Water deficit and induction of summer dormancy in perennial Mediterranean grasses

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Key Results
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Background and Aims
Methods

Background and Aims
Summer dormancy is a trait conferring superior drought survival in Mediterranean perennial grasses. As the respective roles of environmental factors and water deficit on induction of summer dormancy are unclear, the effect of intense drought were tested under contrasting day lengths in a range of forage and native grasses.

Methods
Plants of Poa bulbosa, Dactylis glomerata ‘Kasbah’ and Lolium arundinaceum ‘Flecha’ were grown in pots (a) from winter to summer in a glasshouse and subjected to either an early or a late-spring drought period followed by a summer water deficit and (b) in controlled conditions, with long days (LD, 16 h) or short days (SD, 9 h) and either full irrigation or water deficit followed by rehydration. Leaf elongation, senescence of aerial tissues and dehydration of basal tissues were measured to assess dormancy. Endogenous abscisic acid (ABA) in basal tissues was determined by monoclonal immunoassay analysis.

Key Results
Even under irrigation, cessation of leaf elongation, senescence of leaf bases under water deficit was determined by monoclonal immunoassay analysis.

Conclusions
The results of both experiments tend to invalidate the hypothesis that water deficit has a role in early summer-dormancy induction in the range of tested grasses. However, a late-spring drought tends to increase plant senescence and ABA accumulation in basal tissues of forage grasses which could enhance summer drought survival.

Key words: Poa bulbosa, Dactylis glomerata, Lolium arundinaceum, abscisic acid, water deficit, photoperiod, summer dormancy, perennial grass.

INTRODUCTION

Dormancy, which is defined as a temporary suspension of visible growth of any plant structure containing a meristem (Lang et al., 1987), is a response enabling plant survival during seasons when environmental conditions are most threatening (Vegis, 1964). Dormancy is a common adaptation in plants subjected to harsh winter conditions but also occurs in summer in some species including many geophytes (Alliaceae, Orchidaceae, Poaceae and Liliaceae) subjected to seasonal semi-arid Mediterranean climates (Volaire and Norton, 2006). In Poaceae, species exhibiting summer dormancy include wild grasses such as Poa scabrella (Laude, 1953), Poa bulbosa (Volaire et al., 2001; Ofir and Kigel, 2003), Hordeum bulbosum (Ofir et al., 1967) and some populations of forage grasses such as Dactylis glomerata ‘Kasbah’ (Volaire, 2002; Norton et al., 2006a). All these populations exhibit a complete dormancy since they cease growth completely, most of their aerial tissues senesce, and their meristems are subjected to endogenously induced dehydration even under summer irrigation (Laude, 1953; Volaire and Norton, 2006). Some other populations, such as the cultivar of Lolium arundinaceum ‘Flecha’, exhibit incomplete summer dormancy, with markedly reduced growth associated with partial senescence of foliage but no dehydration of leaf bases under summer irrigation (Norton et al., 2006b; Volaire et al., 2006).

Whether complete or incomplete, summer dormancy was correlated with superior survival, autumn regrowth and high persistence under severe drought (Biddiscombe et al., 1977; Culvenor and Boschma, 2005; Norton, 2007). Therefore, this plant strategy is of great interest since intense drought and heat are likely to increase due to global warming and may affect plant persistence (Lehner et al., 2006). The conditions for induction of summer dormancy have been analysed in a limited number of wild temperate geophytic grasses such as Hordeum bulbosum and Poa bulbosa (Ofir, 1975, 1976, 1981) and require further studies in a larger range of plant material.

