Ecophysiological and morphological parameters related to survival in grass species exposed to an extreme climatic event

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An experiment was performed to elucidate interspecific differences in survival time of grass species subjected to an extreme climatic event. We exposed eight grass species to a simulated heat wave in the field ('free air' temperature increase at 11°C above ambient) combined with drought. We determined whether interspecific differences in survival time were related to the responses of the species to the imposed stress or could be explained by their ecophysiological or morphological characteristics in unstressed conditions. Surprisingly, there was no effect of specific leaf area, but species with a higher total leaf area survived longer. This may arise from a greater water reserve in the plant as a whole, which could delay the desiccation of the meristem, or from reduced evaporation due to a higher leaf area index. Species in which the decrease in light-saturated stomatal conductance (g_s) and photosynthetic CO_2 uptake rate (A_{max}) was strongly related to the decrease in soil water availability (measured as soil relative water content and stress duration) survived longer than species in which g_s and A_{max} likewise declined but responded more to daily fluctuations in irradiance, temperature, and vapor pressure deficit during the heat wave. We, therefore, hypothesize that interspecific differences in stress survival time might be related to the extent to which stomata react to changes in soil water conditions relatively to changes in other environmental and physiological factors. The results suggest that resistance to extremes is governed by other mechanisms than resistance to moderate drought.

Introduction

Climate-change models predict increases in daily and seasonal temperature variation in addition to changes in mean temperatures (IPCC 2001, Wagner 1996). This will yield more extremes (Easterling et al. 2000, Meehl et al. 2000), such as severe heat waves, which may exert

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Abbreviations – A_{max}, light-saturated leaf photosynthetic CO_2 uptake rate (μmol CO_2 m^{-2} s^{-1}); C_i, intercellular CO_2 concentration (μmol CO_2 mol^{-1}); F_0, ground fluorescence of the dark-adapted leaf; F_m, maximum fluorescence of the dark-adapted leaf; F_v/F_m, predawn potential quantum yield of photosystem (PS) II ($F_v = F_m - F_0$); g_s, light-saturated stomatal conductance (mol m^{-2} s^{-1}); PPFD, photosynthetic photon flux density (λ = 400–700 nm) (μmol photons m^{-2} s^{-1}); Ψ_l, predawn leaf water potential (MPa); RWC$_{soil}$, soil relative water content; SLA, specific leaf area (leaf area: leaf dry mass) (m^2 kg^{-1}); TR, leaf transpiration rate (mmol H_2O m^{-2} s^{-1}); VPD, atmospheric vapor pressure deficit (kPa); WUE, water use efficiency of photosynthesis (CO_2 fixed per unit water transpired) (mmol mol^{-1}).

major effects on the distribution of plant species and the composition of plant communities, e.g. by debilitating established plants or by creating gaps through plant mortality (Buckland et al. 2001, Solomon and Kirilenko 1997, Stampfli and Zeiter 2004).

A lot of research has been done on the responses to moderate drought and heat, but until now, there are only a handful of studies in which plants are exposed to the lethal stress levels experienced during extreme events. This is the case despite the fact that extremes play a disproportionate role in shaping the physiology, ecology, and evolution of organisms (Gaines and Denny 1993, Gutschick and BassiriRad 2003). Moreover, most of these studies have focused on community processes (e.g. recolonization, competition, invasion, the balance between C_3 and C_4 species, and the role of species richness during extreme events) (Buckland et al. 1997, Musil et al. 2005, Stampfli and Zeiter 2004, Van Peer et al. 2001, 2004, White et al. 2000, 2001) rather than on the factors responsible for the different responses of individual species. Also, the combined effect of extreme heat and drought has thus far hardly been studied, especially at the ecophysiological level (Van Peer et al. 2004, Wang and Huang 2004), while water deficits in the field rarely act alone (Chaves et al. 2002).

To help predict future changes in global vegetation patterns, we need more knowledge on the mechanisms underlying interspecific differences in survivorship under extremes and on the ecophysiological basis of the sensitivity to extremes in individual species. Which plant species will be more robust and which are prone to decline in a more extreme, future climate? Simple predictors of survival based on easily measurable plant traits are needed to overcome this.

The current study was aimed at finding ecophysiological and morphological plant parameters that correlated with survival time of grass species exposed to a simulated heat wave combined with drought. Both parameters measured on plants not exposed to the heat wave and responses of plants subjected to the extreme were considered. Eight grass species, known to differ in drought sensitivity, were used, and swards of them were subjected to a 'free air' temperature increase of 11°C coinciding with drought. Survival time was derived from exposing the swards to increasing stress duration.

Materials and methods

Plant material

Eight perennial C_3 grass species, known to differ in drought sensitivity, were monitored for various morphological and ecophysiological parameters both in non-stress conditions and during exposure to a simulated heat wave: *Agrostis tenuis* Sibth. (*At*), *Arrhenatherum elatius* L. (*Ae*), *Cynosurus cristatus* L. (*Cc*), *Dactylis glomerata* L. (*Dg*), *Festuca arundinacea* Schreb. (*Fa*), *Festuca rubra* L. (*Fr*), *Lolium perenne* L. (*Lp*), and *Poa trivialis* L. (*Pt*). As a first step towards the understanding of resistance to extremes, we used only species that have many traits in common. Including species belonging to different functional types might blur the underlying mechanisms, because a variety of adaptations and specific defence mechanisms against drought and heat could be introduced. From 1 to 4 April 2003, seeds of the selected species were planted at 2.5 cm interspaces in plastic containers (20 × 15 × 14.5 cm deep) to form monocultures of 30 plants each. Monocultures were used instead of individual plants to avoid unrealistic vertical radiation profiles. At every plant position, the first germinating seed out of five was kept.

