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Ecophysiological adaptations of *Asphodelus aestivus* to Mediterranean climate periodicity: water relations and energetic status

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During the course of a year, we studied the water and metabolic status of *Asphodelus aestivus*, a native geophyte of East Mediterranean, dominant in areas degraded by overgrazing and fire. The species proved to be very efficient in storing water during the long summer drought. At soil water content values around zero, in the upper part of the soil profile (10–20 cm in depth), the roots remained hydrated and turgid; their relative water content was $>60\%$ and water potential > -1.6 MPa. Accumulation of proline during winter in leaves (ca 5 mg g^{-1} dry weight) and tubers (though at significantly lower levels, ca 1.5 mg g^{-1}) might be taken as evidence of a winter cold stress response. Proline accumulation in tubers, under summer drought, was similar to that in winter. Maximal values of caloric content were recorded in expanding leaves (ca 5600 cal g^{-1} dry weight) and minimal before leaf senescence. In contrast, root caloric content remained fairly constant for most of the year (ca 4550 cal g^{-1} dry weight) in spite of drastic changes in the concentrations of soluble sugars, starch and lipids. Long before senescence, photosynthetic products were translocated to the below ground system, where they were stored. Drastic changes of the storage compounds were observed before emergence of the flowering stalk, far greater in magnitude than those before leaf emergence. The below ground part of the species was found to be less susceptible to climatic stress and to constitute an energetically rather stable system. The physiological processes of the species were well synchronised to the fluctuations of the Mediterranean climate.

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Mediterranean ecosystems have a spectrum of plant growth forms, the participation of which differs along environmental gradients. The therophytes, which account for ca 40–50% of species, are the dominant life form (Margaris 1980). Nevertheless, the physiognomy of these ecosystems is dominated by phanerophytes and chamaephytes – the evergreen sclerophyll and the seasonally dimorphic shrubs. Geophytes (cryptophytes) have a considerable, though varying, contribution. In the East Mediterranean region, they become the dominant life form in areas degraded by overgrazing and fire and are represented almost exclusively by *Asphodelus aestivus* Brot. (syn. *Asphodelus microcarpus* Salzm. and Viv.). Such ecosystems, defined as asphodel-geophyte-deserts in Israel (Naveh 1973) or asphodel-semi-deserts in Greece (Pantis and Margaris 1988) correspond to the final stage of desertification processes (Walter 1968, Ayyad and Hilmy 1974). The ability of *A. aestivus*, a native floristic element, to spread all over the Mediterranean region and dominate in areas degraded by over-

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grazing and fire, reflects its capacity to face not only the peculiarities of the Mediterranean climate (Pantis et al. 1994), but also to resist these most common disturbances in its habitat (Pantis and Margaris 1988).

Asphodelus aestivus is characterised by two major life phases in the course of a year: the active phase – from leaf emergence (autumn) to senescence of the above ground structures (late spring), and the dormant – spanning the prolonged dry summer (Pantis et al. 1994). According to El-Ghonemy et al. (1978), the high Si content of mature leaves contributes to its unpalatability, whereas the tubers are protected from herbivores through accumulation of defence substances, such as alkaloids (Hammouda et al. 1971). Occurrence of fires during the hot, dry summer is a common phenomenon in the Mediterranean environment. As it is leafless at that time, with well buried below ground tissues, *A. aestivus* avoids the effect of fire.

Given the importance of *A. aestivus* as a consistent component of Mediterranean vegetation and its dominance over wide areas, we have undertaken several studies focusing on various features and processes of this plant and its communities with the ultimate goal to identify mechanisms that contribute to its remarkable success in the Mediterranean region. We have studied its spatial distribution patterns (Pantis and Stamou 1991), biomass and nutrient allocation patterns (Pantis 1993), phenology (Pantis et al. 1994), flowering characteristics (Pantis and Stamou 1991), litter decomposition (Stamou et al. 1994) and effect of grazing on community structure (Pantis and Mardiris 1992).

The timing of phenophases, exhibited during the annual development of the biological cycle of *A. aestivus* led Pantis et al. (1994) to characterise the plant as an example of synchronisation with the Mediterranean climate. The present study aimed at revealing the underlying metabolic changes that are related to the different phenophases and, concomitantly, to climatic periodicity. It focused on i) water relations, of primary importance under Mediterranean climatic conditions, and ii) the energetic status of the plant given the short growth period and the nutrient limited environment (Margaris et al. 1984).

