A PHYSIOLOGICAL ROUTE TO INCREASED WATER-USE EFFICIENCY IN ALFALFA

By

Vincent P. Gutschick
Principal Investigator
Department of Biology
and
Plant Genetic Engineering Laboratory
New Mexico State University

and

Gary L. Cunningham
Co-Investigator
Department of Biology
New Mexico State University

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ABSTRACT

Alfalfa is the top-ranked cash crop in New Mexico and the most copious user of irrigation water. The water usage per mass of forage yield might be reduced, that is, the water-use efficiency (WUE) might be increased, because it is under some measure of physiological control.

Working from a knowledge of biophysics and plant physiology, we propose that WUE and yield are strongly controlled by two traits that should be heritable and selectable: (1) the CO_2 concentration within the leaf, C_i, and (2) the specific leaf mass. We made a detailed model of alfalfa photosynthesis, transpiration, and growth. We predicted that selection for lower C_i and higher specific leaf mass could improve WUE up to 25% with modest penalties in yield, less than 10%; this indirect selection for WUE appears to have some notable advantages over direct selection. Selection for C_i and specific leaf mass may also be directly extendable to other crop species.

We have grown 80 individual plants of two cultivars, Wilson 9D11A and Mesilla, in controlled environments. We find that (1) within each cultivar there are large ranges in C_i and in specific leaf mass, suitable for breeding, and (2) these two traits are related to WUE and yield substantially as predicted by our model. We will proceed to field trials and heritability tests, as well as to studies of drought tolerance traits.

Key words: water-use efficiency, irrigation requirements, alfalfa, crop yield, plant physiology, photosynthesis

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Dr. James C. Pushnik, postdoctoral fellow employed on the project 6/86-5/88. Ph. D. 1983, Utah State University. Currently postdoctoral fellow, Plant Genetic Engineering Laboratory, New Mexico State University.

Dr. Bruce A. Swanton, postdoctoral fellow employed on the project 6/86-3/88. Ph. D. 1986, University of California, Davis. Currently staff scientist, Environmental Improvement Division, State of New Mexico.

Dr. Lou Ellen Kay, consultant on plant physiology; assisted in operations and data gathering on numerous occasions. Ph. D. 1984, City University of New York. Currently college assistant professor, Department of Biology, New Mexico State University.

Dr. Cliff Currier, consultant on plant growth, plant protection, and breeding; assistant professor, Department of Agronomy and Horticulture, New Mexico State University.

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SIGNIFICANCE OF THIS RESEARCH FOR WATER-RELATED PROBLEMS

Among consumptive users of water, agriculture dominates in the Southwestern states, using 80 to 95%, according to state (Solley *et al.* 1983). In New Mexico alone (U. S. Dept. of Agriculture 1987), alfalfa is the top-ranked cash crop (approx. $91 million value in 1986) with 80% of the hay acreage and 1/6 of the irrigated area. In North America as a whole, 13 million hectares or 32 million acres are planted to alfalfa, and the worldwide total is 33 million hectares or 82 million acres, making it the leading forage crop (Lesins 1976). Alfalfa is also the most copious water user, up to 6 feet (2 m) per year. The rising monetary costs of irrigation water concern the farmer, as does compromised water availability from high water use area-wide, because suboptimal watering is costly in lost yield (Stanhill 1985; Pimentel *et al.* 1982). High water use in any locality also has a distributed effect on water quantity and quality for downstream users. Complementary approaches to these problems are (1) improved water management (use of minimum leaching fraction, lining of waterways, choices of land and crop), and (2) improved water-efficiency by the crop (and better tolerance of water shortages that may become more frequent).

In this research, we take the second approach, that of improving water-use efficiency (WUE). WUE is under the plants' physiological control to a significant degree, and thus is under genetic control. We identify a potentially very effective way to select existing genetic variability in alfalfa to improve its WUE. Specifically, we propose that selection for lower values of C_i (the CO_2 concentration maintained in the leaf) and higher values of SLM (leaf mass per unit leaf area, or *s*pecific *l*eaf *m*ass) should give higher WUE with little or no effect on yield. We estimate that an 11% relative decrease in C_i and a 25% increase in SLM could raise WUE 25% while decreasing yield less than 12%. Our work extends recent success in selecting for WUE by selecting only C_i in peanut (Hubick *et al.* 1986). Our method of indirect selection for WUE by selecting for C_i and SLM could be faster and could have fewer adverse effects on other desirable traits than would direct selection for WUE. Furthermore, the actions of C_i and SLM on WUE and yield may be very generally shared in plants. Thus, our indirect selection technique might be used to improve WUE in a number of other crop species, including seed (grain) crops.

Our research is advancing to field trials under expanded funding (U. S. Geological Survey grant 14-08-0001-G1641). Provided that the field trials also demonstrate improvement in WUE, crop breeders such as our co-investigator C. G. Currier could use our selection procedure to develop alfalfa cultivars of superior WUE. These cultivars would be released for farmers' use, to grow similar yields with less water.

OBJECTIVES

(1) Proof of Principle

We must verify that the genetic variations in internal CO_2 level (C_i) and in specific leaf mass (SLM) alter WUE and yield in the quantitative amounts that our model predicts. (The model is described below in "Methods.") Research by others has clarified the relation of C_i to water-use efficiency (Jones 1983; Nobel 1983; Hubick *et al.* 1986), but the relation of C_i to yield has been studied only peripherally. The role of SLM in yield has been addressed in plant breeding but not clarified (Wallace *et al.* 1972). Physiological modelling (Gutschick 1984, 1987; Gutschick and Wiegel 1988) has yielded detailed hypotheses about how SLM affects yield, but these hypotheses need to be tested. The complete relationships of C_i and SLM to WUE and yield involve a number of feedback processes in physiology and micrometeorology. The accuracy of their description in our model will be tested.

To test our hypotheses, we grow 40 plants of each of two alfalfa cultivars, Wilson 9D11A and Mesilla, in controlled environments at realistic planting densities, light levels, temperatures, and humidities. On each plant we measure C_i (by two independent methods), SLM, yield, and water use, hence water-use efficiency. Regression analyses among individuals in each cultivar reveal the four relations between C_i and SLM on the one hand and WUE and yield on the other hand, and whether or not these relations are statistically significant. We also analyze more detailed predictions of the model, such as the relations between photosynthetic rate and C_i or SLM. We compare cultivars for similarity of responses of WUE and yield to C_i and SLM.

(2) Determining the Ranges of C_i *and SLM Available for Breeding*

Our model predicts that C_i must be changed about 11% and SLM must be changed about 25% in order to increase WUE about 25%. Because alfalfa is an outcrossing tetraploid, it can never be bred to genetic homogeneity (homozygosity) in any given trait. We expect therefore that C_i within any cultivar must range down to about 20% below the cultivar mean and SLM must range up to 50% above the cultivar mean, in order to breed populations with new means respectively 11% below and 25% above the original cultivar mean. The measurements outlined in objective 1 above reveal the available ranges in C_i and in SLM without additional experiments.

(3) Deferred objectives

If selection for C_i and SLM is to be practical, then both traits must be heritable. They must also be stable as the environment varies widely over the season, so that plants selected as superior in one year in one location are indeed superior in general. In the extension of this research with new funding, we are measuring (a) the traits' heritabilities in sexual crosses and (b) their stabilities in four different chamber environments and in field growth. (c) In addition, we are completing tests of the accuracy of leaf isotopic composition as a surrogate measure for C_i. While SLM can be measured rapidly and inexpensively on field-grown plants, C_i requires lengthy measurements with expensive instrumentation. Several research groups (Farquhar *et al.* 1982; Evans *et al.* 1986; Brugnoli *et al.* 1988) have shown that the ratio of the two carbon isotopes, ^{13}C and ^{12}C, in plant tissue can reliably indicate C_i, in several species. We have begun this work and report some results here.

RELATED RESEARCH

Potential Gains in Crop Water-Use Efficiency

The abiotic environment has perhaps the greatest role in determining WUE (Baldocchi *et al.* 1981; Jones 1976; review, Jones 1983). Here, major roles are played by temperature and the water vapor pressure deficit, VPD, which is the difference between the saturated vapor pressure at the crop's leaf temperature and the actual vapor pressure in ambient air. The biological environment can have a major role, too; the transpiration of neighboring plants over large geographic areas affects the VPD and other parts of a plants' local environment (Jarvis and McNaughton 1986). (This coupling is less important when agricultural fields cover restricted areas, as along river valleys in New Mexico.) Though these first two factors generally dominate in determining WUE, nonetheless WUE is under physiological control of the plant in significant measure. Such physiologically control of WUE is subject to improvement by breeding, given the proper guidelines.