Before sowing, the containers were weighed empty and then filled with a fixed amount of air-dried steam-sterilized sandy loam. Three samples of this air-dried soil were oven-dried at 60°C for 10 days and then weighed. Based on the mean ratio of the oven-dried to air-dried mass of these samples, the oven-dried mass of the soil added to the containers was calculated, as a basis for monitoring soil water content (see below). After sowing, the containers were placed in a greenhouse until 27 May and outdoors afterwards. They were well watered until the start of the experiment and were supplied with low amounts of fertilizer (total: 50 kg N ha^{-1}, 50 kg K ha^{-1}, and 80 kg P ha^{-1}) in two equal fractions on 13 June and 31 July. At the start of the experiment (11 August 2003), the containers were randomly assigned to four identical sets: one unheated set and three heated sets in which the plants were exposed to a simulated heat wave in combination with drought stress. Each set contained six replicate containers of each monoculture species or 48 containers in total. Daily rotation of the containers between the heated sets and within all sets minimized possible effects of set and position. The containers assigned to the unheated set were kept close to field capacity, while irrigation of the containers in the heated sets was arrested on 11 August. Soil relative water content (RWC$_{soil}$) in these heated containers was calculated daily on nine randomly chosen containers per species by dividing the actual amount of water in the soil (soil mass including water minus mass of oven-dried soil) by the potential amount of water in the soil (mass of fully wet soil after drainage minus that of oven-dried soil).

Microclimate

A heat wave ('free air' temperature increase and drought) was simulated by irradiating three replicate sets of 48 containers, placed level with the surrounding grassland vegetation, with additional infrared radiation (IR) (0.8–3 μm) using the FATI system (Nijs et al. 1996). Three second-generation prototypes of this device (Van Peer et al. 2004) were used to individually irradiate the three sets that were exposed. Each FATI module consisted of a frame with six 1500-W IR lamps, suspended 1.2 m above the ground, which homogeneously irradiated an area of 1.2 × 1.2 m. On a fourth set of 48 containers, which served to measure plant characteristics in non-stress conditions and was not exposed to drought and heat, a dummy construction was placed with lamp enclosures but no IR lamps. The heated sets were covered with a transparent shelter suspended at 2.5-m height, which eliminated precipitation but did not obstruct direct solar radiation. The experiment lasted until all plants in the heated sets had died (8 September). Because a natural heat wave occurred during the stress period, heating was switched off for 3 days (ambient maximum air temperature (T_a) > 32°C) to avoid unrealistic stress levels in the heated sets.

In each set, type-T copper constantan thermocouples (Omega Engineering, Stamford, CA) measured air temperature shielded from direct sunlight (centre of the set at 5-cm height), and type-T plug-mounted probes (TM Electronics, Goring, Sussex, UK) measured soil temperature at a depth of 5 cm. Canopy temperature of the whole set of 24 containers was measured with non-contact IR semiconductors with a view angle of 90° placed at 60-cm height (Stork Intermes, Naarden, the Netherlands) and photosynthetic photon flux density (PPFD) with JYP 1000 gallium arsenide quantum sensors (SDEC, Reignas sur Indre, France). Sensor output was recorded every 10 min with a DL2e data logger (Delta-T, Burwell, Cambridge, UK).

Average daily maximum, minimum, and mean temperatures [air (T_a), canopy (T_c), and soil (T_s)] during the stress period are summarized in Table 1 for both the unheated and heated sets. The average instantaneous increase in temperature owing to the heating (days when the heating was switched off excluded) equalled $11.27 \pm$ SD 2.15°C, $12.73 \pm$ SD 2.43°C, and $11.79 \pm$ SD 2.15°C for T_a, T_c, and T_s, respectively (n = 600 in all cases).

Plant measurements

Measurements were done on the heated plants to detect an ecophysiological basis for resistance to climatic extremes and on plants in the unheated set to determine potential predictors of stress-survival time.

At the beginning of the experiment, six plants per species (one per container) were harvested from the unheated set to determine total leaf area per plant (for each leaf, the area was approximated by multiplying leaf length by average leaf width; precision 0.5 mm), number of leaves per plant, total plant leaf mass (after oven-drying for 48 h at 70°C), and specific leaf area (SLA). Productivity of the monocultures during summer was estimated by cutting two containers (30 plants) per species to 2.5 cm and harvesting them again after 1 month of regrowth. Biomass was dried for 48 h at 70°C.

On the unheated plants, ecophysiological parameters were measured at the start of the heat wave (11 August 2003) and on 22 August 2003, after measurements on the heated plants had been finished. The plants in the heated sets were measured daily during the stress period [except predawn leaf water potential (Ψ_l), which was measured every two days] until the leaves were desiccated and ecophysiological measurements were no longer possible (after about 10 days of stress). For all measurements, each day the plants were randomly chosen, with the constraint of taking an equal number of plants per set, each from a different container. Plants near the container edge were avoided. All measurements were done on recently expanded leaves.

Ψ_l was measured on six excised leaves per species with a Scholander pressure chamber (ARIMAD-2, ARI Kfar Charuv Water Supply Accessories, Tel Aviv, Israel). Leaves were cut before sunrise, enclosed in plastic bags during transport to the nearby lab, and measured immediately. Instantaneous determinations of light-saturated photosynthetic rate (A_{max}), stomatal conductance (g_s),

Table 1. Average values ± SD of daily maximum, minimum, and mean temperatures (air, canopy, and soil at 5-cm depth) during a simulated heat wave (11 August−6 September 2003) for unheated and heated plants.

	Air temperature (°C)			Canopy temperature (°C)			Soil temperature (°C)		
	Maximum	Minimum	Mean	Maximum	Minimum	Mean	Maximum	Minimum	Mean
Unheated (n = 27)	25.4 ± 3.6	13.3 ± 2.7	18.5 ± 2.5	25.7 ± 4.1	11.7 ± 3.0	17.8 ± 2.9	20.7 ± 2.3	16.0 ± 2.4	18.4 ± 2.2
Heated (n = 27)	35.8 ± 3.6	23.7 ± 2.1	28.8 ± 2.2	36.5 ± 3.6	23.9 ± 2.8	29.3 ± 2.5	32.6 ± 2.5	26.0 ± 2.2	29.2 ± 2.0

transpiration rate (TR), and intercellular CO_2 concentration (C_i) at saturating PPFD (1500 μmol photons m^{-2} s^{-1}) were done around midday with a portable gas exchange system (LI-6400, Li-Cor, Inc. Lincoln, NE) on six leaves per species. CO_2 concentration, air temperature, and humidity during the measurements approximated the ambient conditions. Instantaneous water use efficiency of photosynthesis (WUE) was calculated as A_{max}/TR. Chlorophyll fluorescence parameters were measured around noon using a portable chlorophyll fluorometer (nine replicates per species; PEA, Plant Efficiency Analyser, Hansatech Instruments Ltd, Norfolk, UK). The background fluorescence signal (F_0) and the maximum fluorescence (F_m) were measured following dark adaptation for 1 h (leaf clips) to determine the maximum quantum efficiency of PSII [$F_v/F_m = (F_m − F_0)/F_m$].