Photoperiod and temperature are the main environmental factors to regulate induction and release in winter dormancy (Heide, 2001; Welling et al. 2004; Wang et al., 2008). Symmetrically, summer dormancy in vegetative organs of higher plants such as buds, bulbs, corms or tuberous roots develops under increasing day length and temperature at the end of spring (Ofir and Kerem, 1982; Ofir, 1986). Induction starts during early winter since it is enhanced by pre-exposure

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Moreover, the question of the putative role of water deficit in summer dormancy induction was recently raised. Initial results showed that plants of *Poa bulbosa* were not dormant in winter under a severe drought (Volaire et al., 2001). Conversely, plants of *D. glomerata* ‘Kasbah’ and of *F. arundinacea* ‘Flecha’ when field sown in spring, only expressed a reduced dormancy during the subsequent summer which was nevertheless reinforced by summer drought (Norton et al., 2006a, b). In addition, it was found that summer dormancy in *Poa bulbosa* could be induced by two alternative and probably additive pathways, photoperiodic induction by long days and water deficit (Ofir and Kigel, 2007). In this species, long days increased the endogenous level of abscisic acid (ABA) which was proved to induce summer dormancy (Ofir and Kigel, 1998). ABA was shown to play a key role in a number of cases of dormancy regulation.

In trees, bud dormancy was associated with increase in ABA levels and maximum cold hardiness (Arora et al., 2003). ABA also plays a critical role in tuber dormancy control and is the result of a balance of synthesis and metabolism that increasingly favours catabolism as dormancy ends (Suttle, 2004; Destefano-Beltran et al., 2003; Li et al., 2005). ABA regulates key events during seed formation, such as acquisition of desiccation tolerance and induction of primary dormancy (Kermode, 2005; Finkelstein et al., 2008). In herbaceous plants, biosynthesis of ABA was induced by short days, with a higher concentration in highly dormant varieties of alfalfa (Wang et al., 2008). On the other hand, the decrease in the endogenous ABA level during bulb storage was correlated with dormancy release in bulb plants (Xu et al., 2006). It is also well known that water deficit increases endogenous ABA which mediates many physiological responses contributing to stress adaptation (Blum, 1996; Zhang et al., 2006). However, while cessation of leaf growth and senescence of herbage occurs in all grasses in response to drought, with likely involvement of ABA (Volaire et al., 1998), it is under summer irrigation that these same responses are observed only in summer dormant populations (Norton et al., 2008). The possible role of early water deficit to induce summer dormancy should be clarified to understand better the underlying plant physiology but also to determine the most suitable environmental conditions for assessment of summer dormancy.

Thus, the objectives of the present research were (a) to test the effect of water deficit under contrasting day lengths on summer dormancy induction in a range of dormant grasses and (b) to analyse the effects of the treatments on ABA concentrations in plant tissues and their possible association with summer dormancy.

### MATERIALS AND METHODS

**Plant material**

Three species were compared: two forage grasses, (1) *Dactylis glomerata*, orchardgrass cultivar ‘Kasbah’, a completely summer-dormant, early-flowering population from southern Morocco (Oram, 1990, Norton et al., 2006a), (2) *Lolium arundinaceum*, tall fescue ‘Flecha’, a fine-leaved population of Tunisian parentage with incomplete summer dormancy (Norton et al., 2006b) and (3) a native grass, a local population of *Poa bulbosa*, bulbous bluegrass (‘Poa’), dehydration tolerant and with complete summer dormancy (Volaire et al., 2001), which originates from a poor natural grassland on shallow and calcareous soil near Montpellier. Plants were sown in October 2006 and maintained outside in order to be subjected to low winter temperatures (8-7 °C on average with −5 °C as extreme minimum) since vernalization has been shown to be necessary for subsequent induction of summer dormancy (Ofir and Dorenfield, 1992). All plants received full irrigation with water and nutrient solution alternatively. Plants of orchardgrass ‘Kasbah’ and tall fescue ‘Flecha’ that grew actively were cut at 10 cm every 4 weeks on average before imposing treatments. Conversely, plants of *Poa* which have a prostrate habit, were defoliated less frequently.

