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**INCREASED WATER-USE EFFICIENCY IN
ALFALFA BY SELECTION FOR TWO KEY,
HERITABLE PHYSIOLOGICAL TRAITS**

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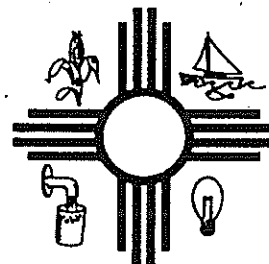
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INCREASED WATER-USE EFFICIENCY IN ALFALFA BY SELECTION FOR
TWO KEY, HERITABLE PHYSIOLOGICAL TRAITS

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ABSTRACT

Physiological breeding to improve crop water-use efficiency is one of several complementary approaches to problems of agricultural water use. These problems include competition with other end-users, soil salinization, and declining or erratic water supplies for agriculture. In previous work, we developed a physiologically based model, which predicted that breeding for two specific traits might confer 10% increases in water-use efficiency (WUE) on alfalfa in large-field growth over the whole season, while only modestly depressing yield. While the predictions likely apply to other crops, we focused on alfalfa as a major crop economically and in water use in the Southwestern states. We report here more detailed tests of the hypothesis about WUE gains and their cost in reduced yield. These tests, in both controlled and field environments, also provided evidence that the two traits (C_i = CO_2 concentration maintained in the leaf interior, and SLM = specific leaf mass or mass per area) are usable for breeding because they are (1) heritable, (2) stable in expression between repeated growth cycles and between modestly different growth environments, and (3) measurable for selection by accurate, cost-effective methods. We recommend that (1) primary attention should be paid to water management to conserve water in agriculture, in view of the rather modest and stringent limits on improving WUE by plant breeding, which is apparent in both comprehensive models and experiments here and elsewhere; (2) while these limits on WUE should be considered in allocating research efforts, a cultivar with low C_i and increased SLM should be developed for field trials; such breeding will be needed for future marginal gains in water conservation; other possibilities for breeding crops of superior WUE, especially selecting lower chlorophyll content of leaves will be more difficult but ultimately useful; (3) in breeding selection, C_i should be measured by mass-spectrometric determination of carbon-isotope discrimination at national facilities; (4) the design and interpretation of WUE in field trials should take careful account of effects of interplant competition and the micrometeorological conditions should be fully defined, particularly the canopy boundary-layer resistance; and (5) future breeding for WUE must account for some demonstrated trade-offs with crop drought tolerance and yield.

JUSTIFICATION

THE WATER PROBLEM ADDRESSED HERE

Among consumptive users of water, agriculture dominates in the southwestern states, using 80 to 95% depending upon the state (Solley *et al.* 1983). In New Mexico alone (U. S. Dept. of Agriculture 1987), alfalfa is the number-two cash crop. It covers 80% of the hay acreage and one-fourth of all irrigated area, and is a copious water user (up to 6 feet/year). Early experiments with potted individual plants indicated that alfalfa has low water-use efficiency, or WUE (Shantz and Piemeisel 1927). More recent work, reviewed by Christian (1977), indicates that its WUE is comparable with other crops, except that it continues growing in times of high vapor-pressure deficit, depressing its seasonal WUE. Bolger and Matches (1990) report that alfalfa's water-production function (slope of yield against evapotranspiration, which they call water-use efficiency) matches or exceeds that of sainfoin. Some comparisons of alfalfa genotypes for physiologically-based WUE differences have been done (e.g., Dobrenz *et al.* 1971; Currier *et al.* 1987), but there latitude remains for studying WUE.

The rising costs of irrigation water concern the farmer, as does simple availability of water because suboptimal watering can be 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 use by the crop. A major route to improve crops' water use is breeding to increase their basal water-use efficiency, that is, the yield of dry matter per unit mass of water transpired under conditions of adequate water supply. Another route is breeding to increase their drought tolerance so they survive suboptimal watering schedules and still give satisfactory yields. Our research focuses on the first route, while pointing out the need to consider drought tolerance simultaneously.

It is worth improving WUE in alfalfa alone, as North America has 13 million ha or 32 million acres of alfalfa; the worldwide total is 33 million ha or 82 million acres (Lesins 1976).

As detailed below, the expected degree of improvement in WUE is modest, approximately 10%. The return on-farm will be correspondingly modest, perhaps amplified by the producer's ability to defer water stress when irrigation water is limited. A 10% gain in WUE can be more significant for the nonagricultural uses of water. All else remaining equal, full implementation of WUE gains could double the water availability for nonagricultural use.

CHOOSING A SPECIFIC APPROACH TO THE PROBLEM

Definitions of WUE and role of WUE in crop productivity

Water-use efficiency is defined as carbon gain per unit water use. Many interrelated definitions of WUE exist, differing in the bases on which carbon gain and water use are specified. Carbon gain may be chosen as short-term CO₂ intake (by leaf or whole plant) or as dry-matter production. In turn, dry matter may be taken as total root plus shoot, total shoot, or harvestable portion only. Water use may be specified as transpiration only, transpiration plus evaporation, or managed supply only, excluding rainfall. All the definitions are related by formal transformations involving plant and environmental processes. For example, multiplication by the harvest index converts WUE based on total shoot dry matter production to WUE based on harvestable portion. Addition of soil evaporation to the denominator of any transpirational WUE converts it to WUE based on total water use. Additional discussion may be found in books by Taylor *et al.* (1983), Jones (1983), Gutschick (1987), and others.

The objectives of a study set a most appropriate choice of WUE definition. Here we consider total forage (shoot biomass) production and total water use over a complete growing season. Improvement of this WUE can allow greater forage production with the same water use, maintenance of forage yield with lower water use, or a continuum of intermediate results that can be optimized to maximize net economic benefit depending on water costs and forage price. If genetic changes for improving WUE also alter forage quality or harvestability, the dimensions of the economic problem increase. Here we address the two major dimensions, WUE and yield, as affected by breedable changes in physiology.

We must state clearly several constraints upon crop production. Greater WUE can confer greater yield only if (1) water is the major limiting factor, rather than, say, soil nutrient availability and (2) the physiological changes conferring higher WUE do not lengthen the maturation time beyond the length of the growing season as fixed by the onset of adverse temperatures or other phenomena. We presume the first condition and adjust for the second in our definition of annualized yield (see RESULTS, section on modelling). The quantitative contribution of WUE may be enhanced if it defers or averts water stress and the attendant tissue damage and dry matter loss (such as leaf loss, or lost opportunity for photosynthesis caused by photoinhibition). This accounting requires knowledge of plant drought tolerance characteristics and much detail on the time course of the environmental conditions. We assume minimal water stress for all our quantitative yield accounting in both modelling and experiments.

Evidence of exploitable genetic (physiological) variation in WUE

The abiological environment has perhaps the greatest role in determining WUE (Baldocchi *et al.* 1981; Jones 1976; reviews: Jones 1983; Taylor *et al.* 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. Although these first two factors generally dominate in determining WUE, nonetheless WUE is under physiological control of the plant in significant measure. Such physiological control of WUE is subject to improvement by breeding, given the proper selection criteria.

The primary physiological determinant of WUE is undoubtedly the photosynthetic pathway. Plants designated as C_4 , such as corn and sugar cane, produce a 4-carbon acid as the first stable product of photosynthesis. They are as much as 1.5-fold superior in the field to C_3 plants such as beans and grains, which produce a 3-carbon acid as the first stable product of photosynthesis. Introgression of the fully functional C_4 pathway into C_3 plants does not seem feasible (see Edwards and Walker 1983; exception: Brown *et al.* 1985).

Fortunately for breeding prospects, WUE varies significantly among genotypes even within a single species (and possibly close relatives) under identical growth conditions. Hubick *et al.* (1986) found 1.6-fold variations in WUE of individual plants growing in isolation, within the peanut genus, *Arachis*. They also found that the principal trait for WUE, the leaf-internal CO_2 concentration or C_i , was highly heritable. Khan and Tsunoda (1970a,b) found similar variations in wheat. Dobrenz *et al.* (1971) found varietal differences in WUE in alfalfa, relating them to some physiological traits without a mechanistic explanation. Wilson *et al.* (1983) and Currier *et al.* (1987; Table 1 here) found implicit variations in WUE in alfalfa, unresolved from variations in drought tolerance. As reported below, we (Gutschick and Cunningham, 1989) have found large WUE variations in alfalfa (up to 2-fold) that we are proceeding to verify. Here we recount other researchers' results on selectable WUE variations in peanut, tomato, and various grains.

Breeding to date has rarely attended to WUE, being much more focused on pest-and-disease resistance (Buddenhagen 1981) and yield. In consequence, much of the genetic variation related to WUE probably remains untapped and exploitable. Note also that the expression of WUE differences is more or less strongly suppressed by canopy boundary-layer or aerodynamic resistance to gas exchange, $r_{b,can}$. Stated simply, when $r_{b,can}$ is large, plants control only a small fraction of the total transport resistance and cannot vary much among themselves in this and in WUE. This dilution of

Table 1. Implicit variations in alfalfa in water-use efficiency (WUE).

Yields of various cultivars at various irrigation levels, indicating some unresolved mixture of WUE with drought tolerance. From data of Currier *et al.* (1987) at one site (Las Cruces) and one time slot (1978-80), among seven such location and time combinations.

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
Least significant difference (0.05)	0.4	0.8	0.7
Entry coefficient of variation (%)	33.5	24.5	26.4

plant control over gas exchange is also expressed as a low degree of coupling of plants to the atmosphere or high Ω factor (Jarvis and McNaughton 1986).

Primary use of indirect, physiological selection rather than direct selection

WUE may be selected indirectly. We propose, in particular, selection for two physiological traits, C_i and specific leaf mass or SLM. Compared to direct selection, such a program may demand extra effort in field measurements and definitely demands a firm knowledge of which traits control WUE and yield, and how they do. However, physiological breeding bears several advantages (cf. Falconer 1983; Wallace *et al.* 1972):

- (1) Fewer inadvertent changes are likely to be made in other aspects of performance. For example, in selecting for WUE defined as shoot dry matter produced per unit water used, direct selection at high water availability might inadvertently select some genotypes with reduced root:shoot ratio that aids WUE but penalizes drought tolerance. Indirect selection for C_i and SLM is unlikely to co-select low root:shoot ratio.
- (2) Less overall genetic variation is carried. Gains in performance might thus be achieved faster and might be more stable. However, the advantage of reduced genetic variability depend upon the selection method design and are not absolute.
- (3) Selection trials are less biased by environment, in general. A genotype with lower C_i because it was selected for lower stomatal conductance will have a shift in WUE relative to a reference genotype, and this shift will be largely predictable in a variety of environments. The predictability is enhanced by the growing knowledge of how stomatal conductance responds to atmospheric humidity (Ball *et al.* 1987). In contrast, a cultivar selected directly for higher WUE in a single environment can have combined changes in stomatal conductance, mesophyll conductance, root:shoot allocation ratio, etc. Mostly different selection environments may select widely different combinations of these physiological changes. The different combinations can give a wide range of WUE changes relative to the reference genotype when the plants are grown in a new environment.

To realize the potential advantages of indirect selection, one must have a fairly complete understanding of how a trait affects WUE. Our understanding is limited, making it beneficial to combine both direct and indirect breeding. Blum (1989) concludes that "physiological criteria must be used in selection." Emphasis should be given to traits that have been shown to contribute to yield under stress as well as to traits with strong theoretical bases (Ludlow and Muchow, 1990). The most important traits are those that match the phenology of the crop to the water supply (*ibid.*), especially distinguishing crops that grow with terminal drought (in Mediterranean climates)

and those that grow with intermittent stress only.

Reasons for focusing upon C_i and SLM

The physiological and biophysical basis of WUE control is understood piecewise, concerning the separate roles of C_i in gas exchange (see below), of leaf size and reflectivity in energy balance (Ferguson 1974; Hiebsch *et al.* 1976; Ehleringer 1980), of canopy development in suppressing “wasteful” soil evaporation (implicit in Dunin 1991; Condon *et al.* 1992), and so on. We have synthesized these ideas into a single model of WUE for whole stands over whole growth cycles. We focused upon the two physiological traits, C_i and SLM, developing our argument as follows:

First, C_i is the physiological variable having highest leverage in what may be termed instantaneous WUE, that is, in short-term gas exchange by single leaves (Jones 1983; Hubick *et al.* 1986; Richards 1991). This WUE_i is the ratio of CO_2 flux into the leaf to water-vapor flux out, and is set by the ratio of concentration gradients for the two species between outside air and the air space inside the leaf,

$$WUE_i = \frac{0.6(C_a - C_i)}{(e_i - e_a)}. \quad (1)$$

The factor 0.6 accounts for the difference in molecular diffusivities of the two gases. The water-vapor concentration in the leaf air space, e_i , is set by leaf temperature. Naively, one may argue that this is not much changed between genotypes differing in C_i , so that WUE differences accrue from differences in C_i . A genotype with $C_i = 0.6 C_a$ would then have $0.4/0.3 = 133\%$ of the WUE of a genotype with $C_i = 0.7 C_a$. We accounted from the start for changes in leaf energy balance (lower C_i comes from lower stomatal conductance and transpiration, less cooling), and estimated that single-leaf WUE_i might improve about 25% by selecting new plants with a ratio $C_i/C_a \equiv \alpha = 0.7$, from an original population with mean $\alpha = 0.85$, as is apparent for alfalfa.

It is promising for breeding that the magnitude of C_i in a plant appears to be controlled genetically and substantially preserved under many environmental variations (Wong *et al.* 1985a, b, c; Küppers *et al.* 1986; Richards 1991; Wright 1992), although some alternative patterns exist (Morison 1987; Raschke 1986).

Complete development of our hypotheses with an extended model

The second piece of our argument is that lower C_i means that the Rubisco enzyme that first fixes CO_2 and often sets the rate of photosynthesis (review: Sharkey 1985) will operate at lower rates. Detailed enzyme kinetics (Badger *et al.* 1984) imply that the light-saturated rate of photosynthesis is approximately proportional to C_i . Growth and yield should vary between genotypes in similar proportion to C_i . Condon

et al. (1991) observed lower growth rates of low- C_i wheat genotypes in the field.

One might compensate for this photosynthetic penalty by selecting plants with more photosynthetic enzyme per unit leaf area. This investment in enzyme is often closely proportional to total dry mass per leaf area, or SLM (*e.g.*, Dornhoff and Shibles 1970; Khan and Tsunoda 1970a,b; Hesketh *et al.* 1981; Oren *et al.* 1986), which is easier and cost-effective to select.

Selection for C_i can be linked to selection for SLM. Variations in C_i can arise from variations in stomatal conductance, g_s , in mesophyll conductance, g_m , or in both. These conductances are related to photosynthesis per leaf area and CO_2 concentrations as $g_s = P_{L,a}/(C_a - C_i)$ and $g_m = P_{L,a}/(C_i - \Gamma)$, where Γ is the compensation concentration. Thus,

$$C_i = \frac{C_a + (g_m/g_s)\Gamma}{1 + (g_m/g_s)}. \quad (2)$$

Only the ratio g_m/g_s matters, so that relative deviations in either have the same effect on C_i , to a close approximation. The value of g_m is proportional to the photosynthetic enzyme investment in common conditions, and so it is often strongly correlated with SLM. Then, for example, selection for high SLM will result in low C_i if g_s remains fairly stable.

The ability of higher SLM to restore light-saturated photosynthetic rate, $P_{L,a}^{max}$, in plants selected for low C_i is compromised by the reduction in leaf area at any given growth stage specified by total leaf mass per plant. The relative effects of higher $P_{L,a}^{max}$ versus lower leaf area in setting total plant photosynthetic rate depend upon growth stage. In late growth with high leaf area index, almost all light is intercepted, so that small changes that occur in leaf area when SLM is changed are unimportant, while in early growth the reverse is true. (These complications underlie the lack of correlation between SLM and yield in reports of Porter and Reynolds [1975] and others.)

Thus, we realized the need for a whole-canopy, whole-season model of photosynthesis, transpiration, and growth, and we proceeded to develop such a model. There are several other reasons for proceeding to a whole-canopy model. For example, genotypes with lower C_i and lower transpiration not only have hotter leaves but they also humidify the canopy less. The resultant higher vapor-pressure deficit tends to drive evapotranspiration back toward the magnitude seen in the original cultivar. The need to consider plants growing in full canopies extends to the experimental program, for all the reasons just presented. In addition, it is only in dense swards that plants will develop their traits, including SLM, in the same quantitative fashion as in field growth that is of the ultimate interest for on-farm application of our ideas.

The model's nearly final version has been reported in several publications (Gutschick 1988, 1991a, b; Gutschick and Cunningham 1989). This model also accounts for the aerodynamic or canopy-boundary layer resistance to transport of CO_2 ,

water vapor, and heat, which resistance suppresses transpiration more than photosynthesis. It raises WUE of all genotypes and suppresses differences between them (Jarvis and McNaughton, 1986). This resistance increases with the length (fetch) of the field. Experimental WUE gains in small plots such as used by breeders must be scaled down appropriately to estimate gains in realistic field sizes (there is experimental evidence that this scale effect is unexpectedly small: Condon *et al.* 1991). During this study, we have improved our model further, to account for soil evaporation in determining WUE as dry matter gain divided by *total* water use, both in transpiration and in soil evaporation. Slower growth of low- C_i plants exposes soil for evaporative loss longer in time than does growth of the original cultivar; this further compromises gains in WUE (implicit in Dunin 1991; Condon *et al.* 1992).

Our model predicts water-use efficiency over the whole growth cycle, as dry matter accumulation divided by water evapotranspired, as a function of C_i and SLM in genotypes that otherwise share a fixed genetic background (root:shoot ratio, maturation behavior, etc.). In the improved form developed during the current research, it also predicts yield, using two alternative premises -- first, that genotypes differing in C_i and SLM all mature at the same shoot biomass, and, second, that the genotypes all mature in the same time interval but different biomasses.

There is a two-dimensional continuum of choices of C_i and SLM and therefore of WUE and yield. We find it profitable to present our model predictions in the form of superimposed contour plots of WUE and yield. One may then seek by geometrical means the combination of WUE and yield that is most desirable for a given economic environment. For example, in Fig. 1, we have identified by an asterisk the C_i and SLM shared by two current cultivars (Wilson and Mesilla). One may interpolate the WUE contours to construct a contour representing a gain of, say, 10% in WUE. One may then search along this contour to find the point (in C_i and SLM) that gives the highest yield (shortest regrowth time, in this case), which is at the end of the arrow labelled 10%. Alternatively, one might seek the point of highest WUE. This is not shown, but represents a gain of about 15%, with a yield loss of about 25%, and a reduction in total water use to $(1/1.15)(1-0.25)=65\%$ of the original cultivar's use.

We have used the improved model to predict the WUE gains to be had from selecting other traits that researchers have considered in the past, such as leaf hairiness or size. These results are given in the section on "Results;" they indicate that C_i and SLM are among the most effective traits to consider, with reduction in leaf chlorophyll content also promising.

We have made model predictions and experimental tests only for alfalfa here. However, the same principles should apply to many other crops, even those grown for reproductive yield, such as grains. In the case of seed crops, the requirement that C_i and SLM are effective selection criteria is that their selection does not alter the partitioning to reproductive growth. There is no evidence for or against this currently.