Among the many definable water-use efficiencies, one that is a near-final measure in agriculture is the ratio of harvested yield (Y) of dry matter (DM) to water used (U), which we term WUE(Y,U). We may analyze this as follows:

$$\mathrm{WUE(Y,U)} = \frac{\text{harvested DM}}{\text{water used}} \qquad (1)$$

$$= \frac{\text{(total DM)(fraction as shoot)(fraction of shoot harvested)}}{\text{(water transpired, evaporated, and leached)}}$$

$$\equiv \frac{\mathrm{DM_{total} f_{shoot} HI}}{\mathrm{T + E + L}}.$$

Here, HI is the harvest index and T, E and L are quantities of water transpired by the plant, evaporated from the soil, and lost to deep percolation into soil, or leaching, respectively. Now, the dry matter derives from the season's cumulative crop photosynthesis, P, times a conversion efficiency, β, from CO_2 gain or from raw photosynthate to final, complex plant matter (carbohydrates, protein, oils, etc.). If we further express $T + E + L$ as transpiration alone divided by the fraction γ that it represents in $T + E + L$, we then have

$$\mathrm{WUE(Y,U)} = \frac{\mathrm{P}}{\mathrm{T}}\beta \mathrm{f_{shoot}HI}\cdot\gamma \qquad (2)$$

$$\equiv \mathrm{WUE(P,T)}\beta \mathrm{f_{shoot}HI}\cdot\gamma.$$

Here, WUE(P,T) is the instantaneous water-use efficiency measured in short-term exchanges of the gases CO_2 and water vapor.

Genetic variation exists in WUE(Y,U) and in most of the five components resolved in Eq. (2). Therefore, breeders can select directly for harvest WUE. However, this has been but a minor part of the breeders' agenda to date, for several reasons: (1) The main job of breeders is still pest and disease resistance (Russell 1978; Buddenhagen 1981). (2) The monetary cost of water resources was more modest in the past. (3) While direct selection for harvest WUE, WUE(Y,U), is feasible, gains may have undesirable side effects. For example, alfalfa has a low f_{shoot} near one-half, compared to values near 0.9 typical of many crops (Loomis 1983). That is, alfalfa has a high investment in roots, appropriate to a perennial plant of semiarid-zone origin (Lesins 1976). In the process of direct selection for harvest WUE, one could easily pick up variant genotypes with a high f_{shoot}, which compromises their drought tolerance, a much more pervasive concern in plant water relations than is simple WUE. In recent work, Wilson *et al.* (1983) and Salter *et al.* (1984; further tests by Currier *et al.* 1987) developed alfalfa populations directly selected for productivity with suboptimal water supply. They thereby gained both WUE and drought tolerance, in an unresolved combinations, and avoided the deleterious possibilities above. In this research, we are attempting to maximize WUE gains in a way that also avoids these deleterious effects, and in a way that is quite general and thus capable of extension to many other crops ultimately.

We focus here, first, on *in*direct selection for improved WUE and, second, on improving instantaneous WUE specifically. In indirect selection, one selects for a few key traits contributing to a final performance measure such as WUE, rather than for the final measure itself. In our case, these traits are C_i and SLM mentioned in "Objectives." The premise is that one has a reasonably complete understanding of how various traits contribute to WUE or whatever is the final performance measure. Indirect selection has a number of potential advantages over direct selection, detailed by Falconer (1960) and Wallace *et al.* (1972): (1) It can avoid or minimize deleterious effects of gains in one measure of performance (*e.g.*, $\hat{\mathrm{WUE}}$) on other measures (such as drought tolerance, forage digestibility, etc.). (2) Less variation of all traits is carried during indirect selection compared to that carried in direct selection. Thus, gains in the final measure may be faster and may be retained better over generations. The limitation of indirect selection is that one's knowledge of how various traits contribute to the final performance measure must be fairly complete, or one will miss much of the potential gain in the final measure.

We focus upon instantaneous WUE, WUE(P,T), both by elimination of other factors in Eq. (2) and by our own research competence. We have noted how it is largely undesirable to breed for increased f_{shoot}. Consider now the factor β, the conversion efficiency from photosynthesis to final dry matter. Gains here are unlikely unless the plant's composition is changed (lower protein and lipid content, *e.g.*), at least according to our current knowledge of plant biosynthetic and maintenance processes (Penning de Vries *et al.* 1974; Penning de Vries 1975). (Wilson [1982] and Robson [1982] have found variants in ryegrass that lose less photosynthate to respiration without apparent composition changes; these are undergoing further investigation.) Gains in harvest index, HI, are inapplicable in alfalfa and other forages, as the whole shoot is used. Even in some seed crops, however, HI is already so high (around 0.4) that it may be nearing biological limits (Gifford and Evans [1981] believe further gains are possible). Gains in γ, the fraction of water transpired in total water use, are probably limited in alfalfa. Because alfalfa is a perennial with dense cover, its water losses to evaporation from soil are small compared to typical crops that are annuals. Admittedly, losses to deep percolation or leaching can be significant in common irrigation management of alfalfa, in which excessive water is applied (Currier *et al.* 1987b). However, this is an area for changes in management rather than in crop physiology.

Exploitable Genetic Variation in Instantaneous Water-Use Efficiency

The most important plant characteristic for instantaneous WUE is its type of photosynthetic pathway. This path may be (1) C_3, in which the first stable product of photosynthesis is a 3-carbon acid; most crops are of this type, including alfalfa; (2) C_4, in which the first stable product is a 4-carbon acid; or (3) CAM, or Crassulacean acid metabolism, chemically similar to C_4 but with a significant time-shift of chemical reactions; these plants are slow-growing, often succulent. The C_3 plants have the lowest WUE, as a class (Jones 1983, Table 10.3; Pearcy and Ehleringer 1984; Table 1 in this report), and alfalfa is reputed to have the lowest WUE of all major crops. Unfortunately, one cannot contemplate a practical effort to change alfalfa from C_3 to C_4 in order to gain WUE. Even within genera having both C_3 and C_4 species capable of crossing (not the case for alfalfa), ingression of the C_4 path into a C_3 species has been rather fruitless (Edwards and Walker 1983), with a few exceptions (Brown *et al.* 1985).

The variation in WUE among species within the C_3 class is significant (Table 1). Again, ingression of many, unresolved traits for WUE into alfalfa from the C_3 species of highest WUE is unlikely.

WUE varies significantly among genotypes within a single species (and possibly close relatives) even under identical growth conditions. Hubick *et al.* (1986) found 1.6-fold variations within the peanut genus, *Arachis*, and the principal trait for WUE, C_i, was found highly heritable. Khan and Tsunoda (1970a,b) found similar variations in wheat. Wilson *et al.* (1983) and Currier *et al.* (1987a; Table 2 here) found implicit variations in WUE in alfalfa, unresolved from variations in drought tolerance. As we report below, we have found more than twofold WUE variations in alfalfa, which we are proceeding to verify in further growth-chamber experiments.

Physiological and Biophysical Bases for Indirect Selection for WUE

At any given moment, the environment presents an ambient water vapor concentration or absolute humidity e_a, an air temperature T_a, direct and diffuse solar irradiance of specified strengths and directions, and a wind velocity profile (also an ambient CO_2 concentration C_a, normally rather invariant). Under the plant's physiological and developmental control are stomatal resistance r_s and mesophyll or internal resistance to CO_2 transport, r_m, composed from many biochemical and biophysical capacities. Stomatal resistance controls both CO_2 influx and water vapor efflux (Fig. 1). The mesophyll resistance, presented only to CO_2 transport, stems from a number of physical and biochemical processes within the leaf. Standard resistance analyses (reviewed by Tenhunen *et al.* 1980; Jones 1985) for CO_2 and water vapor transport indicate that a key measure of both photosynthesis and WUE is the leaf-internal CO_2

TABLE 1

Variations among plant species in water-use efficiency (WUE) on a harvest basis (mg dry matter harvested per g water transpired and evaporated).

Plant identity	WUE	Plant identity	WUE
C_4 plants		C_3 plants	
Cereals	2.63-3.88	Cereals	1.47-2.20
millet cvs.	2.72-3.88	wheat cvs.	1.93-2.20
sorghum cvs.	2.63-3.65	oat cvs.	1.66-1.89
maize cvs.	2.67-3.34	rice cvs.	1.47
Other Gramineae	2.96-3.38	Other Gramineae	0.97-1.58
Other C_4	2.41-3.85	Other C_3 crops	1.09-2.65
		alfalfa cvs.	1.09-1.60
		pulses	1.33-1.76
		sugar beet	2.65
		Native plants	0.88-1.73
Range for C_4 plants	2.41-3.88	Range for C_3 plants	0.88-2.65

Note: This Table is a summarization by Jones (1983) of data of Shantz and Piemeisel (1927) on potted plants grown at Akron, Colorado.

TABLE 2

Implicit variations in alfalfa in water-use efficiency (WUE), mixed with variations in drought tolerance.

Cultivar or germplasm	Irrigation level (inches of water) 16	48	80
	Yield (dry tons $acre^{-1}year^{-1}$)		
C-3	1.7	5.8	5.9
Carrizozo	1.3	5.0	4.5
Cimmaron Hardy	2.1	6.2	6.7
Dawson	2.1	5.3	6.1
Dry Cimmaron	2.1	5.5	6.3
Dryland	1.7	6.2	6.0
Lahontan	2.2	7.4	8.1
Mesilla	2.2	7.0	7.0
NAPB-53	1.3	5.5	5.8
NC83-1	1.9	6.5	6.6
NC83-2	2.1	7.3	6.9
Nomad	1.5	4.4	4.6
Olympic	2.0	6.8	6.6
Ranger	1.8	6.1	6.4
Rhizoma	1.5	5.4	5.4
Salt Lake City	2.1	6.5	6.7
Sandelin	1.7	5.6	6.0
San Jon Dawson	2.5	6.7	6.3
Turkistan	2.3	5.9	6.6
Turkistan Wild	1.0	4.2	4.8
Utterback	2.1	6.5	6.5
Vangard	2.0	7.0	7.9
Vernal	1.6	5.9	5.6
Zia	2.2	7.1	8.2
Mean	1.9	6.1	6.3
L.S.D. (0.05)	0.4	0.8	0.7
Entry C.V. (%)	33.5	24.5	26.4

Note: These are representative data of Currier *et al.* (1987) at one site (Las Cruces) and one time slot (1978-80), among seven such location and time combinations.

