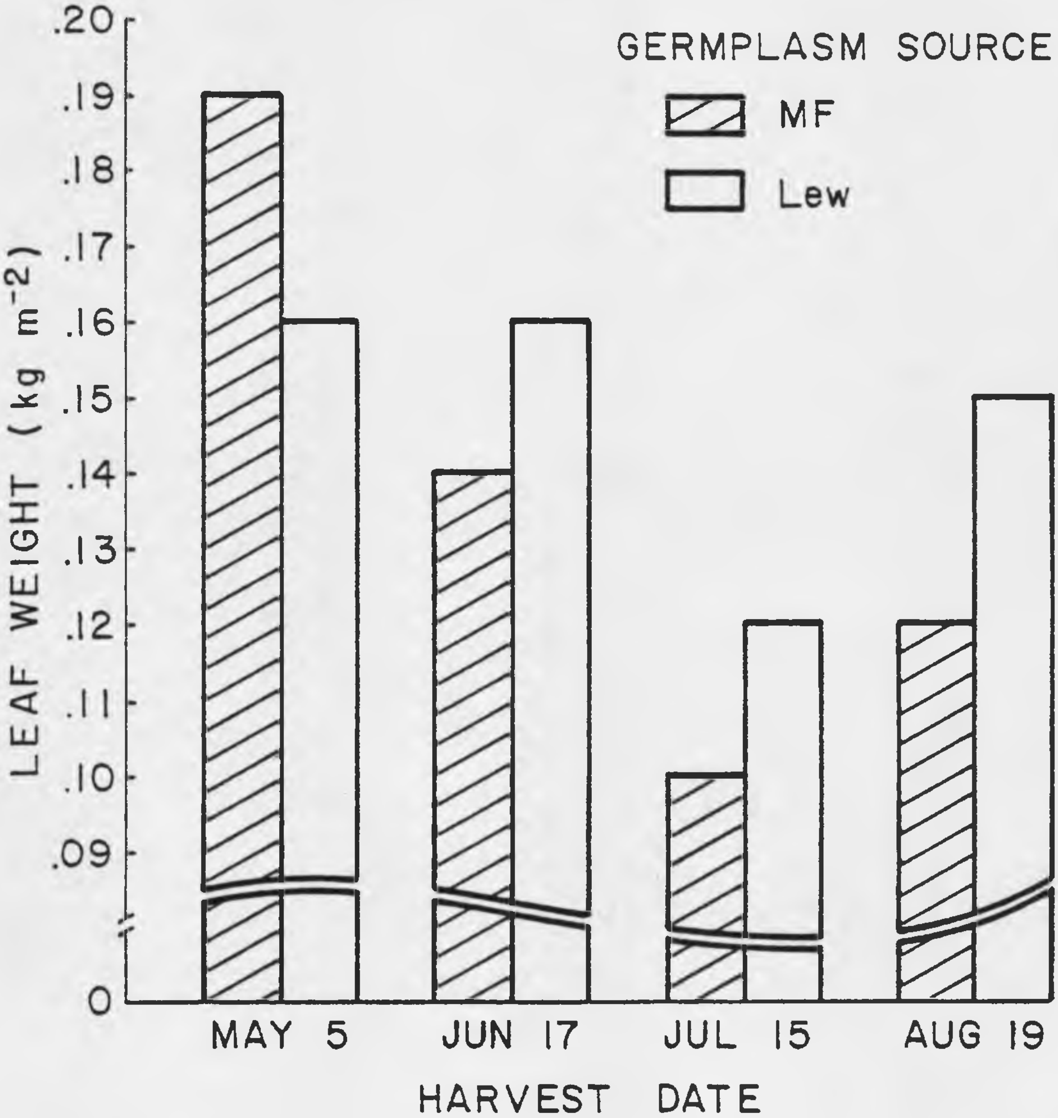

Figure 10. Leaf weight of the MF and Lew germplasm sources at four harvest dates in 1977.

Table 7. Pearson correlations for combined observations on the MF-MF, MF-TF, and Lew germplasm sources among the factors apparent photosynthesis (PS), post-illumination burst (PIB), dark respiration (DR), and specific leaf weight (SLW) over the four harvests.