Survival time of the stressed plants was measured by removing each time two containers per species (=60 plants) from below the FATI constructions, after different exposure times to the heat wave (6, 11, 15, 17, 19, 22, 24, 26, and 29 days of stress). These containers were then rewatered, and the number of individuals that restarted to grow was counted. The drought periods required to reduce survivorship to 50 and 25% (T_{50} and T_{25}, respectively) were used as measures of survival time.

Results

Survival

The survival curves of the eight species were significantly different (survival analysis, Wilcoxon test,

$\chi^2_{7,0.05} = 165.95$, $P < 0.0001$). Percentage survival decreased sharply after around 8 days of stress in the species At and Cc and between days 10 and 14 in the others (Fig. 1A). Species Ae and Dg survived the longest, with live individuals still being found after 19 and 22 days of stress, respectively. The ranking according to decreasing T_{50} was Dg, Fa & Fr, Ae, Lp, Pt, Cc, At. According to decreasing T_{25}, only the position of Ae changed: Ae, Dg, Fa & Fr, Lp, Pt, Cc, At.

Responses of the parameters to the progressive heat and drought

RWC_{soil} decreased progressively during the stress period and reached a minimum after 8 to 10 days, depending on the species (Fig. 1B). Monocultures of At depleted the water supply fastest, whereas RWC_{soil} remained highest in the monocultures of Ae and Fr. Ψ_l declined sharply in all species after about 3 days (Fig. 2). After 7 days, the values had dropped below the maximum range of the equipment of −3 MPa. Ae had the highest average Ψ_l during the first 7 days and At the lowest, but interspecific differences were limited. In all species, F_v/F_m fluctuated around 0.8 at the beginning of the stress and then decreased, indicating photoinhibition (Fig. 2). In Ae, however, there was little decline throughout. Most species were able to recover partially at around days 6–7, which were overcast, except for At, in which F_v/F_m declined from day 4 onwards.

Fig. 3 shows the progressive effect of drought and heat on g_s and A_{max}, which followed a similar pattern
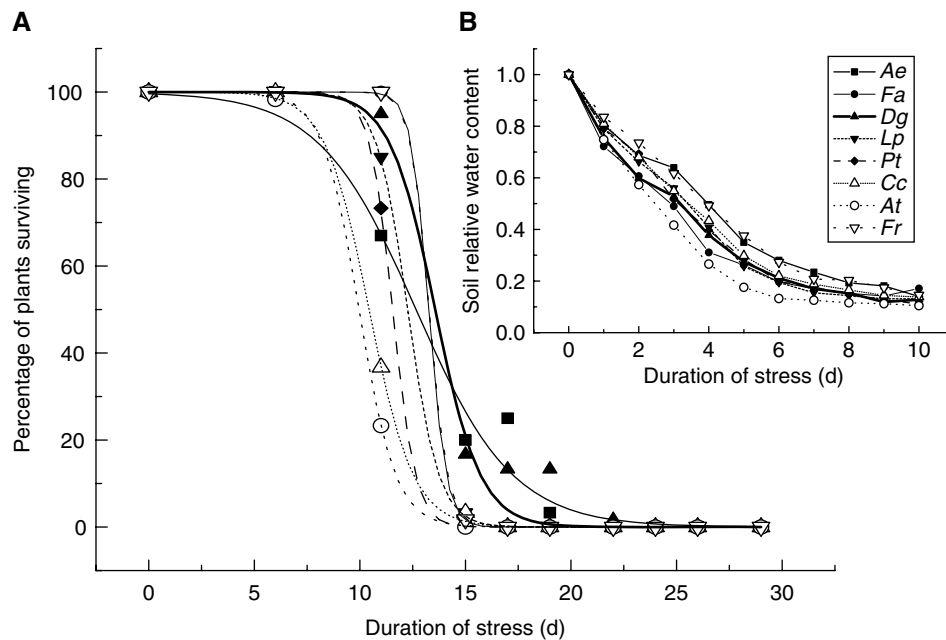

