



Evolution and Agricultural Water Use

INTRODUCTION

The earth is about 4.6 billion years old and photosynthetic organisms appeared around 3 billion years ago, but land plants have existed for only about 0.5 billion years (Chapman, 1985). The invasion of the land probably was initiated by special conditions existing 0.5 billion years ago, including a favorable location of the continents (Bambach *et al.*, 1980; Fischer, 1984; Ziegler *et al.*, 1981), tidal flooding of vast areas of the continents (Fischer, 1982; Hallam, 1984; Parrish, 1983; Ziegler *et al.*, 1981), and moderate temperatures with high humidity and rainfall (Fischer, 1984; Holland, 1978). The early land plants appear to have been relatives of present-day Charaphytes such as *Nitella* and *Chara* that were anchored to bottom sediments with rhizoids and needed only a thin cuticle to resist moderate dehydration (Chapman, 1985; Graham, 1985).

After that time, the land climate became more severe as the continents moved and larger land masses became exposed (Fischer, 1982; Hallam, 1984; Ziegler *et al.*, 1981), and structural and functional adaptations developed rapidly among the plants. These included roots that permitted absorption of water and minerals from large soil volumes, a vascular system that facilitated rapid transport of water and photosynthetic products, and a well-developed cuticle with stomata that permitted CO₂ to enter but controlled water loss from the moist tissues beneath. A major force encouraging the invasion of the land was the availability of light that could support vigorous photosynthesis, but atmospheric CO₂ was consumed until it became only a trace gas in the atmosphere (Holland, 1984), and the photosynthetic apparatus developed special adapta-

tions for operating at a low CO_2 concentration. C_4 photosynthesis appeared in certain species and involved temporary CO_2 fixation in four-carbon compounds followed by its release at high concentrations near the site of more permanent CO_2 fixation in the leaf cells. Crassulacean acid metabolism (CAM) developed in other species and allowed the temporary fixation of CO_2 to occur at night and release the following day. Both adaptations permitted more photosynthesis at declining atmospheric CO_2 levels for the same water loss.

MEASURING EVOLUTIONARY PRESSURES

The expansion of the land area and the increased exposure of plants to environmental extremes created evolutionary pressures for further adaptation, and the fossil record indicates that these pressures have changed as climates changed because many land species have disappeared and been replaced by species better adapted to the new climatic conditions. It therefore seems probable that significant evolutionary pressures are still present. One way to measure evolutionary pressure is to determine the ability to reproduce. The more intense the pressure, the less the reproductive success and the more rapidly unadapted species will disappear from the population. In ideal environments, little evolutionary pressure is present and the maximum potential for reproduction can be approached ($R_{\text{potential}}$). On the other hand, in average environments that represent the usual conditions, evolutionary pressure may be present and reproduction may be suppressed to give (R_{average}). The fraction of the potential that is achieved is the reproductive success $\frac{R_{\text{average}}}{R_{\text{potential}}}$ and the fraction that is lost is $(1 - \frac{R_{\text{average}}}{R_{\text{potential}}})$. The evolutionary pressure P_{ev} is the fraction that is lost

$$P_{\text{ev}} = 1 - \frac{R_{\text{average}}}{R_{\text{potential}}} \quad (12.1)$$

and when R_{average} is as high as $R_{\text{potential}}$, P_{ev} approaches 0, indicating there is little evolutionary pressure, and growth conditions allow the full expression of the reproductive potential of the plants. When $P_{\text{ev}} = 1$, evolutionary pressures are so large that the plants cannot reproduce and will disappear from the next generation. Values of P_{ev} between these extremes indicate evolutionary pressures of various intensities, resulting in varying degrees of adaptation to the environment.

Data for natural communities of plants are sparse but there are numerous data for agricultural communities having economically valuable reproductive structures. Table 12.1 shows that maximum (record) yields were high in eight major crops in 1975 in the United States. Six of these had valuable reproductive structures (maize, wheat, soybean, sorghum, oat, and barley). Because the rec-

Table 12.1 Record Yields, Average Yields, and Yield Losses Due to Diseases, Insects, Weeds, and Unfavorable Physicochemical Environments for Major U.S. Crops^a

Crop	Record ^b yield	Average ^b yield	Average losses ^c			
			Diseases	Insects	Weeds	Physicochemical ^d
Maize	19,300	4,600	836	836	697	12,300
Wheat	14,500	1,880	387	166	332	11,700
Soybean	7,390	1,610	342	73	415	4,950
Sorghum	20,100	2,830	369	369	533	16,000
Oat	10,600	1,720	623	119	504	7,630
Barley	11,400	2,050	416	149	356	8,430
Potato	94,100	28,200	8,370	6,170	1,322	50,000
Sugar beet	121,000	42,600	10,650	7,990	5,330	54,400
Mean percentage of record yield	100	21.5	5.1	3.0	3.5	66.9

Note. Values are kilograms per hectare. Record and average yields are as of 1975.

^aIn the original work (Boyer, 1982), weed losses were considered to be physicochemical because the losses were attributable to competition for light, nutrients, and so on. On the other hand, weeds are of biological origin and it may be argued that the losses should be included with insects and diseases. For simplicity, the latter approach is taken here, which slightly alters the values calculated for each loss in comparison with Boyer (1982).

^bFrom Wittwer (1975).

^cCalculated according to U.S. Department of Agriculture (1965).

^dPhysicochemical losses calculated as record yield—(average yield + disease loss + insect loss + weed loss).

ord yield was measured under conditions that virtually eliminated pests and competing weeds, and nutrients and water were supplied in copious amounts, the record yield should have been an approximate measure of the maximum potential for reproduction ($R_{\text{potential}}$). In contrast, the average yield was obtained on farms under average agricultural conditions and should have been an estimate of the degree of suppression of the potential yield by the environment (R_{average}). All crops showed average yields that were much less than record yields. From Eq. (12.1), the resulting calculated value of P_{ev} was $(1 - 0.215/1.00) = 0.785$ for all the crops and 0.822 for the reproductive crops. Thus, environmental conditions are exerting a marked evolutionary pressure on crops. It seems likely that P_{ev} is substantial in natural communities as well.

From these principles we see that plants probably are evolving rapidly at present and what we perceive as stable species in natural populations appear that way only because the time scale of our observation is so brief. The changes brought about by agriculture are mostly accelerating and modifying change that is occurring naturally, which raises the possibility of modifying plants to allow them to reproduce at higher levels in average environments. Agriculture has approached the problem mostly by raising the genetic potential (e.g., many hybrids) or by changing the environment (e.g., irrigation and fertilization), and to a lesser extent by adapting plants to the existing environment. The latter approach probably will receive increased attention as the magnitude of loss in potential yield becomes better perceived (Boyer, 1982).

ENVIRONMENTAL LIMITATIONS ON YIELD

The causes of losses in potential yield include biotic factors (diseases, insects, and weeds in Table 12.1) that account for about 12% of the genetic potential. This represents the residual pest losses after intense measures for pest control, and a much larger loss would occur if pest control was not practiced. As a consequence, in natural communities where biotic factors are left only to natural defenses, the evolutionary pressure from pests is likely to be larger than in agricultural systems.

After accounting for biotic losses, Table 12.1 shows that nearly 70% of the genetic potential is lost because of physicochemical causes which include water and nutrient availability, temperature, daylength, soil pH, aeration, and excessive salt concentrations in the soil. Thus, these abiotic causes are partly attributable to soil problems and Table 12.2 shows that permanently dehydrated soils and shallow soils subject to frequent dehydration occupy about 45% of the U.S. land area, cold soils are present on nearly 17%, and wet soils cover almost 16% of the surface. Alkaline and saline soils occupy about 7% of the surface and only 12% of the soils are classified as being free of physicochemical problems (Boyer, 1982). The soils of the world (Dudal, 1976) have a similar classifica-

Table 12.2 Area of the U.S. Land Surface Subject to Environmental Limitations of Various Types^a

Environmental limitation	Area of U.S. affected (%)
Drought	25.3
Shallowness	19.6
Cold	16.5
Wet	15.7
Alkaline salts	2.9
Saline or no soil	4.5
Other	3.4
None	12.1

^aFrom U.S. Department of Agriculture (1975).

tion (Table 9.1). Table 12.3 shows that insurance payments to U.S. farmers for crop losses mirror these soil classifications and the biotic and abiotic effects (cf. Tables 12.1, 12.2, and 12.3).