	PS	PIB	DR	SLW
PS+	-	.46**	.65**	.24**
PIB+		-	.11	.15
DR+			-	.61**
SLW++				-

+ mg CO_2 dm^{-2} hr^{-1}

++ mg dm^{-2}

* Significant to 0.05 level

**Significant to 0.01 level

DR and PIB were not significantly correlated when data from germplasm sources were combined over all harvests. However, highly significant positive correlations were found between PIB and DR for the MF-MF (r = .41**) and Lew (r = .41**) germplasm sources. The germplasm sources were analyzed separately (Table 8). Retzinger (37) also observed a positive correlation between PIB and DR. PS vs PIB, and PS vs. DR were also significantly correlated within each variety over the harvest period (Table 8).

Specific leaf weight (SLW) was highly correlated to both PS and DR (r = 0.24** and r = 0.61**, respectively) for the combined observations of the three germplasm sources over the four harvests (Table 7). Delaney (11) found highly significant positive correlation between SLW vs PS at high light intensity and SLW vs DR; however, Retzinger (37) found a less significant (r = 0.28*) correlation between SLW vs DR and no significant correlation between SLW vs PS. Other researchers have found significant positive correlations between PS and SLW (8, 12, 13, 27, 32, 33, 49, 50). Correlations by germplasm source over the harvest period (Table 8) showed highly significant (1% level) positive correlations between SLW x DR for the MF-MF and MF-TF germplasm sources. The correlation between SLW and DR for Lew was non-significant. The correlation between SLW and PS for Lew showed a significant negative correlation (r = -0.32*), while correlations between SLW and PS for the MF-MF and MF-TF germplasm sources were non-significant. The high significance observed for the correlation of

Table 8. Pearson correlations for the MF-MF, MF-TF, and Lew germplasm sources among the factors apparent photosynthesis (PS), post-illumination burst (PIB), dark respiration (DR) and specific leaf weight (SLW) over the four harvests.

		PS	PIB	DR	SLW
MF-MF	PS+	-	0.63**	0.47**	0.19
	PIB+		-	0.41**	0.06
	DR+			-	0.67**
	SLW++				-
MF-TF	PS	-	0.36**	0.46**	-0.10
	PIB		-	-0.17	-0.03
	DR			-	0.37**
	SLW				-
LEW	PS	-	0.77**	0.30*	-0.32*
	PIB		-	0.41**	-0.33*
	DR			-	0.14
	SLW				-

+ mg CO_2 dm^{-2} hr^{-1}

++ mg dm^{-2}

* significant to 0.05 level

** significant to 0.01 level

SLW with PS when data from all germplasm sources were combined was probably due to the larger number of observations used in calculating the correlation coefficient.

Dry matter production (YLD) was significantly and positively correlated with leaf weight (LW) both for the observations from germplasm sources combined ($r = 0.94^{**}$) (Table 9) and for the MF and Lew germplasm sources separately (Table 10).

LW was negatively correlated with dry matter percent (DMP) for both germplasm sources (Table 10), and this correlation became significant ($r = -0.62^{**}$) when the data from the two sources were combined (Table 9). As leaves decrease in their moisture percentage, percent dry matter increases, and leaf weight decreases due to water loss.

YLD was also significantly and negatively correlated with DMP ($r = -0.63$) for the combined germplasm sources over the harvest period (Table 9). As the moisture percentage decreased, so did yield. This could have been due to the increased temperatures as the harvest season progressed.

A highly significant positive correlation ($r = 0.68^{**}$) was noted between the leaflet to stem-petiole ratio (LSP) and LW for the MF germplasm (Table 10). This correlation was not significant either for the Lew germplasm or for the combined data (Table 9). DMP and LSP were also significantly negatively correlated ($r = -0.46^{*}$) within the MF germplasm, but were not significantly correlated within the Lew germplasm or for the combined data.

Table 9. Pearson correlations for combined observations on the MF and Lew germplasm sources among the factors dry matter production (YLD), leaflet to stem-petiole ratio (LSP), dry matter percent (PDM), and leaf weight (LW) over the four harvests.

	YLD	LSP	DMP	LW
YLD+	-	-0.20	-0.63**	0.94**
LSP		-	0.11	0.15
DMP			-	-0.62**
LW+				-

+ kg m^{-2}

* significant to 0.05 level

** significant to 0.01 level

Table 10. Pearson correlations for the MF and Lew germplasm sources among the factors dry matter production (YLD), leaflet to stem-petiole ratio (LSP), dry matter percent (PDM), and leaf weight (LW) over the four harvests.

