STRESS PHYSIOLOGY AND CROP IMPROVEMENT

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ABSTRACT
This paper concentrates on the physiological responses of forages to three major environmental stresses - cold, heat and drought. Methods for identifying genetic variation in mechanisms underlying these physiological responses are evaluated as a basis for developing selection criteria. The implications of climate change on these physiological responses is considered and opportunities for alleviating adverse effects of these environmental stresses on survival and production are discussed.

KEYWORDS
Environmental stress, crop improvement, cold, heat, drought, grasses, clovers

INTRODUCTION

For many agricultural systems such as annual crops, maximizing yield under optimum environmental conditions is a high priority, whereas with perennial crops and natural vegetation the ability to survive periods of environmental constraint is an essential characteristic for success. A survival strategy may be more important than a growth strategy, particularly in more extreme or variable environments. The balance of these two strategies may need to be shifted towards survival in crop improvement programmes as crops are grown increasingly at the limit of their adaptation, while the more frequent occurrence of extreme events predicted with global climate change may mean that the ability to tolerate environmental constraints is essential for a cultivar to give reliable production. As Boyer (1982) points out, there is often a dramatic difference between maximal and average yield for any given crop species. The actual yield achieved is dependent on the environmental conditions the crop encounters. Additionally, genetic variation exists in the ability to survive and grow under stress conditions, and this needs to be combined with increased ability for yield so that not only is the discrepancy between yield under optimal conditions and actual yield reduced but an increased stability of yield obtained. These considerations are important for the development of new cultivars of agricultural and amenity grassland species and are also relevant for natural grasslands. We have chosen cold, heat and drought to illustrate the genetic variation in physiological responses to environmental constraints which is available for selection.

COLD

Although the optimum temperature for growth of temperate grasses is relatively low (Eagles, 1967), plants regularly experience temperatures well below the optimum for leaf growth. In these low temperature conditions, typical of autumn, leaf expansion may be suppressed more than photosynthesis with the result that assimilates accumulate in the leaf bases (Pollock and Ruggles, 1976). This seasonal shift in the balance between growth, quiescence and assimilate storage in response to annual fluctuations in temperature is an important feature of adaptation to a cool temperate environment. Reduced leaf expansion and accumulation of assimilates are frequently associated with both low temperature hardening and increased cold tolerance, leading to reduced risk of freezing damage. It is important to point out that, although a correlation between carbohydrate accumulation and freezing tolerance is well established, the evidence for a role for carbohydrates as cryoprotectants is inconclusive. The consequences of genetic variation in growth at low temperature on cold hardening ability became particularly obvious during the 1960's when germplasm of Mediterranean origin was introduced into *Dactylis* and *Trifolium* breeding programmes with the aim of extending seasonal productivity by incorporating the out-of-season growth characteristics of Mediterranean populations. The negative relationship between growth at low, but non-sub-lethal temperatures and tolerance of lethal temperatures now widely established (Cooper, 1964; Breese and Foster, 1970) proved to be the downfall of the cultivars derived from these breeding programmes. Similar adverse effects on cold hardening ability (Hides, 1978b) and winter survival (Jung and Kocher, 1974; Hides, 1978a) have been reported where out-of-season growth is encouraged by late fertiliser applications, particularly when combined with cutting managements which impose greater stress (Charles et al., 1975). These treatments also have an effect on the carbon balance of the plant (Hides, 1978a), as do dull, warm conditions during winter which reduce cold tolerance while promoting growth at the expense of carbohydrate accumulation (Thomas and Norris, 1979; 1981). Green (1969) has suggested that exhaustion may be a major cause of winter damage in these conditions, which are typical of maritime winter climates.

Although cold can be an important factor in winter survival, either indirectly through hardening responses or directly through freezing damage, other factors, either singly or collectively can influence winter damage. Winter hardiness is a complex character and its expression is determined by the ability to tolerate a range of environmental stresses such as freezing temperatures, fluctuating temperatures, wind, snow cover, ice encasement, heaving, low-light conditions, and low temperature pathogens. The relative importance of these factors will vary with local climatic conditions and management practices, although low temperature is probably the most important single factor which influences winter hardiness across a range of environments. Although the ability to survive under field conditions must be the ultimate assessment of winter hardiness, the choice of site and year can have a major effect since genotype x environment interactions in winter hardiness are highly significant (Habeshaw and Swift, 1978). To overcome this problem protocols have been developed utilising artificial freezing tests for assessment of cold tolerance (Wit, 1952; Pomeroy and Fowler, 1973; Fuller and Eagles, 1978). If these techniques are to be of use to plant breeders, reduction in environmental variation is essential to ensure reliable selection of superior genotypes. In our tests, measurements of LT_{50} values are made either for seedlings grown and hardened under controlled environment conditions or for mature plants grown under natural conditions. Although a protocol utilising seedlings can be criticised since perennial forage crops overwinter as mature plants, measurements of cold tolerance (LT_{50}, defined below) for seedlings of contrasting genotypes of several species are consistent with agronomic assessments of winter hardiness (Fuller and Eagles, 1978).