It is clear from these data that the physicochemical environment, especially the effect of dehydration, is the dominant factor suppressing the productivity of land plants in the present world. As a consequence, desiccation continues as a major force in plant adaptation. Because of the intensive evolutionary pressure, it is likely that plants are still evolving better systems for coping with the land environment. Rice is a likely example because its genotypes extend from deep-water rices that are semiaquatic to upland rices that require well-drained soils

Table 12.3 Distribution of Insurance Indemnities for Crop Losses in the United States from 1939 to 1978^a

Cause of crop loss	Proportion of payments (%)
Drought	40.8
Excess water	16.4
Cold	13.8
Hail	11.3
Wind	7.0
Insect	4.5
Disease	2.7
Flood	2.1
Other	1.5

^aFrom U.S. Department of Agriculture (1979).

and exhibit many characters that are necessary for colonizing the land. The upland rices have thicker cuticle than the deep-water and paddy rices (O'Toole, 1982), and dry atmospheric conditions can desiccate reproductive structures and cause abortion (O'Toole *et al.*, 1984). The upland rices have deep roots that are often more extensive than those of deep-water and paddy rices (Chang *et al.*, 1974). Interestingly, only a few genes appear to control the differing root morphologies (Armenta-Soto *et al.*, 1983; Ekanayake *et al.*, 1985; O'Toole and Bland, 1987). The stems of upland rices have slow stem growth whereas rapid elongation occurs in deep-water rices and keeps part of the shoot and the flowers above rising flood waters in delta areas fed by monsoons (Raskin and Kende, 1985). Thus, it seems clear that in a crop that spans aquatic to upland habitats, extensive genetic adaptation to the land environment is occurring and there is a high degree of variability that could be used to change crop performance.

The traditional solution to water shortages has been irrigation, as pointed out in Chapter 4. Irrigation has made agriculture possible in many otherwise nonproductive areas and has the advantage that water can be made available as needed, and production is more predictable so that investment in other favorable cropping practices also can be undertaken. Large supplies of water are necessary because most of the irrigation water is evaporated by the crop. As a result, the water is consumed and not returned for other uses, and in the United States more water is consumed by irrigation than by all other uses combined (U.S. Department of the Interior, 1977). Salt-laden water cannot be used for irrigation because evaporation removes the water but leaves most of the salt, degrading the soil. New supplies of low salt water have diminished and municipalities compete for the same water, so new irrigation is becoming less possible than in the past, even if farmers can justify the large capital costs of the equipment and expense of moving the water (Boyer, 1982). As this trend grows there is increasing interest in improving the efficiency of water use in irrigation and determining whether plants can yield well in water-deficient conditions. If genetic manipulation or altered cultural practices can contribute to this goal, there could be less degradation of soils and water supplies and more cost effective irrigation.

A number of methods exist for improving the efficiency of water use and have been summarized by Taylor *et al.* (1983) and by Stewart and Nielsen (1990). The methods can be classified in three broad categories: (1) increasing the efficiency of water delivery and the timing of water application, (2) increasing the efficiency of water use by the plants, and (3) increasing the drought tolerance of the plants. The first method is practiced most because it depends mostly on engineering and minimally on the crop. Transporting water with minimal evaporation, preventing runoff, storing water in catchments, delivering water only to the root zone, and timing irrigation to the needs of the plant have been successful in improving productivity per unit of water delivered to the farm.

There are estimates that just by improving irrigation timing, the amount of water can be decreased by half in some crops while maintaining high levels of production (e.g., Bordovsky *et al.*, 1974). The second and third methods depend on understanding the biology of the crop and whether it can be manipulated to achieve the same productivity with less water. The state of knowledge in this area is the focus of the remainder of this chapter.

WATER USE EFFICIENCY

Water use efficiency is defined as the total dry matter produced by plants per unit of water used

$$\text{WUE} = \frac{D}{W}, \quad (12.2)$$

where WUE is the water use efficiency, D is the mass of dry matter produced, and W is the mass of water used. For a field experiment, D and W would be expressed on the basis of land area. For a single plant experiment, D and W would be measured in the same plant and expressed on the basis of the whole plant. One may also consider the water use efficiency for a single leaf and so on. The higher the dry matter production per unit of water use, the higher the efficiency.

There is extensive evidence that WUE of single plants varies among species in the same environment (see Table 7.3) and among climates for the same crop (Briggs and Shantz, 1914; de Wit, 1958; Hanks in Taylor *et al.*, 1983; Tanner and Sinclair in Taylor *et al.*, 1983). Taking advantage of the species and climate effects can help manage limited water supplies in agriculture. For example, alfalfa has a lower water use efficiency than maize when grown in nearby sites in the same year (Hanks in Taylor *et al.*, 1983; Table 7.3). Thus, changing crops can significantly reduce water consumption with little sacrifice in dry matter production. Relocating production to a new climate with lower evapotranspiration is another possible approach. For economic reasons, however, these options are not often employed and probably will not be until the cost of water rises to a level that forces change. What then are the prospects for improving water use efficiency within a species, or protecting against yield loss in a particular climate when irrigation is not possible?

It is first necessary to consider some principles of plant productivity and transpiration. Because land plants fix CO_2 from the air and the C and O atoms of this molecule account for most of the dry mass of the plant (see Chapter 10), D of Eq. (12.2) represents mostly photosynthetic activity. The CO_2 must diffuse into the leaf and dissolve in the wet surface of the cells before it becomes available for photosynthesis. The wet surfaces are exposed to the atmosphere inside the leaf and transpiration is inevitable. The photosynthesizing cells dehydrate

to varying degrees depending on how readily the lost water can be replaced. Land plants generally absorb water from the soil and have a shoot covered by a waxy layer containing stomata that regulate water loss as CO_2 is fixed. Land plants have a much greater control of evaporation and water acquisition than their aquatic counterparts, and depending on the leaf anatomy and physiology, the dry matter produced per unit of water used can vary widely. Nevertheless, water use is affected by physical factors in addition to those imposed by the plant. CO_2 enters the leaf by diffusing down a concentration gradient to the leaf interior, and the water vapor in the intercellular spaces inside the leaf likewise diffuses in the opposite direction. The lower the external humidity, the faster transpiration will be when all other factors are constant. Leaf temperature plays an important role by affecting the vapor pressure of water in the leaf (see Chapters 2 and 7). The higher the leaf temperature, the higher the vapor pressure (Fig. 7.3) and the more rapid the transpiration. Water use will differ among sites and seasons for these reasons and the water use efficiency in Eq. (12.2) thus reflects a complex of plant and environmental factors.

Briggs and Shantz (1914) conducted an extensive survey of the water use efficiency of crops, and they expressed it as the water requirement, that is, the amount of water used per unit of dry matter produced which is the reciprocal of the water use efficiency. They grew the plants in large containers of soil and measured plant dry weight and the water used at the end of the entire growing season. This had the advantage that a large number of crops could be compared in a uniform climate during a single season. In their experiments, the transpiration ratio of maize, sorghum, and millet was less than for the other crops and, although Briggs and his co-workers could not have known at the time, the three crops are C_4 species possessing a special anatomy and biochemistry that allows CO_2 to be concentrated around the site of fixation. This resulted in more photosynthesis per unit of water transpired and accounted for the lower transpiration ratio.