		YLD	LSP	DMP	LW
MF	YLD+	-	0.27	-0.20	0.89**
	LSP		-	-0.46*	0.68**
	DMP			-	-0.38
	LW+				-
LEW	YLD	-	-0.40	-0.23	0.85**
	LSP		-	0.22	0.14
	DMP			-	-0.16
	LW				-

+ kg m^{-2}

* significant to 0.05 level

** significant to 0.01 level

Correlations between dry matter production and LSP were non-significant.

Multifoliolate Expression

During the 1977 growth period, 65% of the stems of the MF cycle-2 syn-1 germplasm displayed the multifoliolate characteristic. Retzinger (37) found only 33% MF expression with his MF cycle-1 syn-1 material. Brick, Dobrenz, and Schonhorst (6) also obtained an average 33% MF expression in the F_1 generation from the initial crosses of multifoliolated Ladak-65 with Mesa-Sirsa alfalfa. Bingham and Murphy (4) obtained 19% and 68% MF expression for the second and third cycles of recurrent selection, respectively, in their study.

Anatomical Characteristics

Leaf thickness (LF) averaged 0.19, 0.18, and 0.17 mm for the MF-MF, MF-TF, and Lew germplasm sources, respectively (Table 11). These means fall within the range of those observed by Delaney (11). Differences in LF among germplasm sources were not significant (Table 11), although the LF of the MF sources was slightly higher than that of Lew. Hesketh and Baker (27), Lewis (31), and Delaney and Dobrenz (13) suggested that apparent photosynthesis on a unit leaf area basis (PS) and specific leaf weight (SLW) were positively associated with LF. Results of this study show that SLW was higher in the MF plants, and that PS averaged for the MF-MF and MF-TF sources was slightly higher at all harvests except the June 17 harvest, as opposed to the SLW and PS of the Lew germplasm source.

Table 11. Mean values and ratios for several anatomical features of the MF-MF, MF-TF, and Lew germplasm sources, and a summary of the analysis of variance among germplasm sources for these variables.

	Germplasm Source			Significance of F
	MF-MF	MF-TF	Lew	
Leaf Thickness (mm)	0.19	0.18	0.17	.298
Palisade Mesophyll Thickness (mm)	0.08	0.07	0.07	.197
Spongy Mesophyll Thickness (mm)	0.08	0.07	0.07	.808
Total Epidermal Thickness+ (mm)	0.03	0.03	0.02	.511
Palisade: Leaf Thickness	0.44	0.41	0.43	.365
Spongy: Leaf Thickness	0.40	0.42	0.43	.526
Palisade: Spongy Thickness	1.10	0.99	1.02	.403
Petiole X-sec Area (mm^2)	0.79	0.63	0.61	.059
Vascular Tissue X-sec Area (mm^2)	0.19	0.14	0.16	.134
Xylem X-sec Area (mm^2)	0.10	0.07	0.08	.064
Phloem + Phloem Fibers X-sec Area (mm^2)	0.08	0.06	0.07	.300
Vascular Tissue: Petiole X-sec Area	0.23	0.22	0.26	.033*
Xylem: Vascular Tissue X-sec Area	0.55	0.52	0.54	.276
Phloem: Vascular Tissue X-sec Area	0.45	0.48	0.46	.276
Xylem: Phloem X-sec Area	1.23	1.11	1.18	.253

+ Sum of upper and lower epidermal thickness
* Significant to 0.05 level

Both palisade mesophyll thickness (PMT) and spongy mesophyll thickness (SMT) figures were 0.08, 0.07, and 0.07 mm for the MF-MF, MF-TF, and Lew germplasm sources, respectively. Differences among germplasm sources were not significant for either characteristic. Figures for PMT were within the range observed by Delaney (11). PMT for the MF-MF germplasm source was slightly higher than that of the MF-TF or Lew sources. Lewis (31) suggested that variation in LF was related to the thickness of the palisade layer. Delaney and Dobrenz (13) showed that PS was significantly dependent on PMT. Gausmann and Allen (24) found that thicker leaves with more complex mesophylls absorb more light per unit leaf area than thinner, less complex leaves.

Both the MF-MF and the MF-TF germplasm sources displayed two layers of palisade cells within the palisade mesophyll. The number of palisade cell layers in Lew varied from one to two.

Total epidermal thickness (TET) was measured as the sum of the thickness of the upper and lower epidermal layers. Mean values were 0.03, 0.03, and 0.02 mm for the MF-MF, MF-TF, and Lew germplasm sources, respectively, and the differences among germplasm sources was non-significant. The indication of a slightly greater epidermal thickness in the MF plants, however, suggests that these plants may have a greater resistance to cuticular transpiration.