Materials and methods

Samples of leaves and tuberous roots of *A. aestivus* were collected randomly from a research site near the Athens University campus (37°57.5′N, 23°48.0′E, altitude 250 m a.s.l.), on a monthly basis during the course of a year. In late February, flowering stalks and flowers were also collected. Each time, a minimum of four plants were sampled.

The soil in the study area is calcareous, rocky and shallow (Fouseki and Margaris 1981). The roots of *A. aestivus* were excavated in the upper soil profile, within a section 10–20 cm in depth. Values of rainfall and air temperature (Fig. 1A) were obtained from a permanent meteorological station, ca 5 km away from the research site.

Water potential (Ψ) was measured on 6 mm diameter discs of leaf and on 6 mm long root apices, in sample chambers (C-52) attached to a microvoltmeter. The sample chambers were kept in a polystyrene box for extra temperature insulation. Solute potential (Ψ_s) was measured on the same samples, which had been frozen previously in liquid nitrogen and thawed. The equilibration time of the plant tissue samples in the psychrometric chamber was found to be 2 h for each of the water potential components. Turgor potentials (Ψ_p) were calculated as the difference between Ψ and Ψ_s. Relative water content (RWC) was determined in both

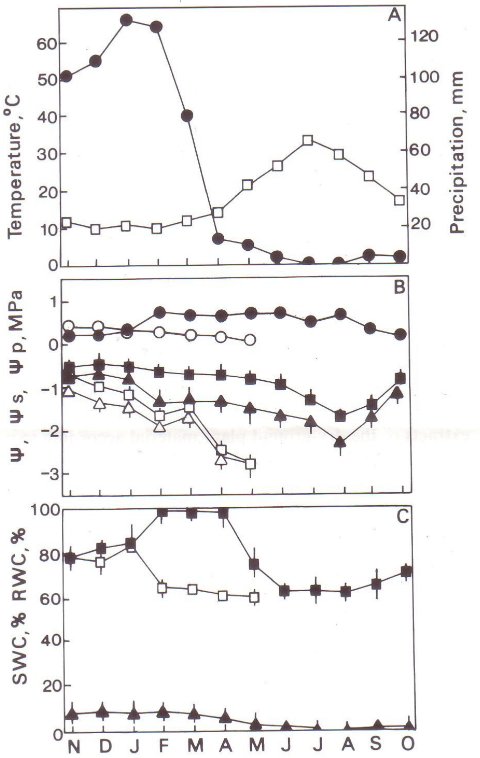

Fig. 1. Water relations of *A. aestivus* during the course of a year. (A) Ombrothermic diagram for a meteorological station, ca 5 km from the research site, during the experimental period (November–October); marked with open squares are the average monthly temperatures and with closed circles the amounts of precipitation. (B) Water potential (squares) ±SE, solute potential (triangles) ±SE and turgor potential (circles); open symbols represent leaves and closed symbols represent roots. (C) Relative water content (RWC), as in B, and soil water content (SWC, closed triangles) ±SE.

tissues by use of Barrs and Weatherley (1962) disc method. Soil water content (SWC) was determined as the percent loss of weight after oven drying (80°C for 48 h) of soil samples, collected from the upper 10–20 cm below surface. The reported values of Ψ and Ψ_s are each the mean of four determinations. RWC is the mean of 15 disc measurements and SWC values are the mean of five replicates.

Soluble sugars were extracted from 1 g dry powdered material (leaves, tuberous roots, flowering stalks, flowers) with acetone, and were determined colorimetrically according to the phenol-sulphuric acid method of Dubois et al. (1956), at 490 nm, using a spectrophotometer. Reducing sugar content was determined colorimetrically using the method of Nelson (1944) as modified by Somogyi (1951). Glucose was used as a standard and absorbance was read at 660 nm. Fructose was estimated according to Whistler and Wolfrom (1962). Sucrose content was calculated as the difference between total sugars and reducing sugars, and glucose as the difference between reducing sugars and fructose. Quantitative determination of starch was made in the residue after the extraction of sugars, using the anthrone method (McCready et al. 1950). Total lipids extracted from dried material with a chloroform/methanol solution (2:1, v/v) were further analysed according to Winter (1963). Sugars, starch, and lipids were determined in triplicate samples.

Free proline content was determined colorimetrically, according to the method of Bates et al. (1973). The sample material was treated as described by Amberger-Ochsenbauer and Obendorfer (1988), dried, and then ground and stored in glass vials. Proline concentration was calculated on a dry weight basis using L-proline for the standard curve. Betaine was determined in aqueous extracts of the dry-ground plant material according to a method described by Grieve and Grattan (1983). Total chlorophyll content was measured on a fresh weight basis, as described by Steubing (1965). Proline, betaine, and chlorophyll contents were determined in triplicate samples.