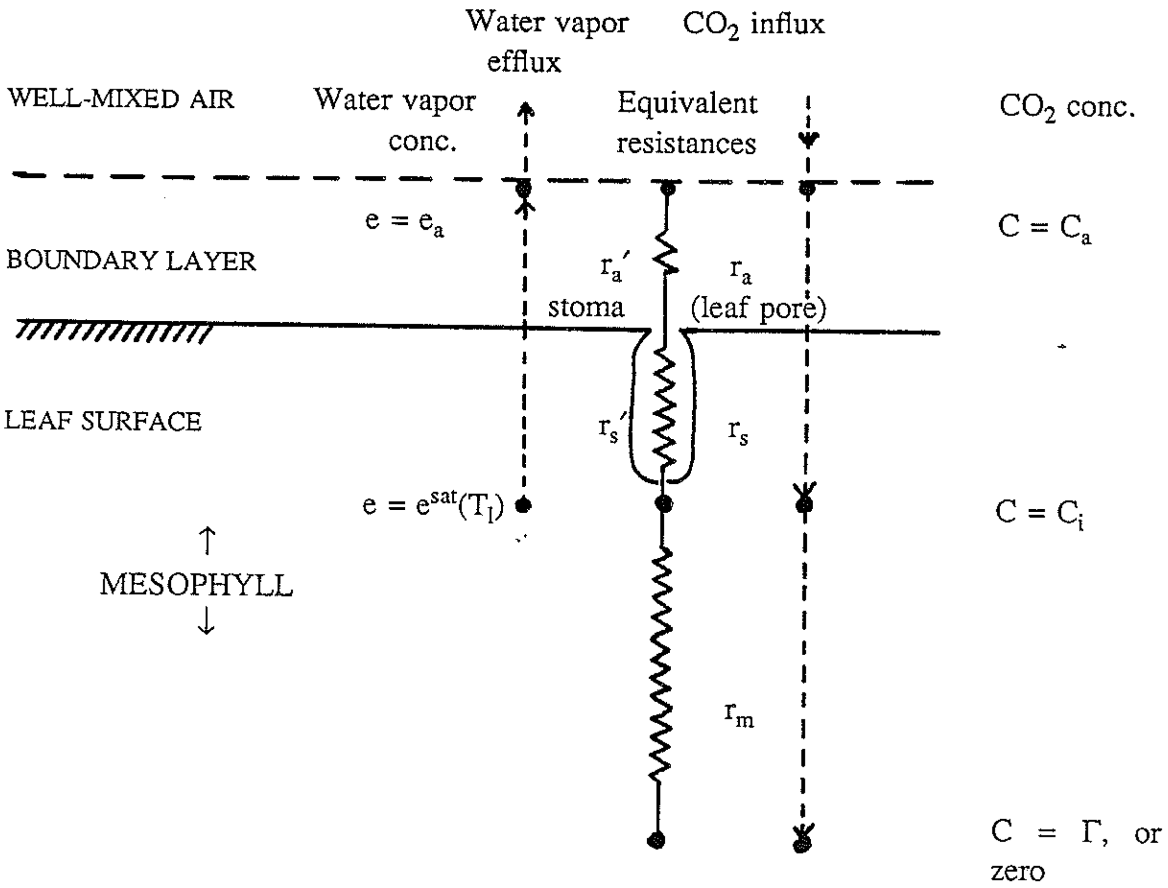

Fig. 1. Simplified resistance analysis of leaf photosynthesis and transpiration. Ambient air has a water vapor content, *e*, defined as e_a and a CO_2 concentration $C = C_a$. Water vapor exits the leaf down the concentration gradient from the stomatal interior, where *e* attains the saturated value $e^{sat}(T_l)$, down to the outside air where $e = e_a$. The diffusive resistances in its path are the stomatal resistance, r_s', and the resistance of the boundary layer, r_a' (often small in value). The rate of water vapor loss (transpiration) is $E = (e^{sat}(T_l) - e_a) / (r_s' + r_{a'})$. Carbon dioxide enters the leaf down the concentration gradient from $C = C_a$ in ambient air all the way to the photosynthetic CO_2-fixing sites where $C = \Gamma$ (the compensation concentration) or zero (depending on the model's resolution). The CO_2 traverses the stomatal resistance r_s, which is a true diffusive resistance, and the mesophyll resistance r_m, an effectiv resistance posed by limitations on biochemical reaction capacities. The rate of photosynthesis is $P = (C_a - \Gamma) / (r_s + r_a)$.

concentration, C_i. Its magnitude is determined by the ratio of r_m to r_s:

$$C_i/C_a = \frac{r_m}{r_m + r_s} = \frac{r_m/r_s}{r_m/r_s + 1}, \tag{3}$$

ignoring for now the generally modest boundary-layer resistance. C_i tends to be stable to changes in irradiance, plant nutritional status, etc. (Wong *et al.*, 1985a,b,c made the most complete studies, though in a C_4, maize.) The stability of C_i implies that r_m and r_s vary in parallel during environmental changes or even during leaf development, as seen by Wong *et al.* (1979).

Photosynthetic rate per unit leaf area, which we denote as $P_{l,a}$, depends upon C_i, and thus upon the resistances that determine C_i:

$$P_{l,a} = \frac{C_a}{r_s + r_m} = \frac{C_a - C_i}{r_s}. \tag{4}$$

Transpiration per unit leaf area, $E_{l,a}$, depends upon r_s strongly (and on r_m only implicitly, in that r_s tends to track r_m closely and thus maintains C_i):

$$E_{l,a} = \frac{e^{sat}(T_l) - e_a}{0.6r_s} \equiv \frac{VPD}{0.6r_s}, \tag{5}$$

where $e^{sat}(T_l)$ is the saturated water vapor concentration at the leaf temperature T_l, and r_s for CO_2 transport is converted to that for water vapor by the factor of about 0.6. Short-term WUE is then

$$WUE = \frac{P_{l,a}}{E_{l,a}} = \frac{0.6(C_a - C_i)}{VPD} = \frac{0.6C_a}{VPD}(1 - C_i/C_a). \tag{6}$$

This relation of WUE to C_i is modified if there is a significant boundary-layer resistance at the leaf or for the whole canopy (Jarvis and McNaugthon, 1986). In such a case, WUE still increases as C_i decreases, but much more slowly; the differences in WUE among plants differing in C_i can become quite small (Fig. 2 in Gutschick, 1989).

The elementary hope is that WUE could be improved by selecting genotypes with low C_i, as indeed Hubick *et al.* (1986) have done. They found significant variations in apparent C_i measured by the elegant and simple technique of $^{13}C/^{12}C$ discrimination, and these variations correlated well statistically (the r-value was sufficiently large and negative) with variations in WUE that covered a 1.6-fold range. Given that C_i is usually about $0.7C_a$ in C_3 plants, the leverage of C_i in WUE is considerable: Eq. 6 predicts that a relative drop of 5% in C_i would give a 17% rise in WUE. (The rise is less in actuality, perhaps 9%. A leaf with lower C_i but with the same SLM and biochemical capacity per unit volume as a reference leaf must have a higher r_s. Thus, it has a lower transpiration rate and less cooling, so that the leaf temperature and VPD are larger.)

Unfortunately, lower C_i also penalizes leaf photosynthesis. This fact is only implicit in Eq. (4), because changes in C_i involve even greater changes in r_s, for example. One must turn to more detailed biochemical observations (*e.g.*, von Caemmerer and Farquhar 1981; Badger *et al.* 1984) and to models of carboxylation-cycle kinetics (Farquhar *et al.* 1980). At constant leaf biochemical constitution, $P_{l,a}$ responds to variations in C_i (achieved experimentally by varying C_a) by a linear rise from zero rate at the compensation concentration Γ, turning to a saturated response at high C_i. Near normal C_i levels, $P_{l,a}$ is almost linearly dependent upon C_i:

$$P_{l,a} \approx (\text{constant})\cdot C_i. \tag{7}$$

In our research described below, we seek to ameliorate the penalty in photosynthesis for decreased C_i by turning to a second trait, SLM, for which the WUE and photosynthesis tradeoffs are different in form.

(If soil water is limiting for a significant fraction of the growth cycle, the high-WUE variant plants will be able to photosynthesize for a greater time than the lower-WUE plants. This advantage in time of function will ameliorate or even reverse the yield penalty for high WUE.)

(There is an intuitive expectation but low potential of improving WUE by breeding for leaves that are more erect. This is a morphological rather than a physiological trait, and is breedable in some species. We do not treat this trait in the proposed research. See discussion in the Appendix.)