Palisade mesophyll thickness to leaf thickness ratios (PLF) were 0.44, 0.41, and 0.43 for the MF-MF, MF-TF, and Lew germplasm sources, respectively. Spongy mesophyll thickness to leaf thickness ratios (SLF) were 0.40, 0.42, and 0.43 for the same three germplasm sources, respectively. Differences among germplasm sources were not significant in either case. The ratio of palisade mesophyll thickness to spongy mesophyll thickness (PSR) was 1.10, 0.99, and 1.02 for the MF-MF, MF-TF, and Lew germplasm sources, respectively. Although differences were again non-significant, the MF-MF germplasm seems to display a greater proportion of its total mesophyll as palisade mesophyll than the Lew germplasm.

Mean petiole cross-sectional areas (PET) were 0.79, 0.63, and 0.61 mm^2 for the MF-MF, MF-TF, and Lew germplasm sources, respectively. Differences among germplasm sources were almost significant to the 5% level (significance of $F = 0.059$) (Table 11).

Vascular tissue cross-sectional areas within the petiole averaged 0.19, 0.14, and 0.16 mm^2 for the MF-MF, MF-TF, and Lew germplasm sources, respectively. Differences in these means among germplasm sources were not statistically significant. Bingham (3) obtained a comparable figure of 0.17 mm^2 for the vascular bundle area of trifolioliate leaves; however, his figures of 0.31 and 0.34 mm^2 for multifoliolate leaves were considerably larger than those found for the MF germplasm in the current study.

Mean xylem cross-sectional areas within the petiole (XYL) averaged 0.10, 0.07, and 0.08 mm^2 for the MF-MF, MF-TF, and Lew germplasm sources, respectively. Phloem and Phloem fiber cross-sectional areas (PHL) averaged 0.08, 0.06, and 0.07 mm^2 for the three germplasm sources, respectively. Although differences in PHL means among germplasm sources were not significant, differences in XYL means verged on significance to the 5% level (significance of F = 0.064) (Table 11). The MF-MF germplasm source displayed a higher XYL than the MF-TF or Lew.

Several researchers (9, 10, 23, 44) suggested that PS and translocation of carbohydrates from the leaf were related. Although PHL was not significantly different among germplasm sources, the PHL of MF-MF was slightly higher than that of the MF-TF or Lew germplasm. Assuming this to be due to a larger phloem as opposed to fiber area, this suggests a greater capacity for translocation of carbohydrates from the MF leaves which would compensate a projected high PS capability due to a greater leaflet area. The fact that the PHL of the MF-MF germplasm was not significantly higher may partially explain the lack of significant difference in PS among germplasm sources (Table 2).

Ratios of vascular tissue cross-sectional area to petiole cross-sectional area (VP) were 0.23, 0.22, and 0.26 for the MF-MF, MF-TF, and Lew germplasm sources, respectively. Differences in these ratios among germplasm sources were significant at the 5% level.

Ratios of xylem cross-sectional area to vascular tissue cross-sectional area (XV) were 0.55, 0.52, and 0.54 for the MF-MF, MF-TF, and Lew germplasm sources, respectively. Phloem cross-sectional area to vascular tissue cross-sectional area ratios (PV) were 0.45, 0.48, and 0.46, and xylem cross-sectional area to phloem cross-sectional area ratios (XP) were 1.23, 1.11, and 1.18 for the three germplasm sources, respectively. Differences among germplasm sources with respect to XV, PV, and XP were non-significant.

Variation in number of vascular bundles in the petiole was noted within the MF germplasm. From three to five vascular bundles were observed (Figure 11). Bingham (3) showed that near the point of attachment of the leaflets to the petiole in MF alfalfa the vascular bundles within the petiole tend to subdivide. As the cross-sections observed in this study were taken approximately midway between the rachis node and the base of the petiole, the vascularization described by Bingham (3) may account for the variability in vascular bundle number.

Correlations

Numerous anatomical features and ratios were correlated, using combined data from the MF-MF, MF-TF, and Lew germplasm sources. A summary of the correlations is presented in Table 12.

Of note, palisade mesophyll thickness and spongy mesophyll thickness were correlated with leaf thickness (r = 0.86** and r = 0.79**, respectively). Lewis (31) had previously reported that variation in leaf thickness was related to palisade layer thickness. Palisade mesophyll thickness was also correlated with spongy mesophyll thickness (r = 0.53**).