Caloric content was determined on samples that were prepared according to Lieth (1975) by using an oxygen bomb calorimeter. The ash content was estimated on 1 g dry-ground samples that were burned at 500°C for 8 h. Each value, given on ash-free basis, is the mean of three replicates.

Results

The active phase of *A. aestivus* in the study area lasted from November to April, corresponding to the period of rainfall (Fig. 1A). Flowering stalks appeared in February and fruiting started in early May coinciding with the onset of leaf senescence.

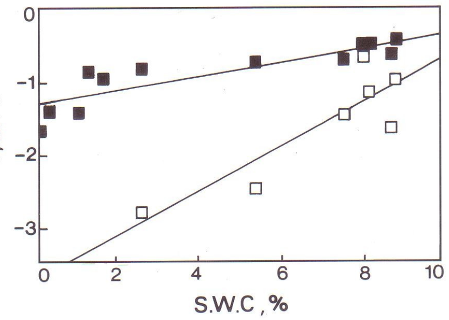

Fig. 2. Relationships between SWC and leaf water potential (open squares, $y = -3.679 + 0.295x$, $r = 0.871$, $p < 0.05$) and SWC and root water potential (closed squares, $y = -1.21 + 0.079x$, $r = 0.768$, $p < 0.05$).

Annual variation in Ψ, Ψ_s and Ψ_p of leaves and roots of *A. aestivus* are given in Fig. 1B. Ψ and Ψ_s of leaves declined throughout the growing period and reached minima (-2.8 MPa) in May. Ψ and Ψ_s of roots decreased as well, though less steeply, reaching minima in August (-1.6 and -2.2 MPa, respectively); Ψ increased thereafter to -0.7 MPa, parallel to Ψ_s, although SWC values (Fig. 1C) continued to be very low. The highest value of leaf turgor (0.4 MPa) was recorded in expanding tissues and the lowest (0.02 MPa) before desiccation in May. Root turgor was maintained at ca 0.65 MPa, between February and June; it remained relatively high even during summer, when soil water availability decreased substantially (Figs 1B and C), implying some root activity. Root RWC was maintained approximately at 100% during late winter-early spring and reached the minimum value (ca 60%) in summer, under zero values of SWC (Fig. 1C). High values of leaf RWC (ca 80%) were obtained from November to January falling to ca 60% before desiccation (Fig. 1C). Ψ_{root} and Ψ_{leaf} were linearly correlated to SWC (Fig. 2); for a given value of SWC, Ψ_{root} was always higher than Ψ_{leaf}.

Root content of starch, lipids and soluble sugars varied considerably over the year (Figs 3A, B and F); in leaves, high values were recorded early in the active phase only. Starch as well as total sugar contents were always higher in roots than in leaves (Figs 3A and F), whereas the opposite held true for lipids (Fig. 3B). The highest values of soluble sugars appeared in roots, in late spring-early summer (Fig. 3F). Sucrose seems to be the sugar most responsible for the particular pattern of total-soluble-sugar change in both leaves and roots (Figs 3D and E); remarkably high values of sucrose were recorded in flowering stalks and flowers (Table 1). Fructose content remained low and fairly stable in both leaves (<10 mg g^{-1}) and roots (~ 35 mg g^{-1}) throughout the year (Figs 3D and E); low values were also found in flowering stalks (Table 1). Glucose concentra-