Fluctuations in cold tolerance of tillers of contrasting cultivars of perennial ryegrass during winter may indicate the physiological basis of genetic differences in winter hardiness (Eagles and Fuller, 1982). In autumn, hardy cultivars begin hardening much earlier than susceptible cultivars, with maximum hardiness recorded in December. In maritime climates,susceptible cultivars often show a rapid loss of hardiness during periods of warmer weather and although some rehardening may occur with the return of colder conditions the earlier hardiness levels may not be achieved. These fluctuations in cold

tolerance, also recorded for stolons of white clover cultivars (Eagles et al., 1994), are a typical feature in maritime winter environments.

Differences in hardiness between contrasting cultivars are not normally expressed in the unhardened state after growth at optimum temperatures and are only observed after a period of exposure to hardening conditions. Several requirements for effective hardening have been proposed, including low temperature, high light, short photoperiods and mineral nutrition, but the answers to several questions about hardening and dehardening have remained unclear in the absence of detailed experiments to address issues such as the range of temperatures which can induce hardening and dehardening, their effective time scales and whether there is genetic variation in temperature responses for these processes.

Temperatures between 2°C and 10°C are reported to induce hardening, with higher temperatures in this range less effective as hardening environments and often causing loss of previously acquired hardiness (Gay and Eagles, 1991). The state of hardiness can be measured in artificial freezing tests as the damage incurred at a single freezing temperature by leaf, tiller or plant, but this technique often fails to discriminate between cultivars with small differences in hardiness. A more sensitive measure of cold tolerance is given by the temperature that causes 50% mortality of a population (LT_{50}) (Pomeroy and Fowler, 1973). Most protocols have used a hardening environment of low temperatures with short day conditions to simulate an autumn environment. We have used 2°C with an 8-h photoperiod (Fuller and Eagles, 1978) while other experimental protocols have used various temperatures from 5°C down to -4°C (Andrews, 1960; Junttila et al., 1990), sometimes with fluctuating temperatures during the hardening period (Tysdal, 1933; Pomeroy et al., 1975). Typically, experimental hardening periods extend to 14-21 days although some have been appreciably longer.

Results from this type of hardening experiment often apply only to the specific conditions used. Hardening parameters of broader application that allow generalisations about hardening and dehardening ability of cultivars would be a significant development in the study of cold hardening. Our recent series of experiments have gone some way to produce comprehensive data sets for temperature responses of hardening and dehardening of cultivars of *Lolium perenne*, *L. multiflorum*, *Phleum pratense* and *Avena sativa* with contrasting winter hardiness ratings (Eagles, 1989; Eagles, 1994; Eagles et al., 1994). In an attempt to quantify these responses, hardening and dehardening have been analysed as quantitative processes dependent on temperature and time (Gay and Eagles, 1991). The resulting model has two components, the first part describes the time course of change in LT_{50} from the unhardened to the hardened state, and *vice versa*, which was best fitted by an exponential relationship. The second part of the model describes the relationship between temperature and the maximum change in LT_{50} which was best fitted by a logistic relationship. The most important feature of this analysis is the derivation of biologically meaningful parameters describing the kinetics of the processes which are a significant development in the analysis of genetic and environmental effects on cold hardiness. These hardening and dehardening parameters offer a simple characterisation of these processes for an individual cultivar and a more rigorous comparison of the physiological basis of differences in cold tolerance than previously possible. They also offer an explanation of fluctuations in hardiness which can occur under natural conditions and may allow predictions of the consequences of climate change on these processes and hence on winter damage.

The parameters derived from the model were (1) initial rates of hardening and dehardening at a given temperature, (2) maximum potential of these processes for a cultivar and (3) the percentage of this potential completed after a given time. The predicted maximum hardiness which can be achieved with optimum hardening temperature was little different from the maximum hardiness predicted at 2°C for cultivars of *L. perenne*. The levels of hardiness predicted from the model were consistent with the agronomic classification of winter hardiness for the cultivars. Initial rates of hardening were very low at all temperatures for susceptible cultivars and were not affected significantly by temperature. In contrast, hardy cultivars hardened rapidly at all temperatures with faster rates at lower temperatures. These cultivar differences in initial rates of hardening were reflected in large differences in predicted percentage completion of maximum hardening potential.

The ability of hardy cultivars to achieve significant hardening at warmer temperatures, compared with the requirement of the susceptible cultivars for relatively long periods at lower temperatures, is consistent with the earlier hardening of a hardy cultivar of *L.perenne* in the autumn (Eagles and Fuller, 1982). The more rapid initial rates of hardening for hardy cultivars suggest that they are better adapted to adjust their hardiness in response to sudden lowering of temperature whereas susceptible cultivars can be very vulnerable to sudden changes in temperature.

Faster initial rates of dehardening were derived for susceptible cultivars than for hardy and intermediate ones with more of their potential dehardening completed with time. There was marked genetic variation in temperature sensitivity of initial rates of dehardening with susceptible cultivars showing significant loss of acquired hardiness even when the temperature was only raised from 2°C to 4°C whereas hardy cultivars were able to maintain hardiness levels, at least for a number of days, when exposed to 8°C. The analysis shows dehardening to be a much faster process than hardening which may indicate that field hardiness is potentially very sensitive to the occurrence of dehardening temperatures. It seems likely that the large variability in cold hardiness of cultivars measured in the field (Habeshaw and Swift, 1978) may be attributed to these rapid rates of dehardening. Cultivar differences in temperature sensitivity of hardening and dehardening may shift the balance between them under the fluctuating temperatures typical of a maritime winter environment, resulting in rapid changes in hardiness similar to those observed for a susceptible cultivar of *L. perenne* (Eagles and Fuller, 1982).