Vascular tissue cross-sectional area showed a high positive correlation with petiole cross-sectional area (r = 0.90**). Clearly, larger petioles tend to have more vascular tissue.

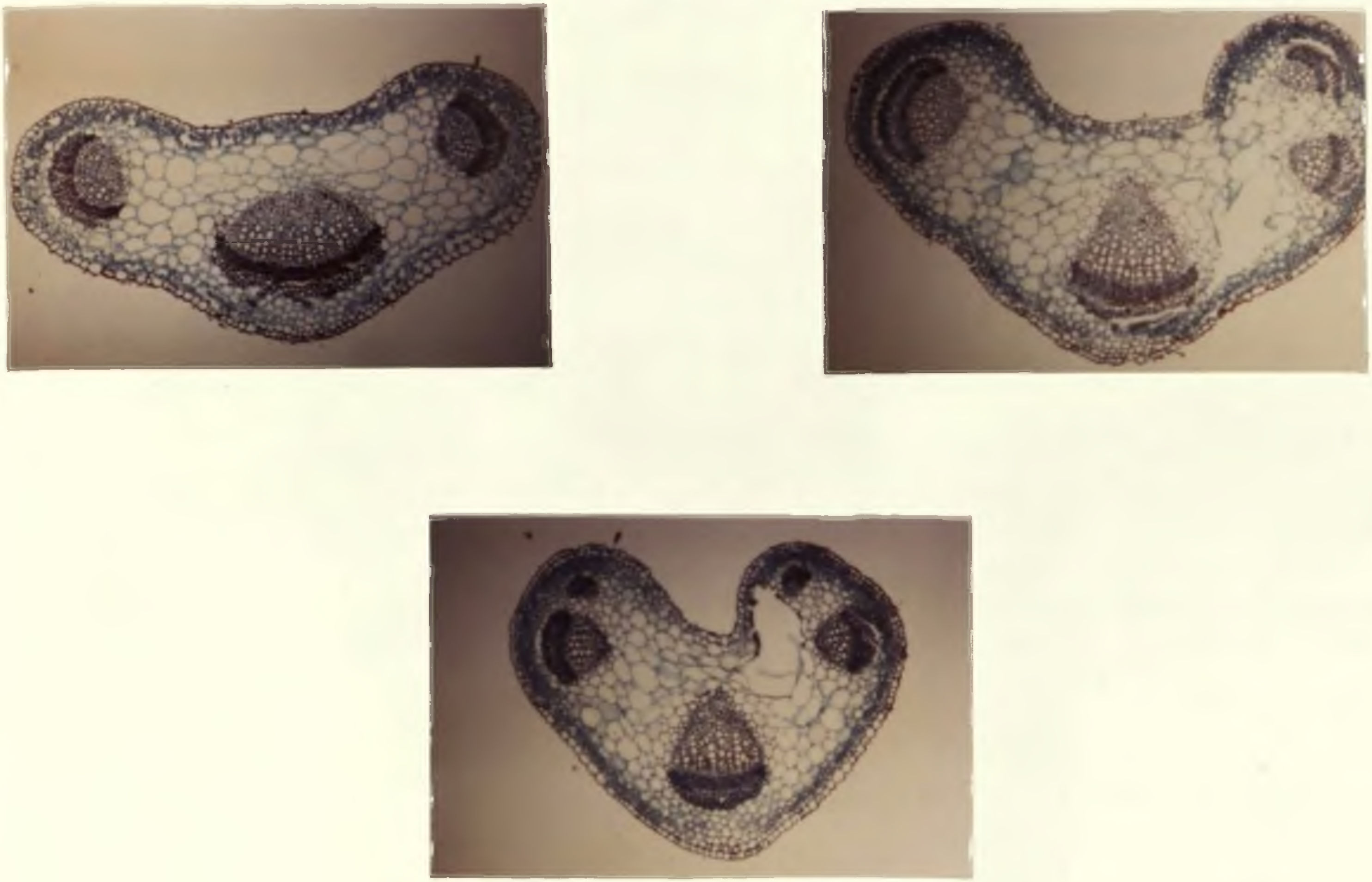

Figure 11. Variation in petiole vasculature within the multifoliolate germplasm source.

Table 12. Pearson correlations among various anatomical characteristics and ratios from the combined data of the MF-MF, MF-TF, and Lew germplasm sources.