**Experimental design**

The first experiment (expt 1) was carried out in a glasshouse at INRA (Montpellier, France) over spring and summer 2007 (Fig. 1). It comprised 96 plastic pots (24 cm height, 20 cm diameter) filled with a similar quantity of substrate (80 % sand, 10 % loam, 10 % clay). On 15 February, four plants (three tillers each) of orchardgrass ‘Kasbah’ and tall fescue ‘Flecha’, respectively, and five tufts (ten tillers each on average) of *Poa* were transplanted into each pot. Three treatments were applied in the spring (Fig. 1). A first set of 32 pots was not irrigated between 17 March and 10 April (early-spring drought) and a second set of 32 pots were not irrigated between 11 April and 7 May (late-spring drought). In both cases, plants were rehydrated when soil moisture reached 3 % (−1-4 MPa) on average for all pots, and irrigation continued until 14 June. A third set of 32 irrigated pots were maintained as controls during the entire spring period. From 15 June (Fig. 1), each of these three groups of pots (three spring treatments) was split into two sub-groups of 16 pots (six treatments). The first sub-group was subjected to a severe summer drought with cessation of irrigation (until 16 July with a final soil moisture of 2-5 %, i.e. −2-8 MPa). The second sub-group was subjected to full summer irrigation (until 24 July). On 24 July, all plants were fully irrigated and maintained under short day length (9 h) and low temperature (20 °C) to measure plant survival (percentage of live tillers) on previously water stressed plants. In spring, the environmental conditions recorded during the early and late drought periods were on average 18/15 °C and 21/19 °C day/night, respectively, with a mean day length of 12 h 34 min and 13 h 40 min, respectively (Table 1).

The second experiment (expt 2) was carried out in a growth room with 80 pots (13 cm height, 17 cm diameter) filled with similar quantities of the substrate previously described. On 16 March, two tillers of both orchardgrass ‘Kasbah’ and tall fescue ‘Flecha’, and around ten tillers of *Poa* were transplanted into each pot. From 4 April, four different combinations of day length and water supply (20 pots for each treatment) were applied: short days (9 h) with...
full irrigation (SDI) or short days with water stress (SDS) and long days (16 h) with full irrigation (LDI) or long days with water stress (LDS). Plants under water deficit were maintained at a soil moisture of between 6.5% and 3.5% by bi-weekly adjustment. On day 40 (12 May), all plants were fully irrigated for 10 d. Mean air temperatures were 2–3°C higher under long days than under short days conditions (Table 2).

**Measurements**

To monitor drought progression, pots under water stress in both experiments were weighed every 2 d.

Lamina length was measured every 2 or 3 d on the fastest growing leaves of 12 tillers per treatment. In exp 1, leaf extension rates were assessed during the periods of rehydration (17 d) following early and late droughts. In exp 2, leaf extension rates were measured (until cessation of elongation for some treatments) for the entire duration of the experiment on plants subjected to the four treatments and during the rehydration stage.

In both experiments, a few destructive samplings (three pots at each date) were carried out. In exp 1, sampling dates were 9 March (before drought imposition), 10 April (end of early drought and irrigated control), 7 May (end of late drought imposition) and 15 June (end of late drought imposition) and 24 July (end of late drought imposition).

**Table 1. Environmental conditions for the glasshouse experiment: means of daily air temperatures, of maxima temperatures, of radiation, of air moisture and of day length for the treatment periods: early drought, rehydration after early drought, late drought and rehydration after late drought**

| Treatment          | Period          | Mean air temperature (°C) | Mean of maxima air temperature (°C) | Daytime radiation (μmol m⁻² s⁻¹) | Air moisture (%) | Mean day length (h, min) |
|--------------------|-----------------|---------------------------|-------------------------------------|----------------------------------|-----------------|--------------------------|
|                    |                 | Day | Night | Day | Night | Day | Night | Day | Night | Day | Night | Day | Night |
| Early drought      | 17 Mar to 10 Apr| 18.2 | 15.1  | 21.2 | 17.5 | 278.2 | 42.8 | 56.0 | 12h34 |
| Rehydration        | 11–29 Apr       | 21.6 | 18.9  | 24.7 | 21.3 | 351.0 | 61.4 | 69.4 | 13h38 |
| Late drought       | 11 Apr to 7 May | 21.2 | 18.8  | 24.1 | 21.1 | 333.5 | 61.5 | 68.0 | 13h49 |
| Rehydration        | 8 May to 25 May | 23.1 | 20.4  | 26.5 | 22.8 | 451.8 | 55.2 | 62.6 | 14h41 |

In both experiments, a few destructive samplings (three pots at each date) were carried out. In exp 1, sampling dates were 9 March (before drought imposition), 10 April (end of early drought and irrigated control), 7 May (end of late drought imposition).
and irrigated control), 22 May and 14 June (all three spring treatments), 16 July (all three spring treatments followed by summer drought) and on 24 July (all three spring treatments followed by summer irrigation). In exp 2, sampling dates were 30 March (before drought imposition), 16 April (d14), 26 April (d24, elongation cessation of plants under water deficit), 4 May (d32), 11 May (d39) and 24 May (12 d after rehydration of plants previously under water deficit).

