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Selection of pasture and forage species adapted to changing environmental conditions in Mediterranean climates

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Abstract. Over the last two decades, many agricultural regions of the Earth with Mediterranean climates have suffered from changing climatic conditions in the form of extended periods of intense drought. These droughts have been associated with an increasing awareness of and concern about climate change and the likely future nature of Mediterranean-type climates. Coping with climate change will be a difficult problem to address because most models for Mediterranean-climate regions predict drier and hotter environments such as, for example, in south-western Australia and the western Mediterranean Basin. In south-eastern Australia, while the overall amount of rainfall is predicted to decline, summers are expected to receive more rain and winters become drier. Assuming these predictions are correct, the pastures and rangelands of these regions will experience stresses that without intervention or altered management may cause their wholesale collapse. Not only would this seriously reduce the production of food and fibre from grazing animals, it would also lead to a decline in the numerous ecosystem services provided by grasslands, such as the services which improve and protect soil structure contributing to the sustainability of cropping through the reduction of nitrate leaching and the maintenance of biodiversity. In Australia, pastures research to adapt to these future climate scenarios has so far primarily focussed on improving plant growth models and assessment of the ability of agricultural soils to sequester more atmospheric carbon. Around the Mediterranean Basin, forage plant research is still mainly focussed on annual crops, cereals and irrigated lucerne. In both regions the focus now needs to shift to the development of more climate resilient perennial plants and agronomic systems able to use these more resilient plants efficiently. Pre-breeding research is needed to identify germplasm resources within the major perennial pasture species with enhanced levels of drought and heat tolerance. This paper describes methods to identify and measure two of the important drought tolerance traits used by perennial herbaceous plants, dehydration tolerance and summer dormancy.

Keywords. Perennial forage species – Dehydration tolerance – Summer dormancy – Climate change – Water stress.

Sélection d'espèces fourragères pour leur adaptation aux conditions environnementales prévues sous changement climatique en climat méditerranéen

Résumé. Durant les deux dernières décennies, la plupart des régions agricoles qui dans le monde ont un climat méditerranéen, ont subi des périodes de sécheresse intense sous l'effet du changement climatique. Ces sécheresses ont contribué à une prise de conscience croissante des risques associés aux changements globaux et notamment aux implications environnementales futures pour toutes ces zones bioclimatiques. Le défi à relever pour s'adapter aux effets du changement climatique est difficile, dans un contexte où la plupart des modèles prédisent des environnements plus chauds et plus secs comme par exemple, dans le sud-ouest de l'Australie et l'ouest du bassin méditerranéen. Dans le sud-est australien par contre, alors que le montant des précipitations devrait déciner, les étés pourraient être plus pluvieux et les hivers plus secs. En supposant que ces prédictions sont correctes, les pâturages et les parcours de ces régions seront soumis à des stress abiotiques qui, dans l'hypothèse d'aucune modification de gestion, pourraient entraîner des dégradations drastiques de la ressource fourragère. En effet, ces contraintes accrues du milieu pourraient...
affecter non seulement la productivité animale mais également les nombreux services éco-systémiques rendus par les communautés herbacées comme l’amélioration et la protection des sols contribuant à la durabilité des systèmes de culture, la protection des nappes phréatiques et le maintien de la biodiversité. En Australie, la recherche pour l’adaptation des systèmes fourragers aux futurs scénarios climatiques s’est surtout concentrée sur l’amélioration des modèles de croissance végétale et l’évaluation des sols agricoles pour séquestrer plus de carbone atmosphérique. Autour du bassin méditerranéen, la recherche en matière fourragère se consacre essentiellement aux cultures annuelles, aux céréales et à la luzerne irriguée. Dans les deux régions, il est maintenant urgent de s’orienter vers le développement d’espèces pérennes et de systèmes agro-écologiques plus résilients. Des efforts de sélection sont nécessaires pour identifier des ressources phyto-génétiques parmi les principales espèces pérennes prairiales avec des niveaux élevés de tolérance à la sécheresse et aux fortes températures. Cet article décrit des méthodes pour identifier et mesurer deux des traits les plus importants de ces espèces pérennes herbacées qui sont la tolérance à la déshydratation et la dormance estivale.