A Combined Goal in Water-Use Efficiency and Yield, by Indirect Selection

From the above review, we conclude that the promise of selecting genotypes with lower C_i to improve WUE is significant. On the basis of detailed model studies and preliminary experimental results described below, we also believe that the moderate penalties in photosynthesis and yield from lower C_i can be ameliorated by selecting concurrently for increased SLM. In fact, the promise is more general. SLM and C_i are predicted to act quite differently from each other in determining WUE and yield (Fig. 2). Thus, there is the potential to achieve a variety of distinct tradeoffs between WUE and yield. If WUE is of paramount importance, the greatest increase is found by following the "fall line" of steepest ascent on the contour plot (Fig. 2) of WUE versus SLM and C_i. For example, a 25% gain in WUE relative to that of the Mesilla cultivar is predicted from an 11% decrease in C_i and a 25% increase in

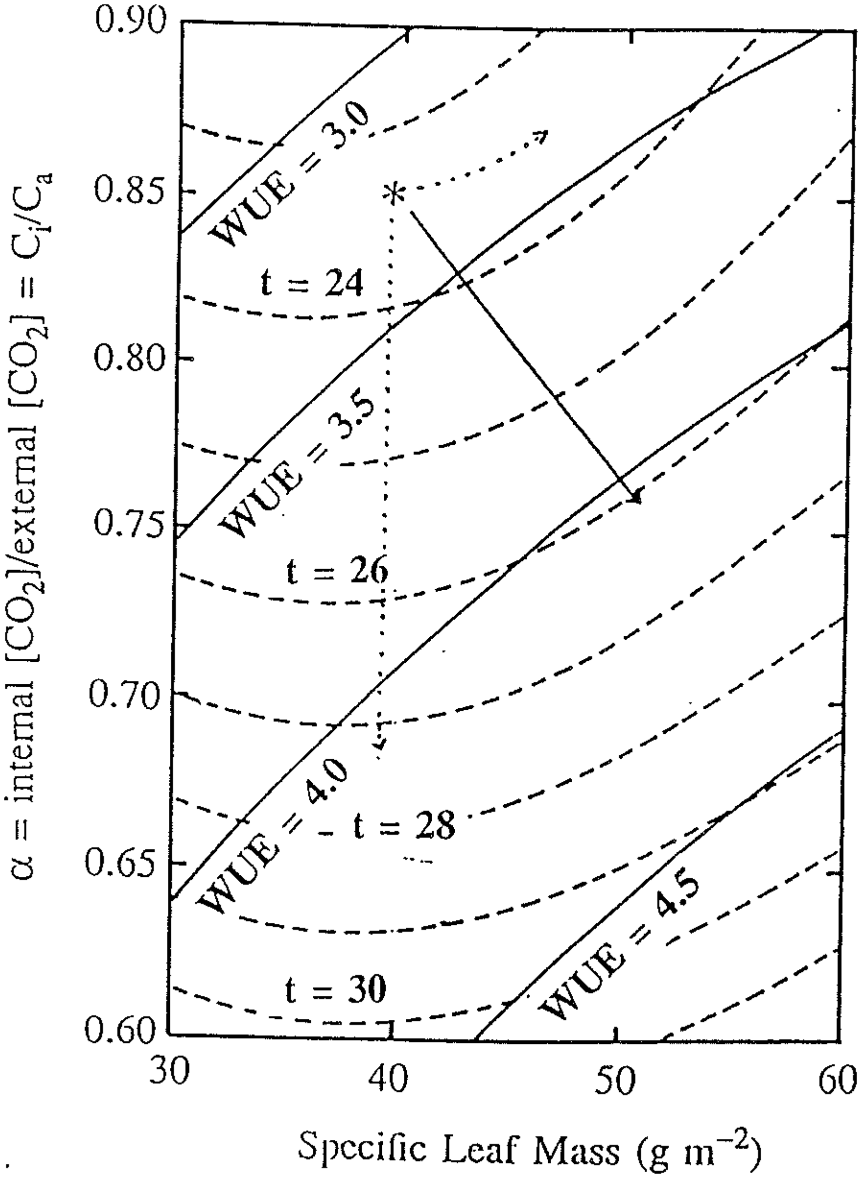

Fig. 2. Predictions of our model for the dependence of water-use efficiency (solid lines) and yield (dotted lines - as cutting cycle time) upon C_i and specific leaf mass. WUE is reported as mg dry matter accumulated in shoot per g water transpired over the growth cycle. Yield is reported as days to grow from 50 g m^{-2} shoot biomass density immediately after previous cut up to 500 g m^{-2} at harvest; shorter growth time implies more harvests and thus greater total yield averaged over years.

The asterisk indicates the C_i and SLM of Mesilla cultivar in the field, and the predicted WUE and growth time. The dotted arc indicates the shifts in C_i and in SLM that should not alter growth time but should increase WUE (by 4%). The solid straight line indicates shifts that increase WUE the most with the least effect on growth time (up to 25% increase in WUE with a 12% increase in growth time). The dotted vertical line indicates the shift in C_i alone needed to bring about the same 25% increase in WUE; the yield penalty is 16%.

SLM. This selection is predicted to drop yield by 12%, while selection for C_i alone to achieve the same gain in WUE is predicted to drop yield 18%. If maintaining yield is most valued, in a different cropping situation, one may "walk" along a contour of constant yield (Fig. 2) to the C_i/SLM combination giving the highest WUE at the same yield. Then, a 25% increase in SLM with no significant change in C_i is predicted to give a modest 5% increase in WUE. Other combinations of WUE and yield changes are also possible. For the reasons outlined in the section on "Significance," we believe such combined goals can be explored similarly in a wide variety of crop species.

Our experimental results to date, described below, are quite encouraging. Nonetheless, for our two-trait selection to be practical in breeding, it is necessary that both traits be (1) heritable to a high degree and (2) stable between different environments, at least so that the rank-order of various genotypes is largely preserved between any two different environments; if so, then C_i and SLM are measurable in the field. Hubick *et al.* (1986) found that C_i in the field correlated well with C_i in the growth chamber among genotypes of *Arachis,* another legume like alfalfa and one of the few species in which C_i genotypic variation has been explored to date. Ehleringer *et al.* (1987), in an oral presentation expanding upon a published abstract, reported a correlation coefficient $r = 0.82$ between C_i of unstressed *Agropyron* genotypes and C_i of the same genotypes when water-stressed. Regarding SLM, Song and Walton (1975) found high heritability in alfalfa. We are thus encouraged further in our experimental program.

METHODS

Developing the Model of Water-Use Efficiency and Yield

We model plant photosynthesis, transpiration, and growth, primarily to predict the potential gains in WUE. The model also helps to identify the relative sensitivity of crop performance (WUE and yield) to various environmental variables (temperature, humidity, windspeed profiles, etc.). The fundamental requirement on the model is that it compute WUE and yield for (a) a specified environment or specified statistical mix of environmental conditions and (b) for a given cultivar and a given new combination of C_i and SLM. The cultivar is specified by values of other physiological variables, such as root:shoot ratio, rate at which increases in SLM increase the leaf photosynthetic rate, etc. A very effective way to use the model is to compute WUE and yield for a whole range of values in SLM (say, from 30 to 70 g m^{-2}, at every increment of 5 or 10 g m^{-2}) and in C_i. (C_i is best specified as its ratio, α, to the CO_2

concentration outside the leaf, C_a. The range from 0.9 down to 0.6 in alpha by decrements of 0.05 is a useful choice.) Then we may graph WUE (or yield) as contours on a plot with α and SLM as its two axes (Fig. 2). From such contour plots we can readily see the effects of changing C_i and SLM. In particular, we can choose a target value for improved WUE, *i.e.*, a new contour, and then see the continuous choices of C_i and SLM combinations that should achieve this target. Simultaneously looking at the contour plot of yield, we can see the tradeoffs of WUE and yield , in order to choose the optimal combination of them, hence the combination of C_i and SLM.

This research both extends the work of Hubick *et al.* (1986) on C_i selection to alfalfa, and adds the concept that selecting specified values of a second trait, SLM, could further improve WUE and maintain yield. As noted earlier, low C_i improves water-use efficiency at a cost in leaf and canopy photosynthesis. However, in many plant species, the saturated rate of leaf photosynthesis, $P_{l,a}^{max}$, is a linearly increasing function of easy-to-measure SLM (*e.g.*, Dornhoff and Shibles 1970; Khan and Tsunoda 1970a,b; Hesketh *et al.* 1981; Oren *et al.* 1986), quite surely because SLM correlates strongly with the leaf content of RuBP carboxylase on an area basis (*e.g.*, Bowes *et al.* 1972; Frey and Moss 1976; Augustine *et al.* 1979; Björkman 1981; Hesketh *et al.* 1981), and it is the carboxylase that limits photosynthesis in most conditions (review: Sharkey 1985; see also Makino *et al.* 1985). Thus, we expect as a strong correlation,

$$P_{l,a}^{max} \approx \text{constant}\cdot C_i \cdot \text{SLM}. \tag{8}$$

Our hope is that increased SLM can compensate photosynthetically for decreased C_i while upholding the gains in WUE. (In fact, high SLM itself should contribute modestly to improved WUE in strong photosynthesizers: increased SLM $\rightarrow$ increased $P_{l,a}$ $\rightarrow$ increased stomatal conductance $\rightarrow$ increased transpirational cooling $\rightarrow$ lower VPD, higher WUE.) The photosynthetic compensation is incomplete and complex, because: (1) Most leaves operate below light-saturation, so that time-averaged leaf and canopy photosynthetic rates increase more slowly than linearly with SLM. (2) Increased SLM still exacts a linearly increasing cost in lost leaf area, and the cost depend upon canopy LAI as noted earlier.