At each sampling and for each population, four tillers were divided into two fractions: the first 20 mm above root insertion and the remaining tissues. This second fraction was divided into green and senescent tissues (lamina and sheaths with visible necrosis) that were weighed after drying (48 h at 80 °C) in order to measure the fraction of senescent tissues in aerial biomass. In the fraction containing the lowest part of the tillers, the enclosed bases of immature leaves (surviving organs), were dissected out, weighed and then dried to determine their water content. The living bases (without senescent sheaths) of immature leaves and bulbs (amounting to 0.5–2.0 g of fresh weight) of the rest of the plant material were collected and frozen (−20 °C) for assays of their ABA content.

ABA assays

The samples were freeze dried, finely ground, extracted in glass-distilled water using about 1.2 mL per 40 mg dry weight, shaken up at 4 °C in the dark and purified through filtration with Whatman no. 5 paper filters. The extracts were assayed in an enzyme-linked immunosorbent assay for ABA using a monoclonal antibody for ABA (AFR MAC252) (Asch, 2000; Bahrun et al., 2002).

Statistical analysis

Experiments were arranged in a completely randomized design. Statistical analysis of variance was carried out with the Statbox Pro 6.3 software package (Grimmersoft). Means between populations and between treatments were compared using least significant differences (l.s.d).

RESULTS

Glasshouse experiment

In exp 1, early- and late-spring droughts had contrasting effects on plant responses. After rehydration following the early period of water deficit, leaf elongation was actively resumed and was 2- to 3-fold higher than that measured on irrigated control plants for all populations (Table 3). In contrast, after the late-spring drought, leaf elongation ceased for Poa and was drastically reduced in orchardgrass ‘Kasbah’. Leaf elongation in irrigated control plants of Poa and orchardgrass ‘Kasbah’ was very low and similar to those of plants previously water stressed. In contrast, leaf elongation in rehydrated or control fescue ‘Flecha’ was similar (around 5 mm d−1) to that measured in irrigated control plants after the early drought period (Table 3).

The proportion of senescent tissues in aerial biomass was 18 % in hardly defoliated Poa, on 9 March before imposition of early drought (Table 4). Senescence reached 43 % to 53 % in orchardgrass ‘Kasbah’ and fescue ‘Flecha’, respectively, due to a large proportion of sheaths remaining after defoliation (Table 4). At the end of the early drought period on 10 April, senescence of aerial tissues increased significantly in drought-stressed plants compared with the irrigated control plants. On 7 May, at the end of the late-spring drought, senescence was over 82 % in orchardgrass ‘Kasbah’ and fescue ‘Flecha’ and 97 % in Poa. At this date, senescence in fully irrigated Poa also reached 89 % and then 100 % for all subsequent sampling dates of all treatments. Senescence in orchardgrass ‘Kasbah’ was generally greater than in fescue ‘Flecha’. Under continuous irrigation treatments (control), it was only from 14 June that plants of orchardgrass ‘Kasbah’ exhibited a high senescence (70 %) which increased on 24 July (86 %). Under this treatment, plants of fescue ‘Flecha’ exhibited a low senescence between 16 % and 32 % from June to July. In orchardgrass ‘Kasbah’ and fescue ‘Flecha’, senescence was higher in summer (14 June) when plants had been subjected to a late-spring drought than to an early-spring drought. The fraction of aerial senescence in orchardgrass ‘Kasbah’ reached 68 % and 94 % (after early- and late-spring drought, respectively), whereas it was lower than 39 % for fescue ‘Flecha’ on 24 July (Table 4).