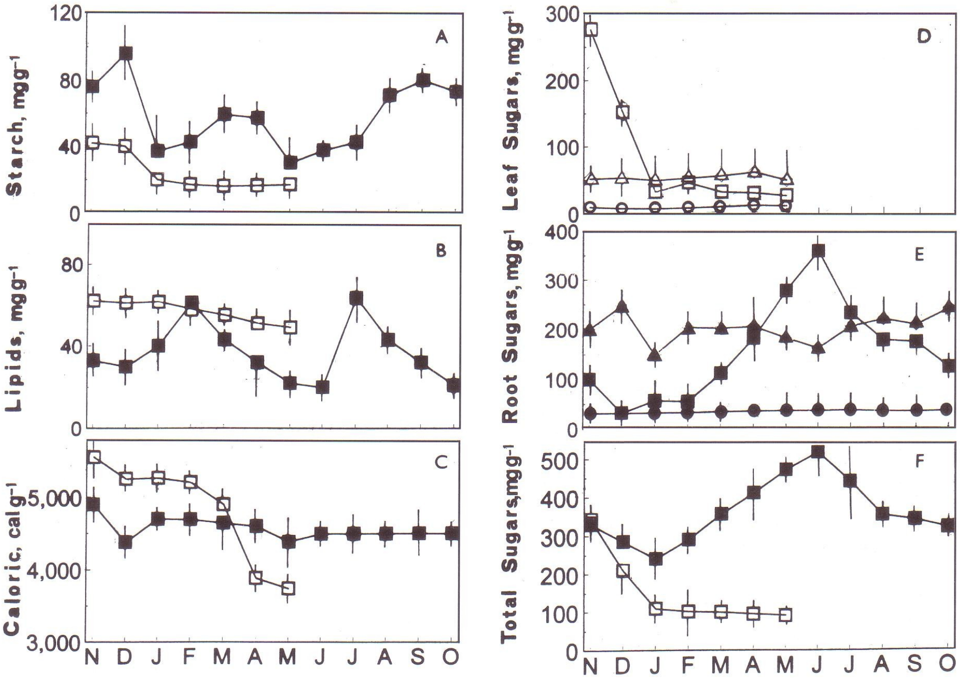

Fig. 3. Annual variations of photosynthates and caloric content in leaves and roots of *A. aestivus*. (A) Starch content ±SE. (B) Lipid content ±SE. (C) Caloric content ±SE. (D) Sucrose (squares), glucose (triangles) and fructose (circles) content ±SE in leaves. (E) Same as D in tubers. (F) Total sugar content ±SE. Open symbols represent leaves and closed symbols represent roots. All values (except for caloric content) are expressed in mg g^{-1} of dry weight.

tion was considerably higher than that of fructose varying from 50 to 80 mg g^{-1} in leaves, and 150–250 mg g^{-1} in tubers (Figs 3D and E); glucose content of flowers and flowering stalks in February were of the same order of magnitude as in leaves (Table 1). Ψ_s of roots was negatively correlated with soluble sugar concentration; the opposite held true for leaves (Fig. 4).

The energy bound in the plant mass of *A. aestivus* is given in terms of caloric values (Fig. 3C). The maximum value of the vegetative parts was detected in young expanding leaves in November (5570 cal g^{-1} dry weight). For most of the active phase, the caloric content of leaves was considerably higher than that of roots; it decreased sharply before desiccation, reaching the minimum value of 3600 cal g^{-1} dry weight. In contrast, the root caloric content remained fairly constant throughout the year, at ca 4550 cal g^{-1} dry weight, in spite of the drastic changes of the storage compounds taking place. Combined with the fact that *A. aestivus* root biomass is 6- to 30-fold higher than that of the above ground (Pantis 1993), this leads to the conclusion that the below ground part consists of a rather stable energy reserve, under continuous replenishment.

The maximum values of total chlorophyll content (ca 10 mg g^{-1} fresh weight) were detected in young expanding leaves in winter. Chlorophyll content decreased abruptly after March (Fig. 5A).

Proline concentration in leaves of *A. aestivus* peaked in mid-February and reached a minimum in November and May, nearly five times less than its highest leaf value (Fig. 5B). Two smaller maxima in proline concentration were recorded in roots (Fig. 5B), in January and June, under totally different water regimes as January-SWC was 5-fold higher than June-SWC (Fig. 1C). The proline content attained the highest overall value in flowers (62 mg g^{-1} d.w.). Considering that proline can be translocated to the flowers (Zhang et al. 1985), we could associate this extremely high concentration to its previous decline in roots and leaves (Table 1, Fig. 5B). Its transfer and further metabolism would serve the demand for nitrogen by reproductive organs.

Betaine content in leaves and roots remained rather unchanged and low throughout the year (Fig. 5C).

Table 1. Free proline, starch and soluble sugar content in the flowering stalks and flowers of *A. aestivus* in February.

		concentration (mg g^{-1} dry weight $\pm$SE)			
Plant part	Proline	Starch	Sucrose	Glucose	Fructose
Flowering stalks	16 ± 0.4	25 ± 0.4	518 ± 3.8	90 ± 0.4	14 ± 0.2
Flowers	62 ± 1.1	67 ± 0.8	383 ± 14.0	44 ± 5.0	12 ± 1.3

Betaine is likely to represent only a small fraction of the total nitrogen and to constitute an inert end-product that is not further metabolised by the plant, as suggested by Bowman and Rohringer (1970).