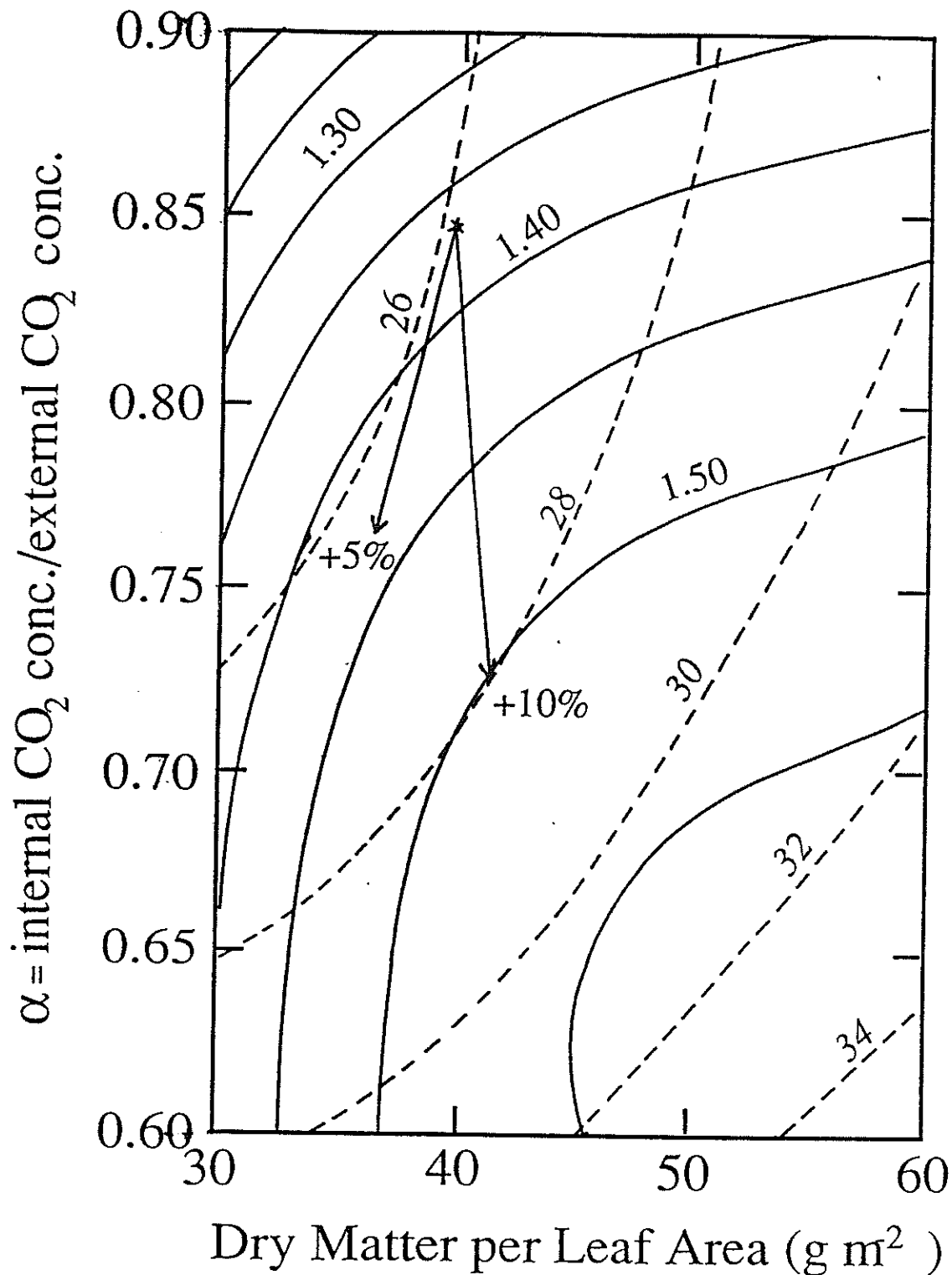


Figure 1. Predicted harvest-basis water-use efficiency (g dry matter/kg water; solid contours) and regrowth time (days; dashed contours) of alfalfa, as dependent upon two physiological traits. All genotypes are assumed to mature at a common value of shoot biomass density, 400 g m^{-2} . The asterisk denotes the physiological status of Mesilla cultivar as measured by us. The two arrows denote predicted changes in α and in SLM required to improve water-use efficiency by 5 and 10% over that of Mesilla cultivar.

PROGRESS IN SELECTING FOR WATER-USE EFFICIENCY

Direct selection

Breeders have paid little attention to WUE *per se* until recently and then for relatively few crops, as noted earlier. Genetic variation in WUE has been observed in several earlier studies that were more physiological than breeding-oriented, for example, Khan and Tsunoda (1970a, b, in wheat). Salter *et al.* (1984) and Currier *et al.* (1987) selected alfalfa in the field directly for productivity under limited water availability. Gains in productivity and in WUE itself showed very limited transferability to other geographic sites. Reasons may lie in inadvertent selection of excess physiological variation that contributes differently in new environments, and in the possibility that high-productivity genotypes exhibited drought escape, tapping a relatively shallow water table available only at the breeding site.

Indirect selection

Ludlow and Muchow (1990) and Blum (1989) have made extensive reviews of WUE breeding efforts and have developed the argument that indirect breeding is generally preferred for water-limited environments. They identified many individual physiological, morphological, and phenological (development-schedule) traits that can be exploited, with many of these related more to drought tolerance than to WUE (see next section here for the distinction).

G. D. Farquhar and coworkers have developed an extensive program of research on the potential of one primary trait, C_i , for improving WUE. They developed the strong mechanistic connection between C_i and the carbon-isotope discrimination ratio, $\Delta^{13}C$, in plant dry matter (Farquhar *et al.* 1982). The discrimination ratio is essentially the difference between plant and air in their ratios of ^{13}C to ^{12}C atoms, each denoted as $\delta^{13}C$. They showed that C_i correlates positively with $\Delta^{13}C$ (or negatively with $\delta^{13}C$) in short-term gas-exchange (Evans *et al.* 1986) as well as in the long-term average behavior that is more valuable for breeding studies. Thus, they established (Farquhar and Richards 1984; Condon *et al.* 1990) that $\Delta^{13}C$ can be used as a surrogate for the determination of C_i by gas exchange, which is both laborious and prone to large variations diurnally and between individual leaves of a plant. Several of their studies show that the discrimination ratio relative to that in the CO_2 of air, or $\Delta^{13}C$, correlates strongly and negatively with WUE. In a study on pot-grown peanut genotypes and related species (Hubick *et al.* 1986), they also showed that variation in both C_i and in consequent WUE was significant (1.6-fold in WUE). Heritability of C_i and WUE was implicit in the retention of rank orders between controlled and field environments. Direct confirmation of heritability was shown by Hubick *et al.* (1988) in this genus. Martin and Thorstenson (1988) and Martin *et al.* (1989) showed that C_i strongly

determined transpirational WUE (soil evaporation being suppressed) in tomato species. They also gave evidence for relatively simple genetic inheritance patterns. Hubick and Farquhar (1989) found significant, negatively correlated variations in both $\Delta^{13}\text{C}$ and transpirational WUE in barley.

Studies in field environments showed that the relation of C_i with WUE is robust. Johnson *et al.* (1990) demonstrated this for range grasses. Much work has been done on wheat. Farquhar and Richards (1984) showed a strong link between $\Delta^{13}\text{C}$ and WUE. Condon *et al.* (1990) extended this and made a larger survey of the potentially selectable variation in $\Delta^{13}\text{C}$, which corresponded to a variation of 0.075 in the ratio C_i/C_a . Dunin (1991) confirmed the relation of $\Delta^{13}\text{C}$ to WUE measured as the ratio of instantaneous fluxes of CO_2 and water vapor. Condon *et al.* (1987) showed that variations in C_i (in $\Delta^{13}\text{C}$) arose from both variations in stomatal and mesophyll conductances. They also found mesophyll conductance correlated with SLM and leaf chlorophyll content.

In field studies on peanut, Wright *et al.* (1988) found that higher values of $\Delta^{13}\text{C}$ (lower WUE) correlates negatively with total dry-matter production when all genotypes use the same amount of water, as expected. In wheat (Richards 1991), the correlation is negative when water is limited (Richards, unpublished data), but positive when water is not limiting (Condon *et al.* 1987). This probably reflects the higher photosynthetic rate of plants with high $\Delta^{13}\text{C}$ and high C_i . Farquhar *et al.* (1989) have discussed the likelihood of field conditions under which $\Delta^{13}\text{C}$ could poorly indicate both the long-term average C_i and WUE.

Jarvis and McNaughton (1986 and following) have emphasized a strong limitation imposed by canopy boundary-layer resistance upon the ability of different genotypes to achieve significantly different WUE. This limitation is most important for short-stature crops, unless they are sparse, as are rainfed crops or pastures in arid areas. The systematic study of this limitation's role in breeding programs is only now in progress, both in our research reported here and in ongoing work in the research groups of G. D. Farquhar, R. A. Richards, A. G. Condon, F. X. Dunin, and A. E. Hall.

With the exception of the research we report here and work on peanut (Hubick *et al.* 1992), little attention has been paid to the distinct role of SLM in determining both WUE and water-unlimited yield. (Dobrenz *et al.* [1971] reported no significant correlation between WUE and SLM, in contrast to our results.) Past studies have shown that SLM has rather high heritability in the field (Barnes *et al.* 1969; Song and Walton 1975).

EXTENDING OUR OBJECTIVES TO ADDRESS DROUGHT TOLERANCE AND CANOPY BOUNDARY-LAYER RESISTANCE

Addressing drought tolerance along with WUE

Our original proposal to the U. S. Geological Survey did not address drought tolerance (DT); it was limited to WUE under conditions of adequate water supply. In 1990, we extended the objectives to encompass DT, for several reasons. First, the projected gains in WUE were only modest. The potential contribution of improved DT to crop productivity under limited water availability is unquantified to date, but may be larger than that from improved WUE. It is also conceivable that drought tolerance, by reducing tissue damage that must be repaired with additional photosynthetic activity, may improve WUE of plants subjected to intermittent stress.

Drought tolerance is broadly the ability to maintain productivity under shortfalls in water availability from current water-use rates. We may distinguish DT from drought avoidance, in which a plant completes its life cycle outside the stress period, and from drought escape, in which a plant taps a privileged water supply such as a perched aquifer. The terminology of tolerance, avoidance, and escape varies among researchers.

DT originates in a wide variety of physiological, phenological, and morphological traits, including root:shoot ratio, osmotic adjustment, appropriate flowering time for seed crops, etc. (see Blum, 1989; Ludlow and Muchow 1990; Turner 1986). On a more aggregated scale, DT involves both aversion of water stress by enhancing access to water supplies or reducing demand and also minimization of damage from developed water stress. In the first aspect, WUE contributes to DT. It is conceivable, however, that low stomatal conductance that confers high WUE can dispose a plant to shut its stomata earlier in a stress cycle and perhaps suffer longer periods of photoinhibition.

Drought tolerance has as yet no universal, quantitative definition -- nor does drought itself. Ideally, we should derive DT as the ratio of a decrease in performance, especially yield, to the intensity of drought stress. Fischer and Maurer (1978) propose that the drop in yield of a reference cultivar is a measure of drought stress, and therefore use drop in a given genotype's yield relative to this as the measure of DT. This method is satisfactory for empirical comparisons but does not allow prediction of yield from the micrometeorological and soil environments. It also biases the measure of DT toward the type of drought (episodic vs. terminal, for example) to which the reference cultivar is adapted. A more widely satisfactory measure of DT awaits a mechanistic formulation of stress responses in the plant. For our study, we take the expedient of setting a uniform droughting protocol (withholding water until a genotype wilts at dawn, recovery, and repetition), not attempting to quantify drought stress. We propose absolute drop in yield from unstressed to stressed treatment as the measure of DT.

Our second reason for addressing DT is that improving WUE may bear physiological costs of reducing the crop drought tolerance. There are physiological and evolutionary/ecological reasons to expect negative correlations between DT and WUE^0 . One physiological hypothesis, about stomatal conductance, was given above. As a second example, consider the example of a high investment in root growth, which can contribute to DT in certain environments. However, by reducing allocation to shoot growth, it would reduce WUE calculated for agricultural or evolutionary purposes (fitness value) as shoot yield divided by total water use. Ecologically, water-saving from high WUE may benefit competitors sharing the soil volume; it may also bear costs in forgone photosynthesis that exceed the benefit of water saved for future photosynthesis (see Jones 1983). There is evidence that coexistence of two different species or conspecific genotypes is stabilized if one has the higher DT and lower WUE^0 and *vice versa* for the other. Such dichotomy is implied or was found, usually as a secondary result, in several studies (Barnes 1986; Thomas 1986; Grieu *et al.* 1988; Monson and Grant 1989; but see Derera *et al.* 1969). Our recent work (see below; additional work on tomato species; manuscript in revision) addressed this question in alfalfa and in two tomato species.

A third reason to address DT in a study of WUE is that drought stress itself commonly induces stomatal closure, which reduces C_i and improves WUE. The effects are most pronounced in the genotypes with lowest WUE/ highest original C_i , that is, in genotypes with the most room for improvement in WUE (Gutschick *et al.*, unpublished data on tomato). We must be aware of this effect that can narrow the WUE gap between genotypes.

In summary, improved WUE may bear a cost in lower DT and a focus on WUE alone may divert us from finding the optimal combination of WUE with DT for a given environment. Further, all crop plantings are likely to experience transient drought stress, even if irrigated and even if they have relatively high WUE (10% above a population norm). This stress might favor greater survival probabilities for genotypes with high DT but lower WUE. In a species such as alfalfa that cannot be bred to homozygosity, the population-mean WUE might drift back towards the WUE of the original cultivar. This limitation on improving WUE is one of several other caveats that we discuss in the "Conclusions."

The ways in which we finally considered drought and drought tolerance in designing our research program are detailed at the end of the very next section.

Surveying canopy boundary-layer resistance in field conditions

Our model of WUE and yield as controlled by C_i and SLM predicts that the canopy boundary-layer resistance, $r_{b,can}$, is important in setting absolute magnitudes of WUE and in determining the relative WUE differences between genotypes. This resistance is predictable from records of wind speed at a fixed reference height and from

some details of the wind profile with height above the crop. We improved the model in the current research to account for the variation of the resistance with each distinct environmental condition sampled over the plant growth cycle. To estimate the various $r_{b,can}$ -values, we have obtained hourly windspeed records for nearby sites, and we measured the wind-profile details for this research.

TESTING OUR HYPOTHESIS OF BREEDABLE GAINS IN WATER-USE EFFICIENCY: STATEMENT OF OBJECTIVES

Requirements for testing our hypothesis

- (1) We must identify individual plants (genotypes) exhibiting genetic variation in C_i and SLM.
- (2) We must measure WUE and yield of these plants, and test that these are correlated with C_i and SLM in the manner predicted by the model. Specifically, WUE should correlate negatively with C_i , down to fairly low C_i , and positively with SLM up to some optimal SLM. WUE should pass through a maximum and decline at very high SLM, because growth in biomass and leaf area declines, allowing cumulative soil evaporation to increase. These correlations may be masked in single-variable regressions because the correlation of the other variable is stronger and sample size is modest, or because C_i and SLM may be correlated with each other. Multiple regression helps reduce some of these problems. We also have evidence that SLM is correlated to early vigor as well as to photosynthesis; this superior yield itself contributes to WUE by increased suppression of soil evaporation. We pursued path analysis to resolve direct and indirect effects of C_i and SLM on WUE and yield. The full predictions of our model are very helpful in designing this analysis. We must use the improved model accounting for soil evaporation, temperature dependence of photosynthesis, etc. to get realistic predictions of the detailed shapes of WUE and yield responses to C_i and SLM. With this we may predict regression coefficients, estimate the adequacy of linear rather than polynomial regressions, and otherwise improve statistical analyses.
- (3) Both C_i and SLM must be examined for stability between repeated growth cycles in the nominally same environment, allowing for small variations. That is, C_i and SLM should be genetically determined in large part.
- (4) We must allow for the inevitable modest differences between the environment in which plants are bred and the environments in which the selections are used. These differences must not substantially alter either the traits nor the expected gains in WUE and acceptable yield tradeoff. Hence:

Both C_i and SLM should be stable when the environment is shifted

moderately, as in temperature or humidity. The population mean may shift moderately but the rank-order of plants should be substantially preserved.

Similarly, ranks of genotypes in WUE itself and in yield must be substantially preserved under modest shifts in environment. We address this with both the model and in experiments.

- (5) Both C_i and SLM must be substantially heritable.
- (6) We must demonstrate that methods exist for selecting plants by C_i and SLM that are both accurate and cost-effective. We have examined both short-term gas exchange and mass-spectrometric measurement of carbon isotope ratios in leaf tissue.

Specific objectives

To test all the hypotheses, we developed the objectives stated in our proposal. We now state these objectives more concisely, adding the objectives concerning canopy boundary-layer resistance and drought tolerance that were developed during the progress of this research:

- (1) Test the selection principles, items 1 to 6 above, in individual plants growing in a controlled environment. The use of a controlled environment increases our ability to detect significant effects without confounding environmental variation, and it can enable us to detect physiological reasons for deviations from the hypothesized behavior. We use 40 individual plants of each of two common cultivars, Mesilla, which is nearing the end of its utility, and Wilson, a new cultivar developed for water-use efficiency by direct selection (Wilson *et al.* 1983; Currier *et al.* 1987). The plants grew at realistic planting densities, irradiances, temperatures, and humidities that represent mean conditions of field growth. Further details of methods are provided later.

In an earlier research program (Gutschick and Cunningham 1989), we achieved partial confirmation of the hypothesis, having developed the hypothesis with a fairly sophisticated but incomplete model. The current research thus had two foci in this first objective:

- (a) To improve the model of WUE and yield and thus improve our insights into the observed behavior of plants in field and controlled environments. In particular, we improved the model to account for:
 - Soil energy balance and soil evaporation
 - Temperature-dependence of photosynthesis
 - Canopy boundary-layer resistance as co-varying with other environmental conditions
 - Light scattered within the canopy, described properly by an integral equation (Gutschick and Wiegel 1984)
 - Similarly, realistic propagation of thermal infrared radiation important

in leaf energy balance and temperature

- Realistic environmental conditions at Las Cruces. This effort included testing the number of discrete conditions needed to simulate the diurnal course of the environment with best economy of computing
- The alternative maturation behavior, equal time for all genotypes rather than equal biomass density
- Possible solar tracking by leaves
- The gradation of photosynthetic capacity (as SLM) downward through the canopy, important in total photosynthesis (Gutschick and Wiegel, 1988)

We also improved computational speed and accuracy and the contour-plot display of results. We are still working on realistic accounting of how alfalfa regrowth is supported by stored carbohydrates.

- (b) To complete many more growth cycles, in order to make firmer statistical tests of the hypotheses about WUE and yield as well as to test the stability and heritability of C_i and SLM.

Concurrently, to improve the methods of growth and measurement (of water use, C_i , etc.) in controlled conditions and thereby to achieve greater realism, particularly in soil evaporation rates, and to reduce artifactual variation in local environmental conditions among different points in the growth chambers.

Further, to use the experience in improving methods for controlled environments in the design of both types of field experiments described below.

- (2) Test that the technique of measuring carbon-isotope discrimination in plant tissue is accurate and effective in identifying genotypes of low C_i and high WUE.
- (3) Test the selection principles under field conditions, in two stages. First, we grew 68 individual plants in separate soil columns, so that we could measure WUE and yield of each plant. The plants were placed densely together and the whole assembly was placed in the middle of an existing alfalfa field (fetch of 30 m (100 ft.) or more). The daily courses of temperature, irradiance, humidity, and other environmental variables thus resembled normal conditions of field growth and incorporated a variety of stresses (particularly water stress and pests) that are not readily simulated in controlled environments but which may compromise the predicted behavior in WUE and yield.

In the second stage, we are currently growing uniform swards of populations differing in C_i and SLM. It is not practical to develop homozygous populations by cloning individual plants identified as exhibiting variations in C_i and SLM, nor is it desirable, since normal alfalfa stands are heterogeneous and this affects performance, which we wished to mimic. We developed populations that were

low, intermediate, and high in C_i and distributed in SLM, and also populations that were low, intermediate, and high in SLM and distributed in C_i . These populations were derived from sexual crosses of plants that are, for example, both low in SLM (expect progeny to have low SLM), or one high and one low (expect intermediate SLM), or both high in SLM (expect high SLM). We cross 4 parent plants of one cultivar with 4 parents of the other cultivar, so that inbreeding depression was avoided. The progeny seed was sown to plots of 1 m² (11 ft²) area. There are three replicate plots and three treatments differing in irrigation level. We are measuring WUE "directly" by dividing dry matter production by water applied, correcting for changes in soil-stored water content as measured semi-quantitatively with neutron probes. We are also estimating WUE indirectly, as the slope of yield vs. water used, including swards receiving different amounts of water. This is not strictly WUE; rather, it is a slope in the water-production function, and it can include unquantified effects of water stress. These sward tests are most realistic in growth conditions, in that roots are free to use unrestricted soil volumes and the populations are heterogeneous.

- (4) Survey the relative frequency of canopy boundary-layer resistances occurring at a typical alfalfa field in the Mesilla Valley. We placed aerial masts bearing five anemometers at different, well-defined heights above the top of the canopy. The wind profile was used to estimate the canopy boundary-layer resistance at frequent intervals.
- (5) Survey the effect of drought stress on the values of C_i and SLM achieved in individual plants, to determine if such stress significantly degrades the rank order of genotypes and thus compromises both the ability to select genotypes and the ability to use genotypes in new environments with different water stress level than in the breeding environment. We investigated this in individual genotypes growing in controlled environments, for which we can set the level of water stress reproducibly. We imposed two episodes of water stress to wilting, followed by recovery. We measured C_i and SLM after recovery and measure WUE and yield for the whole growth cycle at harvest.