I – Introduction

World population has more than doubled in the last 50 years with much of the increase occurring in developing nations. The development of short-straw, high harvest-index cereals through the Green Revolution was instrumental in allowing the feeding of this growing population. Similar to the rest of the World, in regions with Mediterranean climates this population surge is associated with the major and ongoing relocation of people from rural to urban areas. This internal migration has resulted in governments increasingly redirecting their resources, including those associated with agricultural research, away from rural and toward urban-based objectives (Cribb, 2010).

Regions with Mediterranean-type climates have come under increasing climatic stress in the last few decades as the level of precipitation in many of these regions has undergone a decline. This drier trend, indicative of climate change, is predicted to continue (I.P.C.C., 2007) and evidence of this has been the extended droughts that have afflicted the western Mediterranean Basin and southern Australia in the decade 2000-2010. In most regions, rainfed agriculture is likely to face more climate-related risks associated with water shortages (Trnka et al., 2011). In the Mediterranean basin, in particular, the rate of warming may reach +4°C in the summer, leading to an additional month of summer conditions (Giannakopoulos et al., 2009) and greater water shortages (Bindi and Olesen, 2011; Supit et al., 2010). Therefore the decline of grassland production, pasture failures and long-term degradation from drought might gradually become more widespread in some regions (Briske et al., 2003). In some cases the predicted changes in climate are also associated with changes in the distribution of precipitation. For example, in south-eastern Australia while the overall amount of rainfall may change little it is anticipated that the cooler seasons of the year will see a decline in rainfall whereas there may even be an increase in rain over summer. Such climatic change could lead to major alterations in the botanical composition of grasslands and pastures with substantial implications for grazing animal production and the ecosystem services provided by these grasslands (Godfree et al., 2011).

Concerned about these trends, pasture and grassland scientists have made special efforts in the last decade to advance understanding of the traits which improve drought survival of our key pasture species and some plant breeders have begun to incorporate these traits into new cultivars to enhance the resilience of pastures to future climate changes. This paper will briefly present methods to identify and measure two of the key drought tolerance traits of temperate pasture grasses, viz. summer dormancy and dehydration tolerance, and also suggest future research directions to further improve these screening methods.

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II – Traits conferring enhanced survival of drought in temperate perennial grasses

Most research studying the effects of drought on plant growth and production has concentrated on plants of an annual habit with much of it focussing on the maintenance of crop yield or production when subjected to only moderate moisture deficits (Ludlow, 1989; Blum, 2005). Indeed the study of plant response to severe drought and of traits that improve survival particularly for perennial plants has received much less attention, being a more recent development with much work having been stimulated by the droughts of the last decade which killed many plants in grasslands and forests (Volaire et al., 1998a, McDowell et al., 2008). The drought survival enhancement traits found within perennial herbaceous plants of agricultural and industrial importance include dehydration avoidance/delay, dehydration tolerance (Ludlow, 1989; Volaire et al., 2001) and summer dormancy (Volaire and Norton, 2006). Desiccation tolerance, a trait allowing some very highly specialised species termed “resurrection plants” to rehydrate and recommence growth and reproduction after desiccation to air dryness (Gaff, 1971) should be mentioned for completeness. However, it seems unlikely that this trait can be utilised in current agricultural production systems.

1. Identification of dehydration tolerance and summer dormancy

The degree that a plant controls its water potential as it experiences intensifying moisture deficit has been the primary determinant of whether it is considered to express dehydration avoidance (syn. dehydration postponement or delay) or dehydration tolerance (Levitt, 1980). Plants which respond to moisture deficit by employing traits allowing them to maintain a high water potential, e.g. extensive root development to maximise water extraction, high stomatal resistance to minimise water loss, are considered to be dehydration avoiding. Conversely, those that allow water potential to fall while continuing to maintain function are classed as following a dehydration tolerance strategy and are presumed to employ traits conferring cellular tolerance to low water status, e.g. high levels of intra-cellular fructan to maintain cell membrane integrity, enhanced dehydron expression (Turner, 1986; Turner, 1997; Close, 1997; Demel et al., 1998).