Fig. 1. (A) Species-survival percentage measured after different exposure times to a simulated heat wave. Data are fitted with dose–response curves: $y = A/[1 + 10^{(logx0 − x)p}]$. Each symbol is based on 60 plants in two containers. Note that the Fa and Fr curves overlap. (B) Decrease of soil relative water content during the first 10 days of exposure to the heat wave. Data are means of nine containers. Species codes: Ae, Arrhenatherum elatius; At, Agrostis tenuis; Cc, Cynosurus cristatus; Dg, Dactylis glomerata; Fa, Festuca arundinacea; Fr, Festuca rubra; Lp, Lolium perenne; Pt, Poa trivialis.
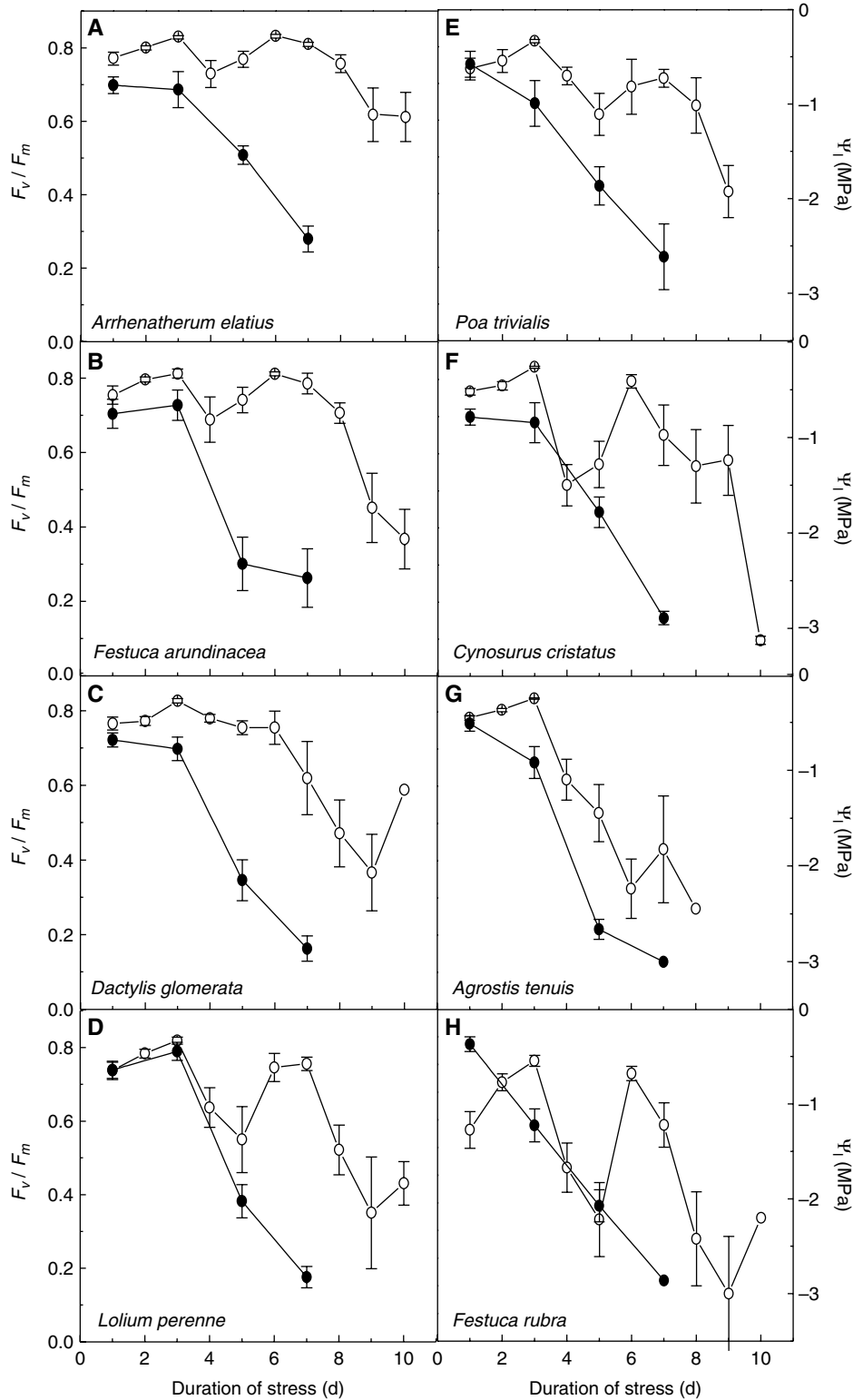
Fig. 2. Time course of maximum quantum yield of photosystem II (F_v/F_m) (open circles) and predawn leaf water potential (Ψ_l) (solid circles) during the heat wave. Means ± SE of nine and six replicates, respectively.

and approximated zero after 10 days of stress. Along with the decline, there were a lot of daily fluctuations in both parameters, except in the species with low values (*Ae* and *Dg*). These fluctuations were most probably due to variation in irradiance, temperature, and atmospheric vapor pressure deficit (VPD) between the days. The
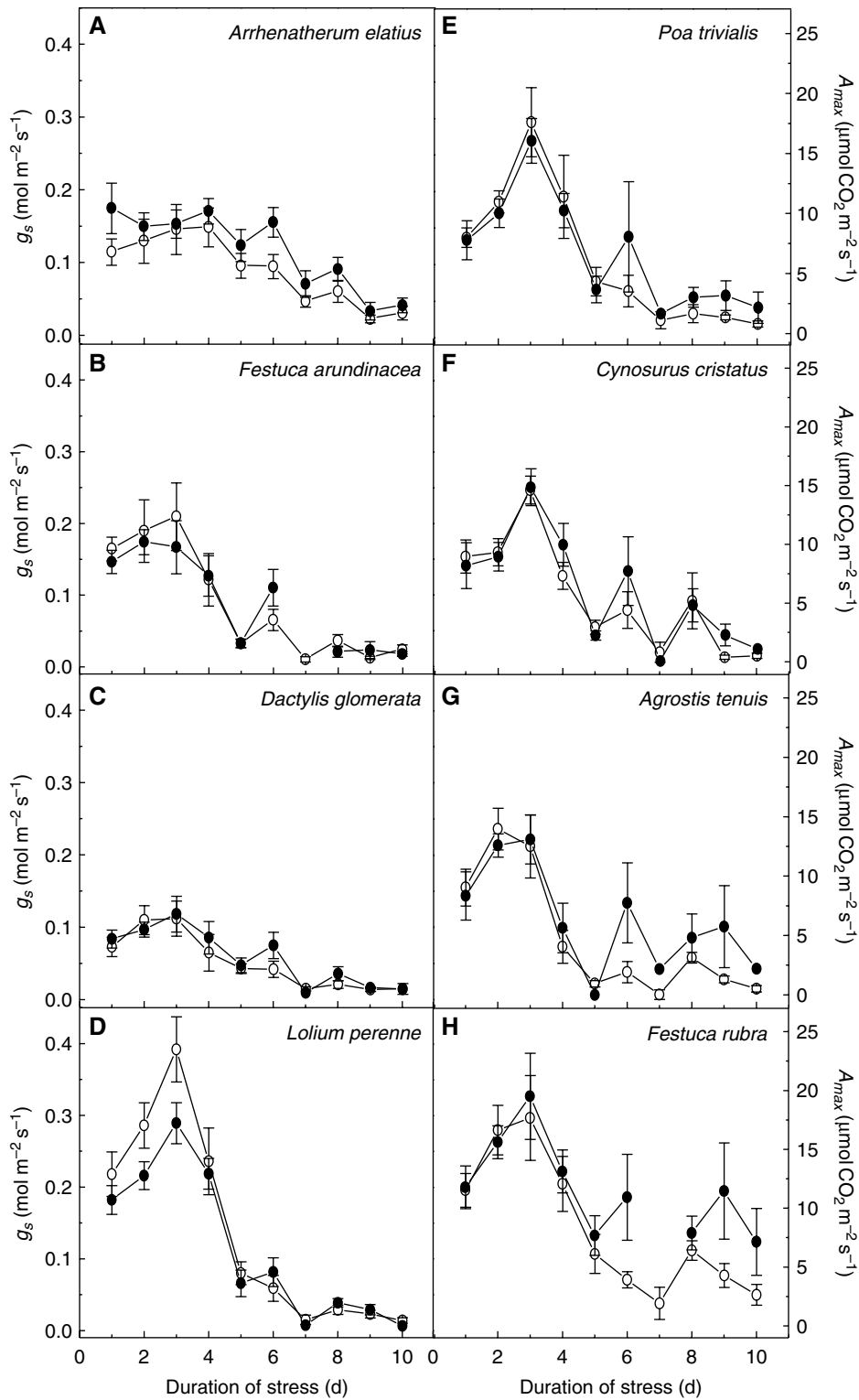
Fig. 3. Time course of light-saturated stomatal conductance (g_s) (open circles) and photosynthetic CO_2 uptake rate (A_{max}) (solid circles) during the heat wave. Means $\pm$ SE of six replicates.