This same survey also contributed to testing the hypothesis that basal WUE correlates negatively with drought tolerance. Because we employed only one of the many conceivable schedules of drought stress, this was likely not representative of the spectrum of stress in the field. Therefore, we did not pursue this further to address questions discussed earlier, such as estimating the optimal combination of WUE and DT for a given environment, or testing the hypothesis that DT may contribute more than does WUE to maintaining crop productivity.)

METHODS

MODELLING THE RELATIONSHIP OF WATER-USE EFFICIENCY AND YIELD TO PHYSIOLOGICAL TRAITS

Model structure and objectives

The model's basic features have been described in other publications (Gutschick 1988, 1991a, b; Gutschick and Cunningham 1989). We give only a brief review here of the model as it now exists, discussing the advances made during this research.

The model treats growth of alfalfa stands that are nominally uniform in plant density and in genotype. We follow growth from immediately post-harvest, specifying an initial value of shoot biomass density, to maturity for the next harvest, specifying a final biomass density. The rate of growth per unit time, hence the total time to re-grow, is modelled as current photosynthesis driving both growth and maintenance respiration. (The lack of accounting for storage and remobilization of carbohydrate [*e. g.*, Feltner and Massengale 1965; Rapoport and Travis 1984] is being remedied at present.) Current photosynthesis is described very mechanistically in terms of performance of individual leaves at all depths, angular orientations, and status as directly sunlit or shadelit. The light interception model treats direct and diffuse sunlight and light scattered from both soil and other leaves. The temperature of each leaf is specified individually to predict both its photosynthetic and transpiration rates. Temperature is determined mechanistically by the leaf's energy balance involving absorption of shortwave radiation (both photosynthetically active radiation, PAR, and near infrared, NIR) and thermal infrared, reradiation of thermal infrared radiation, transpirational loss of latent heat, and convective-conductive transfer of heat from leaf to air. Local air temperature and contents of CO₂ and water vapor are determined interactively, accounting for transport of heat, CO₂, and water vapor through the canopy, with transport described by K-theory (Cowan, 1968), known to be crude but simple; this description is being improved currently by Gutschick at CSIRO, Canberra.

Plant physiological behavior is described by a "background" physiology that is assumed the same in all cases, and by the two traits selectable to any of a variety of values, namely, C_i and SLM. We assume each leaf maintains a constant ratio of C_i to the ambient CO₂ concentration at its location in the canopy. The background physiology includes: absorptivity of leaves for PAR, NIR, and thermal infrared radiation; photosynthetic-rate dependence upon C_i and SLM (resolved, in turn, as the dependence of three parameters in the response to PAR irradiance on the leaf) and upon temperature. One parameter describes the efficiency of dry-matter production from raw photosynthate, and another describes maintenance respiration burden as proportional to total biomass. A constant fraction of dry matter production is assumed to go to root growth. The proportion of shoot biomass present as leaves declines with increasing

plant size, in a way described by a three-parameter equation.

We compute the total-stand photosynthesis (P_{can}), evapotranspiration (E_{can}), and soil evaporation (E_{soil}) typically at four points in the diurnal cycle. We further resolve two levels of soil wetness, generally fully wet and fully dry, and assume they occur stochastically with fixed frequencies, and we average over them. We have recently added a soil hydrological model to predict soil wetness from water balance and irrigation scheduling. We average the daily photosynthesis over the 8 total conditions plus a ninth condition representing overcast conditions. Each of the nine environmental conditions is described by: (1) the direct solar irradiance and solar elevation, and the diffuse solar irradiance; (2) ambient air conditions at the top of the canopy, above the boundary layer: temperature, humidity, CO_2 concentration; (3) windspeed at the top of the canopy, and canopy boundary-layer resistance; and (4) effective radiative temperature of the sky. Numerical values of these descriptors are derived from hourly meteorological records for May and June 1988 and 1989 at the Plant Science Farm of New Mexico State University. Windspeeds are used to compute $r_{\text{b,can}}$ values, using the relations found by C. Bell in this research.

We calculate P_{can} , E_{can} , and E_{soil} at 11 different growth stages (biomass densities, M). These performance measures are each fit to Chebyshev polynomials in M , so that performance at any intermediate M can be interpolated accurately. We formally integrate the growth equation from initial to final biomasses, using a mathematical transformation that saves much computing time. Similarly we compute cumulative evapotranspiration and thus the season-total WUE.

The model is coded in standard FORTRAN 77 and is fully compatible with (portable to) other machines in addition to the Sun 3/75M-4 workstation on which we run it. It uses eleven parameters to control the automatic convergence of the solutions to leaf temperature, leaf photosynthesis, soil temperature, and in-canopy transport; all of these quantities must be solved for iteratively, because all are extensively coupled with each other and have no analytical solutions. Convergence is robust under any plausible choices of physiological and environmental parameters.

In a separate program we process regrowth times and seasonal WUE values to make the contour plots described in the "Justification" section.

The WUE model is described in mathematical detail in a manuscript in revision for *Ecological Modelling*. The current manuscript is available from V. Gutschick, as is the complete program as listing or source-code files. The code is heavily commented for ease of use.

Advances in modelling during this research

Our original model and its predictions (Gutschick 1988; 1991a,b; Gutschick and Cunningham 1989) accounted for some key whole-canopy effects, particularly the statistical distribution of leaf irradiances, temperatures, and conductances with depth and

with sunlit or shadelit status. It also accounted for growth and transpiration integrated over the full season. The model has been much improved since these reports. The combined improvements make the model the best suited of extant canopy models for developing hypotheses about physiological control of WUE and yield. Specific improvements are:

(1) Accounting for the soil energy balance and evaporation. Soil evaporation is expected to vary significantly among cultivars differing in C_i in particular. On an instantaneous basis, lower C_i entails lower transpiration with less canopy cooling and humidification, favoring higher E_{soil} . Over the whole growth cycle, the predicted slower growth rate of low- C_i genotypes (as seen in wheat: Condon *et al.* 1991; less apparent in alfalfa here) would expose bare soil for a greater fraction of the growth cycle. Both these effects compromise the WUE gains of low- C_i plants.

In the energy balance of the soil, we resolve interception of radiation (PAR, NIR, and thermal infrared), reradiation of thermal infrared, soil evaporation, and convective-conductive transfer to the air. We have recently extended the model to parametrize heat conduction to deeper soil layers, with its elaborate dependence upon past thermal history (Campbell 1985). The effects are not included in model results presented here.

(2) Temperature-dependence of photosynthetic responses to irradiance and C_i . Describing these responses is problematic, first, because the net response to temperature is less sharp than that of the composite processes. Second, the net response shows acclimation to temperature during growth, while the component processes show far less acclimation. Ignoring these problems of principle for the moment, we took an empirical approach similar to that used by Farquhar *et al.* (1980) for the electron-transport rate alone. The light-saturated photosynthetic rate, $P_{L,a}^{\text{max}}$ is described as activated by increasing temperature T , up to an optimum, thereafter falling off exponentially. The initial quantum yield (derivative of photosynthetic rate with irradiance, at low irradiances) is taken to decline linearly with temperature (Ehleringer and Björkman 1977).

The temperature response is important for quantitative estimation of growth and, indirectly, of WUE. The response is also potentially important in describing WUE and yield differences among genotypes differing in C_i and SLM. The different genotypes should exhibit differences in leaf transpirational cooling and resultant temperature, hence in time required to regrow from cutting.

(3) Incorporation of stomatal conductance response to atmospheric humidity. Results given here incorporate a simple ramp function in vapor-pressure deficit. The current version uses the model of Ball *et al.* (1987). The humidity response significantly alters the time course and mean value of WUE, compared to a static conductance model.

(4) Accurate description of PAR and other radiation scattered from leaves to other leaves and soil. The PAR scattered from other leaves and soil makes up a minority of total PAR on leaves but can significantly affect P_{can} in particular; changes in

scattered PAR between low-chlorophyll and normal genotypes account for a modest yield gain by the former (model: Gutschick 1984; experimental tests: Pettigrew *et al.*, 1989). We may expect similar importance of accurate description of scattered light in predicting differences among genotypes differing in C_i and SLM. We use the integral equation of Gutschick and Wiegel (1984) to describe scattered radiation. Near-infrared radiation is also modelled carefully, for its effect in leaf energy balance, which determines leaf temperature.

The propagation of thermal infrared radiation (TIR) is modelled by accounting for first interceptions only, because the absorptivity of leaves is high in this region (0.96). A more recent improvement also accounts for second scatterings. The TIR fluxes must be calculated iteratively with the calculations of leaf and soil temperatures, obviously. The TIR portion dominates leaf energy budgets and must be accurate to get realistic leaf and soil temperatures, hence, realistic values of P_{can} , etc.

(5) Consideration of alternative maturation behaviors. Genotypes differing in C_i and SLM may mature at the same biomass density, at the same total time elapsed, or some mixed behavior. We implement the calculation of yield and WUE with maturation at fixed time. We use the same representations of P_{can} and E_{can} as polynomials in biomass density M . We do an explicit (Euler) time-integration and interpolate $P_{can}(M)$ from the polynomial at each time step.

(6) Description of SLM variation with depth in the canopy. The decline of SLM with depth in the canopy is pronounced (reviewed in Gutschick and Wiegel, 1988). This profile affects canopy photosynthesis moderately, and differentially between genotypes differing in SLM. Therefore, specifying SLM as an average in the whole canopy or at the top must be supplemented by specification of the rate of decline of SLM. We use rates found by us in growth-chamber-grown plants (decline of 5 g m^{-2} per unit of leaf area index, with a minimum SLM of 20 g m^{-2}). These rates are similar to those found in other species, as by Tooming and Tammets (1984).

(7) Accounting for heat flux into the soil as a heat sink in summer. This flux significantly reduces the heat source driving transpiration, soil evaporation, and thermal activation and inactivation of photosynthetic enzymes.

(8) Realism in description of environmental conditions. The previous publications concerning the model used educated estimates of average environmental conditions in Las Cruces. The current results use actual hourly weather data, as described earlier. We also added an accounting for soil wetness conditions.

Dr. Christopher J. Bell (sabbatical visitor, La Trobe University, Melbourne, Australia) performed field surveys of windspeeds u in alfalfa fields. We use these results to estimate canopy boundary-layer resistances over 500-meter fetches as being approximately $50/u$.

(9) Accounting for solar tracking by leaves (diaheliotropism). Alfalfa leaves markedly track the sun (Reed and Travis, 1987). The upper leaves (and progressively less so, the deeper leaves) act to maximize interception of sunlight when the plant has

no water stress; when the plant suffers water stress, leaves act to reduce PAR interception (Travis and Reed, 1983). Such solar tracking affects the overall rate of photosynthesis and probably growth in several plant species (see, for example, Ehleringer and Forseth, 1980; Ehleringer and Hammond, 1987), and may differentially affect genotypes differing in SLM because they differ in leaf area index at a given biomass density. We developed a variation of the model that simulates complete solar tracking (diaheliotropism). The differences from no solar tracking were modest, so we did not develop a model in which the degree of tracking varied with depth in the canopy.

(10) Improved speed and accuracy in numerical solutions of the model. We improved the rate of convergence of iterations for individual-leaf energy balance and photosynthesis and of iterations for soil temperature. The convergence is guaranteed for any plausible physiological and environmental description. We improved the accuracy of numerical integration of the growth equation, using knotted spline fits rather than Chebyshev polynomials. In the most recent model version, we implement a rapid, noniterative solution to the simultaneous enzyme-kinetic and humidity-response equations for stomatal conductance.

Improvements 1 through 9 in process description affected the predicted WUE and yield differences among genotypes, as reported briefly in "Results" section.

We modelled the potential benefits in WUE and yield tradeoffs accruing from several physiological changes other than in C_i and SLM, namely, reduced leaf chlorophyll content, full diaheliotropism, more erect leaves (used to improve yield in some crops, with uncertain effects on WUE; review by Trenbath and Angus, 1975), and smaller leaflet size (considered for effect on leaf-air heat transfer: Hiebsch *et al.*, 1976). These results let us estimate if future studies of physiological breeding are merited on these traits.

PLANT GROWTH AND MEASUREMENT IN CONTROLLED ENVIRONMENTS

General considerations

Controlled environments provided a test of the basic hypotheses about C_i and SLM roles in WUE and yield, with least obscuration by environmental effects not yet considered in this hypothesis, such as water stress or differential pest susceptibility of genotypes. Because plants can be grown in individual soil columns, their individual WUE and yield can be measured and correlated with their individual magnitudes of C_i and SLM. We note, however, that the behavior of an individual plant will not directly predict how a uniform sward of this genotype would compare with a uniform sward of another genotype (different C_i and SLM). First, we grew only single individuals or pairs of each genotype, and we had no practical way to control the results for differences in initial size of plants or early vigor, both of which affect a plant's ability

to compete for light in our dense plantings. Second, all the plants are cut at the same time, so that they all experience the same history of progressive light competition. Plants that mature (flower) late will consistently be cut too early to recharge their stores of root carbohydrates, and they will progressively decline in vigor, eventually dying. We have suggestions for better methods of mixed-genotype comparisons, in the section "Conclusions and Recommendations."

A number of the techniques we have developed represent advances derived from this research. We note in particular the design of (1) the high-intensity lighting with plant protection against excess thermal infrared (Pushnik *et al.* 1988), (2) the irrigation system (*ibid.*) and its capability for selective droughting of plants, both in growth chambers and in the field, (3) refinements in use of soil columns for plant growth with water-balance measurement, including control of soil microflora with antibiotics and assurance of drainage, and (4) the protocol and equipment for measuring leaf gas exchange, such as means of averting inaccurate air temperature measurements.

Plant Material

We grew forty individual plants of each of two cultivars, Mesilla and Wilson. Wilson is reputed to have better WUE but not consistently at different geographic sites (see section "Progress in Selecting W.U.E."); it is also superior in leaf-to-stem ratio, leaf retention upon harvest, and several other characters. Each individual plant grown from seed is virtually certain to be a distinct genotype, because alfalfa is an outcrossing tetraploid that cannot achieve homozygosity in sexual propagation. The plants had separate numbers. Some pairs of plants were clones of each other, one of them replacing an original individual that died in chamber mishaps (flooding of a pot caused by blocked drainage) or after continuous decline due to being cut repeatedly with the other plants before its own maturation. Plants that grew large enough to become root-bound were split and cloned, with one clone reintroduced into the growth chamber. Any plant introduced from greenhouse clonal reserves or any split and reintroduced clone underwent physiological acclimation. We therefore did not collect any data on such plants in their first regrowth cycle in the growth chamber.

Growth conditions, water supply, and water-use measurement

Each plant grew in an individual 30-cm-deep soil column (maximum practical soil depth) and was provided with excess water two to three times daily by automatic irrigation with drip emitters (Pushnik *et al.* 1988). Daily frequency of watering and volume per watering were set to give even the highest water-using plants an excess. This averted any water stress that would necessarily vary between plants and which induces plant-specific responses that are properly the subject of separate investigations of drought tolerance. Tap water was purified by reverse osmosis followed by mixed-

bed deionization. Nutrient concentrates were added to water upon delivery by a venturi mixer, to provide the equivalent of 1/4-strength Hoagland's solution at the plants. The high nitrate content suppressed root nodulation that would have added unresolved variability to genotype performances.

The soil columns were placed closely in five rows of eight plants each. They thereby attained a density of 77 plants per m² that results in field-realistic progression of light interception and, consequently, of leaf development. Each column had a drain spout (threaded, barbed fitting) placed as low as is practical (1 cm above the bottom) to give complete drainage. A fine plastic screen overlay the drain spout internally and was then covered with fine gravel; little water puddling occurred, and the small amount of puddled water was not accessible to roots to cause them to grow preferentially at the column bottom. The soil mix, sand with 15% clay, provided high water-holding capacity and good drainage that averts anaerobic puddling. A Perlite evaporation barrier 1 cm thick on top of the soil reduced evaporation from the continuously wet soil surface, to achieve more field-realistic values. (Without this barrier, soil evaporation can attain same magnitude as transpiration, which is quite unrealistic over the whole season.) We drenched the soil with antibiotic solution (40 mg/l each of streptomycin and penicillin G) to reduce root diseases and promote more uniform root growth among genotypes. Infestations of thrips and other insects were treated with soil-surface applications of Di-Syston when they became significant. Water drainage from each plant was measured about once weekly by weighing catchment jars full and empty; the cumulative drainage was recorded. Water use per plant was computed as cumulative input minus cumulative drainage. Cumulative input was estimated from the collected output of three reference emitters, against which each plant emitter was calibrated separately, at the beginning and end of each growth cycle.

The growth chambers provided light at irradiances of 60% or more of full sunlight. Using relatively simple electromechanical programs, the chambers controlled the schedule of lighting (1/2 hour dawn-simulating incandescent lighting, 4 hours at 600 $\mu\text{mol m}^{-2}\text{s}^{-1}$ top-of-canopy flux density, 4 hours at 1200 $\mu\text{mol m}^{-2}\text{s}^{-1}$, 4 hours at 600 $\mu\text{mol m}^{-2}\text{s}^{-1}$, and 1/2 hour of simulated dusk) and top-of-canopy air temperature (basal values 28°C day/15°C night). These stepped conditions simulated field conditions only coarsely, while providing important day-night contrasts and stepped illumination. Humidity was controlled, typically at 50% relative humidity. This exceeded daytime field values but lower values could not be maintained from room air for much of the year, without adding condenser units to the growth chambers. Air circulated continuously with a velocity of 0.5 m s⁻¹ at the canopy top and was replenished from outside air at 1 to 2 m³min⁻¹ to prevent sward photosynthesis from depleting CO₂ more than 15 ppmv. The spectrally balanced light provided by metal halide arc lamps simulated sunlight in its high radiant flux density, high photosynthetic utility, and low potential for photodamage. A continuous glass barrier below the lamps removed much of the thermal infrared radiation in excess of that in sunlight, so that leaf temperatures

et al. (1988) for additional details on lighting, chamber operation, and automated irrigation. We rotated all plants to new positions in the growth chamber twice weekly to average out microenvironmental nonuniformities.

Plants grew together until 10% of the stems among all plants were in flower. We then harvested all the plants, effectively simultaneously. Because harvest procedures are lengthy, all plants remained in holding conditions of low light and low temperature (15°C); this kept changes in leaf morphology small for late-harvested plants. We left 10 cm of stubble for regrowth, part of which derived from these remaining stems, the rest from the crown (see discussion by Leach, 1970).

Measuring yield, WUE, C_i , and SLM

We measured the length of every stem longer than 10 cm and recorded the number of stems. We then partitioned the stem into the top 10 cm, second 10 cm, and remainder down to 10 cm. With a LI-CORTM area meter, we measured the total leaf area of the second 10 cm section, presumed to represent the most photosynthetically active part. We also measured its mass after drying at 60°C, to obtain SLM as the ratio of mass to area. The total dry mass summed over the three sections was recorded as yield, and WUE was computed as yield divided by total water use of the individual plant. Every two growth cycles, we trimmed suberized roots off the unpot-
ted plant to prevent root-bounding and to get estimates of root contribution to total dry-matter yield. Harvesting all genotypes (plants) at a common time ensured that all plants grow their whole life-cycle in a complete canopy. However, some individuals were thereby harvested before physiological maturity. Alternative schemes may be devised to harvest each plant at its own maturity, but extra plants are then needed to maintain canopies, and early-harvested plants must be held in storage up to 10 days to regrow in synchronization with the last-harvested plants.

The dry tissue of the second 10-cm section of stem was ground and sent to the mass-spectrometric facility at the University of Utah (J. R. Ehleringer) for determining the carbon-isotope ratio, $\delta^{13}\text{C}$. This stem section contained the dominant and most recent photosynthate, the isotopic composition of which represented C_i best.

We measured C_i , and other photosynthetic attributes, of each plant by gas exchange, which is a short-term (10 to 30 minute) measure, as opposed to isotope discrimination that averages over days to weeks. For gas-exchange, a leaf was placed in a closed chamber that allowed light entry and air circulation. In the common "closed mode," one measures the rate of depletion of CO_2 and rate of gain of water vapor as measures of photosynthesis and transpiration, respectively (LI-COR 1987; Leuning and Sands 1989). The transpiration rate divided by the water-vapor pressure difference between leaf interior (estimated from leaf temperature) and chamber air indicates the leaf stomatal conductance for water vapor. Standard biophysics (LI-COR 1987; Leuning and Sands 1989) of analogous transport of CO_2 allows estimation of C_i .