The water content in basal tissues was over 74 % for all populations on 9 March (Table 5). As expected, moisture declined in plants subjected to both spring drought periods, but particularly in Poa (reached 19 % and 9 %, respectively, at the end of early- and late-spring droughts). Water content in basal tissues of Poa was also significantly lower than that of fescue ‘Flecha’ under summer irrigation (and summer drought) for all spring treatments except on 24 July, orchardgrass ‘Kasbah’ having intermediate tissue moisture.

From 10 April to 7 May, the ABA concentration in basal tissues of all plants was increased under drought compared with the irrigated control (Fig. 2). It was only in Poa, that ABA concentration was higher under late-spring drought than early-spring drought (Fig. 2B, C). Under irrigation, ABA content in Poa increased from 10 April to 7 May, while it decreased in the two forage grasses. From 14 June to the end of July, ABA concentrations reached 5300–16 000 ng g−1 d. wt in bulbs of Poa, whether under drought or full irrigation (Fig. 2A–C). In contrast, ABA concentrations were significantly lower in other species in summer (Fig. 2D–

| Population | After early drought | Irrigated control | l.s.d | After late drought | Irrigated control |
|------------|---------------------|-------------------|------|-------------------|-------------------|
| Poa        | 1.7                 | 1.9               | 0.5  | 0.0               | 0.0               |
| Kasbah     | 11.9                | 6.4               | 1.7  | 1.9               | 1.1               |
| Flecha     | 19.6                | 5.6               | 1.9  | 5.5               | 4.7               |

Least significant differences (l.s.d) are presented when differences between treatments are significant (P < 0.05).
Plants of orchardgrass ‘Kasbah’ subjected to late-spring drought (Fig. 2F) had a significantly greater concentration of ABA in basal tissues (around 3-fold) in summer than plants having been water stressed early in spring or fully irrigated (Fig. 2D, E).

After rehydration following summer drought, tiller survival in *Poa* was 100% regardless of previous spring treatments (Table 6). Conversely, tiller survival in orchardgrass ‘Kasbah’ and fescue ‘Flecha’ was significantly higher on plants having been subjected to a late-spring drought (67% – 71%) than those subjected to early-spring drought (57% – 66%).

### Table 4. Fraction of senescent tissues in aerial biomass (%) in three perennial grasses, *Poa bulbosa* (*Poa*), *Dactylis glomerata* (‘Kasbah’) and *Lolium arundinaceum* (‘Flecha’) subjected to three treatments in spring: early drought (17 March to 10 April), late drought (11 April to 7 May) or full irrigation; followed with full irrigation until 14 June for all pots

| Spring treatment | Population | Sampled 9 March | Sampled 10 April | Sampled 7 May | Irrigation 8–22 May; sampled 22 May | Irrigation 22 May to 14 June; sampled 14 June | Summer treatment |
|------------------|------------|-----------------|-----------------|--------------|-------------------------------------|-----------------------------------------------|------------------|
| Irrigated control | *Poa*      | 17.7            | 19.7            | 89.3         | 100.0                               | 100.0                                         | 100.0            |
| (9 March to 7 May) | Kasbah     | 43.4            | 29.1            | 17.1         | 43.3                                | 69.6                                          | 100.0            |
|                  | *Flecha*   | 53.1            | 33.9            | 23.7         | 26.6                                | 16.1                                          | 47.7             |
| l.s.d. Pop.      |            | 28.4            | 18.8            | 13.5         | 14.2                                | 12.3                                          |                  |
| Early drought    | *Poa*      | 30.7            | 100.0           | 100.0        | 100.0                               | 100.0                                         | 100.0            |
| (17 March to 10 April) | Kasbah     | 49.8            | 55.3            | 60.3         | 80.0                                | 68.0                                          |                  |
|                  | *Flecha*   | 60.4            | 30.0            | 26.9         | 82.2                                | 32.9                                          |                  |
| l.s.d. Pop.      |            | 20.7            | 12.0            | 14.3         | 11.3                                | 22.7                                          |                  |
| Late drought     | *Poa*      | 97.3            | 100.0           | 100.0        | 100.0                               | 100.0                                         | 100.0            |
| (11 April to 7 May) | Kasbah     | 82.6            | 75.4            | 77.4         | 89.9                                | 93.7                                          |                  |
|                  | *Flecha*   | 82.5            | 66.7            | 78.8         | 76.6                                | 39.0                                          |                  |
| l.s.d. Pop.      |            | 15.6            | 14.8            | 15.9         | 20.3                                | 9.1                                           |                  |
| l.s.d. Treatment | *Poa*      | 8.0             | 6.4             | 4.3          | 2.7                                 | –                                             |                  |
|                  | Kasbah     | 14.1            | 8.9             | 17.9         | 15.2                                | 23.1                                          |                  |
|                  | *Flecha*   | 21.3            | 25.1            | 15.0         | 18.1                                | –                                             |                  |