After the experiments of Briggs and his co-workers, various investigators measured water use efficiency under field conditions where all the adaptations of the crop could express themselves (de Wit, 1958; Hanks in Taylor *et al.*, 1983). Figure 12.1 gives examples for several crops near Logan, Utah, that were grown with varying amounts of irrigation. It is striking that there is a linear relationship between water use and dry matter production (de Wit, 1958; Hanks in Taylor *et al.*, 1983). The linearity is mostly caused by the diffusion link between photosynthesis and transpiration because the visible radiation input is almost completely absorbed by all crops after the canopy closes and, in a given climate, the input is partitioned in a constant proportion between energy for transpiration and energy for photosynthesis. The slope of the linear relationship is the water use efficiency [Eq. (12.2)] and the straight line indicates that the water use efficiency does not change as the availability of water varies. However, it differs among species, especially between maize and the other crops

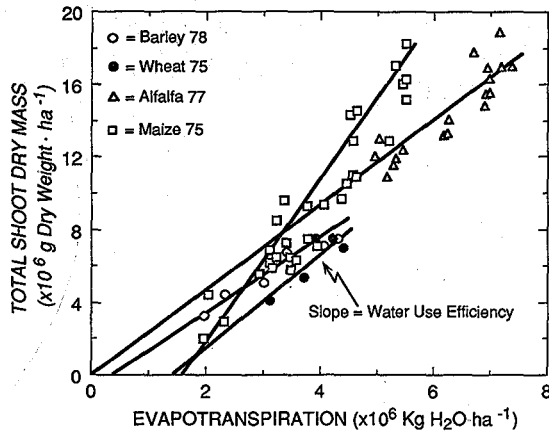


Figure 12.1 Production of aboveground shoot dry matter at various levels of water use in several crops near Logan, Utah. The years in which the crops were grown are shown in the symbol key. Water use was controlled by varying the amount of irrigation and is shown as combined evaporation from the soil and transpiration from the plants. A positive evapotranspiration intercept indicates the amount of water obtained from soil stores. The slope of the linear relation is the water use efficiency which was 2.11 g of dry weight per kg of H_2O for barley, 2.50 for wheat, 2.36 for alfalfa, and 4.49 for maize. Note that maize is a C_4 plant and the others are C_3 . Maize and wheat were grown in the same year. Adapted from Hanks (in Taylor *et al.*, 1983).

(Fig. 12.1). These experiments confirm the differences noted by Briggs and his co-workers and further indicate that water use efficiency does not differ under varying availabilities of soil water. However, it differs among species, climates, and from year to year (Briggs and Shantz, 1914; Brown and Simmons, 1979; Garrity *et al.*, 1982; Hanks in Taylor *et al.*, 1983; Kawamitsu *et al.*, 1987; Pandey *et al.*, 1984a,b; Robichaux and Percy, 1984; Tanner and Sinclair in Taylor *et al.*, 1983). More work may be needed on the constancy of water use efficiency with different mineral availabilities, plant spacing, and other cropping practices.

In this respect, it is important to note that while differences between C_4 and C_3 species are apparent, tests have been made only under limited conditions in species exhibiting CAM. Species such as pineapple exhibit CAM and concentrate CO_2 by temporarily fixing it at night in organic acids from which it is released the next day for photosynthesis. During release, the stomata are closed and water is conserved (see Fig. 8.3). This allows CAM plants to achieve even higher water saving than C_4 plants, and estimates of water use efficiency for pineapple are about 20 g dry mass \cdot kg $^{-1}$ water, for C_4 plants about 3 to 5 g dry mass \cdot kg $^{-1}$ water, and for C_3 plants only about 2 to 3 g dry mass \cdot kg $^{-1}$ water with variations depending on the evaporative environment (Briggs and Shantz, 1914; Hanks in Taylor *et al.*, 1983; Joshi *et al.*, 1965; Neales *et al.*, 1968).

It has been argued that water use efficiencies should not be expressed as

absolute dry mass gained per unit of water mass used but should be normalized for evaporative demand (de Wit, 1958; Tanner and Sinclair in Taylor *et al.*, 1983) and often for the potential productivity of the crop (Hanks in Taylor *et al.*, 1983). Thus, modified expressions of WUE have been used such as

$$\frac{D}{D_{\max}} = \frac{W}{W_{\max}}, \quad (12.3)$$

where the fractional dry mass is D/D_{\max} and is expressed relative to the maximum dry mass produced with optimum water D_{\max} . The fractional water use W/W_{\max} is likewise expressed relative to the maximum transpiration that would occur at the site with optimum water. The approach has the advantage that for a water use of, say, half the potential transpiration, half the maximum dry mass would be predicted. This simplifies the job of predicting the impact of water shortages. However, it requires a knowledge of the maximum dry matter yield and transpiration of the crop for the year, which will vary.

It must be kept in mind that farm income is generally based on the absolute dry mass or economic yield, and expense is based on the absolute amounts of water used. A farmer contemplating whether to irrigate semiarid land needs to have high absolute production of dry mass to justify pumping larger amounts of water than a farmer in a humid region. Even better, he should know the absolute production of marketable yield, which may be only a part of the total dry mass. Figure 12.2 shows that the water use efficiency for producing grain in

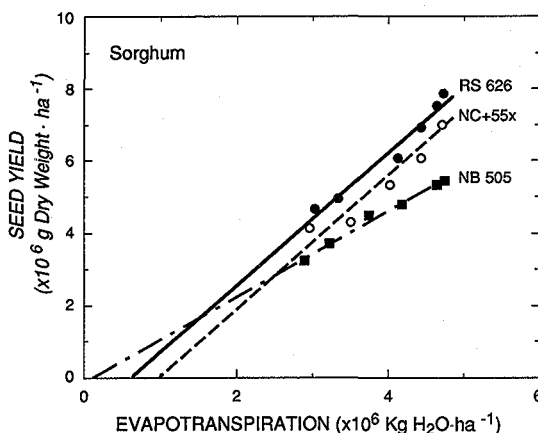


Figure 12.2 Seed yield at various levels of water use by three sorghum genotypes in west central Nebraska. The water use efficiency for seed yield is the slope of the line and was 1.8 g of dry weight per kg of H₂O in RS626, 1.9 in NC+55X, and 1.2 in NB505. Note that the absolute seed yield of RS626 was superior to that in NC+55X even though the water use efficiency was essentially the same. The water use efficiency for total shoot dry matter production was 3.3 in RS626, 3.2 in NC+55X, and 2.0 in NB505. Adapted from Garrity *et al.* (1982).

sorghum was greater in the genotypes RS626 and NC+55X than in NB505. Reducing water use by half in each genotype would give half of the maximum yield but would not distinguish which genotype would give the highest grain production for a particular amount of water used. The farmer would profit more by planting RS626 because it not only had a better absolute water use efficiency than NB505 but also the highest absolute yield (Garrity *et al.*, 1982).

Measuring Water Use Efficiency

Measuring water use efficiency in the field is the most accurate means of determining how dry matter production will be affected by water availability but it is labor intensive and costly. Less expensive methods have been sought, and one has been to measure directly the CO_2 and H_2O exchange of individual leaves (Bierhuizen and Slatyer, 1965; Brown and Simmons, 1979; Robichaux and Percy, 1984). Because the carbon dioxide molecule contributes most of the dry mass, the gas exchange efficiency can be defined as the ratio of the mass of CO_2 gained to the mass of H_2O lost. Figure 12.3 shows an example of water use efficiency measured as gas exchange efficiency in comparison with the actual water use efficiency for the whole growing season measured in the usual way in tomato (*Lycopersicon esculentum* Mill.) and its wild relative *L. pennellii* (Cor.) D'Arcy. The relationship is poor because of the additional factors affecting dry mass accumulation but not gas exchange (Martin and Thorstenson, 1988). The mass of the plant is determined by long-term net dry mass accumulation which is affected by respiratory losses at night and partitioning to nonphotosynthetic organs as well as CO_2 uptake. It is altered by temperature and the molecular composition of the dry mass. Gas exchange for short times during the day does not detect these additional factors. Therefore, while the gas exchange efficiency gives valuable insight into the physiologic and metabolic controls that might operate during photosynthesis and transpiration, the method is being used less frequently than in the past.