Discussion

One of the prominent features of Mediterranean climate is its periodicity, to which *A. aestivus* has responded by synchronising the annual development of its biological cycle (Pantis et al. 1994). Changes in water and energetic status are associated with the particular phenophases and serve a double purpose to the species, i.e. to face climatic stresses and satisfy growth and reproduction needs. The parallel examination of the above ground and below ground structures reveals the contribution of each of these two plant parts to the overall response of the species in this fluctuating environment.

Water relations

The species responds to the prolonged dry summer by avoidance shedding all its above ground structures. Given the lack of transpiring surfaces, the below ground part would not experience soil water deficits. As stated by Evans et al. (1992), survival during drought is ultimately dependent on the maintenance of cell turgor. In *A. aestivus* roots, this was accomplished by decline of Ψ_s parallel to Ψ_{root} during the dry period. Still, in the upper soil profile (~ 10 cm in depth), the root system is likely to be vulnerable to dehydration, as witnessed by a visible shrinkage of older portions of the dahlia-like tuberous roots. Given the maintenance of root apical turgor, this shrinkage probably reflected a hydraulic effect with water moving from the non-growing (upper) to the growing (deeper) parts (Matyssek et al. 1991).

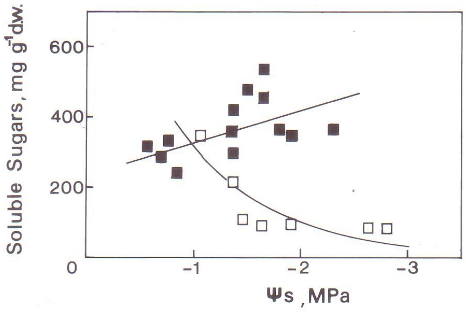

Fig. 4. Relationship between soluble sugar content and solute potential in leaves (open squares, $1/y = -4.48(10^{-3}) + 8.33(10^{-4})x$, $r = 0.802$, $p < 0.05$) and tubers (closed squares, $y = 215.682 - 91.11x$, $r = -0.571$, $p < 0.05$).

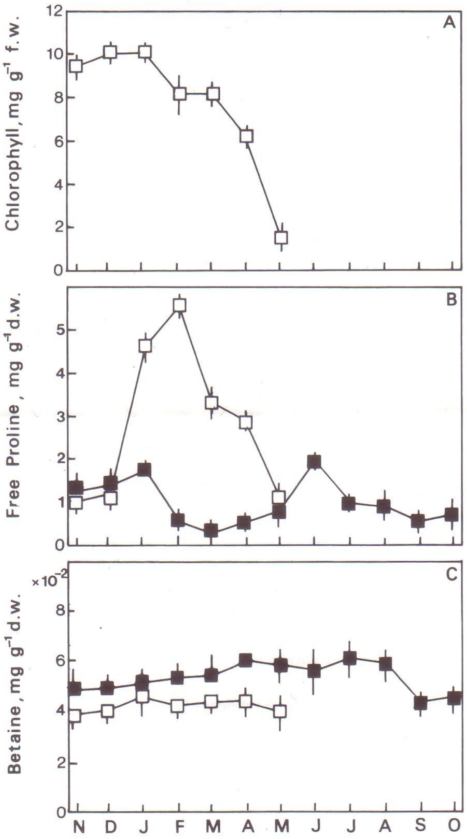

Fig. 5. Annual variation in total chlorophyll, and in proline and betaine content in leaves and roots of *A. aestivus*. (A) Chlorophyll content $\pm$SE. (B) Proline content $\pm$SE. (C) Betaine content $\pm$SE. Open symbols represent leaves and closed symbols roots.

Leaf-RWC was kept at low levels after January and till senescence, despite the saturation level of root-RWC (Fig. 1C). McKenzie et al. (1974) argued that high RWC values during winter would result in intracellular ice formation due to the large amounts of water within the cells of tender tissues. If the low leaf-RWC value in February might be explained in terms of adjustment to cold (February is one of the coldest months), this could not hold true from March to April, when temperatures are moderate. After March, though, as turgor of leaves tended to reach zero and Ψ_s declined to a constant value, we might expect cell walls getting rigid and cell volume decreasing, what indicates leaf ageing (see also Figs 3A, F and 5A).