	LF	PMT	SMT	TET	PLF	SLF	PSR	PET	VAS	XYL	PHL	VP	XV	PV	XP
LF	–	0.86**	0.79**	0.27	0.24	-0.05	0.17	0.32*	0.27	0.32*	0.21	0.03	0.22	-0.22	0.21
PMT		–	0.53**	0.02	0.69**	-0.29	0.55**	0.37*	0.30	0.36*	0.22	0.00	0.27	-0.27	0.28
SMT			–	-0.09	-0.10	0.56**	-0.40*	0.29	0.28	0.26	0.28	0.09	-0.09	0.09	-0.12
TET				–	-0.35*	-0.54**	0.17	-0.14	-0.14	-0.07	-0.22	-0.04	0.37*	-0.37*	0.37*
PLF					–	-0.48**	0.82**	0.22	0.17	0.21	0.12	-0.04	0.19	-0.19	0.22
SLF						–	-0.88**	0.02	0.07	-0.02	0.17	0.11	-0.45**	0.45**	-0.48**
PSR							–	0.08	0.04	0.12	-0.05	-0.08	-0.40**	-0.40**	-0.45**
PET								–	0.90**	0.91**	0.86**	0.01	0.02	-0.02	0.01
VAS									–	0.98**	0.98**	0.41*	-0.08	0.08	-0.08
XYL										–	0.92**	0.36*	0.11	-0.11	0.10
PHL											–	0.45**	-0.27	0.27	-0.28
VP												–	-0.28	0.28	-0.26
XV													–	-1.00**	1.00**
PV														–	-1.00**
XP															–

Note: Variable name abbreviations are explained under 'Anatomical Characteristics'.

** Significant to 0.01 level

* Significant to 0.05 level

SUMMARY AND CONCLUSIONS

Multifoliolate cycle-2 syn-1 (MF) and Lew alfalfa germplasm sources were evaluated for physiological and morphological characteristics, dry matter production, and anatomical features. For the physiological and anatomical measurements, the MF germplasm was further divided into those MF plants displaying the multifoliolated condition (MF-TF).

Apparent photosynthetic rates (PS) and post illumination burst rates (PIB) were not significantly different among germplasm sources. Dark respiration rates (DR) were significantly higher (5% level) for the MF germplasm over the four harvests. Differences in means at each harvest were significant for all three physiological factors. PS and DR showed a declining trend as the harvest season progressed and as mean daily temperatures increased. PIB rates were relatively stable compared with DR and AP.

Specific leaf weight (SLW) was significantly higher for the MF germplasm compared with Lew. SLW showed a decline over the four harvests. Significant correlations were noted between SLW and PS ($r = 0.24^{**}$) and between SLW and DR ($r = 0.61^{**}$).

Dry matter production was not significantly different between germplasm sources over the four harvests; however, MF significantly outyielded Lew at the May 5 harvest when seasonal temperatures were still moderate. At subsequent harvests Lew outyielded MF. Yields declined from the May 5 to the July 15 harvest, but increased slightly at the August 19 harvest. A carryover from winterhard Ladak-65 parentage in the MF germplasm might partially explain the greater 'summer slump' observed in the MF. Incorporation of the multifoliolate characteristic from the present germplasm into Lew might improve the yield during the summer months.

Leaflet to stem-petiole ratios (LSP) were not significantly different between germplasm sources, and LSP did not significantly change over the four harvests. LSP may be used as an indicator of leafiness in alfalfa and thereby as an indicator of one of the quality components of alfalfa hay (45). The results here tend to indicate that there would be no major differences in hay quality between the MF and Lew germplasm.

Germplasm sources showed no significant difference in dry matter percent over the four harvests. A significant increase in dry matter percent was noted at each subsequent harvest. Dry matter percent was negatively associated with yield ($r = -0.63^{**}$).

Percent multifoliolate expression among plants of the MF cycle-2 syn-1 germplasm was 65% over the 1977 harvest period.

Although numerous anatomical features were examined, a significant difference among germplasm sources was noted only for the vascular tissue cross-sectional area to petiole cross-sectional area ratio. MF-MF germplasm displayed slightly thicker leaves than Lew germplasm.

The MF-MF germplasm also had a slightly larger vascular bundle cross-sectional area than Lew. Correlations of 0.86** and 0.79** were observed between the factors leaf thickness x palisade mesophyll thickness and leaf thickness x spongy mesophyll thickness, respectively.

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