Another method involves measuring the relative abundance of natural isotopes in plant tissue. Although most of the CO_2 in the atmosphere is $^{12}\text{CO}_2$, a small amount is $^{13}\text{CO}_2$. Because the $^{12}\text{CO}_2$ is lighter, it diffuses into the leaf faster than $^{13}\text{CO}_2$. Also, ribulose biphosphate carboxylase fixes the lighter isotope faster. The cells accumulate relatively less ^{13}C than ^{12}C , and the unused ^{13}C in the intercellular spaces of the leaf diffuses out according to the extent of stomatal opening. This outward diffusion is correlated with transpiration. Because the inward diffusion and use of $^{12}\text{CO}_2$ correlates with photosynthesis and dry mass but the outward diffusion of $^{13}\text{CO}_2$ correlates with transpiration, the relative uptake of ^{12}C and ^{13}C correlates with the water use efficiency. Farquhar and his colleagues demonstrated differences in water use efficiency between genotypes of wheat (*Triticum aestivum* L.), peanut (*Arachis hypogaea* L.), barley (*Hordeum vulgare* L.), and other crops by measuring the ratio of ^{12}C to ^{13}C

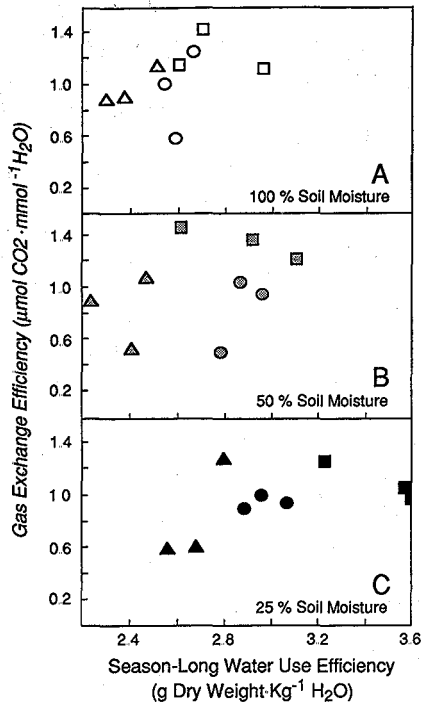


Figure 12.3 Relation between the water use efficiency determined from measurements of CO_2 fixed: H_2O transpired (gas exchange efficiency) and the season-long water use efficiency determined from the total plant dry mass accumulated per water mass used in tomato. (A–C) Plants grown at 100% (open symbols), 50% (half closed symbols), and 25% (closed symbols) of soil field capacity, respectively. Triangles are for *Lycopersicon esculentum*, squares are for *L. pennellii*, and circles are for the F_1 hybrids. Note that the variability is so high that none of the relationships are significant at the $P < 0.05$ level. However, the water use efficiency measured for the whole season is generally higher for *L. pennellii* than for *L. esculentum*. Adapted from Martin and Thorstenson (1988).

isotope content of plant tissue relative to that in a standard (Bowman *et al.*, 1989; Brugnoli *et al.*, 1988; Condon *et al.*, 1987, 1990; Hubick and Farquhar, 1990; Hubick *et al.*, 1986).

This ratio technique makes it possible to survey a large number of plants with a simple analysis of the leaf tissue. Differences integrate the conditions over which the plants are grown. Analyzing the entire shoot indicates the water use efficiency for the time required to grow the shoot whereas analyzing only leaf starch indicates the water use efficiency during the time necessary to accumulate the starch. One may integrate over long or short times with the method and this avoids one of the problems of the gas exchange technique.

Martin and Thorstenson (1988) used this technique to show that differences

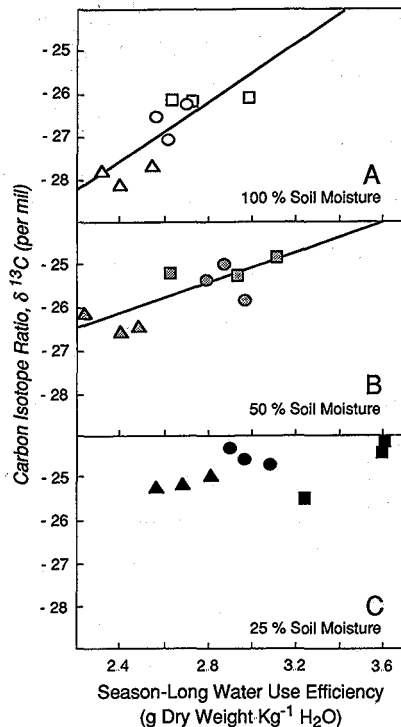


Figure 12.4 Relation between the carbon isotope ratio of leaf tissue and the water use efficiency for the whole growing season in tomato. The tomato species are the same as in Fig. 12.3 and the symbols are the same. Note that the correlations between isotope ratio and water use efficiency are better than for gas exchange in Fig. 12.3. The correlations in (A) and (B) are significant at the $P < 0.01$ and $P < 0.05$ levels, respectively. Adapted from Martin and Thorstenson (1988).

in water use efficiency were present between the domestic tomato species and *L. pennellii* and their hybrids. Figure 12.4 shows that the differences in water use efficiency were detectable in isotope ratio data between the parents and the hybrids particularly when water was optimally available. The domesticated parent had the lowest efficiency, the wild parent had the highest efficiency, and the hybrids showed intermediate behavior. Because the species could be crossed, it was possible to correlate the differences in water use efficiency with restriction fragment maps of the tomato DNA (Martin *et al.*, 1989). Three loci were found to be predictors of the variation in water use efficiency in field-grown tomato. This landmark effort indicates that water use efficiencies are determined by relatively few genetic loci and implies not only that agriculturally relevant differences exist but that they might be genetically manipulated in a simple fashion.

The success of the method suggests that differences in water use efficiency not

only exist within individual species but might be incorporated into breeding programs (Bowman *et al.*, 1989; Brugnoli *et al.*, 1988; Condon *et al.*, 1987, 1990; Hubick and Farquhar, 1990; Hubick *et al.*, 1986), although this is still in its infancy. A significant amount of variability is sometimes present in the data, but it is becoming clearer that selecting for extremes in carbon isotope ratios will select for extremes in water use efficiency. It is surprising that a complex trait like water use efficiency should be controlled by only a few genetic loci. Thus far it has not been determined whether each locus corresponds to more than one gene. It remains possible that the trait is in fact complex. Despite this situation, further studies of the genetic basis for differences in water use efficiency seem warranted, and it is likely that the differences will be heritable.

DROUGHT TOLERANCE

Plants showing improved growth with limited water are considered to tolerate drought regardless of how the improvement occurs. Some species can avoid drought by maturing rapidly before the onset of dry conditions or reproducing only after rain. Examples of these drought avoiders are ephemerals such as California poppy (*Eschscholtzia californica*) that can complete their life cycle in a few weeks or tree crops such as coffee, cacao, and mango that flower and fruit after moderate drought followed by rain (Alvim, 1960, 1985, cover photograph). Others can postpone dehydration by growing deep roots or sealing themselves tightly against transpiration or accumulating large stores of water in fleshy tissues. Examples of dehydration postponers are upland rice with deep roots compared to paddy rice (Chang *et al.*, 1974) or agave or saguaro cactus with thick cuticle or fleshy tissue adaptations. Still other species allow dehydration of the tissues and simply tolerate it by continuing to grow when dehydrated or surviving severe dehydration. *Fucus vesiculosus* is an example of a dehydration tolerator, and the acclimation of sunflower illustrates that dehydration tolerance can be increased as discussed in Chapter 10.