In order to handle the complexities just listed, we developed the model as outlined below:

- The model assumes a homogeneous canopy (even-aged stand).
- At each of 21 discrete growth stages specified by standing biomass density (M), the model computes steady-state canopy photosynthesis (P_{can}) and transpiration (E_{can}) by integrating the performance of individual leaves of 10 discrete angular orientations and 21 discrete depths.

- The model computes steady-state photosynthesis and transpiration of the whole canopy (P_{can} and E_{can}, respectively):
    - ○ at 21 discrete growth stages, specified by the shoot-biomass density per unit ground area (M)
    - ○ for each of several (typically 4) different environmental conditions specified by the solar elevation, direct-beam irradiance, diffuse irradiance, temperature, relative humidity, and windspeed (all at the top of the canopy), the atmospheric boundary resistance, and the effective sky radiative temperature.

  The model then averages P_{can} and E_{can} over the different environmental conditions. (The justification for averaging at each growth stage is not detailed here.)
- Plant growth is integrated in time by a novel transformation of the time-dependent growth equation for biomass density. The growth equation
    - ○ includes maintenance costs
    - ○ includes a fixed average ratio of root:shoot allocation of growth
    - ○ assumes no sink limitations to vegetative growth.
- In computing canopy photosynthesis and transpiration at one growth stage (M) and one environmental condition, the model:
    - ○ uses allometric relations to compute leaf area index as a function of biomass density M
    - ○ emphasizes an accurate description of the interception of diffuse and direct light by individual leaves of different orientations and depths
    - ○ separately computes the heat balance and thus transpiration of each leaf orientation and depth class
    - ○ accounts for in-canopy and canopy-boundary resistances to vertical transport of CO_2, water vapor, and heat.
- The model is coded in portable Fortran with extensive comments and documentation; it currently runs on a Sun 3/75M-4 workstation.

Fig. 2 presents predictions for alfalfa under a mix of environmental conditions approximating summertime in the Mesilla Valley of New Mexico. These are overlaid contour plots of growth time t^* (days to grow from 50 to 500 g dry matter m^{-2} in shoot biomass density) and of season-averaged water-use efficiency $\overline{WUE}$ (mg dry shoot harvested per g water transpired). The independent variables are C_i and SLM. The implications for improving WUE and yield are discussed above.

Experimental Tests on Alfalfa in Controlled Environments

The object of the experiments is threefold:

(a) to measure the variations among individual plants in C_i and in SLM;

(b) to measure the variations in WUE and yield of individual plants; and

(c) to correlate the variations in WUE and yield with the variations in C_i and SLM, in order to test the accuracy of the model's predictions and to estimate potential gains in WUE.

We have chosen two cultivars for study: Mesilla, which has moderate WUE and is in widespread but declining use, and Wilson 9D11A, which has about 1/3-higher WUE and is being phased into agriculture in New Mexico. We grow forty individual plants of each cultivar in growth chambers modified to achieve high light levels (irradiances) that closely simulate field conditions. We (Pushnik *et al.* 1988) solved numerous attendant problems in leaf heat-loading, ultraviolet exposure, and water delivery that arise when high irradiances are provided by metal halide arc lamps, which we chose to simulate the color quality (spectral balance) in sunlight. We achieve field-realistic conditions of:

(a) *Planting density.* Each plant grows in an individual soil column holding 1900 g (dry weight) of sandy soil (15% clay). The columns are placed densely, 77 plants per square meter, to simulate the distribution of light and the consequent developmental patterns encountered in the field.

(b) *Irradiance.* We achieve 1500 μmol $m^{-2}s^{-1}$, comparable to the peak value of 2000 for overhead sun in a completely clear sky. The plants have a 13-hour photoperiod: 1/2 hour of incandescent light, 4 hours of half-maximal irradiance, 4 hours of full irradiance, 4 hours of half-maximal irradiance, and 1/2 hour of incandescent light.

(c) *Temperature.* Day and night temperatures are respectively 27°C and 15°C.

(d) *Relative humidity.* We have used 70% and 35% to date. We will use other values in subsequent experiments.

Vigorous growth of the swards indicates reasonable success in duplicating field conditions. The average shoot yield is 3 to 4 tonnes per hectare (1.3 to 1.6 tons per acre), comparable to very good yields in the field.

At the peak of growth, the swards consume CO_2 rapidly; we maintain chamber CO_2 concentration by rapid exchange with outside air. The individual plants in their soil columns are automatically watered automatically by drip emitters delivering 1/4-strength Hoagland solution in excess. The excess solution that runs through flushes salt accumulations; it is caught and weighed for each plant. The total applied solution volume less the cumulative run-through indicates water consumption by the individual plant.

The sward of each cultivar grows from harvest until 10% of stems have open flowers, as in field practice (Feltner and Massengale 1965). Because plants at the edge of the sward have greater light availability and freer air circulation, they are not representative of a bulk sward. Thus, we measure in any given regrowth cycle the performance of the interior 18 plants:

(a) The value of C_i is measured by two methods:

(i) Gas exchange of single leaves. At mid-cycle and again at harvest, we measure on one top leaf and one leaf at mid-height the steady-state gas exchange (rates of photosynthesis and transpiration per leaf area, from which C_i can be calculated). We use two to four different irradiances between 100 and 1300 μmol $m^{-2}s^{-1}$. As observed by many other research groups, the ratio of C_i to the CO_2 concentration at the leaf surface, C_l, is usually independent of the irradiance. Here, C_l is lower than C_a by the amount $r_{b,l}P_{l,a}$, where $r_{b,l}$ is the leaf boundary-layer resistance. The photosynthetic rate at highest irradiance is used as an estimate of maximal rate and the dependence of rate on irradiance is used to estimate two other photosynthetic parameters entering the model calculations. The maximal rate, P_{la}^{max}, is correlated with SLM to test if the model is correct in detailed physiological mechanisms as well as in overall ties of WUE and yield with C_i and SLM.

The procedure for gas exchange is as follows: We place single trifoliolate leaves in a gas-tight cuvette (LI-COR) irradiated from above with water-jacketed quartz-halogen lamps. We adjust irradiance with neutral-density filters. An open-loop gas flow is fed by a humidity-controlled input air stream. We adjust air flow rate to maintain the desired relative humidity at the leaf and desired CO_2 concentration drawdown. From the steady-state shifts in water-vapor and CO_2 concentrations between input and output air, we calculate transpiration and photosynthesis. We use a sampling and calculation program that we modified from that in the LI-COR LI-6200. The program calculates C_i in standard fashion as

$$C_i = C_a - P_{la} / (g_{total}^{CO_2}) \tag{9}$$

where P_{la} is the photosynthesis rate per *l*eaf *a*rea and $g_{total}^{CO_2}$ is the total conductance (stomatal plus boundary-layer) for CO_2 as estimated from transpiration rate.

(ii) Carbon isotope ratios. We harvest, dry, and grind fresh leaf tissue from the topmost 10-cm section of a given plant containing at least 10% of total leaf area. Dr. James Ehleringer at the University of Utah analyzes the samples by combustion and mass-spectrometric determination of the ratio of ^{13}C to ^{12}C The isotopic ratio is strongly correlated with C_i, as noted earlier. We correlate the ratio directly with WUE and yield.

(c) Specific leaf mass. Using a LI-COR area meter, we measure the area of the individual trifoliolate leaves on which we measured gas exchange. Subsequently, we harvest, dry, and weigh these leaves. The ratio of dry mass to fresh area is SLM. We use these SLM values in correlating maximal photosynthetic rates to SLM. For correlation with WUE and yield, we measure SLM similarly for every 10-cm height section of each plant.

(d) Water use and water-use efficiency. For each plant, we calculate water use as the difference between cumulative water applied and cumulative drainage. The cumulative water application is known from collection of water delivered by three reference drip emitters and from frequent measurements of the ratio of water delivered by each plant's emitter to that delivered by the reference emitters.

We harvest the plants simultaneously, in the sense that we arrest their growth simultaneously. We set the growth chamber to holding conditions (continuous 15°C and 8 hours incandescent light with 1 hour of half-maximal arc-lamp light) and remove the plants sequentially. After harvest, we repot the plants in new soil in their original columns. During repotting, we record the root fresh weights and we harvest, dry, and weigh the root growth below half the column depth (about 20 cm). Root harvesting provides information on allocation of growth between roots and shoots, and prevents root-binding.