peak values in A_{max} and g_s on days 3 and 6, for example, coincided with a significant drop in PPFD and air temperature during these days (not shown).

Transpiration rate correlated significantly with g_s in all species (Pearson correlations, $P < 0.05$ in all cases). Values of C_i approximated 260 µmol CO_2 mol^{-1} during

the first 4 days of stress in all species, but thereafter started to increase (Fig. 4). *Ae*, *Dg*, and *Fa* maintained low values of C_i longer than the other species.

Relationship between ecophysiological responses during the stress and survival time

We investigated whether species-survival time was related to the course of the ecophysiological parameters during the stress. First, for every parameter, we calculated the average value by species during the first 10 days of exposure and tested whether these averages explained a significant fraction of the interspecific variation in survival time (linear regressions). Only the parameters related to the maintenance of photosynthetic capacity, F_v/F_m ($T_{25} = -1.108 + 21.607 \times F_v/F_m$, $P = 0.028$, $r^2 = 0.58$) (Fig. 5A), and C_i ($T_{25} = 19.923 - 0.019 \times C_i$, $P = 0.018$, $r^2 = 0.63$) (Fig. 5B) were significantly related to survival time. Having a high or a low value of A_{max}, g_s, ψ_l, TR, WUE, or RWC_{soil} throughout the heat wave did not determine survival time.

In a second analysis, A_{max}, g_s, and TR were linearly regressed against exposure time, for each species separately. This enabled us to quantify how much of the variation in these parameters was explained by stress duration (by means of r^2) and how much variation was due to other factors, such as daily fluctuations in temperature, VPD, irradiation, etc. ($1 - r^2$). Because there were only four measurements of ψ_l during the stress, this parameter was not considered. All regressions were significant, except A_{max} against time for species *Fr* ($P = 0.066$). We subsequently tested whether the

obtained r^2 values and slopes of the significant regressions were associated with species-survival time. A significant relationship was found between survival time and r^2 of the relationship between g_s and stress duration [$T_{25} = 1.750 + 21.410 \times r^2$ (g_s against stress duration), $P = 0.004$, $r^2 = 0.78$] (Fig. 5C). The r^2 of the relationship between A_{max} and stress duration also explained survival time [$T_{25} = 4.582 + 12.766 \times r^2$ (A_{max} against stress duration), $P = 0.009$, $r^2 = 0.78$] (Fig. 5D). The slopes of the aforementioned regressions were not related to survival time.

In a third analysis, the above regressions by species of A_{max}, g_s, and TR were repeated, but this time against RWC_{soil} instead of against stress duration, to quantify how much of the variability in these parameters was accounted for by the water availability in the soil. All the regressions were significant, except for A_{max} against RWC_{soil} in *Fr* ($P = 0.061$). A significant relationship was found only between species-survival time and the r^2 of A_{max} against RWC_{soil} [$T_{50} = 4.393 + 13.026 \times r^2$ (A_{max} against RWC_{soil}), $P = 0.006$, $r^2 = 0.81$] (Fig. 5E).

Prediction of survival time from plant parameters in non-stress conditions

Fig. 6 shows the interspecific variation in morphological and ecophysiological parameters in non-stress conditions. As there was a clear range in most parameters (except in F_v/F_m; not shown), we investigated whether species-survival time could be predicted from them. Linear regressions with survival time (T_{25} or T_{50}) as dependent variable and the average values of the species characteristics
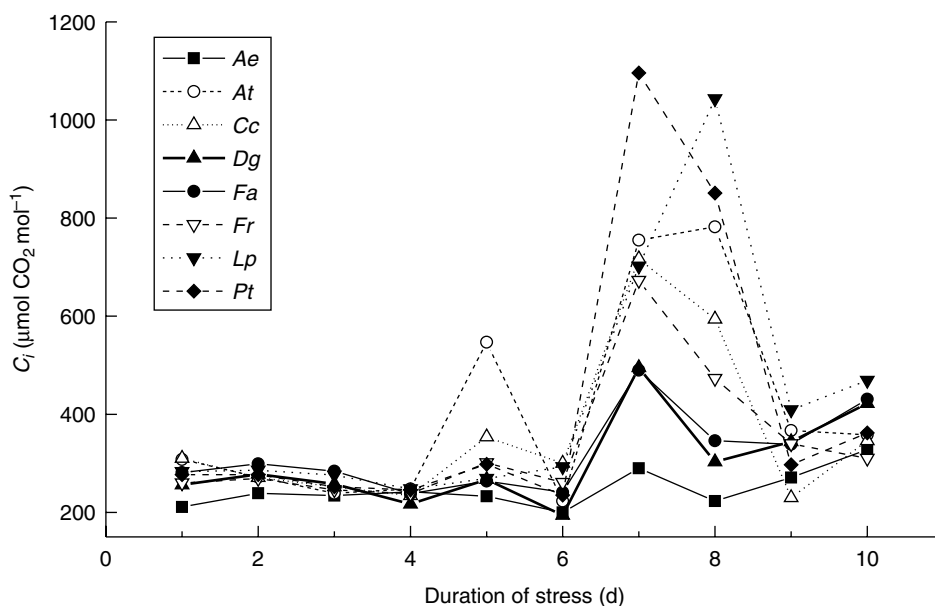