This greater temperature sensitivity of dehardening in susceptible cultivars may be an important factor determining their winter damage in maritime environments and may assume even greater importance in a global warming scenario when the balance between hardening and dehardening may be shifted. With a simple calculation of the net changes in hardiness for different temperatures, based on the assumption that initial rates of hardening and dehardening are additive, it is possible to determine the effect of small changes in temperature on hardiness. This type of analysis suggests that susceptible cultivars would increasingly lose hardiness at temperatures above 4°C (at a rate of $0.3°Cd^{-1}$ at 4°C to $0.75°Cd^{-1}$ at 8°C) whereas hardy cultivars would continue to increase their hardiness, albeit more slowly, at higher temperatures up to 8°C where there would be no net change. This suggests that the cold hardiness of a susceptible cultivar may be seriously affected by warmer winter temperatures making it more vulnerable to freezing damage. Although the cold hardiness of hardy cultivars would also be affected, the consequences for increased risk of winter damage may not be so great. Calculations of the fluctuations in hardiness that would occur

for contrasting cultivars on exposure to the mean daily temperatures of a natural environment, and to these temperatures incremented by 2°C to simulate a response to global warming, offer a more realistic risk assessment.

A comparison of the parameters derived from the models for ryegrass (a species normally considered to be hardy in UK) and for winter oats (a susceptible species at the limit of its winter adaptation in UK) gives a possible clue to the basis of their contrasting hardiness. The maximum potential change in LT_{50} at individual temperatures expressed as a percentage of the maximum predicted hardening potential showed that winter oat cultivars only achieved a very small percentage of their potential LT_{50} in the temperature range 6 - 10°C (20% at 8°C) compared with ryegrass cultivars (80% at 8°C). The poor hardiness ranking of winter oats may be explained by incomplete hardening even at 2°C where only 66% of its potential was realised compared with almost 100% even for susceptible ryegrass cultivars, suggesting that winter oats may require hardening temperatures lower than 2°C to achieve complete hardiness (Eagles, unpublished).

This type of quantitative analysis demonstrates how different thermal characteristics of the hardening and dehardening processes have the potential to modify cold hardiness of contrasting cultivars, particularly in the fluctuating temperatures of a maritime winter environment. The sensitivity to temperature shown by ryegrass cultivars could have a selective disadvantage in some environments and it is reasonable to anticipate that plants which have evolved in an environment with more extreme winters may not depend solely on current temperature to maintain acquired hardiness. Evidence from Scandinavia (Heide, 1982; Hay, 1990) shows that growth and development of grasses from high latitudes is strongly influenced by photoperiod.

There is some evidence that temperature responses for dehardening of more extreme populations of *Phleum pratense* are modified by photoperiod (Eagles, 1994). A cultivar from the north of Norway maintains its hardiness even when exposed to 10°C with photoperiods of 8h and (8+8)h but when exposed to (8+16)h photoperiods significant dehardening occurs at temperatures above 4°C. A less extreme cultivar from south-west Norway and a British cultivar show less ability to maintain their hardiness when exposed to temperatures above 4°C, particularly with photoperiod extension. This modification of temperature responses for dehardening by shorter photoperiods could have important consequences for maintenance of hardiness under natural conditions. Further, any promotion of dehardening which may arise through global warming would only occur in extreme populations when photoperiods exceed 16 hours, by which time there would be reduced risk of severe cold. This degree of photoperiodic control of dehardening would not be necessary in lower latitudes where the seasonal change in photoperiod is much less and the risk of severe cold has declined before photoperiods of 16 hours are reached.

Photoperiodic regulation of dehardening responses may also be critical for survival in some extreme winter climates of high altitude environments. This has been shown with two cultivars of white clover derived from populations collected in the Swiss uplands which show markedly different growth responses in mild winters (Chorlton and Rhodes, 1983). AberHerald, originating from a site with almost continuous snow cover during winter, expands leaves in response to warmer temperatures of a maritime winter but shows little ability to maintain acquired hardiness at elevated temperatures in either long or short photoperiods. In contrast, AberCrest, originating from a site with alternating cold and mild conditions during winter and intermittent snow cover, does not expand leaves in warm periods during the winter although it responds to similar temperatures in a controlled environment when exposed to long photoperiods, suggesting photoperiodic control of leaf expansion. Similarly, its acquired hardiness is maintained at temperatures up to 8°C in short photoperiods while dehardening occurs more readily with photoperiod extension (Eagles et al., 1994).

These interactions of photoperiod and temperature on dehardening of timothy and white clover suggest that cultivars from more extreme winter climates have developed an effective survival mechanism to avoid any possible damaging effects of dehardening. This photoperiodic regulation of dehardening could also prevent any adverse effects of global warming on winter hardiness. The early cessation of growth in autumn typical of photoperiodic control makes this mechanism unsuitable for achieving a breeding objective of an extended growing season for cultivars aimed at a more maritime climate. However, if global warming produces a significant shift in the balance between hardening and dehardening then the incorporation of photoperiodic control of dehardening into cultivars for these fluctuating winter climates should be considered.