We employed an open mode, in which fresh air of controlled humidity is provided continuously. Photosynthesis and transpiration are measured from differences between gas contents (CO₂, water vapor) of input and output streams. Such a system is highly preferred when a plant cannot be accessed -- readily or without mechanical damage - where it grows, such as in a growth chamber. Taken to a measurement site, it must be acclimated to new lighting conditions. With the open mode, the plant may be maintained indefinitely and its stabilization can be monitored.

The gas-exchange measurements were performed in mid- growth cycle, when the canopy was closed and near maximal photosynthesis but before any plants had flowered. We found that additional measurement times add little useful information.

We used a LI-COR[™] LI-6200 portable photosynthesis system, modified as follows: (1) We used it in the open mode. We re-plumbed the air stream, also including a branch of the input stream which is fully humidified. This stream could be blended in to control humidity at approximately 80% relative, so that responses of stomatal conductance to relative humidity (Ball *et al.*, 1987) were averted. (Care must be taken to avoid humidities much higher than 80%, above which inaccuracies in sensors and methods of calculating C_i become much larger: Leuning and Sands 1989.) Outside air was drawn from 10 m above the ground, dried with magnesium perchlorate, and compressed. Air flow rate was regulated by a needle valve. The air drying was necessary to prevent water condensation in the compressor chamber that adsorbs CO₂ in time-varying fashion. An alternative was using compressed gas, though this added considerable operational expense and variation in CO₂ level unless research-grade air mixture was purchased. The perchlorate is expensive but may be regenerated by heating to approximately 140°C, comfortably below the melting point of 147°C. Airstream CO₂ content varied slowly over the day. We interpolated values in time; subsequently, we (a) reported C_i as the ratio $/(ambient\ CO_2\ concentration)$ and (b) scaled measured photosynthetic rates linearly with CO₂ to a reference value of 350 μl l⁻¹ (parts per million by volume). (2) We revised the calculation program in the console. (3) The chamber temperature was controlled by an external stream of air chilled by a heat exchanger in a temperature-regulated water bath; the volume of air flow was adjusted with a variable-speed control on a blower motor. We found this the best method of control for the LI-COR cuvettes. The alternative of incorporating a Peltier cooler to the chamber bottom requires greater temperature excursions that risk condensation of water vapor in the cuvette (chamber). Leaf temperature was held within one degree of a common temperature representative of growth-chamber mean conditions, typically 28°C.

Light was provided by two quartz-halogen lamps mounted in water jackets to reduce thermal infrared radiation on leaves. Neutral density filters allowed selection of irradiance levels on the leaf. We found that care must be taken to avoid substantial direct radiation falling on and raising the temperature of the thermistor that senses chamber air temperature; the artifactually higher indicated air temperature causes an

erroneously high estimate of chamber absolute humidity and correspondingly low estimates of conductance and high estimates of C_i . A system description is available from V. Gutschick.

We measured gas-exchange of fully-expanded single leaves, usually two separate leaves at the canopy top and two at mid-height. We used two different irradiances, typically 400 and 1600 ; C_i varies modestly with irradiance.

Methods of imposing drought stress and measuring physiological changes

To study the effects of developed water stress on C_i , SLM, WUE, and yield of genotypes, we developed a complete protocol that is likely to elicit full adaptive responses comparably among all genotypes. We imposed two episodes of withholding water to wilting. The first episode induced some acclimation, so that fuller expression of stress-tolerance characteristics occurred in the second episode. In each episode, water withholding was phased in, starting with the reduction to one watering per day. This allowed stress to develop gradually over 5 days or more, as may occur in the field. (With the limited soil volumes usable in columns, sudden, complete cessation of watering would have led to plant wilting in as little as three days.) This protocol involved different water-deprivation times for each individual plant, which can be managed by having separate valves in the automated irrigation system for each plant. We switched water flow to a second emitter that dumped the water to drain. This kept total water flow independent of the number of plants under withholding, so that emitter delivery rates were stable. Each morning we surveyed the plants and restored water to those plants that were visibly wilted. We imposed water stress on 20 different clones within each cultivar. Each clone had a matched partner not subjected to stress, acting as a control.

In addition to measuring C_i (by both gas-exchange and carbon-isotope discrimination), SLM, WUE, and yield, we also measured a number of traits possibly related to drought tolerance. Among these were photosynthesis per leaf area, leaf water potential, and leaf osmotic potential, both before and after each drought cycle. The interpretation of these traits' relation with drought tolerance goes beyond the original proposal's objectives. We discuss only shifts in C_i here, for its potential relevance to WUE and especially to stress-induced changes in the rank-order of genotypes in WUE and yield. We wished to assess our hypothesized negative relation between WUE and drought tolerance (DT). Therefore, we defined DT quantitatively as (yield of droughted clone) - (yield of undroughted clone). Higher retention of yield under stress was equated to higher DT. Several alternative definitions exist (see, for example, Fischer and Maurer, 1978), including the above divided by undroughted-clone yield. This tends to weight more heavily the plants with low yield with consequently little margin for loss under stress. No definition is fully satisfactory for the wide variety of objectives (plant breeding, economic assessment, etc.) that are implicit in calling a

quantity DT. We used the noted definition as a first, useful estimator.

A possible objection to the protocol we used is that plants with high WUE may take longer to deplete soil water and may be penalized (deprived of carbon gain) longer than low-WUE plants. We have shown (manuscript in preparation) that this is unlikely. Several alternative protocols of inducing water stress can be considered. For one, all plants may be kept unwatered for the same time. This protocol, like the one we use, makes the deprivation time dependent upon absolute plant size. Also, to avert high mortality rates it requires that water be restored when the first plants wilt; late-wilting plants will have experienced little stress and acclimation. In the field, there is substantial sharing of soil water among adjacent plants, tending to bring them toward a common soil (and plant) water potential. This tendency toward the mean is not simulated in this alternative protocol. No single protocol is fully satisfactory, we note.

PLANT GROWTH AND MEASUREMENT IN FIELD CONDITIONS

General considerations

As described in the objectives, we have designed two separate field experiments. First, we grew 68 individual plants in separate soil columns, so that we could measure WUE and yield of each plant. The plants were placed densely together and the whole assembly was placed in the middle of an existing alfalfa field (fetch of 30 m (100 ft.) or more). The daily courses of temperature, irradiance, humidity, and other environmental variables thus resembled normal conditions of field growth and incorporate a variety of stresses (water stress, pests, etc.) that were not readily simulated in controlled environments but which could compromise the predicted behavior in WUE and yield.

In the second stage, we are currently growing uniform swards of populations differing in C_i and SLM. The swards have a realistic environment in daily courses of temperature, irradiance, etc. They also have an unrestricted rooting volume and the normal degree of interplant variation of an alfalfa cultivar. All the plants shared soil water significantly, as in farm plots. In addition, a whole sward is harvested at its average maturity, not at a common time with other plants differing in C_i and SLM. Finally, the sward determines to some degree its own microclimate, as might a real farm planting of greater area. Therefore, these populations represent our most realistic simulation of performance of cultivars that might be developed by our selection principles. These populations were derived from sexual crosses of plants that are, for example, both low in SLM (expect progeny to have low SLM), or one high and one low (expect intermediate SLM), or both high in SLM (expect high SLM) There are three replicate plots and three treatments of irrigation level. WUE is measurable by water balance but the soil volume is not strictly closed. Deep percolation can occur out the bottom of the profile, though we take care to avert this.

Soil-column experiment: plant material

Each plant in the 68 soil columns was a clone of plants involved in the growth-chamber experiments, with a few exceptions of plants grown from seed to replace plants lost to disease or decline from consistent pre-maturation harvesting.

Growth conditions, water supply, and water-use measurement

The plants grew in 1-meter-long PVC columns of the same diameter (4 in./ 10.2 cm) as used in the growth-chamber experiments. Columns contained the same soil mix and drainage provisions as used in the latter experiments. No Perlite evaporation barrier was used on the soil surface, as it was difficult to prevent its loss to wind while allowing normal surface air transport. The columns were placed densely (77 per m²) in a hexagonal packing in a pit excavated to 1.3 m in the middle of an existing alfalfa field. Tops of the columns closely matched the soil surface in the surrounding field. The space inbetween soil columns was covered by a wooden cutout, so that the roots and soil did not reach unrealistically high temperatures because of warm air or strong sunlight penetrating below. The cover also prevented rain from entering the catchment jars receiving drainage from each soil column. The entire pit was enclosed in with wood and concrete to stabilize the surrounding soil against washing in. In the pit below the columns was a space for drain tubes and catchment jars, one for each soil column. This bottom space was accessed by a 10-m tunnel sloping down from a part of the field that was typically downwind, so that personnel did not disturb the local environment.

Water was provided, along with one-quarter-strength Hoagland's nutrients, by individual Tygon tubing lines to each plant. Because pressurized, deionized water was not available in the field, we delivered premeasured volumes of water under gravity head through a funnel and blew out held-up solution after delivery. Each plant was given water in sequence. (Attempts to provide water to all plants rapidly, simultaneously from a common reservoir, failed. While equal division of flow to all plants could be enforced initially by using equal lengths of capillary tubing running to each plant, algal growth in sunlight rapidly introduced large, erratically distributed resistances.) The catchment jar contents were measured by aspirating their contents up a Tygon line going up the access tunnel. A portable vacuum pump operated by an auto battery was connected to a manifold that allowed 5 jars to be aspirated at a time. The jar contents were measured with a graduated cylinder and discarded. A rain gauge in the field at the pit measured meteoric water input, pro-rated as volume delivery per soil column in computing total volume of applied water.

It was not practical to rotate the positions of these large columns to average out microenvironments. However, microenvironmental differences were much less pronounced in the field, given uniform sunlighting and wind ventilation, plus surrounding

alfalfa kept at a comparable stage of growth.

Plants were harvested when 10% of all stems have open flowers, as in the growth-chamber experiments and as is standard in field practice. The surrounding alfalfa field was cut at the same time, as closely as possible, to maintain synchrony of growth in height. The plants in their soil columns were placed in the field from May through October. They were maintained in a greenhouse over October through April, because the air spaces in the pit made subfreezing temperatures more common than in the field. In April, the plants were placed outdoors near the greenhouse to acclimate to outdoor conditions.

Measuring yield, WUE, C_i , and SLM

We harvested plants in three sections, as for the growth-chamber experiments. Yield and SLM were derived as in the latter. Yield and cumulative water use gave estimates of WUE, as in the growth-chamber experiments. Field measures of C_i by gas-exchange were impractical, both because it would have required closed-mode gas-exchange that is less accurate than steady-state open-mode gas-exchange and because the plants would have been seriously disrupted as we reached for other plants. Therefore, we only used $\delta^{13}\text{C}$ of leaf tissue samples as an indirect but reliable estimator for C_i . No heritability tests were performed in this field experiment; the sward field experiment should give indications of heritabilities of C_i and SLM.

Sward experiment: plant material

We have derived four populations graded in C_i and another four graded in SLM. They represent crosses of Mesilla parents selected as high or low in C_i (or SLM) with Wilson parents similarly selected. The crosses were described therefore as hi-hi, hi-lo, lo-hi, and lo-lo in each of the two traits. The crosses were made between and not within cultivars in order to avoid inbreeding depression. Initially, crosses were made by hand pollination, but seed production was too slow for the twelve 1-m² plots required for each population. We then progressed to crossing in "bee boxes," in which interspecies crossing is less certain. We produced about 6 g or more of seed for each cross and germinated seed in soil containers. Seedlings were transplanted to the field at a density of 75 plants m⁻² in September, 1991 (the experiment is being carried past the termination of this grant). This method assured high survival so that 6 g of seed would suffice to cover 12 m².

Growth conditions, water supply, and water-use measurement

The swards have been established at the Plant Science Farm of New Mexico State University on clay loam soils near the Rio Grande, as used in routine alfalfa breeding.

Water is being supplied by sprinkler irrigation from a line source. This supplies water differentially at different distances, in amounts estimated with rain gauges. For each of the 8 populations of C_i and SLM crosses plus the two parental lines, we planted swards of 1 meter square at three distances from the source. Each sub-plot was replicated four times, twice on each side of the source. The differential water treatments that extend below the amount supporting maximal yield will provide estimates of water-stress effects on C_i , SLM, and WUE. Thus, we will test if stress reorders the genotype ranks in WUE in particular. Ultimately, continued low water availability reduces plant density and mean stress level. Differences in yield regressed against water delivery then determine the water-production function, related to WUE but far from identical with it.

Total water use is being computed as the sum of applied water (irrigations plus rainfall) minus the gain in soil-stored water between beginning and end of the growth cycle. The soil-stored water is determined by neutron-probe measurements, one per sub-plot, integrated over the soil depth to 2 m. The profile of water content with depth will also indicate if deep percolation is occurring. As this is difficult to estimate from water-content profiles alone, we will reject data from plots showing deep percolation and revise irrigation rates to minimize it in future cycles.

Measuring yield, WUE, C_i , and SLM

Each sward is being harvested at its own physiological maturity, at 10% flowering. Each plot has a guard strip of Mesilla cultivar, so that its harvest does not introduce marked edge-effects in neighboring swards. The total dry matter harvested down to 10 cm is deemed yield. Ten selected stems are harvested for the second 10-cm height section, to measure both SLM and $\delta^{13}C$ as in the growth-chamber and soil-column field experiments.

CANOPY BOUNDARY-LAYER CONDUCTANCE VALUES

Theoretical background

The formal definition for the canopy boundary-layer resistance, $r_{b,can}$, between the canopy top z_h and a reference height, z_r , is $r_{b,can} = \int dz/K$. Here, the integral limits are z_h and z_r and K is the eddy diffusivity, which depends on windspeed, $u(z)$. Using the logarithmic approximation (*e. g.*, Monteith and Unsworth, 1990) for the windspeed profile,

$$u(z) = \frac{u^*}{k} \ln \frac{(z-d)}{z_0}. \quad (3)$$

Using $z_0 \approx 0.63z_h$, we find that $r_{b,can} = u(z_r)/(u^*)^2$. Experimentally, u^* is found from

the slope of measured $u(z)$ against $\ln(z-d)$. If the flux of sensible heat to or from the crop is significant, the equation above should be corrected to account for the effects of buoyancy. One suggestion (*ibid.*) is to replace $u(z)$ by

$$u(z) - n(z-d) \frac{dT}{du} \frac{g}{T}, \quad (4)$$

where n is a constant (approximately 5), dT/du is the gradient of temperature in the boundary layer, g is the gravitational constant (9.8 m s^{-2}) and T is the absolute temperature (K). The slope of this corrected $u(z)$ plotted against $\ln(z-d)$ then gives a corrected value of u^* . In stable atmospheric conditions, this will be greater than the uncorrected value, and lower in unstable conditions.

Field methods

We placed a mast supporting our micrometeorological sensors in a crop of alfalfa (cultivar Doña Ana) in its second year after sowing. The field was located about 15 km north of Las Cruces, New Mexico, in the Mesilla Valley. The mast was about 150 m north of the south-eastern boundary of the crop, which totalled about 12 ha. The crop was grown as part of a normal rotation with cotton and chiles on a commercial farm, and was subject to normal farm practice, including a grazing by sheep in March, followed by low-rate fertilizer at 30 kg/ha (unspecified N:P:K), and irrigated approximately every 15 days. The site was quite open, and the nearest obstacle to air flow was the irrigation ditch on the southeastern boundary. The prevailing wind was from the southwest, with a fetch of at least 200m. The land was laser-levelled into bays approximately 10 m wide, but differing in elevation by only a few centimeters.

Measurements were started on April 5, 1989, some days after the sheep were removed, but before the first irrigation on April 13. The first cut was taken on April 28, and measurements were resumed on May 12. The final measurements were made on May 30, shortly before the second cut.

The meteorological mast supported four cup anemometers (Weather Measure Corporation, three of type W203-DC and one W103-B) at various heights, the highest being 2.4 m above the ground, the lowest about 20 cm above the canopy. In May, four thermistors were added to measure the temperature profiles, supported about 8 cm away from the mast on clamps and with radiation shields to the south. The thermistors were at the same heights as the lower three anemometers, and at approximately 0.6 of the canopy height, within the canopy.

The outputs from each sensor were connected using shielded cable to a datalogger (Campbell Scientific, model 21X) which read each sensor every 15 seconds and recorded the averages each half hour. The data was transferred to a microcomputer every 4-6 days and analyzed using Minitab.

The anemometers were calibrated relatively by placing them all at the same height

and recording windspeeds for 3 days. The regressions of output from each anemometer were used to relate output of the others to one particular sensor. The accuracy of calibration was $\pm 0.3 \text{ mV} \pm 0.6\%$ for each anemometer. One anemometer was calibrated absolutely against the speedometer of a car (itself calibrated by measuring the time taken to cover an accurately measured mile) by holding the anemometer out the sun-roof of the car while travelling at various speeds on a day when windspeed was low. The absolute calibration did not differ from the nominal calibration supplied by the manufacturer, 1 mile per hour = 10mV. The accuracy of calibration was estimated as $\pm 5\%$.

The thermistors were calibrated by placing them inside a polystyrene drink container, along with a small fan. With the fan running, the air inside the container gradually increased in temperature but remained uniform. The sensor outputs were regressed against the mean output and corrections calculated. These were of the order of 0.2 K, with an uncertainty of less than $\pm 0.01 \text{ K}$ and $\pm 0.1\%$ of the reading. No attempt was made to provide an absolute calibration, since it was only the temperature difference that was needed, but the distributor provides an estimate of $\pm 0.25 \text{ K}$.

Friction velocity was calculated from the difference in windspeeds of pairs of sensors using Equation 3 and a correction based on Equation 4. The slope of temperature against windspeed was calculated similarly, from the pairs of thermistors and anemometers. In each case, a mean was calculated and the individual estimates compared to the mean. The estimates from pairs of sensors usually agreed within the random error of measurement. Best agreement was found using the top and bottom sensors only (least scatter), so gradients derived from these readings were used in further calculations to estimate u^* and $r_{b,can}$.

RESULTS

MODELLING THE RELATIONSHIP OF WATER-USE EFFICIENCY AND YIELD TO PHYSIOLOGICAL TRAITS

Form of the results from the model

Basically, the model predicted the season-total water-use efficiency, WUE, and the annualized yield per ground area, Y , for genotypes ranging in C_i and SLM between specified limits. We assumed that C_i was maintained as a constant fraction, α , of the external CO_2 concentration, and we considered α in the range 0.6 through 0.9. (This is modified in the current model when using the model of Ball *et al.* [1987] for humidity response; a genotype is assigned an α at the limit of high humidity.) Similarly, we considered that SLM was fixed over time and was described by its average over the whole canopy, ranging between 30 and 60 . The value of SLM was taken to decrease with depth in the canopy by 5 per unit leaf-area index. The season-total WUE was computed as total shoot dry biomass per unit ground area, divided by total water use

(evaporation plus transpiration) per ground area.

Predicted yield bears further discussion, which also bears on WUE. It is clear in our model, as well as in field experiments on other plant species (Condon *et al.*, 1991), that genotypes differing in C_i and SLM are predicted to have different growth rates. It is less clear if these growth-rate differences are linked to differences in time to mature, t^* . We ran the model with two distinct alternatives: (1) All genotypes matured (reach 10% flowering) at the same final value of biomass per ground area, M_f . Let us call this the constant- M_f alternative. (2) All genotypes matured at the same chronological time, the constant- t^* alternative. In the first alternative, each genotype matured at a distinct time t^* ; longer maturation time translated to fewer cuts per growing season of length t_s . The annualized yield was estimated as $Y = (\text{shoot biomass per cutting}) \times (\text{mean number of cuttings per season}) = M_f t_s / t^*$. In the second alternative, the same formula was used, but it was M_f rather than t^* that varies among genotypes. The two alternatives gave quite different predictions of the yield consequences of changing C_i , for example, from the C_i value of an original cultivar. The two alternatives also predicted quite different changes in WUE. Even though the instantaneous WUE at a growth stage (shoot biomass density) was identical in both maturation alternatives, this WUE was a strong function of growth stage, and the two alternatives predicted differences in final biomass density.