RESULTS AND DISCUSSION

Variations of Traits Within Cultivars

The most accurate data have been collected for Mesilla cultivar grown at 70% relative humidity and for Wilson cultivar grown at 35% humidity. (Wilson has since been grown at 70% humidity but the data have yet to be analyzed.) Usefully large ranges exist in each cultivar in each trait. Under the chosen growing conditions, the specific leaf mass (of the topmost 10-cm section with 10% or more of the plant's leaf area) varies as follows:

Mesilla	Wilson
Range 17.2 to 26.5 g m^{-2}	Range 17.3 to 33.9 g m^{-2}
Mean 21.4±2.9 g m^{-2}	Mean 24.0±3.8 g m^{-2}

The values of C_i measured in gas exchange are most informative if presented as the ratio

$1 - \alpha = 1 - C_iC_l$, for two reasons: (a) The plant physiologically regulates the ratio C_i/C_l. (b) The ratio C_i/C_l, or the closely related ratio C_i/C_a, is the most direct determinant of WUE (Eq. 6). The variability in the ratio C_i/C_l is as follows:

Mesilla	Wilson
Range 0.070 to 0.170	Range 0.150 to 0.270
Mean 0.120±0.028	Mean 0.190±0.037

The second measure of C_i, more practical for breeding, is the isotopic ratio, $\delta^{13}C$, which is the ratio of the atom-percent of ^{13}C to that of ^{12}C in the sample, minus the same ratio in a reference material (PeeDee Belemnite). We measured this ratio only on Wilson plants. The ratio ranged from -28.54 to -32.07 per mill, in the normal range for plants with C_3 photosynthesis. The ratio should correlate negatively with $\alpha = C_i / C_l$ measured in gas exchange. We find a weak, positive correlation, perhaps resulting from some systematic errors in these early gas-exchange measurements.

Interestingly, C_i and SLM appear to be coupled. As SLM increases, C_i declines. This is just as one would expect if the biochemical capacity and hence mesophyll conductance $1/r_m$ (Eq. 3) increased while stomatal conductance changed much less. For the Mesilla cultivar, the correlation was moderately significant:

$$\alpha = -0.0061 \text{ SLM} + 1.007$$

$$r = -0.576 \ (N = 13;\ P \approx 0.04)$$

For the Wilson cultivar, the correlation of the isotopic ratio with SLM was quite strong:

$$\delta^{13}C = 0.198 \text{ SLM} - 35.10$$

$$r = 0.771 \ (N = 16;\ P < 0.001)$$

Variations of WUE and Yield Within Cultivars

WUE varied twofold or more in each cultivar. We reduced our data to apparent WUE in producing shoot dry matter, which is shoot dry mass divided by mass of water used (transpired plus evaporated). It varied as follows:

Mesilla	Wilson
Range 2.23 to 3.63 g/kg	Range 1.46 to 2.92 g/kg
Mean 2.75±0.33 g/kg	Mean 2.30±0.48 g/kg

The difference in means reflects the difference in relative humidities during growth, partly ameliorated by the higher inherent WUE of Wilson cultivar.

Yield per plant varied more than fivefold:

Mesilla	Wilson
Range 2.40 to 12.25 g	Range 1.99 to 10.34 g
Mean 6.67±2.34 g	Mean 4.65±2.50 g

The difference in mean yield between the two cultivars is primarily due to age and we do not interpret it here in terms of C_i or SLM. The large ranges for individual plants far exceed relatively small shifts in yield with changes in C_i and SLM predicted by the model. The ranges reflect more than differences in compounded photosynthetic performance as affected by C_i and SLM. They reflect also the inherent vigor, the starting sizes, etc. While these yields correlate with the two traits, the regression coefficients' signs and not their absolute magnitudes are relevant for predicting field performance.

Correlations of Traits with WUE and Yield

Water-use efficiency correlates with C_i at modest levels of statistical significance:

Mesilla	Wilson
$WUE_{shoot} = -7.23\ \alpha + 9.14$	$WUE_{shoot} = 0.214\ \delta^{13}C + 8.79$
$r = -0.532$ ($N = 14$; $P \approx 0.04$	$r = 0.435$ ($N = 16$; $P \approx 0.10$)

For Mesilla, WUE also correlates with SLM at approximately the same level of significance as above:

$$\mathrm{WUE}_{\mathrm{shoot}} = 0.0633\ \mathrm{SLM} + 1.441$$

$$r = 0.481\ (N = 17;\ P \approx 0.04)$$

The regression coefficient is greater than expected, perhaps because SLM and C_i covary to reinforce each other's effect on WUE.

Yield correlates significantly with C_i, but only in Wilson and there in the opposite direction from expectations. That is, yield is higher in plants with lower C_i values:

$$\mathrm{Yield} = 39.58\ \alpha - 27.39$$

$$r = 0.662\ (N = 10;\ P < 0.05)$$

$$\mathrm{Yield} = 1.996\ \delta^{13}\mathrm{C} + 65.22$$

$$r = 0.776\ (N = 16;\ P < 0.001)$$

A possible explanation of the anomaly is that the plants of high yield also tend to have high SLM that draws down C_i, as seen earlier. The yield enhancement from high SLM outweighs the simultaneous yield depression from low C_i.

Yield correlates positively, as expected, with moderate to low significance with SLM:

Mesilla	Wilson
$\mathrm{Yield} = 0.529\ \mathrm{SLM} - 4.30$	$\mathrm{Yield} = 0.221\ \mathrm{SLM} - 0.46$
$r = 0.556\ (N = 18;\ P \approx 0.01)$	$r = 0.451\ (N = 16;\ P < 0.10)$

Intriguingly, yield is positively correlated with water-use efficiency, even though these plants are in no sense water-limited:

Mesilla	Wilson
$\text{Yield} = 3.32\ \text{WUE}_{\text{shoot}} - 2.15$ $r = 0.508\ (N = 37;\ P \approx 0.001)$	$\text{Yield} = 3.90\ \text{WUE}_{\text{shoot}} - 4.34$ $r = 0.746\ (N = 16;\ P < 0.001)$

Again, the causal link may be that higher SLM directly improves both yield and water-use efficiency and correlates by plausible physiological mechanisms with lower C_i and the attendant higher WUE.

Causal Links of the Traits with WUE and Yield

In the model, the causal link between the general improvement of yield with increasing SLM is the large improvement in photosynthetic rate per unit leaf area. Specifically, we expect that the maximal or light-saturated rate of photosynthesis, P_{la}^{max}, should correlate positively and very significantly with SLM. This expectation is borne out in the Wilson cultivar:

$$P_{la}^{max} = 0.640\ \text{SLM} - 4.43$$

$$r = 0.741\ (N = 10;\ P < 0.01)$$

However, the correlation is not significant in Mesilla, for reasons we have not been able to pursue. (The correlation is significant in many species, including alfalfa, as noted earlier, but not all: see the results of Crosbie *et al.* [1977] on maize, a C_4 plant.)

Additional Physiological Traits and Their Relations with WUE and Yield

In our model, we estimated values of a number of physiological parameters in addition to C_i and SLM. In the process of measuring leaf gas exchange, we derived values for two of these parameters: (a) The so-called rectangularity parameter (Johnson and Thornley 1984) in the response of photosynthesis to irradiance. Our value of 0.8 to 0.9 agrees very well with the value 0.9 used in the model. (b) The coefficient of proportionality between P_{la}^{max} and SLM, which we measured as having significant ranges in each cultivar and a mean value in each near 0.7 $\mu\text{mol g}^{-1}\text{s}^{-1}$. In the model we used the value 1.07 $\mu\text{mol g}^{-1}\text{s}^{-1}$. Field-grown plants often have greater photosynthetic competence and we feel no need to revise the model significantly.

While measuring plant yield and SLM, we also measured two quantities that reflect investment in photosynthetic tissues relative to investment in nonphotosynthetic tissues:
(a) The ratio of leaf mass to whole shoot mass, L:S. This ratio varied from 0.301 to 0.394 in Mesilla cultivar (it has not been computed yet for Wilson cultivar). Surprisingly, yield has no

significant correlation with L:S, despite the very strong effect that L:S is expected to have on plant photosynthesis. (b) The ratio of standing root mass to harvested shoot mass. This ratio varied from 1.8 to 5.0 in Mesilla cultivar. Despite the expectation that higher investment in roots is at the expense of shoot photosynthesis and growth, this root:shoot ratio had a weak correlation with yield.

CONCLUSIONS AND RECOMMENDATIONS

Proof of the Principle

The experimental data substantially support the model's predictions of how C_i and specific leaf mass control water-use efficiency and yield. In particular, the directions and magnitudes of the observed correlations agree with model predictions:

(a) WUE increases with decreasing C_i in both cultivars.

(b) WUE increases modestly with SLM. The effect is statistically significant only in Mesilla cultivar; in Wilson, the greater variation in WUE from variation in C_i may mask this correlation.

(c) The yield per plant increases with SLM. The rate of increase is greater than the model predicts. Two plausible explanations are that: (i) The inherent vigor (ability to produce new stems, etc.) may be coupled physiologically and genetically to greater SLM. (ii) The predicted modest differences in growth rate among plants differing in C_i and SLM may be compounded by competitive growth. In swards of plants sharing the same average C_i and SLM, the gains in yield compared to the parent cultivar should be more modest, in line with model predictions.