The above terminology was developed primarily to describe the drought response strategies of crop plants with an annual habit, so it is not surprising that these terms become inadequate when confronted with perennial plants, not least those that express traits solely in perennial plants such as summer dormancy. Indeed, the key factors to differentiate and identify drought response strategies of perennial plants include, season of expression and moisture environment. Thus, because summer dormancy can only ever be expressed in the field in summer it can only ever be observed in that season. Indeed, after the induction under low temperatures and short photoperiods of winter, summer dormancy is expressed under the high temperatures and long photoperiods of summer and in the genotypes which have the trait, expression will occur independent of soil moisture level (Ofir, 1975; Ofir and Kigel, 1999; Volaire and Norton, 2006).

Dehydration tolerance can be expressed in any season of the year although it is only ever expressed in response to moisture deficit. To avoid confounding with summer dormancy when testing perennial plants it is therefore advisable to test for expression of this trait in the cooler seasons of the year when photoperiods are short.

2. Measurement of dehydration tolerance

Two methods to measure dehydration tolerance will be described here. The first considers that the minimum soil moisture conditions in which the plant is just able to survive is the primary index to rate dehydration tolerance (Volaire et al., 2005). The second method considers that the length of time that a plant can survive after it has entered the stage when stomata are permanently closed is an important component in dehydration tolerance (Bolger et al., 2005) although it also includes a factor accounting for differences in final plant survival.
Within one series of testing and using a scale developed with this second method, differences in dehydration tolerance (scale of 10 to 0, where 10 is maximum dehydration tolerance) and associated plant mortality varied substantially. For example, across three cultivars (cvv) of cocksfoot dehydration tolerance ranged from 10 to 5.8, across two cvv of tall fescue ratings of 6.8 and 3.3 were measured, while across four cvv of phalaris scores varied from 4.5 to 3.5 (Norton, 2007). Within cocksfoot the different dehydration tolerance ratings equated to cultivar mortality ranging from 0 to 42%, in tall fescue from 18 to 59% and in phalaris from 18 to 38%.

Typically dehydration tolerance is measured in pots where it can be assured that plant roots have fully exploited the available soil volume prior to the imposition of the drying cycle. Ensuring full exploitation of the pot soil prior to drying cycle imposition overcomes concerns voiced about the artificial soil physical constraints associated with some pot experiments where the failure to achieve full root exploitation can compromise experimental results (Passioura, 2006).

In both methods, pot soil water content is measured by weighing at regular intervals over the duration of the drying cycle to illustrate the rate of plant water use over the period of drying. This measurement also determines the commencement of the stage (commonly termed stage 3) from which plants have permanently closed stomata to the point of plant death (Sinclair and Ludlow, 1986).

To remain alive a perennial grass plant must maintain its youngest vegetative tiller. This tissue is usually in the form of a meristem enclosed by older leaves. As a drought intensifies and lengthens it is typically difficult to determine the exact point of plant death because the youngest meristem is normally enclosed by older leaves which themselves senesce more and more as the drought progresses. This problem is addressed by undertaking sequential rehydration of certain pots assigned for this purpose at short and regular time intervals, to examine the subject plants for regrowth and thus life, while allowing the drought to continue in other pots within the trial. By this means it is possible to determine the soil moisture content and time of death of the desired proportion (usually 50% of the sample) of the test genotype.

For Method 1, the soil moisture content at which 50% of the sample of the test genotype is dead is the criterion of dehydration tolerance and this figure is simply quoted. Alternatively for method 2 (Norton, 2007),

\[
\text{Dehydration tolerance} = \text{Stage 3 duration (days) of cv y} \times (1 - \text{proportion of y death}).
\]

Both methods rank the relative level of dehydration tolerance of one genotype with respect to the others only within the suite of genotypes being tested. A thorough measurement of environmental parameters, including ambient aerial and soil conditions should occur to enhance comparability of one series of dehydration tolerance measurements with another. Moreover, the development and use of a suite of “control” genotypes within the species being studied of high, moderate and low levels of dehydration tolerance is essential because it will further enhance the comparability of different series of assessments.

3. Summer dormancy

A. Nature and identification

Dormancy, whether expressed in the summer or winter, is an adaptive physiological response state primarily aimed at ensuring survival of perennial plants, allowing the plant to minimise exposure to the season when the most stressful, life-threatening conditions prevail (Vegis, 1964). It is therefore, a form of stress avoidance as during the period of dormancy, the metabolic rate of plants declines greatly (Volaire et al., 2005) and those life processes which are most adversely affected by unfavourable environmental conditions, e.g. growth and reproduction, are halted (Hoffman and Parsons, 1993). Summer dormancy is defined as an endogenously controlled and
coupled series of processes comprising the cessation or reduction of leaf growth, the complete or partial senescence of herbage and in some cases the endogenous dehydration of meristems expressed even under non-limiting moisture conditions. However, it is always expressed under the environmental conditions typical of Mediterranean summers (Volaire and Norton, 2006).