We predicted WUE and Y for a set of 28 different combinations of C_i (7 values of α) and SLM (4 different values). We fitted the results to bivariate Chebyshev polynomials to interpret WUE or yield at any intermediate values of α and SLM.

The consequences of changing C_i and SLM upon WUE and yield were somewhat complicated but perhaps most easily grasped in contour plots such as Fig. 1 given earlier. First, one may locate the predicted WUE (WUE^\dagger) and yield (Y^\dagger) (or regrowth/maturation time) of the current cultivar (asterisk). It is apparent that a line going to lower α at fixed SLM traverses contours of increasing WUE - that is, lower α (lower C_i) confers increased WUE. Naive models of WUE of single leaves, discounting energy balance and in-canopy transport of heat and water vapor (see "Justification" section), imply much greater gains in WUE are possible. (In addition to this defect, these models cannot address yield costs.) The primary reason that WUE gains are diluted in the realistic simulation is that leaf temperature increases as C_i decreases: all else equal, especially SLM, a lower C_i can only be achieved by lower stomatal conductance and consequently lower transpirational cooling. The naive models predict a high value for what we define as S_α , the fractional change in WUE with fractional change in $\alpha = C_i/C_a$, namely, $S_\alpha = -\alpha/(1 - \alpha)$. This equals about -5.7 for the Mesilla cultivar ($\alpha = 0.85$). Accounting for the leaf temperature change reduces S_α to approximately -3. Accounting for the canopy boundary-layer resistance and for soil evaporation increases from slower growth at low C_i brings S_α down to -0.9, about one-sixth the naive value.

The initial derivative, S_{α} , is not a complete indicator of potential WUE gains: as apparent in Fig. 1, WUE has a quadratic or higher dependence upon C_i (or α , equivalently). For low SLM, dropping to very low C_i values makes WUE approach an asymptote. Gains in instantaneous WUE of gas-exchange (Equation 1) are counterbalanced by higher leaf temperature and greater time-integrated soil evaporation. The intermediate output from the model (not shown) indicates that very low α slows the predicted growth rate; the slower development of foliage cover over the soil allows high rates of evaporation from nearly bare soil to persist longer, which inflates the total water use. At high SLM, moving to low α even gives a reversing trend to lower WUE, because soil evaporation is more important when leaf area development is slow. The rapidity with which WUE gains slow and then reverse going to lower α is enhanced at high canopy boundary-layer resistances. Contours of WUE become closed ellipses. Thus, there is a maximum WUE at any SLM. In the constant- t_f case, it is primarily increased soil evaporation that strongly limits potential WUE gains (detailed analysis not shown).

Similarly, higher SLM at constant α conferred higher WUE, though to a lesser degree over the allowed ranges of α and SLM. At any fixed value of α , greater SLM connotes greater absolute transpiration rates per unit leaf area and a lower leaf temperature. This improves instantaneous WUE of gas exchange (Equation 1) by decreasing the leaf-to-air vapor pressure deficit. The rate of gain of WUE is smaller as one goes to high SLM, where leaf area development is slow and time-integrated soil evaporation is consequently larger.

The consequences of changing C_i and SLM for yield were similarly grasped from inspection of Fig. 1 and some intermediate model output on processes. In the case that all genotypes mature at the same shoot biomass density (Fig. 1), regrowth time increases as C_i (α) decreases. Simply, carboxylation rates per unit mass of enzyme decrease. Plant relative growth rate declines in parallel, because it equals photosynthetic (carboxylation) rate per unit mass of plant, multiplied by a biosynthetic conversion efficiency. The rate of photosynthesis per mass of plant is proportional, in turn, to the rate per unit leaf mass, at any given shoot biomass (fixed ratio of leaf to shoot mass). The regrowth rate also increases as SLM increases, because slower leaf area development is more important than greater diurnally averaged photosynthetic rate per unit leaf area (see "Justification" section). The optimum SLM, where regrowth time shows zero derivative with respect to SLM, is predicted to be at SLM values much lower than attained in normal physiology.

One can locate the point of maximal WUE graphically on Fig. 1. For quantitative estimates, we found it by analytical mathematical method, using the Chebyshev polynomial representations of WUE as $WUE(\alpha, SLM)$. The C_i and SLM values at this point simultaneously determine the regrowth time and thus the yield penalty for this WUE increase. One may also consider a fixed percentage gain in WUE, say, 5% above the basic cultivar's WUE, and estimate the least penalty in yield to achieve this

gain. Any value of WUE defines a curved contour traversing various combinations of α and SLM. One may find numerically the combination (α^\dagger , SLM †) along this WUE contour where yield is highest. The estimated penalty in yield is then $((Y^\dagger - Y)/Y^\dagger) \times 100\%$. This yield penalty varies with the percentage gain in WUE desired, increasing faster than linearly with the latter.

One may also estimate the value of co-selecting SLM with α , rather than selecting lower α only as in most crop-breeding programs to increase WUE. Simply, one finds the point of lower α but same SLM as the current cultivar that reaches a WUE contour that is, say, 5% above the current cultivar's WUE. One then uses the yield for this new- α genotype to estimate the yield penalty. For comparison, one may find the point of least yield penalty as noted above. The lessening of the yield penalty for the given WUE gain is attributable to co-selecting for SLM.

All the predictions of WUE and yield were sensitive to the assumed environment, particularly humidity and temperature. Thus, it was important to have realistic estimates of the environment. It was also useful to predict how much WUE advantage a new genotype retains over the old cultivar, when both would be grown in a new environment, perhaps a site distant from the breeding site. The absolute WUE of both genotypes could change, perhaps markedly; what is important for the current research is the change in estimated percentage gain by the new genotype, *e. g.*, does a 5% gain at the breeding site decrease to a 3% gain in a more humid environment? We evaluate this "transferability of gains" by rerunning the model with the new description of the environment, for both the original and new genotypes.

In summary, we estimated WUE and yield for the original cultivar and a wide range of new genotypes. We thereby estimated the maximum potential gain in WUE and its associated yield penalty; we also estimated the yield penalty for any specified WUE gain. We estimated how the percentage gain in WUE and percentage yield penalty for a new genotype relative to the original might change when both are grown in a new environment. In all these estimates, we used two plausible alternatives for how maturation time depends upon the genotype.

Predicted absolute magnitudes of WUE and yield

We ran the model with environmental descriptors (temperature, humidity, length of photoperiod, etc.) for conditions in the growth chambers. We used gas-exchange and other experimental measurements on our plants to set the parameters of plant physiology and morphology, *e. g.*, SLM, C_i , leaf area:shoot weight ratio, rate of decline of SLM with depth in the canopy, and proportionality constant between light-saturated photosynthetic rate and SLM. (A complete listing of environmental and plant parameters, and of the model, is available from V. Gutschick.) Over five cycles of regrowth and harvest, the measured mean WUE of all 40 plants ranged from 1.53 to 1.86 gDM / (g water). The model predicted a value of 1.75, well within the range. Raising the

daytime growth temperature from 28 °C to 33 °C while maintaining the relative humidity at 35% decreased WUE relatively by 35%; the model prediction was 33%. When the model was run to a fixed final biomass density of 400 g m⁻², it predicted a regrowth time of 28 days. We did not time the sward harvest by its attaining a fixed biomass density - rather, by its attaining 10% bloom. Nonetheless, the biomass density averaged 370 g m⁻², with a regrowth time ranging from 24 to 31 days. Later growth cycles involving plants of Wilson cultivar showed much delayed flowering, up to 42 days, with consequent attainment of very high biomass densities.

Predicted potential gains in WUE and of yield changes

We simulated field conditions for the Mesilla Valley in May and June, using hourly weather records. These conditions are broadly representative of those in alfalfa fields in the arid Southwest (vs. the humid Southeast, for example, where WUE responds less strongly to changes in C_i). Figure 1 presents the results graphically. The quantitative estimates of WUE changes and associated yield costs, obtained by numerically processing the contour data in Fig. 1, are summarized in Table 2.

We shall first discuss gains if genotypes with new C_i and SLM values maintain the same biomass density at harvest as the original cultivar (constant-M_f behavior). The maximal gain in WUE was significant, 13% or 15% depending upon cultivar (Wilson or Mesilla, respectively) from which one starts. The associated yield costs were high, 14% or 23%; that is, there would be fewer cuts per growing season. Costs were much smaller for more modest WUE gains. A 10% gain in WUE from Mesilla cultivar was predicted to cost only 7% in yield. WUE gains from the cultivar with a higher WUE were more costly in yield (11%). WUE gains were up to 2% greater at the same yield if both C_i and SLM are selected, rather than C_i alone. Alternatively, yield penalties for attaining the same WUE gain were reduced, but by negligible amounts, 0.2% to 0.8%. Because SLM requires very low experimental effort to measure, relative to measuring C_i, it is worth co-selecting.

No field tests have yet determined if genotypes of altered C_i and SLM mature at constant M_f or at a constant time t_f. We modelled the latter as a more extreme case. Potential gains in WUE were much lower, only 2% to 5%. Predicted yield costs for a 5% WUE gain (possible only in Mesilla cultivar) were 4% greater than in the constant-M_f case.

The model predicted good transferability of WUE gains from a given breeding environment to an environment differing modestly in temperature or humidity. We simulated an environment in which both air and sky-radiative temperatures were 5 °C higher than our base case. WUE of the original Mesilla cultivar was predicted to drop by 8.5%, that of Wilson cultivar by 10.6%. *Relative* to the Mesilla cultivar, an improved genotype (lower C_i, higher SLM) that was 5% superior in WUE was predicted to be 3.5% superior in the new environment. Its predicted yield penalty

TABLE 2. Predicted potential gains in harvest-basis WUE.

Predicted percentage gains, above original genotype, in WUE of alfalfa growing in Mesilla Valley conditions by selecting for new values of α (ratio of external/internal CO₂ concentration maintained in leaves) and specific leaf mass, SLM.

	Constant M_f : If all genotypes mature at same biomass density		Constant t^* : If all genotypes mature at same regrowth time	
	Gain in WUE	Change in yield	Gain in WUE	Change in yield
<u>Gain relative to Mesilla cultivar</u>				
If both α and SLM are selected				
Maximal gain in WUE	+14.7%	-22.9%	+5.1%	-8.1%
5% gain in WUE	≡ +5.0%	-1.1%	≡ +5.0%	-5.2%
10% gain in WUE	≡ +10.0%	-6.9%	-	-
Maximal gain at same yield	+3.8%	≡ 0%	+4.5%	≡ 0%
If only α is selected				
Maximal gain in WUE	+11.6%	-13.8%	+5.0%	-10.4%
5% gain in WUE	≡ +5.0%	-1.9%	≡ +5.0%	-9.4%
10% gain in WUE	≡ +10.0%	-7.1%	-	-
Wilson cultivar	+1.8%	+0.9%	+3.2%	+3.0%
<u>Gain relative to Wilson cultivar</u>				
If both α and SLM are selected				
Maximal gain in WUE	+12.7%	-23.6%	+1.9%	-10.8%
5% gain in WUE	≡ +5.0%	-3.9%	-	-
10% gain in WUE	≡ +10.0%	-11.1%	-	-
If only α is selected				
Maximal gain in WUE	+3.4%	-10.5%	+0.2%	-3.0%

increased from 1.1% to 2.3%. The genotype with a predicted 10% WUE gain over Mesilla in the original environment had a predicted gain of 7.5% in the hotter environment, and a yield penalty increased from 6.9% to 8.3%. For Wilson cultivar, the derived genotype with a predicted 5% WUE advantage increases its advantage slightly, to 5.3%, in the hotter environment, and has a slightly lower yield penalty of 3.5%, compared to 3.9% in the original environment. Those genotypes derived by selecting only lower C_i , leaving SLM unchanged, have similar degrees of WUE gain transferability, about 70%, and similar small changes in yield penalty. Therefore, the transferability of improved WUE-yield combinations is not predicted to be problematic in either physiological breeding strategy. Less satisfactory are predictions for changing to an environment with double the relative humidity (for example, 88% at dawn, 30% at midday). For Mesilla and its derived genotypes, a WUE gain of 5% drops to 2.5% in the more humid environment, while yield penalty rises from 1.1% to 2.5%.

We also predicted the effects of alternative magnitudes of the canopy boundary-layer resistance, $r_{b,can}$. This resistance is a function of the turbulent-diffusion coefficient (dependent on windspeed and canopy "roughness;" see Monteith and Unsworth 1990) and the distance over which transport must traverse from canopy top to "bulk atmosphere." This distance depends upon the scale of the cropped field, or fetch, in ways that are loosely defined to date but approximate scaling as $x^{1/7}$ (Sutton 1953). Moving from the scale of Mesilla Valley fields, hundreds of meters, up to kilometers, the value of $r_{b,can}$ might double. Our simulations with doubled $r_{b,can}$ showed a strong suppression of WUE differences among genotypes and thus of potential WUE gains. In terms of gain transfer, the genotype with 10% WUE advantage over Mesilla in the original environment had only a 4.8% advantage in the high- $r_{b,can}$ environment. The yield penalty increased a small amount, 0.6%.

Estimates of WUE gains from selecting other traits

Table 3 summarizes studies on altered leaf angle, solar tracking, leaf size (boundary-layer resistance), and chlorophyll content (absorptivity for PAR). Only lower chlorophyll content has significant promise, and then only for large decreases in chlorophyll (50%, in the Table). In fact, larger WUE gains might accrue from this than from selecting C_i and SLM, and with less yield penalty. Evidence that Gutschick's (1984b) prediction of yield gains is correct (Pettigrew *et al.* 1989) bolsters the promise of this path to raise WUE. The major barrier is genetic. Genotypes with reduced chlorophyll content but unaltered carboxylation capacity and unaltered SLM are known in many species but tend to be non-true-breeding heterozygotes of a recessively lethal mutation. Some true-breeding homozygotes are known (*ibid.*), but not in alfalfa.

TABLE 3. Predicted potential to increase WUE by selecting other traits.

Predicted percentage gains in harvest-basis water-use efficiency of alfalfa growing in Mesilla Valley conditions by selecting for new values of other physiological traits as well as α and SLM. Yield changes in last column given in parentheses are evaluated using the regrowth time for genotype with new trait.

Change in other trait	Change relative to Mesilla cultivar in base environment			
	Constant M_f : If all genotypes mature at same biomass density		Constant t^* : If all genotypes mature at same regrowth time	
	Change in WUE	Change in yield	Change in WUE	Change in yield
30% reduction in PAR absorptivity				
With unchanged $\alpha = 0.85$, SLM = 40 g m ⁻²	+3.9%	+5.5%	+4.1%	+5.5%
Choosing α , SLM for max. WUE	+18.6%	-16.4%	+8.3%	(-6.0%)
Choosing α , SLM for 5% gain	≡+5.0%	+7.3%	≡+5.0%	+11.7%
Choosing α , SLM for 10% gain	≡+10.0%	+3.2%	-	-
Choosing α , SLM for 15% gain	≡+15.0%	-3.0%	-	-
Leaf erectness (zenith angle $\rightarrow 70^\circ$)				
With unchanged $\alpha = 0.85$, SLM = 40 g m ⁻²	-2.3%	-4.7%	-2.6%	-4.7%
Choosing α , SLM for max. WUE	+8.4%	-20.2%	+5.6%	(+5.3%)
Diahleiotropism as observed in field				
With unchanged $\alpha = 0.85$, SLM = 40 g m ⁻²	-0.8%	+0.5%	-0.8%	+0.5%
Choosing α , SLM for max. WUE	+15.6%	-22.4%	+5.2%	(-12.7%)
Narrow leaves (half size)				
With unchanged $\alpha = 0.85$, SLM = 40 g m ⁻²	-2.0%	+0.6%	-2.0%	+0.6%

Sensitivity of predictions to accuracy in process modelling

The requirements for accurately modelling the roles of $r_{b,can}$, maturation behavior, leaf energy balance, and soil evaporation are apparent in the discussion above. Some further discussion is merited. Gutschick (1991a) has noted that some simplified and plausible models of diffuse-skylight interception by leaves lead to very inaccurate predictions of how canopy photosynthetic rate, P_{can} , depends upon leaf angle. Gutschick and Wiegel (1988) have noted how the profile of SLM with depth in the canopy alters predicted photosynthesis and makes P_{can} resistant to changes in average SLM, clearly important for the current modelling effort. A model not incorporating soil energy balance (evaporation and sensible heat generation that alters air temperature around leaves) gives misleadingly high estimates of WUE gains from altered leaf angle (10% to 20%; compare to Table 3). A good model must include scattering of PAR from leaves; this scattered radiation drives about one-fifth of total photosynthesis and at favorable transpiration ratios. Finally, propagation of thermal infrared radiation deserves accounting. It is significant for soil temperature affecting leaf temperature, especially in early growth when soil occupies a large fraction of geometrical solid angle in the view from any leaf.

EXPERIMENTS IN CONTROLLED ENVIRONMENTS

Normal morphology and phenology; mean WUE and yield

Our growth conditions were reasonable simulations of field conditions, not in detailed time courses but in average temperature, photoperiod length, irradiance, and such. As evidence, we note that the plants showed rather normal growth, morphology, and phenology. The sward typically matured in 28 to 35 days, attained yields of 300 to 400 g m⁻² that are equivalent to 3 to 4 metric tonnes per hectare, and reached a height of 75 to 100 cm (blowers that move plant stems reduced the spindliness typical of other growth chamber experiments). The average values of SLM were low, about 60 to 75% those of field-grown plants. A hypothesis we present is that growth-chamber lighting, coming from 6 separate lamps, is more diffuse in character than most sunlight. Lower SLM is adaptive in diffuse light, which does not much exceed light-saturation levels of leaves over most of the canopy as does direct sunlight. A detailed analysis of canopy light distribution is worth pursuing, both theoretically and experimentally. The research experience of Gutschick is good background for such a study, which is not directly relevant to present purposes and is not presented here.

It is interesting that the mean WUE of Wilson cultivar was not significantly different from that of Mesilla cultivar. This was seen in comparison of means of the cultivars in all growth cycles. Wilson seemed to be superior in water-production function, WPF, which is $\partial \text{Yield} / \partial (\text{water use})$ (see, for example, Currier *et al.* 1987). The

WPF involves more than WUE, however. This lack of WUE difference is not important to our hypothesis, particularly since the two cultivars did show small but statistically significant differences in mean carbon-isotope ratio, $\delta^{13}\text{C}$, within any one cycle. Wilson has a $\delta^{13}\text{C}$ value that is about 0.4‰ higher when all cycles are pooled as standard scores. This is equivalent to a α that is lower by about 0.013, which might be expected to confer a marginally higher WUE - about 2.5%, too small to be significant in our scale of experiments.

Adequate genetic variation in C_i and SLM

Experimental tests in growth chambers with simulated swards comprised the second, critical part of our first objective, the first test of proof of principle. Within this test, the first mandate was verifying the existence of significant, presumably genetic variation in both C_i and SLM. Table 4 shows that significant variation always existed and that it was both genetic and environmental. For example, short-term variation in C_i (measured by gas exchange) always exceeded the long-term variation in C_i inferred from isotopic ratios, $\delta^{13}\text{C}$ (column " σ_α ," in quotes). That a significant portion was genetic is implied by (1) the strong correlation of SLM from one cycle to the next and across treatments differing in temperature, and similarly for $\delta^{13}\text{C}$; and (2) the more direct tests of heritability, at least for SLM; these are discussed later. Table 5 demonstrates the inter-cycle correlations of SLM and $\delta^{13}\text{C}$. There were also developmental or phenological trends in SLM, in particular: SLM decreased sharply toward maturity, causing the notable variations in mean SLM between cycles, while the rank order of genotypes was largely preserved.

Rotating the plants among positions within the growth chamber to average out microenvironmental differences significantly improved the reproducibility of WUE, yield, and traits between growth cycles. Older data obtained without rotating plant positions typically showed values of r^2 about 2/3 as large as in Table 5 (analyses not shown for brevity).

The degrees of variation in C_i and SLM were very much adequate to drive large variations in single-plant WUE and yield, as will be apparent in a section below.

Origins of C_i variations in conductance variations

Variations in C_i can arise from variations in stomatal conductance, g_s , in mesophyll conductance, g_m , or in both; see Equation 2, earlier. Only the ratio g_m/g_s matters, so that relative deviations in either have the same effect on C_i , to a close approximation. Among peanut genotypes, it is principally g_m that varies (Wright 1992), while among Australian wheat genotypes both g_s and g_m contribute about equally to variations in C_i (Condon 1992).

TABLE 4. Significant variation in C_i and SLM among genotypes.