Fig. 4. Time course of intercellular CO_2 concentration (C_i) during the heat wave. Data are means of six replicates. See Fig. 1 for species codes.
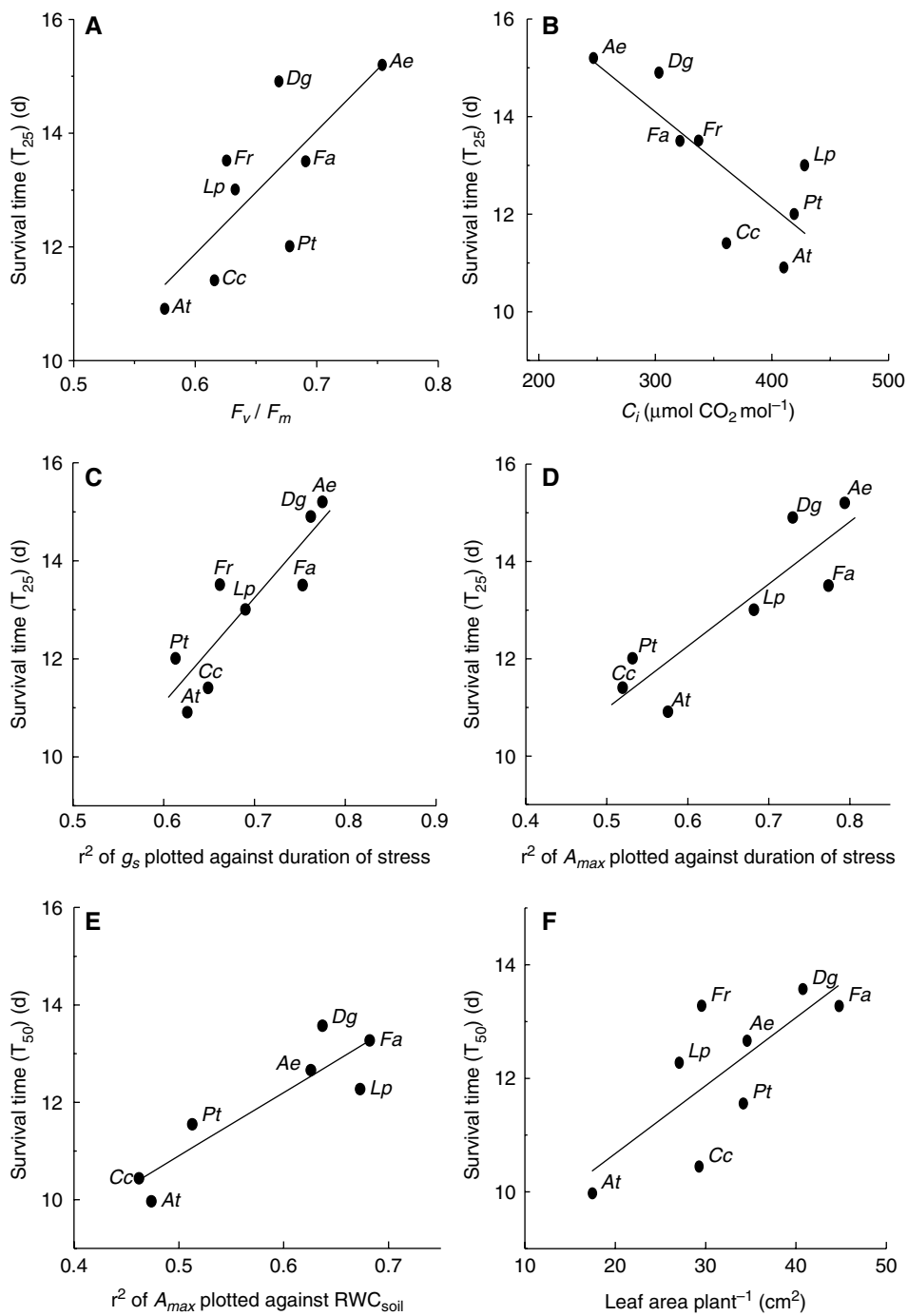
Fig. 5. Relationship between species-survival time and (A) maximum quantum yield of photosystem II (F_v/F_m), (B) intercellular CO_2 concentration (C_i), (C) r^2 of the regression between light-saturated stomatal conductance (g_s) and time, indicating the amount of variation in g_s explained by stress duration, (D) r^2 of the regression between light-saturated photosynthetic CO_2 uptake rate (A_{max}) and time, indicating the amount of variation in A_{max} explained by stress duration, (E) r^2 of the regression between A_{max} and soil relative water content (RWC$_{soil}$), indicating the amount of variation in A_{max} explained by RWC$_{soil}$, (F) total leaf area per plant ($n = 6$). In (A) and (B), x-values are averages per species during the first 10 days of stress. In (D) and (E), there is no data point for Fr, because regressions of A_{max} against time or RWC$_{soil}$ were not significant in this species. Survival times T$_{50}$ and T$_{25}$ are the drought periods required to reduce survivorship to 50% and 25%, respectively. See Fig. 1 for species codes.