Most studies of cold tolerance have concentrated on the acclimation process and the freezing process with the recovery period receiving relatively little attention and even excluded from some protocols where damage is assessed by techniques such as electrical conductivity (Jenkins and Roffey, 1974), release of ninhydrin-reactive metabolites, chlorophyll fluorescence (Barnes and Wilson, 1984) and vital staining. Eagles et al. (1993) have identified the recovery period after freezing as a critical stage in the analysis of cold tolerance of winter oats and perennial ryegrass. The main apex appears to have little ability to harden and survival depends on the development of viable regrowth from dormant tiller buds during the recovery period after the main apex has been killed by freezing. This recovery regrowth may be affected by differences in assimilate availability which could be established either during low temperature hardening when production exceeds utilisation or as the result of post-freezing photosynthetic activity. Manipulation of assimilate availability by light level during hardening and during recovery after freezing has significant effects on LT_{50} achieved, with a high light treatment during hardening giving 3.5°C greater hardiness. Plants previously exposed to low light during hardening show increased hardiness in response to high light during recovery, indicating that assimilate availability influenced by post-freezing photosynthesis may be a factor in regrowth from tiller buds. Although this result does not prove the existence of post-freezing photosynthesis, infra-red gas analysis measurements have established that the capacity for photosynthesis exists in *Lolium* following our freezing protocol (Tonkinson and Eagles,1994). The time course of photosynthesis in unhardened and hardened plants of hardy and susceptible cultivars after a range of freezing temperatures was consistent with a role for assimilates in tiller bud regrowth during recovery after freezing. Photosynthesis was unaffected by non-lethal freezing temperatures, but at -6°C and below photosynthetic competence declined sharply coinciding with the temperature for apical death. Unhardened plants show no recovery of photosynthetic competence whereas hardened plants display different patterns of recovery of photosynthetic rates, with the magnitude and time course of recovery related to the cultivar and the severity of freezing stress. Stomatal conductances to CO_2 and water vapour, and internal CO_2 concentrations indicate that photosynthetic rates are not limited by CO_2 availability but by damage to the photosynthetic machinery which in unhardened plants shows no sign of recovery. In contrast, hardened plants of hardy cultivars are capable of CO_2 assimilation after freezing to lower temperatures

than susceptible cultivars.

Higher carbohydrate contents in leaf tissue of hardened compared with unhardened plants, and in hardy compared with susceptible cultivars when frozen to temperatures below -6°C, are consistent with the hypothesis that assimilate availability is a critical factor in survival after freezing. The ability to translocate these assimilates to the recovering apex is a key factor in establishing the validity of this hypothesis and preliminary results from $^{14}CO_2$ feeding experiments indicate that this does occur in *Lolium*.

Future research to elucidate basic mechanisms of plant adaptation to departures from optimal temperatures should include studies of genetic variation in hardening and dehardening under both controlled environment conditions and field conditions so that better models can be developed to consolidate our knowledge of those aspects of the processes which are most sensitive to environmental change. This may offer plant breeders more appropriate selection criteria for the development of new cultivars with suitable cold hardiness characteristics to exploit future grassland systems in a changing climate. It would also focus plant collection on climatic regions where appropriate characters or combinations of characters have evolved thus presenting suitable potential breeding material. If extension of the growing season and improved winter hardiness are maintained as dual breeding objectives then greater effort will be required to sever the negative relationship between growth at low temperature and ability to harden, either genetically or by introducing new germplasm from different climatic regions.

It is somewhat discouraging that physiology is becoming unfashionable just when a better understanding of the physiology of cold hardening, dehardening and recovery may identify critical events in the control of hardiness thus allowing the new technologies to be exploited in the manipulation of genes controlling these processes. It is essential that efforts directed towards genetic manipulation are based on sound physiological knowledge of the processes involved and that the physiological consequences of these manipulations are assessed.

HEAT

As discussed above, low temperature is one of the main environmental risks associated with mortality of temperate grasses in northern temperate regions, whereas, in warmer areas, such as Mediterranean environments, summer drought associated with supra-optimal temperatures can be the factors most limiting to production and survival of perennial grasses. Analysis of the effects of high temperatures on temperate grasses focuses on the development of screening techniques to identify heat tolerant germplasm for incorporation into breeding programmes with three main objectives; (a) to extend the geographic range of species such as perennial ryegrass (Wilkins, 1991), (b) to improve quality which frequently declines during periods of high temperature stress (Minner et al., 1983), and (c) to improve survival by determining the relative importance of drought and supra-optimal temperatures (Wardlaw and Wrigley, 1994).

Screening in the field in the target environment has the advantage of using relevant stress levels. However, the natural climate is both variable and unreliable. Moreover, in the field the interactions of water stress, high temperature and other edaphic constraints on plant responses complicate the assessment process. Dissecting a complex process such as survival into component traits that are under more simple genetic control should permit rapid and precise crop improvement. An understanding of the physiology of the response to heat is required to enable the development of such tests. Most laboratory tests are therefore based on analysis of whole plants or plant segments in controlled environments. Recently, molecular techniques have been used to detect cultivar differences in levels of thermotolerance. It is essential, however, that a laboratory test has a significant relationship to field performance.