Variation is within cultivars Mesilla (M) and Wilson (W) or combined (C). Cycle dates are as months/year. SLM is in $g\ m^{-2}$, C_i is given as $\alpha = C_i/C_a$ for two irradiance levels (1=high, 2=low), and $\delta^{13}C$ is in per mil relative to PDB standard. Means are indicated by superior bars and standard deviations are indicated with σ . Variation in $\delta^{13}C$ has been converted to equivalent variation in α , using $\delta^{13}C = -4.4\text{‰} - (23\text{‰})\alpha - \delta^{13}_{air}$, or " σ_α " = $\sigma_\delta/23$. To reduce artifactual variation, we culled all outliers exceeding 2.5 σ . Sample sizes range from 8 to 20.

Cv.	Cycle	\overline{SLM}	σ_{SLM}	$\bar{\delta}$	σ_δ	" σ_α "	$\bar{\alpha}_1$	$\sigma_{\alpha,1}$	$\bar{\alpha}_2$	$\sigma_{\alpha,2}$
C	4-5/89	21.9	6.4	-30.37	0.56	0.024	0.805	0.032	0.824	0.040
C	5-6/89	27.8	5.5	-30.50	0.88	0.038	0.801	0.044	0.821	0.040
W	2-4/90	17.4	5.4	-	-	-	0.794	0.041	0.839	0.057
M	2-4/90	23.8	4.4	-	-	-	0.756	0.051	0.829	0.029
M	4-6/90									
W	7-8/90	31.2	4.2	-31.09	0.33	0.014	0.726	0.095	0.726	0.096
W	9-10/90	24.5	3.5	-30.94	0.24	0.010	0.726	0.082	0.722	0.049
M	10-11/90	27.9	5.9	-31.28	0.617	0.027	0.706	0.101	0.813	0.058
M	11-12/90	24.1	2.8	-32.06	0.653	0.028	0.758	0.035	0.801	0.091

TABLE 5. Stability of traits, WUE, and yield between growth cycles.

Values of WUE (shoot only), Yield (shoot only), SLM, and $\delta^{13}\text{C}$ (both, for second 10-cm section from top) in each of 5 growth cycles are regressed against same values in next growth cycle. Cycles are identified by months spanned in 1989. Sample size N, not shown for brevity, is 9 to 15, except N = 5 to 8 for $\delta^{13}\text{C}$. Data from plants with yield < 1 g were culled, as also outliers at 2.5 σ . Significance $p < 0.05$ shown in boldface.

Cycles	WUE		Yield		SLM		$\delta^{13}\text{C}$	
	r	p	r	p	r	p	r	p
	4-5 vs. 5-6	0.748	0.0033	0.788	0.0008	0.416	0.1575	0.480
5-6 vs. 6-7	0.775	0.0011	0.907	0.0001	0.579	0.0486	-	-
6-7 vs. 7-9	0.712	0.0043	0.668	0.0065	0.447	0.1456	-	-
7-9 vs. 9-10	0.595	0.0247	0.684	0.0070	0.751	0.0031	0.800	0.1040
9-10 vs. 10-11	0.944	0.0001	0.863	0.0028	0.940	0.0002	0.938	0.0185
Wilson								
4-5 vs. 5-6	0.636	0.0081	0.766	0.0003	0.847	0.0001	0.436	0.2800
5-6 vs. 6-7	0.779	0.0028	0.906	0.0001	0.734	0.0102	-	-
6-7 vs. 7-9	0.895	0.0001	0.347	0.1725	0.847	0.0005	0.722	0.0280
7-9 vs. 9-10	0.803	0.0003	0.887	0.0001	0.651	0.0063	0.823	0.0060
9-10 vs. 10-11	0.838	0.0001	0.878	0.0001	0.667	0.0067	0.822	0.0036
Combined								
	$\delta^{13}\text{C}$							
	r	p						
4-5 vs. 5-6	0.556	0.0254						
5-6 vs. 6-7	-	-						
6-7 vs. 7-9	0.753	0.0047						
7-9 vs. 9-10	0.712	0.0043						
9-10 vs. 10-11	0.867	0.0001						

Variations in both C_i and g_m matter for determining WUE, because higher g_m at a given C_i implies higher g_s and greater transpirational leaf cooling, hence, higher WUE. Restated, both C_i and SLM (as a surrogate for g_m) matter. We tracked both variables and our models explicitly address the effect of SLM (and g_m) upon WUE. Nonetheless, for comparison to other species, it was of interest to assess the origins of variation in C_i . We did regression analyses of $\alpha = C_i/C_a$ upon g_s and g_m obtained in gas exchange, with g_m approximated at $P_{L,a}/C_i$, that is, ignoring the unmeasured Γ . For one representative growth cycle (February-April, 1990), g_s explained 68% of the variance in α , while g_m explained 0%. For Wilson cultivar in the same growth cycle, the corresponding figures were 59% and 1%. (The values do not sum to 100% because Γ also varies and because we are using linear regression on a relationship that is weakly nonlinear over the data ranges.) Thus, alfalfa represents the other extreme from peanut.

Stability and transferability of C_i and SLM between environments

Concerns about stability and transferability were partly answered affirmatively above. We note that three of the inter-cycle comparisons discussed above involved shifts up or down in temperature between 28°C and 33°C. A further test is the stability of genotype rank order, in either trait, between growth chamber and the field experiment with 68 soil columns. We obtained SLM data for field-grown plants only for two growth cycles later in the season (July-September and September-October). These SLM values show no significant correlation with SLM values of the same genotypes grown in the growth chamber (any of four cycles in 1989). The $\delta^{13}\text{C}$ values of field-grown plants, currently available for only the July-September cycle, show no significant correlation with $\delta^{13}\text{C}$ values for the same genotypes grown in the growth chamber (two cycles).

Tests of fundamental relations of traits to WUE and yield

Our model predicts quantitative relationships between the two traits, C_i and SLM, on the one hand and WUE and yield on the other hand. That is, it predicts that such quantities as $\partial\text{WUE}/\partial C_i$ and $\partial\text{WUE}/\partial\text{SLM}$ have opposite signs (respectively, negative and positive) and different magnitudes (greater for the first). (The model also predicts absolute magnitudes, but in the field these may be altered by a host of "smaller," unaccounted factors, *e.g.*, soil factors affecting rooting depth.) The predictions are specifically for WUE and yield of uniform stands of a genotype. The model does not predict variations of WUE and C_i within a stand in which C_i and SLM vary among individuals. However, it is just this intra-stand variation that we can analyze in our growth-chamber experiments, which cannot be run for many, separate, sizeable groups of plants all at once. Our regression analyses on individuals within a stand will

magnify the slopes of some relationships. For example, a plant with low C_i will achieve higher WUE at the leaf level; at the canopy level, it will not suffer the whole-stand compromising factor of lower canopy humidification, because the rest of the plants, of higher mean C_i , will be humidifying the canopy. Similarly, the yield penalty for having low C_i may be magnified: the generally faster-growing plants of higher mean C_i will progressively shade the slower-growing plant, further slowing its growth. Nonetheless, the algebraic sign of the regression coefficient and of the beta coefficient (slope) should agree with that of the sward model. The proportion of variance explained by a given factor will differ between within-sward and between-sward analyses, because the magnifications of slopes will vary with the particular relationship. Only the field sward experiments are definitive tests of the fundamental WUE and yield relationships. The growth chamber experiments serve as preliminary tests. They also serve to identify variation in C_i and SLM, test heritability, and identify parents to breed plants to establish the field swards.

With these caveats in mind, we proceed to regression analyses on individuals within swards in the growth chambers. We use only $\delta^{13}\text{C}$ as a measure of long-term C_i or α , rather than values obtained in short-term gas exchange. The superiority of $\delta^{13}\text{C}$ as a whole-cycle indicator of C_i or α is discussed later. We also note that we present only shoot yield and shoot-basis WUE in our analyses. Although root growth represents a significant part of total biomass, about 25%, the regression results are qualitatively just the same using shoot mass only. We avoid inaccuracies in assigning root growth to individual cycles thereby, as well.

We present here three levels of analysis: single regressions, multiple regressions, and path analyses. The single regressions in Table 6 test the model-predicted relationships only insofar as the effects of C_i and SLM act directly to determine WUE and yield. Indeed, we see that C_i does determine WUE quite strongly. Its effect on yield, however, is opposite to model predictions: higher $\delta^{13}\text{C}$, which means lower C_i , correlates with higher yield. The third relationship in Table 6 gives a clue, that higher SLM also seems to drive higher WUE, by its ties to higher $\delta^{13}\text{C}$ (lower C_i) seen in the fourth relationship. This, however, is puzzling in itself: the discussion above on origins of variations in C_i seems to establish that C_i varies because stomatal conductance varies, not because mesophyll conductance - which is closely related to SLM - varies.

Continuing with the single regressions, we note that higher SLM is strongly related to higher yield. The slope of the regression, not reported here, is equivalent to a 10% change in SLM driving a 20% gain in yield - notably more than our model, or any model, predicts. The multiple regressions show that the more direct effect of SLM on yield is weak. Finally, WUE is strongly and positively correlated with yield. This is contrary to model predictions and cannot be explained by the plants being water-limited: in fact, they always had excess water, for our method of measuring water balance. The multiple regression sheds little light on the origin of this relationship.

TABLE 6. Relationships of traits to WUE and yield, from single regressions.

“Expected algebraic sign” is relative to canopy model, assuming that direct effect is being measured in regression; (Yes) in parentheses indicates support may be fortuitous, from linkage of traits examined in next regression below (indented column); “-” indicates that model has no prediction of linkage. Significance values $p < 0.0001$ are given as 0.0001. Boldface indicates $p < 0.05$. Order of growth cycles is same for all relations.

Relation	r	Expected algebraic sign?	N	p	Cycle
$\delta^{13}\text{C} \rightarrow \text{WUE}$	0.485	Yes	23	0.0191	Apr-May
	0.845	Yes	20	0.0001	May-Jun
	0.647	Yes	17	0.0050	Jul-Sep
	0.749	Yes	16	0.0008	Sep-Oct
	0.846	Yes	16	0.0001	Oct-Nov
$\delta^{13}\text{C} \rightarrow \text{Yield}$	0.592	No	24	0.0191	
	0.762	No	21	0.0001	
	0.866	No	15	0.0001	
	0.422	-	16	0.1039	
	0.665	No	16	0.0049	
SLM \rightarrow WUE	0.855	(Yes)	40	0.0001	
	0.689	(Yes)	36	0.0001	
	0.823	(Yes)	33	0.0001	
	0.699	(Yes)	29	0.0001	
	0.800	(Yes)	25	0.0001	
SLM $\rightarrow \delta^{13}\text{C}$	0.643	-	40	0.0001	
	0.757	-	21	0.0001	
	0.814	-	17	0.0001	
	0.902	-	16	0.0001	
	0.892	-	16	0.0001	
SLM \rightarrow Yield	0.885	Yes	40	0.0001	
	0.746	Yes	35	0.0001	
	0.821	Yes	33	0.0001	
	0.552	Yes	29	0.0019	
	0.767	Yes	26	0.0001	
Yield \rightarrow WUE	0.912	No	40	0.0001	
	0.846	No	33	0.0001	
	0.924	No	33	0.0001	
	0.935	No	30	0.0001	
	0.863	No	25	0.0001	

However, we realized that larger plants gained some WUE by virtue of size alone, independent of their C_i and SLM values. They shielded the soil more extensively than small plants and suppressed more of the soil evaporation. Evaporation amounts to 10% to 25% of mean water use, even with a vapor barrier on the soil, because soil is constantly wet in our protocol. The path analyses given later verify this linkage, while the quantitative relationship is not firmly established thereby.

The multiple regressions of WUE on $\delta^{13}\text{C}$ (for C_i) and SLM, Table 7, further establish that C_i is actually the primary determinant of WUE. The multiple regression of yield on $\delta^{13}\text{C}$ and SLM compounds the puzzle that low C_i appears related to high yield. High SLM is seen as weakly related to higher yield, more in line with model expectations.

The path analysis diagrammed in Fig. 2 gives the most satisfactory explanation of the relationships of traits to WUE and yield, if not wholly satisfactory. The path coefficients are derived by the methods of Sewall Wright (see, for example, Sokol and Rohlf 1981) and represent as well as possible the direct effects of one quantity on the other. Path analysis requires that mechanistic links be either known *a priori*, as in our case, or established on multiple data sets. We implemented the analysis in the SASS package, with the help of D. Clason at our university and R. Wasserstein at Washburn University.

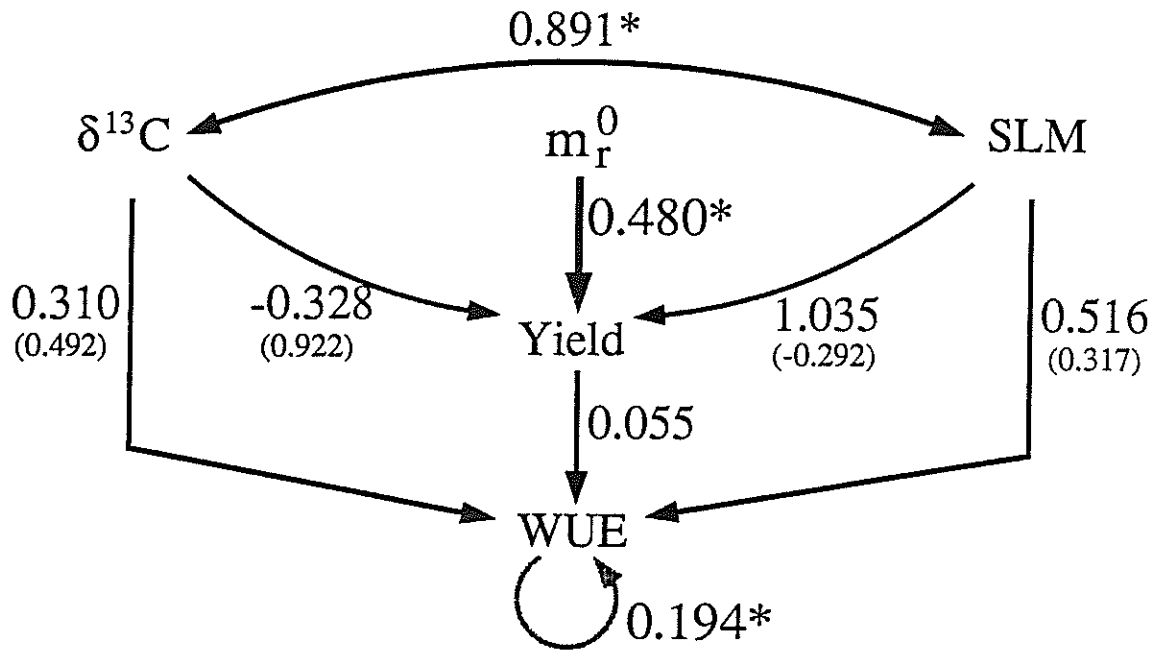
Our initial path analyses, which used only C_i (as measured by $\delta^{13}\text{C}$) and SLM as determinants of WUE and yield, had large residuals. Moreover, these analyses did not indicate strong determination of WUE and yield by C_i and SLM. Typical values of path coefficients for $\delta^{13}\text{C} \rightarrow$ or for SLM \rightarrow WUE were only 0.1 to 0.2 in data sets for single cycles and even for all cycles pooled. We realized that initial plant size was the strongest determinant of yield and of yield's indirect effect on WUE. This size is uncorrelated with C_i or SLM; it reflects primarily the vagaries of plant replacement. We therefore added the mass of root plus crown at the beginning of the growth cycle as a third determinant of yield. We denote this as m_r^0 in Fig. 2. This figure shows that m_r^0 is indeed a strong determinant of yield. Moreover, accounting for its effects makes clearer the roles of C_i and SLM. The path coefficients for $\delta^{13}\text{C} \rightarrow$ WUE are typically of magnitude 0.3 for both the direct path and indirect path (in which C_i , as measured by $\delta^{13}\text{C}$, affect SLM and yield, which in turn affect WUE). The positive sign is satisfying, indicating that high $\delta^{13}\text{C}$ or low C_i confers increased WUE. The path coefficients for SLM \rightarrow WUE are also typically large but variable, from 0.2 to 0.9, and positive.

In all the path analyses, an unsatisfying aspect is the variability of path coefficients from one data set to another. The pooled data set might be expected to give the most reliable answers. However, the path coefficients for SLM \rightarrow WUE are -0.124, direct, and 0.376, indirect. The occasional variability in growth conditions, deliberate and inadvertent, undoubtedly contributes to the problem. Later analyses will

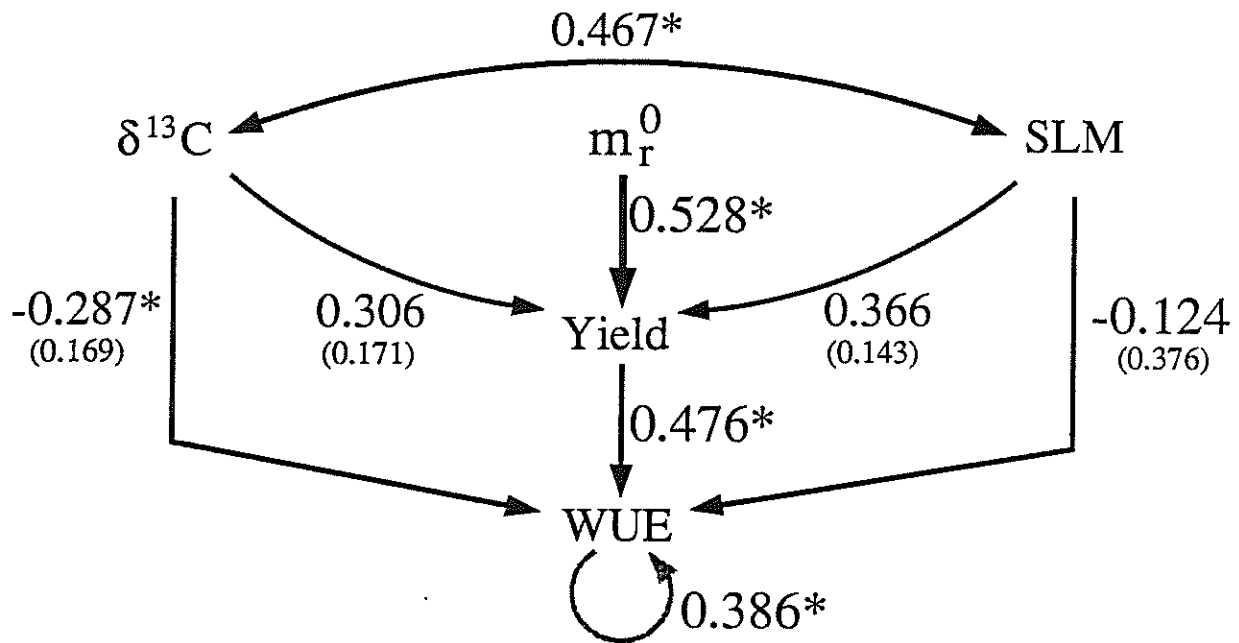
TABLE 7. Relationships of traits to WUE and yield, from multiple regressions.

“Expected algebraic sign” is relative to model predictions; possibilities are as noted in Table 6. Boldface indicates significance $p < 0.05$.

Cycle	$\delta^{13}\text{C}$			and	SLM	\rightarrow	WUE(shoot)
	Beta slope	Expected algebraic sign?	p	Beta slope sign?	Expected algebraic	p	Overall p
Apr-May	0.027	-	0.76	0.024	-	0.068	0.0370
May-Jun	0.243	Yes	0.0156	0.012	-	0.49	0.0004
Jul-Sep	0.120	-	0.5337	0.038	-	0.1117	0.0061
Sep-Oct	0.308	Yes	0.0239	-0.014	-	0.4200	0.0034
Oct-Nov	0.385	Yes	0.0082	-0.010	-	0.5871	0.0002
	$\delta^{13}\text{C}$			and	SLM	\rightarrow	Yield (shoot)
Apr-May	1.083	-	0.2082	0.249	Yes	0.0482	0.0023
May-Jun	3.791	No	0.0018	-0.092	-	0.6302	0.0004
Jul-Sep	2.316	-	0.2510	0.326	-	0.1835	0.0030
Sep-Oct	3.424	-	0.1463	-0.281	-	0.3918	0.1916
Oct-Nov	4.323	No	0.0253	-0.274	-	0.2793	0.0123



Path coefficients in single growth cycle, July-August 1990



Path coefficients for pooled data of 10 growth cycles, 1989 and 1990

Figure 2. Path analyses to estimate direct and indirect effects of factors on water-use efficiency, shoot basis (WUE). Larger coefficients correspond to stronger effects. Numbers in parentheses are coefficients for indirect effects. Number in parentheses by closed cycle at WUE is a coefficient of nondetermination of WUE.

use data converted to standard scores within a growth cycle before pooling. In any event, the residual in WUE, indicative of the degree of nondetermination by our three chosen physiological factors, is typically modest in magnitude, about 0.2 to 0.35, which is desirable.