These effects are generally distinct from the factors controlling water use efficiency. Drought avoiders often reproduce themselves after a minimal accumulation of dry weight and their success ensures that they are represented in the next generation. Their adaptation centers on timing development and thus is under internal control. Dehydration postponers having deep roots may have a water use efficiency identical to that of other species but will accumulate more dry weight because of their ability to gain access to a larger amount of water than shallow rooted types. In effect the slope of the water use efficiency relation in Fig. 12.5 may be the same but the deep rooted species work farther out on the curve. Their adaptations are mostly structural and take time to build, requiring the expenditure of photosynthetic products. Finally, dehydration toler-

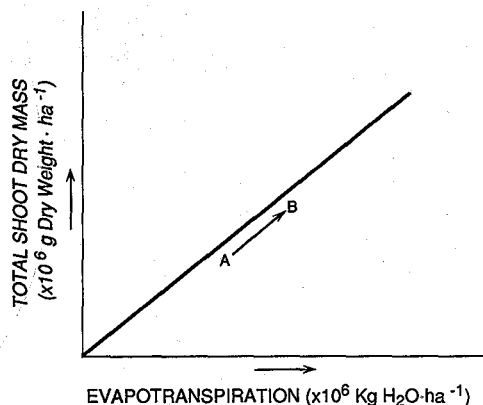


Figure 12.5 Effect of increasing the amount of water available to a crop without changing the water use efficiency. Production moves from A to B. An example might be increasing rooting depth.

ators may have the same water use efficiency as dehydration sensitive species when water is available but the tolerators can grow at tissue hydration levels at which the other species cannot.

Of the three forms of drought tolerance, dehydration tolerance is most intriguing because it often requires only slight repartitioning of dry mass. An example is osmotic adjustment (Morgan, 1984; Munns, 1988) which occurs because dry mass normally used to synthesize new cells instead accumulates in the cells as solute (Meyer and Boyer, 1972, 1981) or is deposited in fewer or smaller cells (Fraser *et al.*, 1990; Sharp *et al.*, 1990). Only a brief decrease in biosynthesis of tissue is necessary to accomplish this (Meyer and Boyer, 1981), but the increased concentration of solutes can markedly increase the ability of the cells to extract water from the soil. The increased solute is present only under dry conditions. In other words, there is little cost to the plant when water is scarce and no cost when water is plentiful.

Improvement of Drought Tolerance

From these examples it can be seen that crop improvement under conditions of limited water involves more than water use efficiency. Often, breeding programs for drought tolerance are based on the productivity of plants when water is plentiful. The idea is that, for a given climate, water use efficiency will be highest when dry matter production is highest. Because productivity is linearly proportional to water use (Figs. 12.1 and 12.2), the high productivity of dry matter should carry over to drought conditions. However, it is clear that many opportunities will be missed if superior selections are based only on this concept

of water use efficiency. Characters such as osmotic adjustment are called into play only during a water deficit. Roots may penetrate deeper soil layers or leaves may persist better during a water deficit in some genotypes than in others, and so on. Without plant selection under water-deficient conditions, these beneficial traits will be missed.

The design of a crop improvement program for drought tolerance seems difficult at first because water is so ubiquitously involved in growth and metabolism that identifying targets seems impossible. Moreover, the multitude of possible targets implies that effects might involve enormous numbers of genes and that improvements might be only incremental or, worse still, may cause problems at other genetic loci. However, there are examples of successful approaches that have resulted in significant improvements in the drought tolerance of plants. Jensen and Cavalieri (1983) described the improvement in drought tolerance they achieved by breeding maize after field testing at a large number of locations varying in water availability. Genotypes were identified that had all the combinations of yield performance under optimum and water-deficient conditions: a high yield in both conditions (Hybrids 3377 and 3358 in Fig. 12.6), a high yield in optimum conditions but a low yield under deficit conditions (Hybrid 3323 in Fig. 12.6), and a low yield in optimum conditions but a high yield under deficit conditions (Hybrid 3388 in Fig. 12.6). The first kind of response is the preferred one but the last response seems worthy of some consideration.

The study by Jensen and Cavalieri (1983) is particularly important because it tested whether improved yield under water limited conditions sacrificed yield under optimum conditions. Because grain yield from about 500 field replications was used to evaluate the germplasm and whole season yield performance, characters associated with particular environments or parts of the life cycle were included. Their experiments give the strongest possible evidence that improvement under water limited conditions need not sacrifice yield under favorable conditions. The number of replications, genotypes, and field sites was so large that this principle now seems beyond doubt (Fig. 12.6).

The principle was confirmed by Morgan (1983) in a completely different experiment. He selected wheat for superior osmotic adjustment under dehydrating conditions and observed improved yields that were at no cost to yield in optimum conditions. An important feature of this study was that the test genotypes had essentially the same genetic background. Therefore, osmotic adjustment was the main difference between the standard commercial genotype and the genotype with superior drought performance. Quisenberry *et al.* (1984) also tested the effectiveness of osmotic adjustment in cotton but concluded that it had little benefit. However, they failed to compare plants of similar genetic backgrounds and the results could have been caused by features other than osmotic adjustment. Therefore, the work of Morgan (1983) provides the better

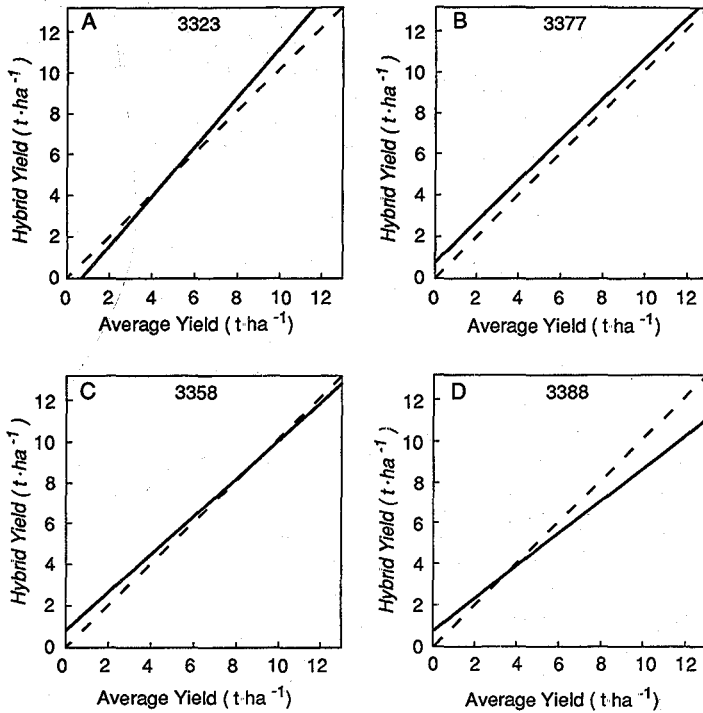


Figure 12.6 Regressions of seed yield of four maize hybrids grown at various locations in the United States over 3 years. The dashed line indicates the average yield for all hybrids at each location (1:1) and the solid line shows the yield of an individual hybrid for comparison. A solid line above the dashed line indicates a better than average yield for the hybrid. (A) Hybrid 3323, (B) hybrid 3377, (C) hybrid 3358, and (D) hybrid 3388. Except for (B), the slopes of each hybrid regression differed significantly from the slope of the dashed line ($P < 0.01$). The r^2 values were between 0.67 and 0.82 for the regressions of the four hybrids. Regressions were formed for 399 genotypes and, in most instances, for over 500 sites. Adapted from Jensen and Cavalieri (1983).

test and indicates that there can be a benefit of osmotic adjustment without sacrificing yield under optimum conditions. This probably is explained by the low metabolic cost of osmotic adjustment together with the lack of osmotic adjustment under optimum conditions.

Another example of genetic improvement of drought tolerance is the selection for improved seedling establishment in native range grasses in the western United States. Wright and Jordan (1970) showed rapid improvement in the establishment of boer lovegrass (*Eragrostis curvula* Nees) selected for seedling growth in dehydrated soil. The character that appeared most improved was the thickness of the cuticle covering the shoot tissues of the young seedlings (Hull

et al., 1978). These selections allowed the establishment of grasses to become more reliable when rooting was shallow, rainfall was sporadic, and germination had to occur with limited water.

Burton *et al.* (1954, 1957) showed that deep rooted Bermuda grass [*Cynodon dactylon* (L.) Pers.] exhibited increased pasture productivity compared to more shallow rooted types in humid regions subjected to sporadic drought. Deep rooted rice is another example of this approach to improving drought tolerance (Chang *et al.*, 1974), which works best in deep soils that allow deep rooting.