Imada et al. (1993) identified heat tolerant cultivars of perennial ryegrass by increasing temperature in a growth cabinet to impose heat stress (36 to 48°C) and estimating survival visually. Recovery of *Lolium perenne* and *Poa pratensis* after immersion in a water bath (36 to 42°C) rapidly detected heat tolerant cultivars (Minner et al., 1983). By studying the recovery of *Poa pratensis*, *Poa annua* and *Lolium perenne* after exposure to 41 to 49°C for 30 min, Wehner and Watschke (1981) showed that initial injury occurred at 41 to 43°C with complete kill at 47 to 49°C and that *P. pratensis* was more heat tolerant than the other species tested.

A cell membrane system that remains functional during heat stress appears central to adaptation of plants to high temperatures (Raison et al., 1980). Thus electrolyte leakage has been proposed as a way to quantify thermal tolerance (Sullivan and Ross, 1979; Blum, 1988) but care must be taken since injury to membrane stability is influenced by age of the leaf and the part of the leaf sampled (Wardlaw et al., 1989), and the expression of heat tolerance is dependent on acclimation (Martin and Wehner, 1987; Blum, 1988). Wehner and Watschke (1984) found no significant differences between various turfgrasses in efflux of cell solutes from tissue sections heated to 43-49°C. They concluded that disruption of some other physiological process might account for the heat tolerance rankings of the grasses. White et al. (1982) found greater electrolyte leakage from perennial ryegrass leaf segments subjected to 55°C than to slightly lower temperatures (51-52°C). The effect of acclimation to a high but non lethal temperature (40°C) for 7 days before the treatment was stressed. However, it was unclear whether the evaluation could provide reliable information on thermal tolerance of the cultivars tested due to variation in electrolyte leakage associated with length of acclimation and timing of the measurements. Selection of appropriate test and acclimation temperatures are critical. The modelling approach described by Gay and Eagles (1991) for cold acclimation would provide a useful tool.

Julander (1945) emphasised the interaction between water stress and heat acclimation and suggested that heat resistance could be an indication of the drought resistance of perennial grasses. Wallner et al. (1982) showed that quantitative differences in heat tolerance, measured by electrolyte leakage of leaf segments, were consistent with qualitative descriptions of drought resistance for most species and that the best criterion to illustrate differences of heat tolerance among species was the killing time (LT_{50}). However, Becwar et al. (1983) could not ascribe any variation of *in vitro* tolerance of several grasses to levels of water stress imposed either by reducing water supply or by using PEG solutions.

It is clear from the literature that greater emphasis has been placed on the role of drought tolerance than of heat tolerance as a factor in survival of temperate grasses in warm climates. However, this is not the situation for tropical grasses which are cultivated near their high temperature maxima for growth and where an increase in temperature can have dramatic effects on growth and survival. These high temperatures can cause considerable damage when they coincide with vulnerable stages of plant development, particularly seed germination and seedling establishment (Howarth, 1991). The risk of hot, dry seedbed conditions during crop establishment is high in much of the

semi-arid tropics, with soil surface temperatures often greater than 50°C at midday (Peacock et al., 1993) recurring for many days following sowing which causes both thermal injury and death of young seedlings. Injury depends on the absolute temperature, the duration of exposure and the frequency of exposure to such conditions.

Plants are able to acclimate to changes in environmental conditions; periods of moderate stress induce tolerance to more severe conditions. However, there is a limit beyond which plants cannot acclimate. Acclimation involves both short-term chemical, molecular and physiological responses and longer-term physiological, structural and morphological modifications. Successful acclimation results in some form of change in metabolism or structure so that the plant not only survives the stress but recovers from the consequences of the stress. Identification of stress-induced changes in gene expression is one approach to understanding the ability of plants to acclimate to, and tolerate, environmental stresses (Howarth and Ougham, 1993). It is hypothesised that particular proteins whose synthesis is induced by stress conditions are critical in the survival of that stress. For example, above a threshold temperature the normal pattern of protein synthesis is repressed and a new set of proteins are synthesised from newly transcribed mRNA. These are the heat shock proteins (HSPs) and their synthesis shows a strong correlation with the acquisition of induced thermotolerance although the precise function of HSPs in thermotolerance is not understood.

The temperature at which HSP synthesis occurs is positively correlated with optimal growth temperature so that HSPs accumulate maximally at 45°C in pearl millet seedlings (Howarth, 1989) and 35°C in *Lolium temulentum* (Ougham, 1987). The response of gene expression to an increase in temperature is very finely modulated and is dependent on the severity of the heat treatment. In comparison to changes induced by low temperature, the heat shock response is very rapidly induced and is detectable within minutes of the onset of high temperature conditions. Although HSPs are relatively long-lived, induced thermotolerance does not persist from one day to the next. A subsequent heat shock, during which HSPs are again synthesised, returns the tissue to a thermotolerant state (Howarth and Skt, 1994). The ability to survive repeated high temperature exposure on a diurnal basis is of critical importance in parts of the world where high midday temperatures are prevalent and genotypic differences exist in this ability (Howarth, 1991).