Path coefficients for $\delta^{13}\text{C} \rightarrow \text{yield}$ and $\text{SLM} \rightarrow \text{yield}$ are commonly quite reasonable in individual growth cycles, as in the top of Fig. 2. The path coefficients in the pooled data cycle are not satisfactory, perhaps reflecting the variability in growth conditions between cycles.

In summary, the model prediction most commonly used in other breeding programs, that lower C_i confers higher WUE, is clear at all levels of regression analysis. The other three predictions of our model relating SLM to WUE and SLM and C_i to yield, are readily obscured in simple and multiple regressions, only becoming clear in path analyses; the predicted relationship of SLM to WUE is not well supported, also. The net effect is that selection for lower C_i , via higher $\delta^{13}\text{C}$, and higher SLM, does confer higher WUE, as the model predicts. It also confers higher yield in the competitive-growth conditions we are analyzing. It remains to see if these relationships hold in the field. Field results will be available in late 1992 and will be issued in an addendum to this report.

Heritability of C_i and SLM

We estimated heritability from the following formula,

$$h = \frac{\bar{x}_{\text{HH}} - \bar{x}_{\text{pop}}}{\bar{x}_{\text{PH}} - \bar{x}_{\text{pop}}}, \quad (5)$$

where the mean values \bar{x} of the trait are subscripted HH for progeny of high x high parent crosses, HP for high-trait parents, and *pop* for the population. In each of the four crosses, high x high, high x low, low x high, and low x low, the seed was inadvertently bulked during production of seed for swards. It was impractical to grow sufficient numbers of the original population simultaneously with the crosses and parents. We therefore estimated the population mean as that of all the four crosses (respectively having 6, 6, 4, and 5 plants represented) plus the parents (7 high, 5 low). With this substitution, we obtained $h = 0.62$. Dropping the parents from the population pool, we obtained $h = 0.69$.

The second estimator of heritability, the regression coefficient of the progeny trait value upon the parental trait value, could not be derived because the progeny had been bulked inadvertently.

Regrettably, we cannot estimate the heritability of C_i as $\delta^{13}\text{C}$. We chose parent plants for high and low C_i from early gas-exchange measurements, because the crossing process required a long time and $\delta^{13}\text{C}$ measurements imposed an additional 6-

month leaf time in 1989. We subsequently verified that C_i from gas exchange has high non-genetic variability. One consequence is that our designated high- and low- C_i groups did not differ significantly in C_i . This problem emphasizes the need to use $\delta^{13}\text{C}$ rather than gas exchange in all stages of breeding selection.

Assessing gas-exchange vs. isotopic ratio to measure C_i

Gas exchange measures C_i quite directly and the results can be available quickly. Isotopic analysis of plant tissue for $\delta^{13}\text{C}$ requires some sample preparation and waits for mass-spectrometric analysis. It also is costly, from \$6 to 45 per sample, depending upon the accuracy desired, the individual laboratory, and whether one provides the laboratory with dry tissue or gas from combusted tissue. One must not neglect costs for gas exchange, of course, for which the instrument costs between \$12,000 and \$20,000. Operating it accurately and efficiently requires much training and considerable expenditure, so that salary is a significant part of the budget for a selection program using gas exchange. From data analyses given here and from related experience of other researchers, we argue that $\delta^{13}\text{C}$ is the more cost-effective method. We argue later that $\delta^{13}\text{C}$, while somewhat costly, is probably more cost-effective than lengthy direct selection for WUE.

We demonstrated rather directly that C_i obtained by careful gas exchange at any one time is not related closely to C_i averaged over the growth cycle, nor, therefore, to whole-cycle WUE:

- (1) First, $\delta^{13}\text{C}$ but not C_i (α) values of plants correlated well between growth cycles.

Even between two growth cycles at the same nominal growth conditions, the C_i values (as α , to reduce noise from C_a variations) showed poor correlation. In the April-June 1989 cycle with both cultivars mixed together in the sward, we found $r^2 = 0.16$ ($N=10$) between Mesilla plants, and 0.11 ($N=12$) between Wilson plants. Both values were for α in high light. For low light ($400 \mu\text{mol m}^{-2}\text{s}^{-1}$), the respective values were 0.06 and 0.35. Between the February-April 1990 and April-June 1990 cycles, with cultivars growing separately, the Mesilla plants showed $r^2 = 0.06$ ($N=23$) for α in high light and $r^2 = 0.12$ ($N=21$) for α in low light. The other growth cycles showed the same pattern.

This lack of consistency in α between cycles was not simply from measurement error. The α values in high and low light were well correlated with each other, *e. g.*, $r^2 = 0.51$, $N = 28$, $p = 0.0001$ for Mesilla plants within the February-April 1990 cycle, and $r^2 = 0.627$, $N = 27$, $p = 0.0001$ for Mesilla and Wilson plants together in the June-July 1989 cycle.

In contrast to low inter-cycle stability of α in short-term gas exchange, $\delta^{13}\text{C}$ values were significantly correlated for the same plant between different growth

cycles; see Table 5, earlier.

These distinct patterns between C_i and $\delta^{13}C$ have been found by other researchers, such as Hall (1992).

- (2) Second, $\delta^{13}C$ but not C_i (α) values correlated with WUE.

We considered five growth cycles in 1989. In single regressions against WUE, $\delta^{13}C$ was almost always significant, as noted above. In contrast, α in either high or low light was significant in a limited number of cases. For example, α , but only in high light, was significant in the June-July cycle when the Mesilla cultivar was considered separately ($n = 15$, $p = 0.04$). When both cultivars were pooled, α in either high or low light was significant, at $p = 0.014$ and 0.026 , respectively. In the September-October cycle, α was never significant.

- (3) Third, values of C_i or α correlated poorly with $\delta^{13}C$ values.

For five growth cycles in 1989 (as in Table 5), the regression of $\delta^{13}C$ against α for either high or low light was significant at $p < 0.05$ in only one cycle, May-June. In this case, $\delta^{13}C$ became more negative as α increased, as expected ($r = -0.595$ at high light, -0.635 at low light).

FIELD TESTS WITH INDIVIDUAL PLANTS IN SOIL COLUMNS

Normal morphology and phenology; mean WUE and yield

The plants in soil columns were virtually indistinguishable in height and morphology from plants of the surrounding fields, when they were harvested and regrown in synchrony with the latter. We took data from such synchronous cycles. The mean WUE of Wilson (1.749 ± 0.502 gDM / (kg water)), did not differ significantly ($p = 0.554$) from that of Mesilla (1.652 ± 0.614). Both means were in the ranges seen in field studies with swards (implicit in data of Currier *et al.* 1987). Shoot yields per plant did not differ significantly (6.6 ± 4.5 g for Mesilla, 8.8 ± 3.94 g for Wilson; $p = 0.0935$). On an areal basis, with 60 plants per m^2 , the mean yield was equivalent to 4.6 metric tonnes / ha, in the range of good commercial yields. Mean SLM in the July-September growth cycle with best condition of plants was 33.3 ± 6.1 g m^{-2} for Mesilla and 35.2 ± 4.5 g m^{-2} for Wilson. These values were not significantly different from each other ($p = 0.25$) and were in the normal range for field plants. The mean $\delta^{13}C$ values (July-September cycle) in units of per mil were 28.30 ± 0.78 for Mesilla and -28.18 ± 0.75 for Wilson. These are not significantly different from each other. They are about 2 per mil higher than in the growth chamber because air in the latter has significant admixture of human-respiratory and fossil-fuel CO_2 .

Adequate genetic variation in C_i and SLM

The variability of $\delta^{13}\text{C}$ is about 1.5 times larger than in the growth chamber, and likewise is adequate for breeding. The variability in SLM, noted above, is very close to the variability seen in the growth chamber (see above), and is adequate for breeding by our proposed scheme. We did not propose to test heritability of either trait in the field, in this study. Results of other researchers support significant heritability. The recent results for $\delta^{13}\text{C}$ are notable (Hubick and Farquhar 1989; Johnson *et al.* 1990; Hall 1992; Wright 1992).

Stability and transferability of C_i and SLM between environments

The values of SLM correlated poorly between the two growth cycles for which we have SLM measurements: $r = 0.049, 0.163$ and $p = 0.87, 0.52$ for Mesilla and Wilson, respectively. The cause of variation is unknown. The value of SLM in the field in 1991 was also poorly correlated with its value for clones of the same plants in the growth chamber: for Mesilla alone, Wilson alone, or both pooled, the value of p never was less than 0.20. Because $\delta^{13}\text{C}$ measurements are in progress, we cannot assess their stability. Yield was significantly preserved between the two cycles: $r = 0.758, p = 0.0003, N = 47$.

Tests of fundamental relations of traits to WUE and yield

SLM was positively related to yield in single regressions. In the July-August 1991 cycle, we found $r = 0.662, N = 13, p = 0.0137$ for Mesilla alone, and respectively 0.331, 19, 0.16 for Wilson and 0.535, 32, 0.0016 for both cultivars pooled together. SLM was also marginally significantly related to WUE. For all plants pooled together, $r = 0.295, N = 45, p = 0.049$. These are the only tests possible until $\delta^{13}\text{C}$ data are obtained.

FIELD TESTS IN SWARDS

These tests are all in progress. Results will be reported in an addendum to this report, to be issued in about one year. Ninety-six plots have been established for the 3-factor experiment (8 populations x 3 water treatments, 4 replicates each). Seedlings were sprouted from seed derived from bee-box crosses and then transplanted into the field at a density of 100 plants m^{-2} . Border strips of Mesilla cultivar were established from seed. The swards appear to be well established and will be measured in summer, 1992. Neutron-probe access tubes have been placed to measure soil water balance.

SURVEYS OF CANOPY BOUNDARY-LAYER RESISTANCES

During April, 1989, we measured only windspeed, so were unable to correct for atmospheric stability. Nevertheless, there was a good relationship between u^* and u , at least for higher windspeeds, as shown in Fig. 3. For $u > 2.5 \text{ m s}^{-1}$, u^* is approximately proportional to u , with proportionality constant 0.116 ± 0.001 for the period 14-18 April 1989.

In May, we corrected for stability using Equation (3) above. Figure 4 shows the windspeed and Richardson number Ri for May 21st, showing that the air above the canopy was unstable in the early morning when windspeed and VPD were both low, then became stable by 10 a.m. as the windspeed and temperature increased, and remained so during the afternoon. This pattern was quite regular, only changing if the air temperature was high early in the morning, when stable conditions would precede the short period of instability during mid-morning.

Figure 5 shows the corrected value of u^* plotted against u for the period 12-15 May 1989. The effect of the stability correction has been to produce a marked bifurcation of the graph at low windspeeds, the upper branch corresponding to unstable conditions, the lower to stable conditions. For $u > 3.0 \text{ ms}^{-1}$, these branches came together, and u^* was again proportional to u with proportionality constant 0.095 ± 0.001 . Later in the month, this bifurcation was less obvious but still present (not shown). For windspeeds above 2.0 ms^{-1} , u^* was given by $0.099 \pm 0.001 u$ for the period 18-23 May, and $0.113 \pm 0.001 u$ for 26-29 May.

Figure 6 shows the boundary-layer resistances, $r_{b,can}$, for all daylight measurements in May. Although very scattered, particularly at low windspeeds where the stability correction was often large, they followed a similar trend to that shown by the line $r_{b,can} = 100/u$ as plotted. This corresponds to $u^* = 0.1 u$, as indicated by the data at higher windspeeds. This value is twice as large as the one we used in our model simulations but corresponds to a wind path length or fetch larger than the alfalfa fields we have modelled. The fetch requirements remain controversial. When the resistances were plotted against time-of-day, there was little obvious trend, despite there being large differences in average windspeed during the day (from 1.5 m s^{-1} just after dawn to 5.5 m s^{-1} in mid afternoon). The line shows the resistance calculated from the average windspeed over the same period in two-hour intervals during the day. Obviously, there have been many occasions when unstable conditions have reduced the resistance, particularly in the early morning and late afternoon, and conversely stable conditions during the day have greatly increased $r_{b,can}$.

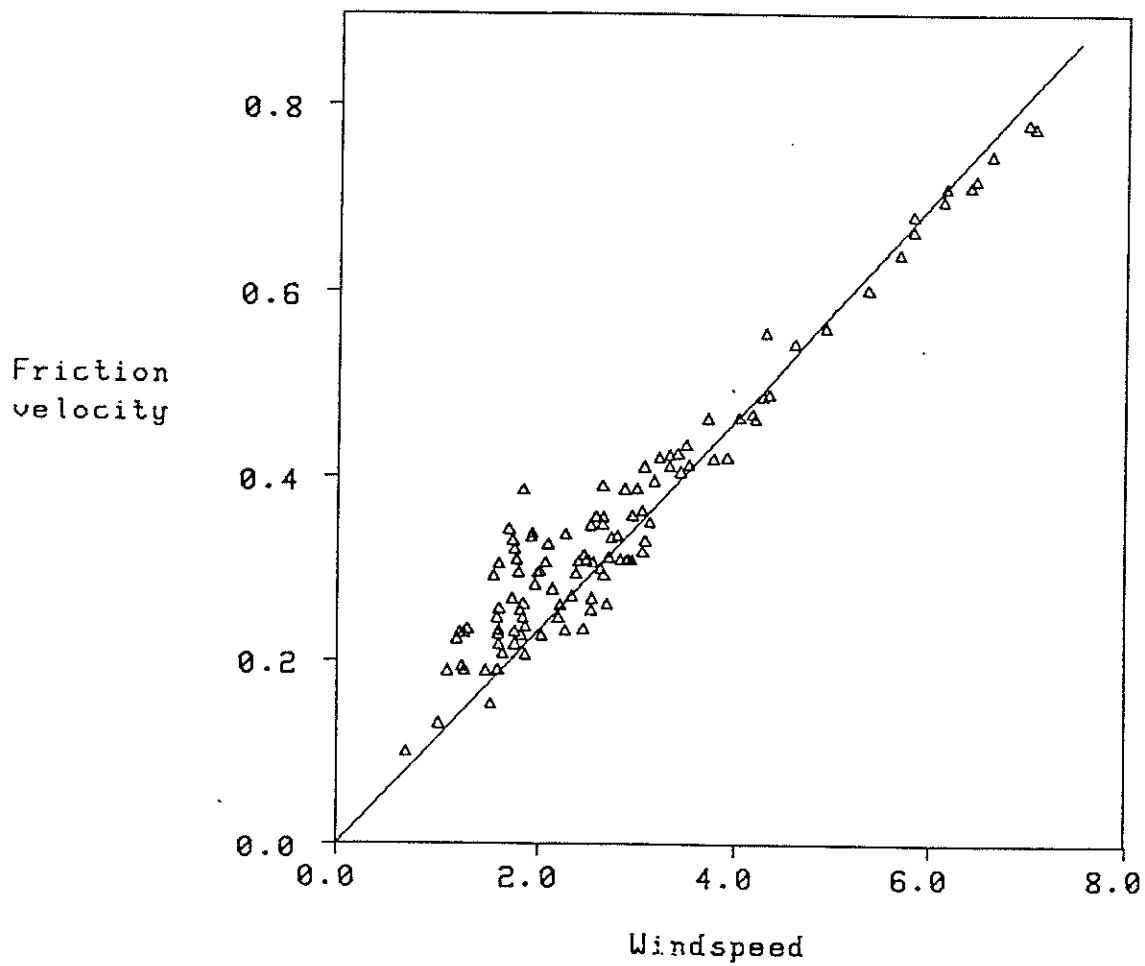


Figure 3. Friction velocity (m s^{-1}) as dependent upon windspeed (m s^{-1}) at reference height above an alfalfa canopy in the Mesilla Valley, New Mexico.

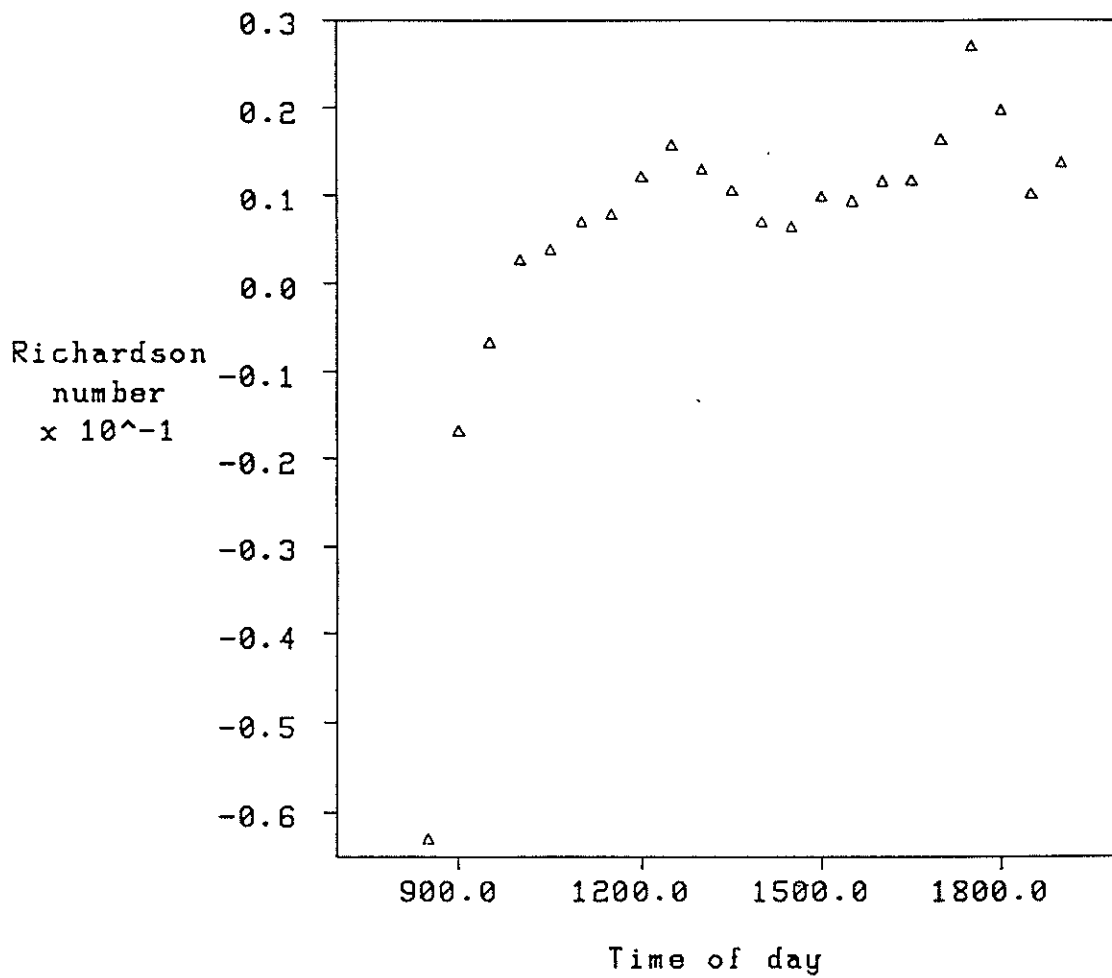


Figure 4. Dimensionless Richardson number for wind flow above the same alfalfa canopy as in Figure 3.