#### Table 5. Water content (%) of bases of last enclosed leaves in three perennial grasses, *Poa bulbosa* (*Poa*), *Dactylis glomerata* (‘Kasbah’) and *Lolium arundinaceum* (‘Flecha’) subjected to three treatments in spring: early drought (17 March to 10 April), late drought (11 April to 7 May) or full irrigation; followed with full irrigation until 14 June for all pots

| Spring treatment | Population | Sampled 9 March | Sampled 10 April | Sampled 7 May | Irrigation 8–22 May; sampled 22 May | Irrigation 22 May to 14 June; sampled 14 June | Summer treatment |
|------------------|------------|-----------------|-----------------|--------------|-------------------------------------|-----------------------------------------------|------------------|
| Irrigated control | *Poa*      | 83.8            | 68.1            | 53.6         | 50.8                                | 59.5                                          | 8.4              |
| (9 March to 7 May) | Kasbah     | 74.3            | 79.5            | 62.4         | 57.2                                | 54.2                                          | 31.4             |
|                  | *Flecha*   | 91.7            | 83.0            | 67.8         | 69.3                                | 66.4                                          | 35.2             |
| l.s.d. Pop.      |            | 13.4            | 6.5             | 9.7          | 5.5                                 | 8.3                                           | 5.8              |
| Early drought    | *Poa*      | 19.4            | 58.7            | 67.2         | 7.9                                 | 68.0                                          |                  |
| (17 March to 10 April) | Kasbah     | 52.0            | 58.0            | 50.7         | 31.5                                | 53.9                                          |                  |
|                  | *Flecha*   | 55.7            | 68.5            | 53.6         | 33.6                                | 61.9                                          |                  |
| l.s.d. Pop.      |            | 14.2            | 7.4             | 6.8          | 5.3                                 | 5.5                                           |                  |
| Late drought     | *Poa*      | 8.7             | 54.6            | 59.7         | 7.8                                 | 64.0                                          |                  |
| (11 April to 7 May) | Kasbah     | 39.2            | 64.1            | 57.8         | 27.8                                | 62.3                                          |                  |
|                  | *Flecha*   | 42.1            | 80.0            | 72.8         | 35.4                                | 60.5                                          |                  |
| l.s.d. Pop.      |            | 9.0             | 6.6             | 9.5          | 18.2                                | 5.9                                           |                  |
| l.s.d. Treatment | *Poa*      | 13.9            | 2.9             | 5.5          | 3.6                                 | –                                             |                  |
|                  | Kasbah     | 6.1             | 10.1            | 6.1          | –                                   | –                                             |                  |
|                  | *Flecha*   | 5.1             | 8.4             | 7.6          | –                                   | –                                             |                  |

Half of the pots subjected to each spring treatment were then subjected to a summer drought (15 June to 16 July), the other half of the pots were subjected to summer irrigation (15 June to 24 July), i.e. six treatments in summer in a glasshouse experiment.

Least significant differences (l.s.d.) are presented when differences between populations (Pop.) or treatments for each population are significant (*P < 0.05*).

–, Data unsuitable for analysis of variance.

I). Plants of orchardgrass ‘Kasbah’ subjected to late-spring drought (Fig. 2F) had a significantly greater concentration of ABA in basal tissues (around 3-fold) in summer than plants having been water stressed early in spring or fully irrigated (Fig. 2D, E).
and 41%, respectively, for the two populations) than to full irrigation or early-spring drought.