Dormancy was not observed in the earlier drought tolerance work of the annual crop physiologists (Ludlow, 1989) so that its role in enhancing drought tolerance of perennial plants has, in contrast to other strategies, been particularly neglected. The summer dormancy trait commonly occurs in those temperate perennial grass species of Mediterranean origin found in regions usually experiencing summer dry periods of four months or more (Cooper, 1963). In the Mediterranean Basin most of these regions are found in North African and Middle Eastern countries (Neal-Smith, 1955). Other research has shown that the trait also exists in North American species growing in similar environments (Laude, 1953). Observations suggest that the trait exists at least in the Australian grasses, *Austrodanthonia caespitosa* and *Elymus scaber* (C. Waters and J. Virgona pers. com.) from summer-dry environments although this still requires experimental confirmation.

Summer dormancy is a powerful trait that enhances plant survival over hot, dry summers. Several field trials conducted over a range of seasons and environments and in different continents showed that grasses with the trait had better survival invariably over dry summers than grasses of the same species without the trait (Malinowski et al., 2005; Norton et al., 2004). Other more detailed, field-based physiological studies have quantified this advantage, showing that the highly summer-dormant cocksfoot (*Dactylis glomerata*) cultivar Kasbah had 28% greater survival than the non-dormant, Mediterranean cv Medly through a prolonged and intense summer dry period during which 913 mm of evapotranspiration accumulated (Norton et al., 2006a). Under similar conditions the summer-dormant tall fescue (*Festuca arundinacea*) cv Flecha had 30% greater survival than the non-dormant cv Demeter (Norton et al., 2006b).

Breakthroughs in our understanding of the anatomical basis of dormancy are also quite recent. These studies have focussed most intensively on birch (*Betula* spp.) expressing the trait in winter (Rinne and Schoot, 2003; Rinne et al., 2001) and on the herbaceous geophytes, *Tulipa* spp. and *Ranunculus asiaticus*, which express summer dormancy (Kamenetsky, 2009). The centre piece of these studies has been the microscopic imaging of dormant apical meristems. This has clearly depicted the isolation and consequent inability of adjacent cells within the apex to communicate with one another due to the plugging of inter-cellular plasmodesmata which imposes a complete absence of growth and greatly reduced cellular activity.

Although this research has not occurred in those temperate perennial grasses which express summer dormancy, the physiological nature of the trait in these species is sufficiently similar to the geophyte subjects to assume that comparable anatomical mechanisms to those observed in the geophytes operate also in the grasses. However, as the complexity of the natural world continues to astound biologists telling us that it is unwise to make too many assumptions, this is a field of research which truly beckons.

**B. Measurement**

Several approaches to the measurement of summer dormancy are cited in the literature. These methods are based on: (1) the level of herbage production under full, continuous irrigation over the summer (Laude 1953); (2) assessment of production of new tillers or “greening-up” after a mid-summer storm (or mid-summer irrigation) occurring in the middle of an extended summer dry period (Oram 1983), and (3) measurement of herbage senescence after an extended summer dry period (Silsbury 1961; Lorenzetti et al. 1981; Ceccarelli and Somaroo, 1983). This third technique has been shown subsequently to be quite unreliable often giving inconsistent results and therefore is not recommended (Norton et al., 2008). It was also noted that summer dormancy is
most reliably expressed in the field under the correct environmental factors of both induction and expression, i.e. vernalisation followed by long days and increasing temperature (Norton, 2007). Summer dormancy has been reliably reproduced and studied in long pots (<1.1 m deep) in which substantial soil moisture tension could be developed when water was applied from the bottom of the pot (McWilliam and Kramer, 1968).

Within any temperate perennial grass species it can be difficult to differentiate the responses plants use to avoid or tolerate dehydration from those associated with the expression of summer dormancy. This is because under summer moisture deficit these traits are expressed in a similar way, viz. cessation of growth, senescence and shedding of herbage, tolerance of low tissue water potential in some species.