DiMascio et al. (1994) detected differences in levels of HSPs between a thermal-tolerant and a thermal-sensitive cultivar of *Lolium perenne* and they concluded that it should be possible to test the heat shock response and the induction of thermotolerance in grass cultivars by evaluating gene products or polymorphism of thermal tolerance genes. Differences in gene expression in relation to level of thermotolerance have been found in cell cultures of *Lolium temulentum* (Bettany, 1995a,b) and of heat tolerant and non-tolerant variants of *Agrostis palustris* (Park et al., 1996). In addition, Robertson et al. (1994) showed that thermostability of cell suspensions of *Bromus inermis* was conferred by ABA-responsive, heat stable proteins and cell osmolytes such as sucrose .

Seedling vigour measurements have also been used to assess their significance on seedling survival of heat stress. Fast seedling growth and consequent early seedling establishment could be one strategy to escape a stressful environment, particularly as conditions for seedling establishment often become increasingly less optimal with time after sowing. However, rapid development of a greater leaf area might result in faster depletion of limited soil water resources. In fact, measurements of leaf growth in the field indicate that a conservative growth strategy may favour survival in extreme environments.

Adaptation to a given trait is complex. Plant physiology can be used to identify not only critical components but also to identify genes or regions of chromosomes linked to a given trait. This is done using molecular markers,such as restriction fragment length polymorphisms (RFLPs), combined with physiological screening and permits the mapping, identification, manipulation and combining of specific genes involved in tolerance. The challenge is to identify specific physiological or biochemical processes and to develop rapid, high-throughput screening techniques based on them. Molecular marker maps have now been developed for many species and these can be used to identify quantitative trait loci (QTLs). Once identified, marker assisted selection can be used precisely to improve the required character by following closely the movement of desired and undesirable gene segments. Mapping potential physiological and biochemical components of a trait also provides information on their involvement in that trait and is a new way into elucidating the mechanism of plant responses to the environment. Genetic mapping not only shows in a much clearer fashion how traits are genetically correlated but how they are linked on the chromosomes. Active collaboration between geneticists, molecular biologists, physiologists, breeders and germplasm collectors is required to ensure success. Considerable genetic variation exists for survival of high temperature conditions and identification and exploitation of this variation is essential for crop improvement, particularly if production is expanded into more marginal lands as the result of increased population pressure and climatic change.

DROUGHT

The importance of drought. Drought is a complex phenomenon: it involves not only dry soil, but also high evaporative stress, high temperature (discussed above), high irradiance leading to photo-oxidation, unavailability of mineral nutrients (especially phosphate) in dry soil, toxic concentrations of other minerals, and increased soil hardness. Drought resistant plants have to tolerate many or all of these stresses. In this section we will concentrate on the effects of water availability on temperate forage species.

Leaf growth is very sensitive to drought because water is needed to expand the young cells. Consequently, foliage crops such as forages are often seriously affected by even slight drought. To ensure maximum yield temperate forages may have to be irrigated whenever soil moisture deficit reaches 25 mm (MAFF, 1982), a deficit which can be achieved in 3 days without rain in a Mediterranean environment, or 6 days in a more maritime one. Leaf growth during drought may continue for about 25 – 100 days, depending on the environment.

What are the options for improving forage production under drought? Since irrigation is usually not economically justifiable, the most obvious option is to grow species which are naturally drought-resistant (described by Humphreys, 1981; Tueller, 1988), but unfortunately, they often have low yield and digestibility. Another option is to carry out empirical field trials of the more agronomically-desirable species in dry environments in order to identify or breed good, drought-resistant cultivars. Some successes in this area have been reported (e.g. Wedderburn et al, 1990; Johnson and Asay, 1993; Kemp and Culvenor, 1994), but the difficulty lies in establishing the generality of the drought resistance which has been determined from trials in a particular area over a limited number of years under one or two management regimes. Hence, a third option has been adopted

by teams working on most agricultural species: to try to identify drought resistance strategies, to understand the underlying mechanisms, and to use this knowledge to develop selection criteria that are fundamental, robust, and likely to be valuable in a wide range of environments.

The most convenient classification of drought resistance strategies and the component mechanisms evolved by plants was developed by May and Milthorpe (1962), and has been much elaborated since. Fundamentally drought-resistant plants may (a) *evade* or *avoid* drought, by surviving the summer as seed, or as resting organs (bulbs, etc.); or (b) *tolerate* drought, by either (i) *delaying* dehydration or (ii) *tolerating* dehydration. Note their careful use of the words *dehydration* and *drought.*

Every drought is unique, but for the purposes of this discussion we will consider two categories of drought: (a) moderate or spasmodic drought, when rain is variable from year to year, but soil moisture deficits are rarely severe enough to kill grassland outright (e.g. temperate maritime), and (b) severe or terminal drought when periods of several months with zero or trivial rainfall are common (e.g. Mediterranean) and where no forage may be available for long periods.

Resistance to moderate or spasmodic drought. For environments that suffer less severe droughts, it should be possible to maximize growth during dry periods without prejudicing persistence after drought or production during wet seasons or years. Support for this view comes from data by Hughes (1977) who showed that varieties of *Lolium perenne*, *L. multiflorum*, their mutual hybrid, and tall fescue (*Festuca arundinacea*) which grew well during drought also recovered rapidly in autumn.