If we accept that proline accumulation is closely associated with survival in the face of a severe environmental stress (Bradford and Hsiao 1982), then proline accumulation in leaves in winter suggests that the low temperatures are also important in influencing Mediterranean plants, as already argued by Mitrakos (1980) and Larcher (1981), besides the ubiquitous summer drought. The winter peak of proline concentration in tubers, of considerably lower value than in leaves, shows that the cold winter effect in the root/soil continuum is milder than that of the leaf/atmosphere interface. Proline deposition in the leaves of phrygana and evergreen sclerophylls grown in the same area (Rhizopoulou et al. 1990, 1991, Diamantoglou and Rhizopoulou 1992) was lower than that recorded in the leaves of *A. aestivus*, implying that the latter might experience more severely the winter cold. The osmotically active proline seems to play a role in the decline of Ψ_s in leaves during winter as has been reported for summer deciduous perennials in California (Calkin and Pearcy 1984).

Comparing the values of water, solute and turgor potentials and the proline content between roots and shoots, we could argue that the two plant parts experience and respond differently to the two stress factors associated with the major phenophases of the species. The above ground part responds by avoidance of summer water stress and by tolerance to winter cold stress whereas the below ground part experiences mildly the winter cold stress and responds by tolerance to summer water stress.

Energetic status

Comparing the annual variation of starch, lipid and sugar content in shoots and roots of *A. aestivus*, it is deduced that leaves do not accumulate sugars or starch, except for a two-month period, early in the active phase (Figs 3A, B and F). Around January, the substantial decline of soluble sugar and starch contents in both leaves and roots could be linked to the oncoming emergence of the flowering stalk and the energetic requirements associated with it. Lipids do not seem to have a substantial contribution at that stage.

Changes in the leaf and root contents of these compounds from January to the time of leaf senescence suggest a massive translocation of soluble sugars taking place from the above ground to the below ground tissues over that period. Similar results have been published for seasonally dimorphic species growing in the same area (Meletiou-Christou et al. 1992). This type of response can be associated with an osmotic adjustment, which functions in roots but not in leaves and which may result in reduction of Ψ_s in roots, parallel to the substantial decline of SWC (Figs 1B and C).

The contrasting patterns of change in root starch and soluble sugar contents (Figs 3A and F) after May, the month of leaf senescence, indicate soluble sugar conversion into starch over this period, as also suggested by Nerd and Nobel (1991) for other arid-zone plants; starch has been associated with low root permeability (Passioura 1988) and with hardening and survival of tuberous roots (Vartanian 1981) during the unfavourable period. Seasonal storage of carbohydrates in non-photosynthetic tissues is an essential feature of deciduous perennials (Chapin III 1980, 1991, Miller and Rose 1992), a major reason being that stored reserves enable perennial plants to start vegetative growth more quickly than annuals at the onset of the growing season (Schulze 1982).

The absence of any major change of storage compounds in roots prior to leaf emergence (late October), contrasting the remarkable ones before emergence of the flowering stalks, suggests that the energetic requirements for the production of the non-photosynthetic flowering stalk exceed those for the production of leaves, which from the very beginning have a high chlorophyll content (Fig. 5A) and, hence, photosynthetic activity.

According to Pantis et al. (1994), processes associated with the reproductive effort of *A. aestivus* might be distinguished as two phases; emergence of inflorescence stalks seemed to be entirely based on the amount of energy and materials stored in the tubers in the previous years, whereas elongation and flowering to be achieved as a result of the current period productivity. Results of this study broadly agree with the above statement except that energetic requirements for the emergence of inflorescence stalks are covered both by compounds stored in roots during the previous year and by ones produced during the current growth period.

Given the above, we could argue that the low flowering percentage (10–25%) observed in *A. aestivus* populations (Pantis and Stamou 1991), could be explained in energetic terms, besides soil nutrient availability. If the energy pool were not sufficient to support both vegetative growth and reproduction – necessitating previous construction of a sizeable, non-photosynthetic structure

– the plant would postpone anthesis till the time vegetative growth supplied the energy required.

In concluding, we should note those features of the below ground part of *A. aestivus* that contribute to its success in a fluctuating environment; i) it is less susceptible to the climatic stresses responding differently in magnitude and/or in character to the above ground, ii) it constitutes an energetically rather stable system and a regulating structure, responsible for the synchronisation of the plant to oscillating climatic variables and iii) it is able to withstand the prolonged dry period by efficiently storing water. These features, combined with those related to *A. aestivus* ability to avoid grazing and fires, may explain the species' occurrence and often dominance in a wide array of arid environments, from Mediterranean to desert.

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