(d) Yield is not significantly correlated with C_i in Mesilla cultivar. Anomalously, yield increases as C_i decreases in Wilson cultivar; that is, benefits are doubled - both yield and WUE increase together. We hypothesize that a causal linkage may be: (i) Increased SLM directly confers increased yield and increased WUE, as the model predicts (Fig. 2). The increase in yield is inflated in competitive growth, above the gains predicted by the model for growth in a sward of more uniform physiology. (ii) Increased SLM also increases mesophyll conductance; if stomatal conductance varies little, then C_i decreases as SLM increases. (iii) The decrease in C_i confers additional gains in WUE, while the detriment to yield is more than offset by the direct (and inflated) yield increase from SLM. Thus, this double benefit of increased yield and increased WUE may reduce to the single benefit of increased WUE in field growth of more uniform populations.

There are a few caveats about these results. First, it is difficult to rationalize the positive correlation between SLM and yield in Mesilla cultivar: increased yield should come from increased photosynthetic capacity in the whole plant, but the data show no significant correlation of SLM with maximal photosynthetic rate. Second, competition for light probably magnifies the yield differences among individual plants, to give differences greater than one might see in comparing whole, uniform swards. Small differences in growth rates confer gains in light interception; these gains are compounded exponentially in further growth. We maintain the full diversity in growth rates in our test populations, while field populations undergo self-thinning that reduces the variation in growth rates and hence in per-plant yield. Thirdly, we have not accounted for differences among plants in their allocation patterns, such as between root and shoot or between vegetative growth and reproduction. We have measured root growth approximately and summed it with shoot growth for each plant. The modified yields and WUEs show very similar correlations with SLM and C_i as do the shoot-only yields and WUEs. Fourth, a possibly larger complication arises from differences between plants in the timing of reproduction. As in field practice, we cut all the plants at once, while individuals are at different growth stages; some have not begun flowering, others are past flowering. It is around the time of flowering that large changes in allocation of photosynthate occur (Feltner and Massengale 1965). If one individual plant is consistently late in flowering and in recharging root carbohydrates, it will be cut consistently before root reserves are replenished. Therefore, its long-term yield will be depressed strongly relative to early-flowering neighbors. Thus, we expect that, like simple competition, the variations in flowering times also accentuate yield differences. (The same accentuation occurs in the field, but the low-yielding plants will die out, leaving a more uniformly yielding population.)

Survey of Ranges of Traits Within a Cultivar

The variations in C_i are large. Expressed as the quantity $1 - \alpha = 1 - C_i / C_l$, the variations are typically twofold in a cultivar. SLM varies 1.7-fold among individuals in a cultivar. These large ranges are quite adequate for breeding 11% changes in the population-average C_i and 25% changes in SLM.

Additional Insights

The ranges observed in root:shoot ratio are dramatic and should be related closely to drought tolerance. Both WUE and drought tolerance are relevant to alfalfa productivity where water availability is limited episodically. A very basic question that is yet unanswered in any crop is, What is the linkage either physiologically or genetically between WUE and drought tolerance? There are limited overlaps in physiology, *e.g.*, a high root:shoot ratio should increase drought tolerance while decreasing apparent WUE based on shoot harvest. Genetically, WUE and drought tolerance might be driven in opposite directions by selection pressures. Compared to a reference plant, a plant with lower WUE will likely deplete its water reserves earlier and suffer water stress. It will also deplete the reserves of its neighbors, with some time lag. The low-WUE plant will require greater drought tolerance to survive in competition with the reference plant. Barnes (1986) has observed the stable coexistence of two tree species, in which one species has lower WUE and higher drought tolerance.

One problem in such an inquiry is that there is no single number quantifying drought tolerance. We are working to develop better measures of drought tolerance so that we may develop testable hypotheses related to crop improvement. In particular, we have developed research proposals with several other investigators (R. Zartman at Texas Tech University and T. C. Hsiao at the University of California, Davis) that build upon the information derived from our current research. The proposal with Dr. Zartman has recently been funded.

A tantalizing prospect that remains highly speculative has recently emerged. Researchers at Native Plants, Inc. (Martin *et al.* 1988) have reported studies of WUE in tomato. They find that WUE increases as C_i decreases, in agreement with our research and that of other groups. On the same plants, they have also made restriction-enzyme digests (Martin *et al.*, 1989). They find that a small number, perhaps three, of the restriction fragments correlate well with low C_i. While they avoid speculation at this time, it is conceivable that a few genes control the development of SLM and thus of C_i. Thus, major control of WUE may lie in relatively few genes, which may be amenable to classical breeding.

Further Action and Recommendations

(a) We are continuing research on how C_i and specific leaf mass control water-use efficiency and yield. In particular, we are pursuing the deferred objectives, testing the stability of C_i and SLM between different environments and also their heritabilities. Another extension of this work is testing the effects of episodic water stress on C_i and SLM and on their relations with WUE and yield. All field crops undergo occasional water stress, which is known to alter C_i in some species (*e.g.*, sunflower: Matthews and Boyer 1984) but not in others (implicit in data of Bunce 1981 on five species).

(b) We are designing field trials of our principles, to begin in the second year of our expanded program funded by the U. S. Geological Survey. Prof. C. J. Bell of La Trobe University, New South Wales, Australia will be working with us while he is on sabbatical leave. He will help particularly in micrometeorology, defining the local environment and the means of measuring its potentially large effects on crop performance.

Field trials will reveal how much several complicating factors affect our ability to improve WUE by selecting for C_i and SLM. Among these factors are:

(i) Altered rooting patterns: The relative allocations of photosynthate (and growth) to roots vs. shoots is expected to be somewhat different in the field, where root extension is unrestricted, compared with that in the growth chambers. Although the direct effects on apparent yield and WUE may be modest, the new rooting patterns may affect developmental schedules and therefore cumulative photosynthesis by individual plants.

(ii) The large excursions in temperature and humidity and the occasional periods of water stress will undoubtedly alter the C_i and SLM values of individual plants. As we noted in our deferred objectives, our selection procedure is only practical if the rank-order of the individual plants (in either C_i or in SLM) is not significantly changed by environmental changes.

(iii) Ventilation of the canopy by wind is highly variable in the field. In periods of low windspeed, there is a high resistance to moving CO_2 into and water vapor out of the canopy. Photosynthesis can be modestly depressed . WUE is raised for all plants, but differences between plants are much reduced (Jarvis and McNaughton 1986; see also Fig. 3 here), thus reducing the relative gains from breeding.

(iv) Light interception in the field is more complex. First, wind induces leaf movements and consequent slow to rapid fluctuations in the irradiances on individual leaves. The response of photosynthesis to fluctuations is fairly complex (Gross 1982). We propose that the differential effect of fluctuations between plants differing in C_i and SLM will not be very significant. Second, alfalfa leaves track the sun when well watered (Scott and Wells 1969; Travis and Reed 1983). Our model indicates that this changes canopy performance only slightly.

(v) Maturation behavior may change. In estimating the changes of yield and WUE attending changes in C_i and SLM, our model assumes that all populations of whatever C_i and SLM reach the same biomass density at harvest. The differences in yield come only from having fewer or greater number of harvests in a season. The other paradigm is that they all mature in the same time, and yield differences show directly. Until we perform field trials with cloned or sexually bred populations, we do not know which alternative will be closer to correct. We estimate that the yield changes will be greater if harvest biomass density is conserved rather than if maturation time is conserved. That is, we took the worse case for our estimates of potential yield effects.