Since leaves are the major economic part of the plant, the water status must be maintained for as long as possible to permit leaf extension. To do this, plants must have vigorous root systems that explore the deeper soil horizons for water. For example, Garwood and Sinclair (1979) showed that tall fescue owed its drought resistance to the ability to root more deeply than other more susceptible species. Similarly, amongst white clover populations deep roots confer drought resistance (Woodfield and Caradus, 1987). A problem is that deeper soil horizons may be low in nutrients and contain toxic levels of other minerals.

One objection to diverting more dry matter into roots is that less will be available to produce harvestable herbage, but this is not likely to be a problem for forages in summer since they generally fix more carbon than they need for immediate growth and store the rest as fructans (grasses) or as starch (clovers). Indeed, photosynthesis is much less sensitive to drought than leaf growth, and after a few weeks without rain unconsumed sugars start to accumulate in the leaf sheaths of grasses (Thomas 1991) and may eventually reach 50% of dry matter (Volaire, 1995).

The main problem of using root growth as a selection criterion is, of course, the practical one of evaluating expression in the field, especially in genetically diverse outbreeding species such as forages. One possibility is to add immobile markers to specific soil horizons, and determine when the marker is detectable in the herbage. Another possibility is to develop an observation made on wheat: Morgan and Condon (1986) found a correlation between osmotic adjustment in leaves and depth of rooting. We know that there is significant heritability for osmotic adjustment in at least one species of forage (perennial ryegrass; Thomas and Evans, 1989), and it would be worthwhile exploring the generality of this relationship. Perhaps the most efficient way would be to select on the basis of molecular markers associated with root depth in other species, such as rice (O'Toole and Bland, 1987).

Once water is absorbed, plants have to achieve a balance between use for evaporative cooling, for maintaining water status and for expanding growing cells. This is achieved in well-irrigated or moderately water-stressed plants through adjusting stomatal aperture, and a number of attempts have been made to select for low stomatal conductance in ryegrasses in order to improve water status and prolong growth during drought (Wilson, 1975; Gay, 1994). Unfortunately, while this does improve performance in pot-grown plants, it is not translated to field conditions because of the poor coupling between transpiration and stomatal conductance in aerodynamically "smooth" crops such as forages (Jarvis and McNaughton, 1986).

Other leaf characteristics that might reduce water loss include leaf rolling (*Festuca* spp, *Ammophila*, etc), low cuticular conductance (in wheat; Clarke and Townley-Smith, 1986) and reflective wax deposits (in *Eragrostis*; Jain et al., 1989; and *Leymus angustus;* Jefferson, 1994).

The net effect of the balance between transpiration and growth is measured as water-use efficiency (WUE) - for grassland agronomists, this is the mass of herbage produced per unit of water transpired (about 2 – 5 g_{DM} kg^{-1}_{water} for well-fertilized grasses). WUE is a very attractive concept, but has so far proved of little use to plant breeders (Johnson and Asay, 1993). To take an extreme example, Thomas (1994) calculated WUE of Italian ryegrass swards during drought from the data of Garwood and Sinclair (1979), and found it to be higher than that of several other species. The swards achieved this high WUE by high levels of production in early summer but very low water extraction, and subsequently died of drought. The time-scale of calculating WUE is obviously important.

An indirect way of estimating WUE is by comparing carbon isotope discrimination (the ratio of ^{12}C and ^{13}C in dried herbage samples compared with that in atmospheric CO_2) in irrigated and droughted plants. The work led by DA Johnson (e.g. Johnson et al., 1990) and RA Johnson (e.g. Johnson and Bassett, 1991) has shown significant levels of broad-sense heritabilities of WUE and discrimination in a wide range of temperate forages, but the usefulness of this trait as a selection criterion for drought resistance has yet to be established (Read et al., 1993).

Even the most efficient "water saver" will eventually become dehydrated if drought progresses. Drought-tolerance mechanisms will then have to come into play to preserve the integrity of tissues, organs, membranes and organelles if healthy green leaf area is to be maintained in anticipation of rain. Blum (1988) has pointed out the difficulty of evaluating these mechanisms in crops because their expression depends very much on the efficiency of drought-delaying mechanisms. Fortunately, moderate drought seems to delay the natural senescence of grass leaves (Reekie and Redmann, 1991), and we have found (unpublished data) that photosynthetic capacity of *L. perenne* recovers rapidly on re-watering even after 8 weeks of drought. It may be that, for moderate drought, existing cultivars already have adequate expression of drought tolerance.

Resistance to severe or terminal drought. Attempting to improve herbage production during high summer in drier climates is unrealistic. Rather, we should aim for drought-avoiding plants that

are able to survive drought in a dormant or quasi-dormant state, but recover rapidly as soon as the rains return. There is no hard and fast demarcation between the drought avoidance and tolerance strategies, and a number of the traits contributing to growth maintenance during moderate drought (described above) will be advantageous in laying down the foundations of drought avoidance.