Another approach has been to control the life cycle so that growth only occurs when water is most available. Hall and Grantz (1981) selected early flowering cowpeas [*Vigna unguiculata* L. (Walp.)] that escaped late season drought. Because the reproductive tissues were the valuable structures, genetic selection for earliness restricted growth to the part of the season when water was available. Similarly, Passioura (1972) demonstrated that wheat using only water stored in the soil produced grain if the roots were pruned to reduce early season water use by the shoot but not if the roots were unpruned and the plants consumed most of the soil water by the time of grain fill. This experiment suggests that genetic means of controlling root morphology might be sought in wheat that matures grain on water stored in the soil. These kinds of genetic manipulation of development are valuable for climates where late season drought is predictable.

Although each of these approaches is unique, there are some common concepts among them. First, each investigator made selections under conditions of realistic water limitation in soils. Approaching the problem this way ensured that drought-adaptive factors were called into play and had an opportunity to express themselves. Important traits for drought performance were identified because they were present. This avoided the problem of selecting only crops yielding well in favorable environments and hoping they will not "crash" in water-limited environments. Second, in most cases, there was an intimate knowledge of the soil, climate, and physiology and biology of the crop. This knowledge increased the rate at which superior genotypes could be found. Morgan (1983) was aware that osmotic adjustment could benefit the crop. Without that knowledge, he would have been restricted to selections for yield alone. Third, selections often were for single traits. Reducing the problem to a few specific traits simplifies the selection effort. The number of selections and range of conditions are fewer and the program more easily fits into the available resources.

The complexity of drought tolerance has tempted many to take shortcuts such as selecting seedlings for growth in osmotica or using single biochemical tests for performance. In general, the results do not carry over into field situations. For example, Sammons *et al.* (1978, 1979) showed that physiological

tests on plants grown in controlled environment chambers gave tolerance rankings that differed from those for yield in the greenhouse. These differed again when ranked for field performance (Mederski and Jeffers, 1973). Therefore, for drought tolerance there seems to be no substitute for growing the plant under realistic field conditions or carefully simulated field conditions.

WATER DEFICITS AND REPRODUCTION

The principles discussed in the preceding section can be applied to any aspect of plant development and have the potential to improve individual features of performance. However, reproductive development holds particular interest because a large part of agricultural production is devoted to the reproductive parts of plants. In the United States, reproductive crops (grain, fruit, nut, vegetable) account for about 78% of the harvested area of land. Moreover, early stages of reproduction are more susceptible to losses from limited water than any other stage of development in reproductive crops (Claassen and Shaw, 1970; Salter and Goode, 1967). A good example is maize where part of the problem is caused by a high susceptibility of floral parts to inhibition of cell enlargement (Herrero and Johnson, 1981; Westgate and Boyer, 1985b). This susceptibility exists in part because the cells enlarge dramatically in the floral tissues during normal development, and water deficit can prevent the enlargement (see Chapter 11). However, more than cell enlargement is involved because Dampney *et al.* (1978) observed losses in inflorescence development in maize treated with abscisic acid during floral initiation before most enlargement of reproductive structures had begun. Losses in reproductive activity also were reported because of megagametophyte sterility (Moss and Downey, 1971), asynchronous floral development (Herrero and Johnson, 1981), and nonreceptive silks (Lonnquist and Jugenheimer, 1943), depending on when dehydration occurred. When gamete and floral development are normal and plants are hand-pollinated, reproductive failure still occurs and can be induced by only a few days of dehydration (Westgate and Boyer, 1985b, 1986b). The loss is caused by irreversibly arrested embryo development (Westgate and Boyer, 1986b). This indicates that, provided there is good floral development, pollination and fertilization can be successful. Nevertheless, a complete block in embryo growth can remain even when everything has been normal up to the time of cell division in the newly formed zygotes.

In other crops such as wheat and barley, drought during microsporogenesis caused pollen sterility (Morgan, 1980; Saini and Aspinall, 1981, 1982; Saini *et al.*, 1984). Well-watered plants whose stems were fed ABA (Saini *et al.*, 1984) or whose shoots were sprayed with ABA (Morgan, 1980) showed a similar pollen abortion, thus implicating high ABA levels during dehydrating conditions. However, the high ABA may have acted by closing stomata and inhibiting pho-

tosynthesis. Increasing CO₂ pressures around wheat plants overcame some of the reproductive losses (Gifford, 1979), which supports an involvement of photosynthesis. In rice (*Oryza sativa* L.), dehydration of the soil caused especially severe dehydration of reproductive tissues, and death and bleaching of florets followed (O'Toole *et al.*, 1984). The cuticle is only poorly developed on the floral tissues of rice and may be inadequate to prevent excessive dehydration (O'Toole *et al.*, 1984). Therefore, in various crops, there is increasing evidence for metabolic and growth regulator effects and some direct dehydration effects that might account for the susceptibility of early reproduction to water limitation. CO₂ and ABA are involved, and photosynthesis also may play a role but each might act in concert or separately, depending on the crop.

More insight may be possible from studies of embryo development in maize. Westgate and Boyer (1985a) found that the block in embryo development was correlated with low photosynthetic reserves in the maternal plant. Because photosynthesis was inhibited during the treatment, the lack of reserves could have caused embryo starvation. Westgate and Thomson Grant (1989) observed that the sugar content of maize embryos was not significantly different in hydrated and dehydrated plants but concluded that the flux of sugar might differ. Schussler and Westgate (1991a,b) found that the uptake of sugars was less in maize ovules isolated from dehydrated plants, even though the sugar content was high which further confirms that the flux of sugars was more important than the sugar content of the developing grain. Myers *et al.* (1990) showed an inhibition of endosperm cell division in maize when high ABA levels were present 5 to 10 days after fertilization.

Boyle *et al.* (1991a,b) took advantage of the finding of Westgate and Boyer (1985a, 1986a) that a few days of low water potentials can prevent embryo growth and developed a system to feed stems a complete medium for embryo growth during this time. This allowed photosynthetic products and other salts and metabolites to be supplied to the plants at normal levels without rehydrating the plants. The controls yielded well (Fig. 12.7A), but withholding water for a few days virtually eliminated grain production (Fig. 12.7B). Nevertheless, production was almost fully restored when the plants were infused with the complete medium as low Ψ_w developed (Fig. 12.7C). Infusing the same amount of water alone showed no restorative activity (Fig. 12.7D). Therefore, sufficient water was available to the embryos so that water itself was not the limiting factor. Reproduction was maintained by feeding other substances normally supplied by the parent plant during embryo development that the parent plant failed to supply. Thus, reproductive loss is a biochemical problem.

This type of experiment offers the promise of eventually identifying components that are required for reproductive development in plants and which may be lacking when dehydration occurs. Supplying these nutrients might be possible in superior genotypes or with cultural conditions that would allow large

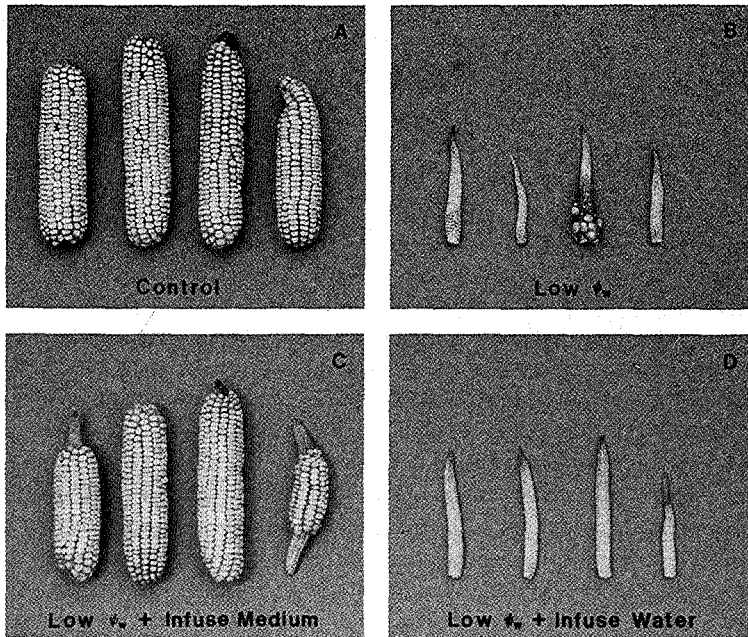


Figure 12.7 Grain yield at maturity for soil-grown maize plants subjected to a water deficit during pollination. Treatments were adequately watered controls (A), water-deficient plants from which water was withheld for 6 days (B), plants treated as in (B) but with stem infusion of a complete medium for embryo culture (C), and plants treated as in (B) but with stem infusion of water (D) in the same amount as in (C). All plants were rewatered on the sixth day. Plants were hand-pollinated. The grain weight of the plants infused with medium at low water potentials was about 80% of the weight of the controls. The infusion did not change the water potential or photosynthesis of the plants. From Boyle *et al.* (1991b).

amounts of the missing constituents to be present at the right time. This form of dehydration tolerance might protect against losses in reproduction, at least for short periods of water deficiency.