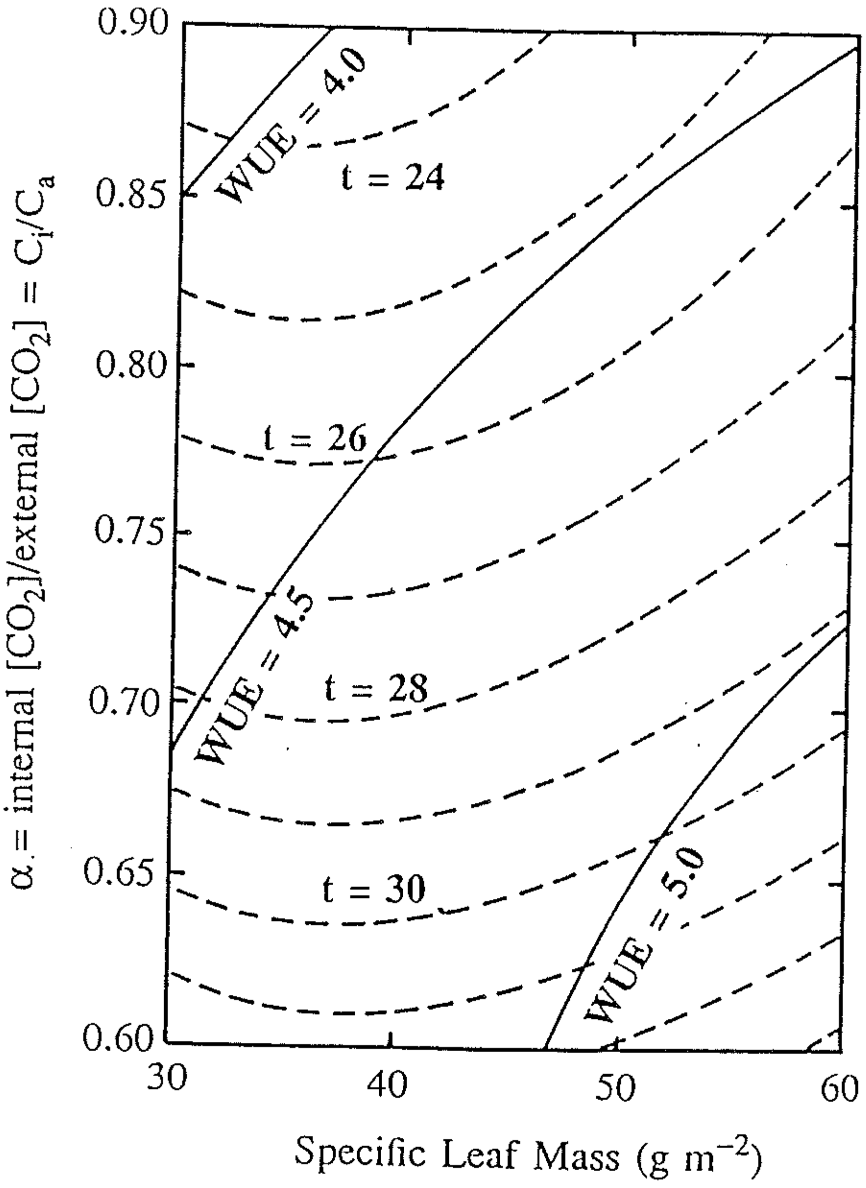

Fig. 3. Predictions of our model for the dependence of water-use efficiency (solid lines) and yield (dotted lines - as cutting cycle time) upon C_i and specific leaf mass. As for Fig. 1, except that the environmental conditions include a canopy boundary-layer resistance of 25 s m^{-1}, which retards gas exchange.

Initial Estimate of Direct Value to Breeders

The principle of selecting for C_i and SLM to improve water-use efficiency appears very useful, based on growth chamber results to date. Provided that field tests confirm that significant gains can be made, the new selection procedure could be adopted in breeding programs at once. The techniques for measuring SLM and C_i appear to be practical for the field: They take only a modest time (principally preparing leaf tissue for isotopic analysis) and investment (comparable to Kjeldahl digestions), and they are expected to be accurate.

SUMMARY

This research is an effort to reduce water use by alfalfa, and perhaps other crops, by genetic selection of plants having higher water-use efficiency (greater productivity of dry matter per unit of water used). This approach complements the improvement of water management in attempting to reduce crop water use, which accounts for 80 to 90% of consumptive water use in Southwestern states.

Within a crop species, plants may be selected directly for high water-use efficiency (WUE), but this has several potential disadvantages. We propose indirect selection for improved WUE with least effect on yield by selection for two readily measured and apparently heritable traits. Working from a knowledge of biophysics and plant physiology, we propose that WUE and yield are strongly controlled by (1) the CO_2 concentration that the plant maintains in its leaves, C_i, and (2) the leaf mass per unit area, or specific leaf mass, SLM. In particular, with a comprehensive model of plant performance, we propose that selection for 11% lower C_i and 25% higher SLM in two cultivars might yield 25% higher WUE with only a 12% decline in yield. (The required changes in traits and potential gains depend on the specific environment in which the crop is grown.) We suggest that the actions of C_i and SLM on WUE and yield are widely shared among crop plants. Thus, our indirect selection method might apply to many other crop species, including grains.

Our specific objectives are: (1) To develop a comprehensive model of alfalfa WUE and yield as dependent on C_i and SLM. The model provides initial estimates of how large are the potential gains in performance; it also helps in analyzing the effect of various environmental and experimental factors. (2) To test our hypothesis that genetic variations in C_i and in specific leaf mass alter water-use efficiency and yield in the quantitative amounts that our model predicts. Among the four pairwise relations involved, only the relation of C_i to WUE has been studied adequately by other researchers. (3) To determine if the genetic variations in C_i and SLM within a given cultivar cover ranges wide enough to breed new populations with means that are respectively 11% lower and 25% higher than in the parent cultivar. In addition to these objectives, we have several deferred objectives that are integral to the success of the project and which are being pursued with further funding. (4) To test that both C_i and SLM are strongly heritable, for utility in breeding. (5) To test that C_i and SLM are stable, that is, that individual plants substantially retain their rank order when the environment changes. Again, this is necessary for traits to be selectable in uncontrolled field conditions. (6) To test the accuracy of the carbon isotope ratio as an indicator of C_i that is both cost-effective in breeding and less sensitive to plant condition during measurement.

Our experimental methods may be briefly summarized. We grew 40 individual plants of each of two cultivars, Wilson 9D11A and Mesilla, in controlled environments. We set the planting density, irradiance, temperature, and humidity to simulate field conditions. On each plant we measured C_i (by both direct gas exchange and by carbon isotope ratios), SLM, yield, and water use, hence WUE. Regression analyses among individuals in each cultivar correlate C_i and SLM on the one hand with WUE and yield on the other hand, and test for statistical significance. We also analyzed more detailed predictions of the model.

Our results to date are highly encouraging: (1) WUE increases as C_i decreases. (2) WUE increases modestly as SLM increases (statistically significant only in Mesilla cultivar). (3) Yield per plant increases strongly as SLM increases. The rate of increase is more than the model predicts for uniform stands but appears explicable by competitive effects absent in purer stands. (4) Yield has either no significant correlation with C_i (Mesilla) or else (Wilson) increases as C_i decreases - so that both WUE and yield increase together. Again, we ascribe this extra benefit as a partial artifact of growth in mixed populations, and we predict that only the benefit of increased WUE with decreased C_i will persist in field swards. (5) The ranges of C_i and SLM in each cultivar are large, quite adequate for our breeding goals.

We discuss caveats about the results and their extension to field growth. We conclude that the results merit the completion of controlled-environment tests and progression to field studies up through tests of the breeding procedure. We also discuss additional insights into drought tolerance and into possible few-gene control of C_i and WUE that we have derived in the course of our experiments.

PUBLICATIONS AND PRESENTATIONS RESULTING FROM THIS RESEARCH

Publications:

Pushnik, J. C., Swanton, B. A. and Gutschick, V. P. 1988. Plant growth chambers at high irradiance. *BioScience* 38:44-47.

Gutschick, V. P. 1988. Optimization of specific leaf mass, internal CO_2 concentration, and chlorophyll content in crop canopies. *Plant Physiol. Biochem.* 26:in press.

Gutschick, V. P., Pushnik, J. C. and Swanton, B. A. 1989. Optimizing photosynthesis and water-use efficiency with the aid of models. In *Proc. Int. Cong. on Plant Physiology*, ed. K. Sinha. New Delhi: in press.

Other manuscripts in preparation.

Presentations:

24 November 1986. Evapotranspiration Laboratory and Dept. of Agronomy, Kansas State University. Seminar: "Modelling vegetative growth and water use in a canopy."

29 April 1987. Systems Ecology Research Group, San Diego State University. Seminar: "Modelling canopy growth and water use."

22 July 1987. Annual meeting, Amer. Soc. Plant Physiologists, St. Louis, MO. Poster: "Increased water-use efficiency in alfalfa. I. Physiological and micrometeorological model."

24 July 1987. OECD Workshop on Photosynthesis, Cambridge, England. Talk: "Optimization of specific leaf mass, internal CO_2 concentration, and chlorophyll content in crop canopies."

19 February 1988. International Congress on Plant Physiology, New Delhi, India. Invited presentation: "Optimizing photosynthesis and water-use efficiency with the aid of models."

14 July 1988. Annual meeting, Amer. Soc. of Plant Physiologists, Reno, NV. Poster: "Physiology of tradeoff of water-use efficiency with yield in alfalfa." Gutschick, V. P., Pushnik, J. C. and Swanton, B. A.

29 November 1988. Annual meeting, Amer. Soc. Agronomy/ Crop Science Soc. Amer./ Soil Science Soc. Amer., Anaheim, CA. Symposium talk: "Modeling photosynthesis and water-use efficiency of canopies as affected by leaf and canopy traits."

APPENDIX: Potential Gains in Water-Use Efficiency from Leaf Erectness

At times of peak irradiance near noon in dense crop stands, greater leaf erectness lets sunlight be distributed over greater leaf area at lower average intensity. Leaf photosynthesis is more efficient at lower irradiances and hence canopy-total photosynthesis, P_{can}, improves in some crops (review: Trenbath and Angus 1975). Because the heat load on more erect leaves might be lower, they might have lower temperatures and higher WUE. This potential has been inadequately tested (*ibid.*). Forseth and Teramura (1986) extrapolated to but did not measure improvements in WUE from leaf erectness in the legume kudzu. Recently, Gutschick and Wiegel (1988) re-estimated the effects of leaf erectness using full models of light interception, photosynthesis, heat balance, and transpiration, with improved accounting of diffuse sunlight. Subsequently (manuscript in prep.) they also accounted for solar tracking by alfalfa leaves, which is significant (Travis and Reed 1983; Reed and Travis 1987). Neither P_{can} nor WUE was predicted to be significantly altered (<5%) by leaf erectness in crops of high leaf photosynthetic capacity such as alfalfa.

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