Some "wild" grasses such as *Poa bulbosa* and *Hordeum bulbosum* show an extreme expression of the drought-avoidance response by forming dormant bulbs or corms (Ofir and Kerem, 1982). For more agronomically-valuable species, true dormancy may be less desirable than the ability to survive whilst growth is arrested by water deficit.

The first requirement of survival is to conserve water during the dry summer months, and this is most effectively done by shedding transpiring leaf area through senescence and recycling resources to surviving tissues, or at least by not regrowing after defoliation. The second requirement is to maintain a supply of water to the surviving tissue, but only a few deep roots may be needed, as shown by the work of McWilliam and Kramer (1968) on *Phalaris*. As the water status of the surviving tissues declines, mechanisms must come into play to protect membranes and proteins against dehydration damage. Proline is an osmoprotectant that is produced by dehydrating grass and clover tissue, and its production in *L. perenne* is highly heritable (Thomas, 1990), but the evidence that genotypes with extreme production of proline have enhanced dehydration tolerance is equivocal. As with heat shock, an enormous range of proteins and polypeptides are produced during desiccation, and are presumed to have a role in scavenging potentially damaging polypeptides or (as dehydrins) in stabilizing membranes and proteins (Bray, 1994). If these processes are to be used to develop selection criteria, it will be necessary to determine which are genuinely acclimatory, and which are merely responses to damage or to altered metabolic rates.

Since severely dehydrated plants are leafless and not able to fix carbon, a third requirement is that earlier in the year they must have built up substantial food reserves to provide the surviving tissues with carbohydrates and amino acids. Cultivars that flower early in the season before drought occurs are likely to tolerate drought better because they partition less carbon into seed production and more into producing deep roots and accumulating reserve compounds. Volaire (1994) showed that several management practices that reduced carbohydrate reserves in *Dactylis glomerata* also adversely affected survival, and that early-flowering cultivars accumulated more carbohydrate and were more drought resistant. Therefore, a low maintenance respiration rate should be advantageous, particularly since temperatures are often very high during drought.

The net effect of these traits should be to preserve tiller apices and buds in a healthy state. Volaire (1995) has shown considerable diversity in tiller survival between *D. glomerata* populations of Mediterranean and continental origin, which is related to both carbohydrate status (above) and to root depth (Volaire and Thomas, 1995). Unlike the effects of cold stress, however, under severe drought the main tiller apex and the bases of existing leaves survive, at least in resistant types, and after re-watering regrowth may resume within a day, and all surviving tillers start growing within the week. Norris and Thomas (1982) found that the constitutively high tiller densities of small-leaved experimental *L. perenne* populations conferred rapid recovery from drought; perhaps the greater density of nodes provided more sites for the production of new adventitious roots which are more efficient than old ones. The ability to produce new tillers from axillary buds is more important in swards where tiller mortality has been high, but our observations (unpublished) show that these secondary tillers are produced from already-growing ones, rather than from buds on tillers with dead apices.

How can we prove the worth of putative traits? Much more work has been carried out on improving drought resistance of cereals than of forages, and it is tempting to extrapolate from one to the other. There are, however, several dissimilarities that can invalidate this approach. Firstly, cereals are annuals, and traits concerning establishment and vegetative growth are relevant only to the seeding year of perennial grass swards, and then only to those sown at the wrong time of year. For example, seedling root characteristics do not necessarily represent those of mature perennial plants. Secondly, much cereal breeding has concentrated on maximising the grain yield through manipulating the harvest index, which is much less relevant to a forage crop, unless grown for seed.

Another approach is to develop lines having divergent expression of a putative drought-resistance trait, and to compare their performance under drought. This has been done for stomatal conductance (Gay, 1994), water-use efficiency (Johnson et al, 1990), and osmotic adjustment (Thomas and Evans, 1989), which were described above. The disadvantage with outbreeding crops such as forages is that such lines are heterozygous for other characters, and one cannot compare traits in an isogenic background.

Alternatively, one can adopt the analytic approach and compare species or cultivars with proven resistance or susceptibility to drought. The problems here are that species will differ in many traits as well as the target ones, and it is difficult to know which ones are relevant. In comparisons of cultivars we have the same difficulty to a lesser extent, but are often unable to identify resistant or susceptible cultivars because of confounding with such factors as ontogeny, management and timing of drought.

Recently, a new opportunity has emerged. By using intergeneric hybrids between a resistant species (*F. arundinacea*) and a susceptible one (*L. multiflorum*) drought resistance has been transferred by introgression from the fescue into the ryegrass (Humphreys and Thomas, 1993). By the process of genomic in-situ hybridisation it is possible to visualise, under the microscope, recombinant chromosome segments that have been transferred from the fescue to the ryegrass. Therefore, in elite drought-resistant genotypes we can identify the chromosome segment that confers drought resistance, and quantify associated traits (physiological, morphological, developmental, molecular) that are likely to contribute to drought resistance in the phenotype. Early studies (Thomas, 1994) indicate that elite genotypes have (surprisingly) the high adaxial stomatal conductance of the fescue parent and the low abaxial conductance of the ryegrass parent. Other traits are currently being explored. We believe that by combining this approach with QTL analyses we will be able to understand both the physiology and genetics of drought resistance, and produce probes and sequences that can be applied, through synteny, to other members of the Gramineae.

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