It also demonstrates that the reproductive fraction of the plant can vary from zero to nearly normal during a drought. This implies that successful protection of reproductive development may be possible under otherwise inhibiting drought conditions. It will be important to determine the active ingredients in the medium and whether any other aspects of reproduction also can be protected in the same manner.

From this work, it seems that through genetic manipulation the impact of dehydration could be minimized during the early stages of reproduction. For

example, Edmeades and his co-workers found that the time between pollen shed and silking can be changed by genetic means in maize (Bolaños and Edmeades, 1993a; Bolaños and Edmeades, 1993b; Bolaños *et al.*, 1993; Edmeades *et al.*, 1992, 1993), and early silking during drought may indicate vigorous development of the ear perhaps because the plant supplies more of the biochemical requirements for ear growth. Genetic selections in this direction might then increase the ability of the plants to maintain grain during a drought.

DESICCATION

When seeds mature, it is common for them to dehydrate as part of the maturation process. Barlow *et al.* (1980) found water potentials as low as -5 MPa in maturing wheat grain. Westgate and Boyer (1986c) observed water potentials of -7 to -8 MPa or lower late in the growing season in maize grain. These seeds are exposed somewhat to the atmosphere and are known to desiccate to a large extent by evaporation to the air. Seeds surrounded by a fleshy fruit show a similar but less severe desiccation. Welbaum and Bradford (1988) observed that water potentials of melon seeds decreased to about -2 MPa during maturation, and the surrounding fleshy fruit decreased similarly in water potential. Bradford (1994) considers high solute concentrations to be present in the apoplast surrounding embryos and proposes that structures may exist to keep the solutes localized there. The low osmotic potential of the apoplast solution may explain how the seeds are dehydrated inside fleshy fruits. Regardless of whether the seeds air dry or are dehydrated osmotically inside a fruit, it is clear that embryos become exceptionally tolerant of desiccation late in maturation despite their susceptibility to the effects of water limitation when they are young.

Plants lower in the evolutionary scale than seed plants sometimes show a similar tolerance to desiccation. Some fungi, algae from the intertidal zone, and a few mosses and lycopods can be desiccated to the air-dry state without losing viability (Bewley, 1979). Some specialized seed plants (*Craterostigma* species, *Myrothamnus flabellifolia*, *Xerophyta* species) that can tolerate desiccation also exist (Gaff, 1971, 1977; Gaff and Churchill, 1976). However, desiccation tolerance is virtually nonexistent in most agricultural species except for the seeds. It is curious that most seed plants, which are descendants of plants that crossed the intertidal zone, should have lost the ability to tolerate the desiccation that is so prevalent in that zone. In land plants, desiccation tolerance often evolved as part of the seed habit because an aqueous medium generally was absent and the embryo was exposed to drying conditions during dispersal. In agriculture, this property makes it possible to store seeds and allows uniform planting times.

An important aspect of severe desiccation is that water contents can become so low in the cells that enzyme activities can be directly inhibited by the lack of water, as described by Vertucci and Leopold (1987a,b). As discussed in Chap-

ter 9, enzymes equilibrated in humidities around 60% or below are affected directly because the hydration shells next to the protein are necessary for catalytic activity and may become modified. Substrates probably are unable to reach the active site of the enzyme because the aqueous medium is no longer continuous (Skujins and McLaren, 1967). Seeds desiccated to the air-dry state are likely to be affected by these phenomena. Most can return to activity when they are rehydrated, provided water contents have not become so low that the tightly bound water required for viability is lost (Vertucci and Leopold, 1987a,b).

On the other hand, leaves generally are susceptible to desiccation damage and in most crop species show a breakdown of compartmentation that releases cell constituents to the apoplast (Leopold *et al.*, 1981), and the plasmalemma and tonoplast show breakage followed by a loss of organelle structure (Fellows and Boyer, 1978). In leaves of tolerant species, the membranes and organelles remain intact although they often are distorted (Hallam and Gaff, 1978a,b). Therefore, an important distinction between tolerance and sensitivity to severe desiccation appears to be the maintenance of membrane structure and an ability of enzyme activity to return upon rehydration.

It has been proposed from work with desiccation tolerant animals that a possible mechanism to account for preservation of enzymes and cell structure might be an accumulation of specific sugars such as trehalose or sucrose whose structure resembles water in certain respects (Crowe and Crowe, 1986). Sugars having the appropriate stereostructure might form hydrogen bonds with cell membranes where water would ordinarily bind. Because the sugars would remain as water is removed, the bonding would be stable and membrane structure might be maintained where otherwise it would become disorganized.

Evidence that the sugar replacement hypothesis may have merit is the accumulation of sugars such as sucrose and raffinose in developing seeds (Caffrey *et al.*, 1988; Koster and Leopold, 1988). Species such as maize have seeds that can tolerate desiccation to the air-dry state, and their sugar concentration, while not high for the seed as a whole, becomes high in the remaining residual water and could have a stabilizing influence at local sites. As germination proceeds, the stabilizing sugars are metabolized to nonstabilizing ones such as glucose and fructose, and desiccation tolerance is lost (Koster and Leopold, 1988). A related hypothesis is that certain sugars may be converted to the glassy state during dehydration (Williams and Leopold, 1989). The glassy state is common in sugars such as sucrose used to make candy, and evidence for the existence of glassy sugars is accumulating for embryos of dehydrated seeds (Williams and Leopold, 1989).

A similar role has been proposed for certain proteins in seeds (Crowe and Crowe, 1986; Dure *et al.*, 1989). The developing seeds of a range of crops accumulate hydrophilic proteins in the embryo as normal desiccation begins (Dure *et al.*, 1989). The proteins have been variously called dehydrins, embryo

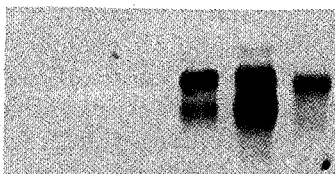


Figure 12.8 mRNAs for wheat dehydrins. A barley cDNA was used to detect the level of dehydrin mRNA in a total mRNA extract from severely dehydrated seedlings and in hydrated controls. al, aleurone tissue; sh, shoots; and root, root system. From Close and Chandler (1990).

maturation (Em) proteins, or late embryogenesis abundant (LEA) proteins (Dure *et al.*, 1989). Common to all of them is a high content of hydrophilic amino acids so that the proteins as a whole are highly water soluble. In some of them, an α -helix is present that could remain structurally stable during desiccation and it has been proposed that this portion of the protein could act like a membrane-stabilizing sugar (Crowe and Crowe, 1986).