**Growth chamber experiment**

In expt 2, leaf elongation differed markedly between the populations subjected to the combination of two day lengths and two levels of irrigation applied during the first 40 d (Fig. 3). After 15 d, leaf elongation ceased for all populations under water deficit at both SD and LD. Under long days and irrigation (LDI), leaf elongation also ceased after 15 d for plants of *Poa* (Fig. 3A) and after 25 d for orchardgrass ‘Kasbah’ (Fig. 3B). By contrast, plants of fescue ‘Flecha’ maintained leaf growth under irrigation irrespective of the day length (Fig. 3C). In the second stage of the experiment, after rehydration of plants previously water-stressed, it is only under short days that plants of *Poa* and orchardgrass ‘Kasbah’ resumed leaf growth (Fig. 3A, B). Conversely, all plants of fescue ‘Flecha’ previously under water deficit, exhibited a high leaf regrowth exceeding that of irrigated control, under both long and short days (Fig. 3C).

The fraction of aerial senescent tissues reached 90% in *Poa* after 32 d under long days whatever the water supply and remained below 40% for plants under short days (Fig. 4A).
between populations are significant (Flecha 1.21 0.04 0.83 0.2).

Periods shorter than 12 h were shown to be non-inductive (Fig. 3). These results confirm previous studies since photoperiodic treatments tend to invalidate the hypothesis that water deficit has an alternative factor to day length and temperature in inducing summer dormancy remains unclear. The results of both experiments tend to invalidate the hypothesis that water deficit has a role in summer dormancy induction for the range of grasses tested. After a severe drought imposed under short days, either 12 h 30 min on average in early spring (Table 2) or 9 h in controlled conditions (Fig. 3), all populations resumed leaf growth at rehydration demonstrating they were not dormant.

In the range of temperatures likely to be inductive since they correspond to average means found from May to August in Mediterranean areas (18–26 °C during the day and 15–20 °C at night), summer dormancy was mainly induced by long days, either at day length higher than 13 h 30 min at the end of spring (Table 2) or under controlled 16-h day length (Fig. 3). These results confirm previous studies since photoperiods shorter than 12 h were shown to be non-inductive while 14- and 16-h days were fully inductive for Poa bulbosa (Ofir and Kigel, 1999).

In spring, since levels of ABA were generally higher during the early-spring drought than the late one, no clear association was shown between populations and ABA.

The three species analysed in this study exhibited patterns of dormancy according to their expected summer adaptations. Poa bulbosa is desiccation tolerant with an early onset of summer dormancy. The reported experiments confirm that the onset of dormancy of Poa occurred after a fortnight of long days at high temperatures (Ofir and Kerem, 1982), corresponding to the period between mid-April and the beginning of May.
of May when leaf growth ceased even under full irrigation. At this date, all aerial tissue senesced and remained senescent until the end of July irrespective of the water supply. It is only under severe water deficit that bulbs desiccated to water content lower than 10%, while under irrigation bulbs remained hydrated (50–60%). Whatever their hydration in summer, bulbs of Poa exhibited higher ABA concentrations than those of forage grasses. An increase in ABA in both leaf blades and in the developing bulbs under an inductive photoperiod was related to dormancy imposition and to increased desiccation tolerance of the bulb tissues during their dehydration under summer dormancy (Ofir and Kigel, 1998). Poa had no plant mortality after severe summer drought whatever the water supplies in spring, confirming the reliable role of summer dormancy and desiccation tolerance on viability of over-summering buds in this species.

Orchardgrass ‘Kasbah’ is not desiccation tolerant but is completely dormant in summer. The results show that the

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**Fig. 4.** Time course of mean fraction of senescent tissues in aerial biomass (%) and water content in basal leaf tissues in three perennial grasses, Poa bulbosa (A, D), Dactylis glomerata ‘Kasbah’ (B, E) and Lolium arundinaceum ‘Flecha’ (C, F) under four combinations of water supply and day length applied for 40 d, as indicated: short days of 9 h + full irrigation (SDI), short days + water deficit (SDS), long days of 16 h + full irrigation (LDI), long days + water deficit (LDS) and followed by 10 d of full irrigation for all treatments in a growth chamber experiment. Water deficit was applied between day 1 and day 40 for SDS and LDS. Bars indicate least significant difference (l.s.d) when differences between treatments are significant at $P < 0.05$. 