(measured on unheated plants) as independent variables revealed a significant relationship for leaf area per plant: survival time was longer in plants with a greater total leaf area [T$_{50}$ = 8.265 + 0.120 × (leaf area per plant), $P = 0.033$, $r^2 = 0.56$] (Fig. 5F). None of the other parameters were related to survival time, although the species that survived longest, Ae and Dg, had low values of A_{max}, g_s, and TR and a high WUE compared with the other species.

Discussion

Relationship between ecophysiological responses during the stress and survival time

Although high averages of A_{max}, g_s, and TR during the stress might indicate high water use, these values were not related to survival time. The reason for this
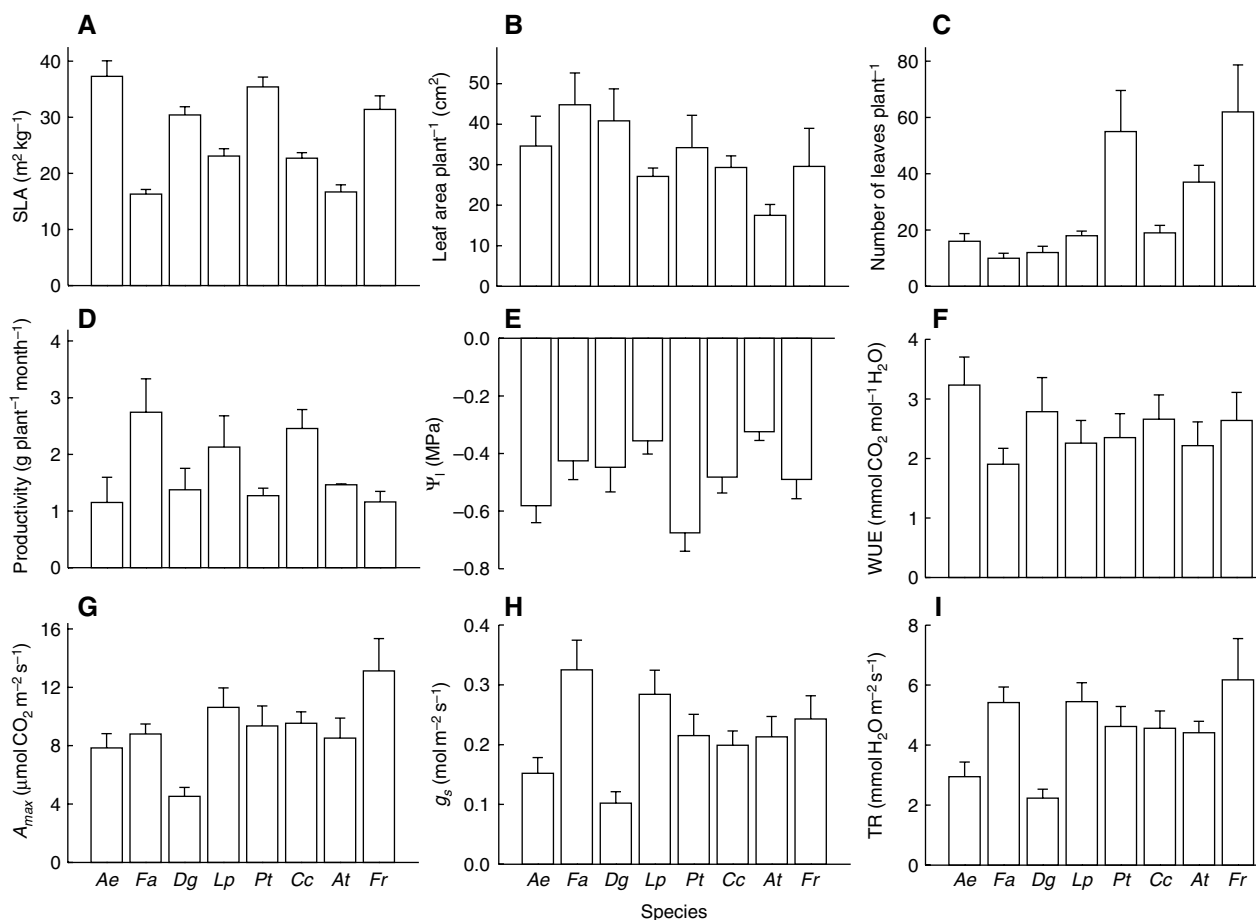
Fig. 6. Plant characteristics of the grass species measured in unstressed conditions (not exposed to the heat wave and well watered). Means ± SE of six replicates for specific leaf area (SLA), total leaf area per plant, and number of leaves per plant, and means ± SE of 12 replicates for water use efficiency (WUE), predawn leaf water potential (ψ_l), light-saturated photosynthetic CO_2 uptake rate (A_{max}), stomatal conductance (g_s), and transpiration rate (TR). Productivity was measured on two containers per species. See Fig. 1 for species codes.

might be that such averages do not distinguish whether a species has a high photosynthetic rate at the beginning of the stress which sharply decreases when water becomes scarce or a moderate initial photosynthetic rate which is maintained throughout the stress period. Therefore, information on how rapidly physiological parameters change with developing stress or on the magnitude of these responses might be more relevant. This was confirmed by our study of the relationship between the ecophysiological parameters and the imposed stress. By quantifying how much of the daily variation in the parameters was accounted for by the progressive drought (r^2 of the regressions), we could explain 78 (r^2 of g_s or A_{max} against duration of stress) to 81% (r^2 of A_{max} against RWC_{soil}) of the variance in survival time (Fig. 5C,D,E). This suggests that, despite the important role of root parameters (e.g. root biomass, root/shoot

ratio) for survival during drought, there might also be an effect of ecophysiological and morphological (see further) factors on the survival of species under extreme climatic events.