The mRNAs for these proteins are not readily detected in leaves or roots of hydrated plants but can be induced by severe desiccation in very young rape (Harada *et al.*, 1989) and maize and barley seedlings (Close *et al.*, 1989; Close and Chandler, 1990). Figure 12.8 shows the marked increase in dehydrin mRNAs when young wheat seedlings were dehydrated soon after germination (Close and Chandler, 1990). The mRNA expression was especially increased in shoots, which are most exposed to dehydration under natural conditions. Also, the mRNAs can be induced by treating hydrated seedlings or immature embryos with high ABA concentrations (Galau *et al.*, 1986; Hong *et al.*, 1988; Mundy and Chua, 1988). ABA levels normally increase in plants subjected to dehydration and they become high in maturing dehydrating seeds (Ihle and Dure, 1972). The induction of these mRNAs suggests that the new dehydrin-Em-LEA proteins may be involved in the development of desiccation tolerance of young seedlings and embryos.

ANTITRANSPIRANTS

The possibility of reducing evaporation from plants and plant parts is attractive, and covering stem ends with protective coatings to prevent splitting or covering grafts with waxes to preserve viability has been practiced for many years. It has been possible to decrease the evaporation from large bodies of water by covering them with suitable films such as cetyl alcohol and, in principle, similar effects on transpiration could save considerable water and might reduce the inhibitory effects of dehydration on leaf metabolism. However, the plant carries on many activities besides transpiration and the effectiveness of an antitranspi-

rant is determined not only by its water-saving capability but also by the way in which it alters other aspects of plant performance.

Most antitranspirants act on the stomata by closing them or by covering them with a substance that decreases diffusion. Other approaches such as increasing the reflectiveness of leaves for infrared radiation or increasing the CO_2 partial pressure around leaves have been tried, but these have not proven practical because the infrared reflectance is already high for most leaves and increasing the CO_2 is too expensive. Therefore, most antitranspirants center on stomatal function, which was discussed in Chapter 8, and Gale and Hagan (1966) reviewed those types.

Stomata control not only the loss of water vapor but also the diffusion of CO_2 into the leaf. When an antitranspirant is applied that decreases water loss there is also the probability that the diffusion of CO_2 into leaves will be reduced, and most interest in side effects of antitranspirants has centered on photosynthesis and growth (which reflects photosynthesis to some degree as described in Chapters 10 and 11). At times when photosynthesis is not limited by the availability of CO_2 in the leaf such as in low light intensities, it should be possible to decrease transpiration without inhibiting photosynthesis. Similarly, under other conditions in which the photosynthesis rate depends on the internal partial pressure of CO_2 , it was argued that decreasing stomatal apertures should inhibit transpiration more than photosynthesis (Gaastra, 1959; Gale and Hagan, 1966; Slatyer and Bierhuizen, 1964b). As explained in Chapter 7, transpiration is determined mostly by the resistances to water vapor diffusion from the evaporating surface to the bulk air outside the leaf [$r_{\text{leaf}} + r_{\text{air}}$ in Eq. (7.2)], but CO_2 encounters an additional resistance to diffusion in the liquid of the mesophyll cells. The argument pointed out that because the total resistance to diffusion is thus smaller for transpiration than for photosynthesis, increasing the resistance of the leaf with an antitranspirant should have less effect on photosynthesis than on transpiration (Gaastra, 1959; Gale and Hagan, 1966; Slatyer and Bierhuizen, 1964b). Recognition of this fact gave considerable impetus to antitranspirant work (Gale and Hagan, 1966).

Nevertheless, it has been difficult to identify a chemical that reduces transpiration by closing the stomata without inhibiting photosynthesis or growth at least as much. Slatyer and Bierhuizen (1964b) tested several chemicals that decreased transpiration but all except phenylmercuric acetate caused photosynthesis to decrease as much as transpiration. Other workers found variable effects on photosynthesis and growth with this compound (Shimshi, 1963a,b; Zelitch and Waggoner, 1962a,b).

Film-forming chemicals such as hexadecanol can inhibit transpiration when coated on leaf surfaces containing the stomata. An ideal film would inhibit water vapor diffusion more than CO_2 diffusion or at least have equal effects on

both. However, Woolley (1967) measured the diffusive characteristics of a number of film-forming polymers and found that all inhibited the diffusion of CO_2 more than water vapor, some by large amounts. CO_2 is a larger molecule than H_2O and this result may reflect that fact. Woolley (1967) concluded that favorable polymers may not exist. Therefore, film-forming polymers have had their largest use in protecting overwintering ornamentals from desiccation damage or transplanting stock from water loss during storage. In short, the polymers work mostly in cases where photosynthesis is not important.

Most tests of the effectiveness of antitranspirants have been made on single leaves or plants, but crop canopies have much larger overall dimensions than the leaves making them up because the diffusive resistance of the air in the unstirred boundary layer and the turbulent boundary layer above is much larger in a canopy than when the individual plants and leaves are measured separately. As a consequence, the crop boundary layer is generally more limiting than the diffusive resistance of the leaves, and the stomata need to close much more to increase the diffusive resistance of the crop than in a single leaf of the same crop. An additional factor is the energy budget for the crop canopy (see Chapter 7) which absorbs radiation and partitions it between latent heat loss (energy lost in evapotranspiration) and sensible heat loss (energy lost because of a temperature difference between the leaf and air). According to the energy budget, decreasing stomatal apertures by large amounts to decrease transpiration will force more of the radiation to be partitioned to sensible heat loss which results in increased leaf temperature. The increased temperature increases the vapor pressure of water in the leaf, reversing somewhat the effects of stomatal closure. Therefore, canopy transpiration can be inhibited only by marked stomatal closure under many conditions, and this causes marked inhibition of CO_2 uptake and photosynthesis. With the demonstration of this principle by investigators such as Van Bavel (1966, 1967), Van Bavel and Ehrlar (1968), Brown and Rosenberg (1973), and Johns *et al.* (1983), research with antitranspirants was largely abandoned.

SUMMARY

The development of the land habit was a dramatic phase in evolution and caused major changes in plant form and function. These appear to be continuing amid strong evolutionary pressures imposed by the inherently dehydrating conditions presently existing on land. As a consequence, land plants are not yet optimally adapted to meet dehydration and we would likely see large improvements in dehydration performance if this chapter could be written a few hundred million years hence. However, modern methods of plant breeding and genetic modification can speed the transition to increased tolerance of dehydration so that it can occur within a few years.

Indeed, considerable success has already been achieved. Water acquisition can be improved by deep rooting and strong osmotic adjustment, earliness in reproduction can be used to avoid late season droughts, and cuticular characters can be modified to conserve water in some cases, although antitranspirants have not proven generally practical. It also appears increasingly possible to improve water use efficiency by genetic means using new techniques for screening for this trait. Central to these efforts is the use of realistic drought conditions, and selection for a small number of traits that might be valuable for crop performance with limited water. It is now clear that successful improvement of drought performance can come at no sacrifice to performance under favorable conditions.

Water is required for biological activity, and studies of plant water use efficiency show that total dry matter production is linearly proportional to the amount of water used. Total water acquisition can be enhanced by means such as larger deeper root systems with concomitant increases in dry matter production. However, it also is possible to partition dry matter production differently to valuable plant parts. This is relatively independent of the overall water use efficiency and provides a means for maintaining a fraction of plant development that may be important without having to improve the productive capacity of the plant as a whole. This approach could lead to large benefits.

Metabolic changes have developed during the course of evolution that have improved the ability of plants to withstand limited water supplies, particularly in photosynthesis. The recent evolutionary development of C_4 photosynthesis and Crassulacean acid metabolism are clear examples of this type of metabolic adaptation, leading to increased water use efficiency in those species possessing it. The demonstration that reproductive losses usually associated with drought often are a biochemical problem instead of a direct water deficiency problem further supports the notion that metabolic modification may be important for improving plant performance with limited supplies of water.

The molecular mechanisms of reproductive losses and desiccation tolerance are being elucidated, and the work with desiccation tolerance shows that changes in expression of specific genes are correlated with a decreased lethality of severe desiccation at least during late seed development. Plant growth regulators such as abscisic acid can trigger this protective mode and it will be interesting to determine whether the impact of desiccation can be ameliorated by manipulating this regulation chemically or genetically.

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