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cessation of leaf elongation, i.e. the onset of dormancy in orchardgrass ‘Kasbah’ occurred around 10 d later than Poa in the growth chamber. This onset of dormancy occurred at the beginning of May, both under drought and irrigation, in the glasshouse experiment. Compared with early-spring water deficit, a late-spring drought increased senescence of aerial tissues of orchardgrass ‘Kasbah’ that reached 80–94 % in summer under irrigation. Basal tissues dehydrated between 50 % and 60 % and to a threshold of 30 % at the end of summer drought that was lower than the mortality threshold found in most previous experiments (Volaira et al., 2001, 2005; Volaira, 2002). Although undoubtedly dormant in summer, potted plants of orchardgrass ‘Kasbah’ could not fully withstand severe drought. The lowest mortality (33 %) was obtained on plants previously subjected to a late-spring drought.

Tall fescue ‘Flecha’, with incomplete summer dormancy in the field, was unaffected by day length since its leaf growth actively resumed after each spring drought and under short days. However, fescue ‘Flecha’ survived better after a summer drought following a late-spring water deficit. This treatment also increased its aerial senescence, without affecting water content in basal tissues which remained high as expected in a case of incomplete dormancy.

It can be argued that, for both cultivars of forage grasses that represent model cases of levels of summer dormancy without desiccation tolerance, the imposition of a late water deficit, at a period when environmental conditions were conducive to summer dormancy, could possibly have had a hardening effect resulting in a higher tolerance to summer drought and possibly higher dormancy with greater senescence of aerial tissues. Indeed, in this case, ABA concentration was up to three times greater in basal tissues of orchardgrass ‘Kasbah’ and also higher in tall fescue ‘Flecha’. Moderate water stress at or near the end of the growing season in tree seedlings accelerates budset, induces early dormancy and increases cold hardiness (Kozlowski and Pallardy, 2002). Whether better survival can be ascribed to more complete dormancy or greater tolerance to the environmental stress (drought) is always difficult to unravel since these responses are closely associated (Arora et al., 1997; Brummer et al., 2000; Cunningham et al., 2001). However, the relationship between ABA and dormancy levels should be investigated further. These results confirm that initial soil moisture stress in early summer may be an additional factor required to enhance dormancy in cultivars like fescue ‘Flecha’ which have incomplete dormancy (Malinowski et al., 2006).

The present results showing no direct effect of water deficit under short days to induce dormancy in the tested contrasting grasses, tend to contradict the findings that water deficit was a possible pathway for summer induction in Poa (Ofr and Kigel, 2007). It can be argued, however, that the native population of Poa used in the present study had a different response to that found in Israeli ecotypes of Poa. First, the plants were a few months old, grown from seed, whereas bulbs of the Israeli population were collected from the field from older plants which may have been more reactive to environmental factors triggering summer dormancy. Secondly, large genotypic differences among populations adapted to different climatic conditions could affect responsiveness to factors of dormancy induction such as critical photoperiod, temperature or soil moisture. Indeed, according to the aridity of the site of origin, the date of onset of dormancy varied in a range of Poa populations (Ofr and Kigel, 2003). It was shown that autumn dormancy of saltgrass (turfgrass) was related to longitude of clone origin and minimum winter temperature (Rukavina et al., 2008). In a lilioid geophyte, for phenotypes originating from sites where summers were consistently hot and dry, summer dormancy was ‘obligate’ and invariably induced by environmental cues. On the other hand, phenotypes originating from sites subject to variable summer conditions, showed facultative summer dormancy induced by drought (Vaughton and Ramsey, 2001). Both the variability of the summer dormancy trait and its factors of induction are likely to be associated with the ecological variability found between and within each species. Further investigation of environmental factors regulating induction and release of summer dormancy are required to better understand and manage temperate perennial grasses in lower rainfall, summer-dry environments (Harris et al., 2008).

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