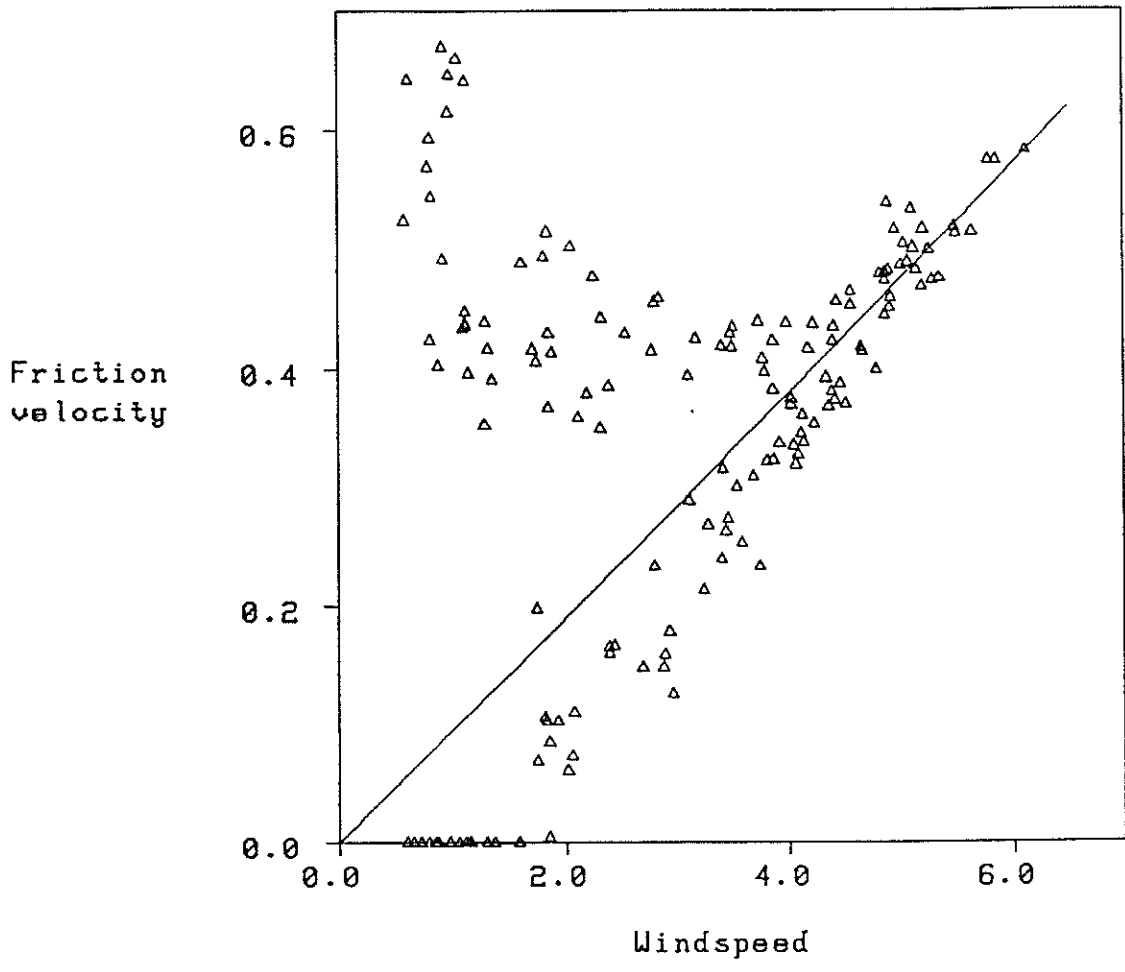


Figure 5. Friction velocity (m s^{-1}) corrected for atmospheric stability, as a function of windspeed. Daylight measurements only.

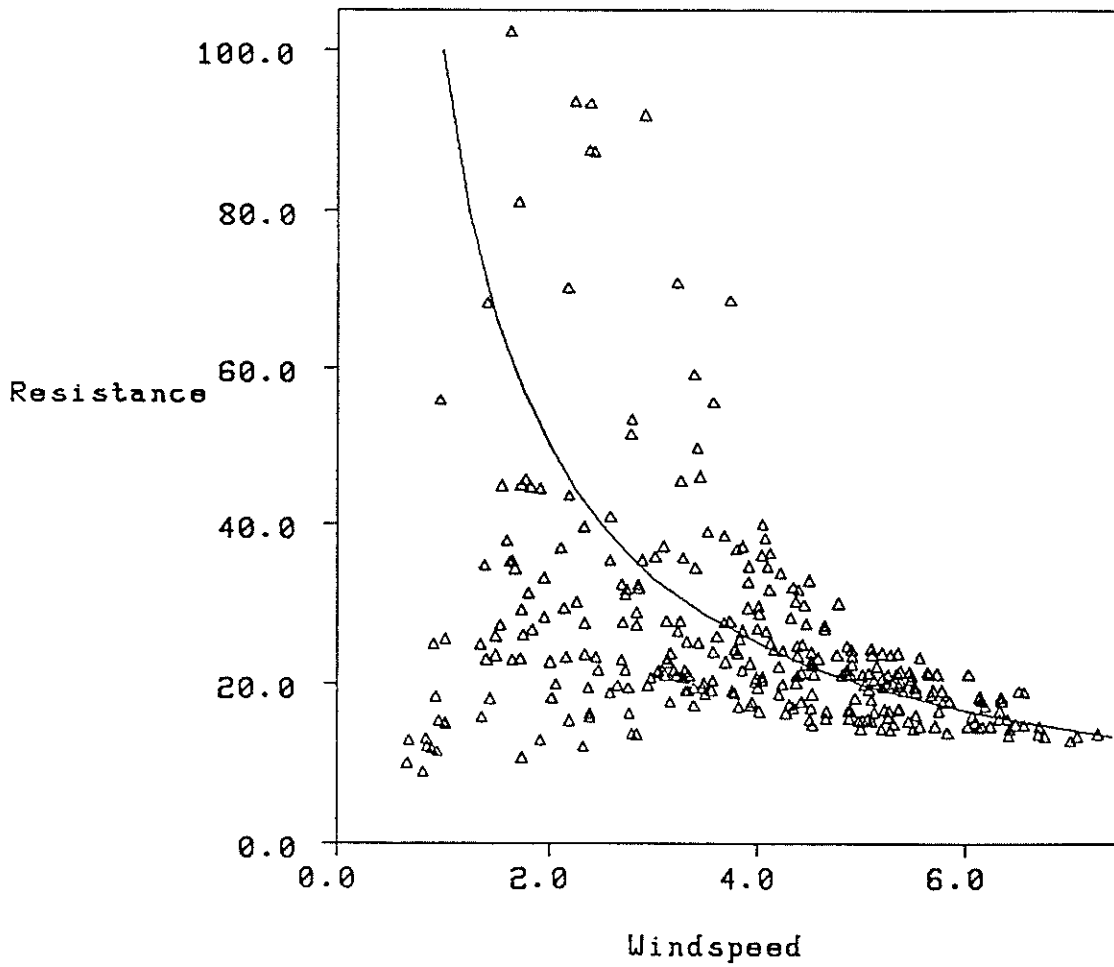


Figure 6. Computed canopy boundary-layer resistance (s m^{-1}) from canopy top to reference height, as a function of windspeed (m s^{-1}) at reference height above alfalfa canopy.

EFFECTS OF DROUGHT STRESS

Effects of drought stress on C_i and SLM in growth chambers

Drought stress, imposed as two episodes of water withholding to wilting, induced shifts in $\delta^{13}\text{C}$ and SLM. The value of $\delta^{13}\text{C}$ did not show a uniform trend under stress. One might have expected it to rise, that is, for C_i to decrease and WUE to rise. Neither within a cycle nor in pooled cycles was the regression of $\delta^{13}\text{C}$ (droughted) against $\delta^{13}\text{C}$ (undroughted) significant, nor was the difference in means significant. Drought did induce a marginally significant increase in SLM in Wilson in one of the two growth cycles we ran ($r = 0.709$, $N = 8$, $p = 0.049$).

Relation of WUE to drought tolerance

Drought tolerance (DT), defined as in "methods" above, was negatively correlated with WUE of the unstressed genotype (Fig. 7), in the two cycles analyzed to date. The physiological origin of this negative relation suggested in the "Justification" section does not apply: the genotypes with higher DT did not have larger root:shoot ratios. In current research, we are testing an alternative hypothesis: genotypes with high WUE and low C_i from low stomatal conductance may suffer earlier and more prolonged stomatal closure under water stress. Earlier closure is predicted by the model of Ball *et al.* (1987), augmented with the hypothesis that water stress decreases assimilation capacity directly without amending slope and intercept parameters of the conductance model (J. G. Collatz, pers. commun.). Consequent longer closure could allow photoinhibition to proceed longer. Indeed, we have initial evidence that lower DT is associated with longer time to recover positive carbon balance of leaves. Another possible origin of negative correlation between unstressed WUE and DT is ecological, as suggested in the earlier discussion.

PRINCIPAL FINDINGS, CONCLUSIONS, AND RECOMMENDATIONS

POTENTIAL GAINS IN WATER-USE EFFICIENCY ARE MODEST AND HAVE A RESTRICTED NICHE IN SOLVING WATER PROBLEMS

Our model helps indicate the theoretical limits to improving water-use efficiency. Earlier efforts have also contributed, with a less comprehensive accounting of the various biophysical and physiological processes determining WUE. Most of the earlier models were not whole-canopy models (*e. g.*, those of Farquhar and coworkers). Rough theoretical upper bounds to improving WUE have been set via the important discussion of canopy boundary-layer or aerodynamic resistance (Jarvis and McNaughton 1986 and a number of recent commentaries, *e. g.*, Paw U and Gao 1988).

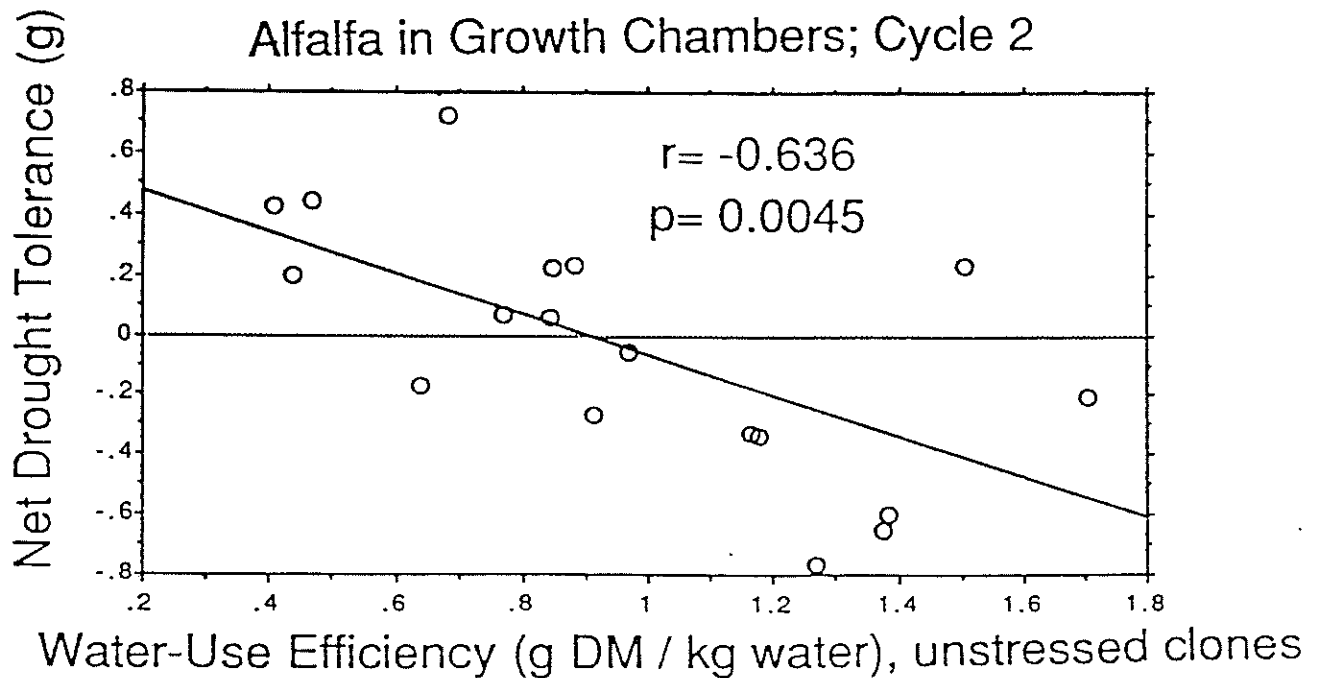
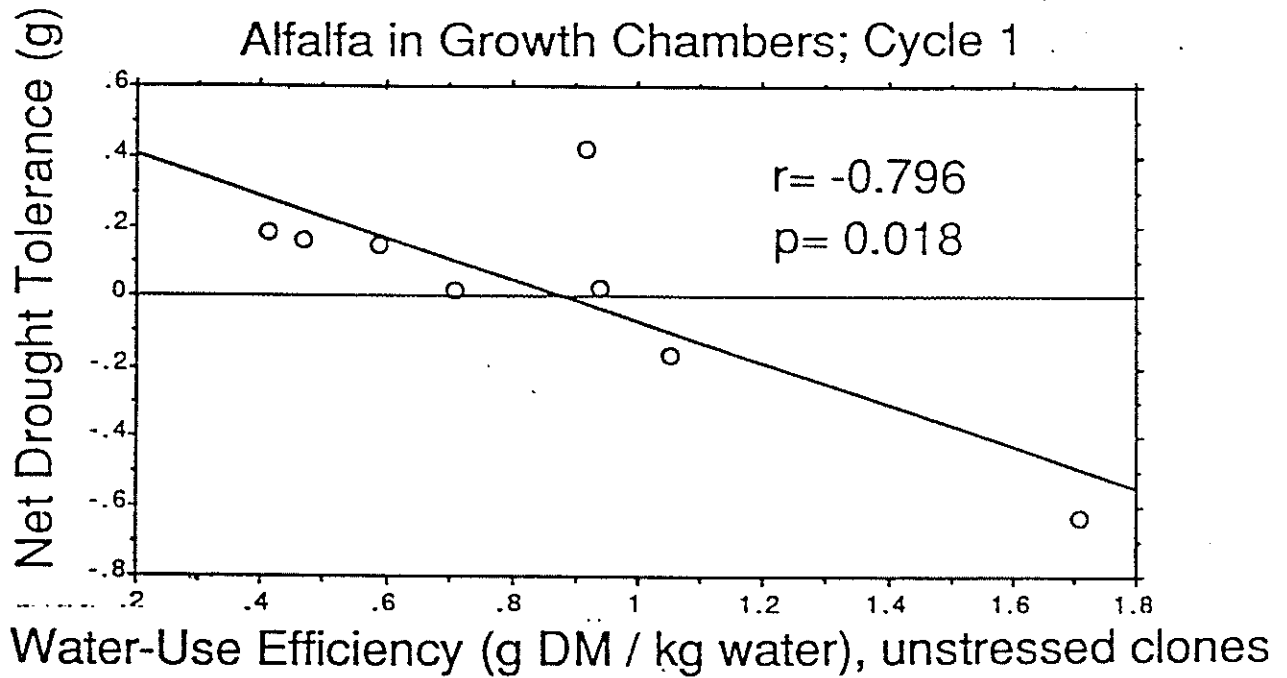


Figure 7. Indication of negative relation of drought tolerance with water-use efficiency of unstressed plant of same genotype. Droughting method and definition of drought tolerance are given in text.

The experiments reported here give preliminary confirmation to our model and thus to its prediction of WUE gains being practically limited to 10 to 15% for alfalfa and similar short, dense crops. We propose to publish an addendum to this report with additional experimental tests of our model. Field experiments on wheat (Condon *et al.* 1991; Richards 1991, 1992; Condon 1992) are confirming the stringency of limits to WUE gains. Forty percent differences in stomatal conductance between genotypes confer only 10% differences in WUE. Further, these gains are only expressed under restricted conditions for a seed crop such as wheat rather than a forage crop such as alfalfa. In wetter conditions, low-C_i wheat may have unchanged or even modestly lower WUE than a base genotype, because its slower growth seems to allow more soil evaporation. Experiments are in progress to confirm this hypothesis; one of us, Gutschick, is contributing to interpretive models. Field experiments on other crops, such as cowpeas (Hall 1992) and beans (White 1992) show even more restriction to expression of WUE gains by low-C_i genotypes. (However, more careful attention to drought tolerance as a separate measure from WUE, and modelling effects of stress on phenology, may clarify the issues.) We note that rainfed crops in arid areas may be sufficiently sparse to have high roughness and low aerodynamic resistance (Hatfield 1989). Thus, they may have greater potential for WUE gains, though the absolute yields will remain small.

We propose that at least one other type of indirect, physiological selection might improve WUE significantly, namely, selection for lower leaf chlorophyll content. The effort to breed this trait may be quite large, if a mutant genotype is not found fortuitously as in several other plant species. As with breeding for yield, or, in fact, many other performance measures such as disease tolerance, progress promises to be incremental, if ultimately very significant.

In the medium term of a decade or so, we project that much greater contribution to water-use problems could come from attention to water management methods. Losses of water in irrigation canals, flood irrigation, sprinkler application, etc. can often be reduced twofold, virtually dwarfing gains in water saving afforded by breeding crop WUE for irrigated agriculture. Until the marginal benefits of management improvements become small, there will be little incentive to apply plant-breeding gains in WUE. Plant breeders themselves (Richards 1992; Acevedo 1992) point out that current, weak market incentives to use of high-WUE genotypes are one of several significant barriers to the plant-breeding approach. Reform of water laws, particularly in the western United States, will almost surely be required to provide incentives for breeding greater crop WUE.

Once water management has improved sufficiently, it will be worth pursuing the marginal benefit afforded by breeding for crop water-use efficiency. In the interim, efforts to breed WUE should be pursued, given that proof of their efficacy may take a decade, making high-WUE crops available in timely fashion.

INDIRECT SELECTION APPEARS PRACTICAL FOR IMPROVING WATER-USE EFFICIENCY

Our own experiments offer some piecewise verification of our hypothesis that selecting C_i and SLM can improve WUE, particularly while minimizing yield penalties. The extension of our tests to field conditions in realistic sward growth is in progress. We have evidence that there is adequate genetic variability, heritability, and environmental stability in SLM for a breeding program. This confirms earlier studies for purposes unrelated to WUE, *e. g.*, Song and Walton (1975). Other researchers have provided evidence of similar adequacy of variability, heritability, and especially environmental stability in C_i (Richards 1992; White 1992; Wright 1992). The major caveat is that developed water stress reduces the stability of genotype rankings in C_i (as $\delta^{13}C$).

Our experiments and those of other researchers whom we have just cited strongly confirm that selecting $\delta^{13}C$ should be the central focus in breeding WUE. It is C_i that exerts the predominant control over WUE. Our experiments in particular demonstrate the need for care in specific aspects of experimental design: (1) Full swards must be tested, not individual plants, and in fields rather than controlled environments, in order to get realistic estimates of WUE gains. The first point is confirmed by Condon *et al.* (1990) most concisely. Individual-plant studies and/or controlled-environment studies are required to identify usable genetic variability in parent genotypes. (2) Carefully designed experiments with mixed genotypes, such as we employ, can give initial confirmation of gains and can lead to insights on biophysical/ physiological mechanisms determining WUE. Nonetheless, they cannot supplant field methods with uniform swards. Even for initial tests of our hypotheses, path analysis methods are required to resolve C_i and SLM effects when the traits are correlated with each other and when plants unavoidably differ in initial size. Controlled environments must use realistic irradiances and any microenvironmental differences must be compensated, as by rotating plants to various positions. (3) The role of soil evaporation in whole-season WUE must be accounted in the design of the experiments. Likewise, the role of canopy boundary-layer resistance is critical.

Comprehensive models of plant and meteorological processes may be valuable tools in estimating the potential to improve WUE and yield, and other performance measures. Models may also aid in the design of experiments by highlighting important variables to measure or control. They may also suggest forms of regression analyses, particularly nonlinear forms or path analyses.

Carbon-isotope discrimination is the most reliable measure of C_i among various genotypes, for the average over the growth cycle that is relevant to season-long WUE. Work of other researchers just cited abundantly confirms the superior reliability of $\delta^{13}C$ over gas exchange for breeding selection. The significant unit cost of mass-spectrometric analyses of $\delta^{13}C$ is counterbalanced by the savings in technical staff

salary compared to direct selection for WUE. SLM can be selected rather straightforwardly within a stand of plants sampled at one time. Ontogenetic drift in SLM makes comparison of SLM sampled at different times problematic.

Two significant tradeoffs in plant performance may attend the breeding of improved WUE. First, modest yield losses are predicted by our model and borne out in field experiments on wheat (Condon *et al.* 1991). Our experiments in mixed-genotype swards superficially indicate the contrary, but deeper analyses are indecisive. Our full field experiments, in progress, should be decisive. Second, tolerance to at least some types of drought, such as the episodic drought we enforced, may be reduced. It is imperative that drought tolerance and WUE be carefully distinguished and that we seek quantitative definitions of drought tolerance -- and of drought itself.

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