This superficially similar behavior under summer drought is well illustrated by comparing the response to varying levels of summer moisture of the cocksfoot cultivars Medly and Kasbah, known for their contrasting expression of summer dormancy. Indeed, in fully irrigated and summer storm trials where plants were well-hydrated, dormancy scores of cultivar (cv) Medly were quite low (range 0-2.4 where 10 is maximum dormancy) suggesting a summer-active grass able to produce new herbage whenever moisture was non-limiting (Norton et al., 2008). In contrast, when the assessment was made 47 days after the commencement of a summer drought, with plants that were no longer well-hydrated, the same cultivar had a dormancy score of 6.2, a value normally indicating a moderately high level of dormancy. However, while Medly is a plant with a quite high level of drought survival ability (Volaire et al. 1998) this drought survival is due both to dehydration avoidance and tolerance, with avoidance expressed by the shedding of foliage (Norton et al., 2006a) leading to reduced transpiration with the associated conservation of soil water while dehydration tolerance expressed through tolerance of low tissue water content in its meristems increases as drought intensifies (Volaire and Lelievre, 2001). The strong growth response of Medly to non-limiting summer moisture occurring under either irrigation or in response to a summer-storm clearly shows that it is not summer-dormant. In contrast, cv Kasbah showed no growth response to non-limiting summer moisture irrespective of how the moisture was applied. In summer Kasbah expressed a consistent and similarly high level of dormancy (range of 8.7-9.9), characterised by complete cessation of growth, full senescence of herbage and substantial dehydration of meristems under either full summer irrigation or after a simulated summer storm thus confirming the strong expression of this trait (Norton et al., 2006a).

III – Conclusions

Substantial progress has been made recently in the characterisation and measurement of summer dormancy. The same cannot be said about dehydration tolerance and there is an urgent need for further research to compare, refine and develop methods for the measurement of this trait. Only two methods have been described here but these should not be considered as fulfilling all the potential requirements to measure this trait. Method 1 is attractive because of its simplicity, although the degree of precision that it provides would probably vary depending upon the moisture holding capacity of the soil used and it may be inadequate for some situations and depend upon the plant material being assessed. Method 2 provides potentially for a greater degree of precision but it is more complex with all the attendant technique problems that are associated with greater complexity.

The great ability of summer dormancy to improve survival over hot, dry summers makes this a potentially attractive trait even in environments where summers are only occasionally dry. However, when a summer dormant grass is removed from a Mediterranean climate, the degree of expression of the trait may be much reduced. For example, during recent abnormally cool and wet summers at Canberra in south-eastern Australia, on-going, although slow growth of cocks-
foot cv Kasbah has been observed throughout the summer (Norton, 2011). Therefore, in climates where substantial summer rainfall is common it may be imprudent to use summer dormant forages because summer active weeds are likely to invade the pasture to exploit any moisture not used by the forage. Alternatively, it might be possible to develop a stable pasture comprising both summer dormant and summer active genotypes where any moisture available throughout the year can be utilised by one of the forage species. However, this “ideal” pasture needs substantial research and development input before it can be used in agriculture (Norton, 2010).

In Australia the primary selection criterion used by perennial pasture plant breeders to improve survival in hot, dry environments remains field persistence. Persistence is determined by measuring density in the field of the subject plant. The longer the subject maintains an acceptable density in the field, the more persistent it is considered. However, while persistence must always be measured it is not a trait within itself. Rather persistence is the product of a number of traits which when combined in interaction with environmental factors lead to the outcome we subsequently recognise as persistence.

Recently Australian breeders have sought to improve the tolerance to the acidic soils so widespread across south-eastern Australia particularly in phalaris and lucerne, species reputed to be acid soil intolerant (Culvenor et al., 2011). If this breeding objective is attained it is possible that superior drought survival will also be obtained in these species because acid soil tolerance should confer enhanced root growth and make available to plants a greater volume of soil for water extraction (Norton et al., 2012).

Given the major improvement that dehydration tolerance and summer dormancy can make to survival of periods of intense drought and heat together with the increasing frequency of these events we suggest that plant breeders should consider selecting for these traits, prior to field screening, as this is highly likely to improve overall persistence.

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