Stomatal conductance depends simultaneously on multiple factors such as light, temperature, VPD, intercellular CO_2 concentration, guard cell and epidermal turgor, and water flow through the soil and plant (Franks et al. 2001, Netting 2000, Tuzet et al. 2003). The degree to which these factors influence g_s varies between species (Tardieu and Simonneau 1998). For example, stomata of different species vary in their sensitivity to leaf water potential (Henson et al. 1989, Leuning et al. 2003, Tuzet et al. 2003), which in turn is a function of soil water potential, the rate of flow through the soil and plant, and the xylem hydraulic resistance. In our data, we found interspecific differences in the amounts of daily variation in g_s and A_{max} that were explained by

stress duration or RWC_{soil}, which were indicators for water availability of the soil. In the best-surviving species (Ae and Dg), g_s and A_{max} correlated strongly with the decreasing soil water supply, while only little daily variation in these parameters could be attributed to other factors. In the other species, the dependence of g_s and A_{max} on stress duration was lower, which suggests that the stomata in these species were more influenced by the aforementioned microclimatic factors. Much higher peaks in the values of g_s and A_{max} in these species compared with Ae and Dg at days 3 and 6 (when there was a significant drop in PPFD, air temperature, and VPD; data not shown) support this (Fig. 3). We therefore hypothesize that interspecific differences in stress-survival time might be related to the extent to which stomata react to changes in soil water conditions relatively to changes in other environmental and physiological factors. The reason why this has an effect on survival time is that peak values in g_s and A_{max} are not immediately compensated by equally large drops in these parameters, such that after a few days of stress the consumed amount of water is greater in the species with higher fluctuations, although their average values of g_s and A_{max} might be comparable with less fluctuating species. A similar division of stomatal responses is mentioned in a study of Gutschick and Simonneau (2002), who state that stomatal conductance responds to two distinct environments: (1) the local and aerial environment of the leaf, defined by irradiance, temperature, humidity, CO_2 concentration, and boundary-layer condition and (2) the distal environment, particularly that of the roots, which generates root-sourced signals of water stress [abscisic acid (ABA)] and contributes to determining leaf water potential. However, more research on the relative importance of aerial vs. root signals (ABA) for reducing g_s, with respect to survival of extremes, is needed.

Species that maintained high values of F_v/F_m (around 0.8) (Figs 2 and 5A) and low values of C_i (around 260 μmol CO_2 mol^{-1}) (Figs 4 and 5B) during the stress survived longer. This was not surprising, because both parameters are related to the condition of the photosynthetic apparatus (electron transport capacity, reflected in F_v/F_m, and carboxylation capacity, reflected in C_i), which is a good proxy for overall stress level (Srivastava et al. 1995). These parameters therefore rather indicate symptoms of drought injury than mechanisms of tolerance. An increase in C_i when stress becomes severe often indicates non-stomatal limitations to photosynthesis, involving progressive downregulation or inhibition of metabolic processes (Flexas and Medrano 2002, Lawlor 1995). However, caution must be taken, because C_i calculation under drought might be inaccurate, because patchy

stomatal closure and changes in mesophyll conductance to CO_2 often tend to overestimate C_i (Bota et al. 2004, Buckley et al. 1997).

Although only eight grass species were used, a range in survival times and different responses to the stress were observed. When more species and functional types are considered, the diversity of plant responses, special adaptations, and specific defence mechanisms against extreme heat and drought is likely to further increase (Chaves et al. 2002). The use of species belonging to the same functional type, however, has the advantage that underlying mechanisms might be easier to detect, because interference of different mechanisms is limited. Even though our results apply only to the studied grass species, we believe that the detected relationships might also be relevant for other species, because increasing water loss due to fluctuations in g_s might adversely affect all species under progressive drought stress.

In this experiment, we chose to do the gas exchange and fluorescence measurements around midday, because we wanted to compare the species at the moment when they suffered the most from the imposed stress. Differences between the species might, however, be more pronounced when fluorescence measurements are done predawn to remove the effect of preillumination and when gas exchange measurements are done before noon, because at midday, photosynthetic depression may occur. Future research may reveal whether the results might be improved by measuring the optimal physiological status of plants during a heat wave instead of midday values of gas exchange. As the plants in this study had no stress history, acclimation to stress as a result of previous exposure was not considered. Exposure to subsequent stress episodes can influence the stomatal reaction to Ψ_l, VPD, irradiance, etc. in a way that water loss is scaled down (Bohnert and Sheveleva 1998, Tabaei-Aghdaei et al. 2000); hence, the survival times recorded in the current study may well represent a lower limit for the stress levels used.

Relationship between survival time and plant characteristics in non-stress conditions

Total leaf area per plant was the only morphological characteristic related to survival time, with longer survival in species with more foliage (Fig. 5F). This was surprising, because a greater transpiring surface evidently enhances water loss, and many plant species react to drought by partial or complete abscission of the leaves (Clifton-Brown et al. 2002, Rascher et al. 2004, San Jose et al. 2003). The above finding is also

in contradiction with the idea that slow growth enhances resistance to moderate drought (Polley et al. 2002, Tardieu and Simonneau 1998).

However, when due to extreme conditions the stomata are almost entirely closed, plants with a large leaf area might have a greater water reserve, which will be depleted first before the basal meristem desiccates, and the plant eventually dies. This might explain the longer survival time in the grasses with a larger leaf area. Whether this mechanism occurs also in other life forms remains to be seen, although it would not be unexpected to find it in other hemicryptofytes than grasses. Another possible explanation is the relatively high leaf area index (LAI) in species with a high leaf area per plant. The leaf area in the studied species ranged from 18 to 45 cm^2, and the area per plant in the containers was approximately 10 cm^2. This produces a LAI between 1.8 and 4.5 depending on the species. Because a canopy with a LAI of 4.5 is relatively dense, this may produce a boundary layer of still air reducing evaporation and conserving water in the species with a higher total leaf area. There was no correlation between SLA and survival, although a low SLA is often associated with plants developed under conditions of poor water supply (Fernández et al. 2002). Resistance to extremes thus seems to be governed by other mechanisms than resistance to moderate drought.

None of the ecophysiological plant characteristics measured on the unheated plants were related to stress-survival time; the main reason for this being that the behavior in unheated conditions was a poor predictor of the behavior during the heat wave. For example, while most species reduced g_s by a factor of two in response to the stress, the most 'water wasting' species in non-limiting conditions (Fa) reduced g_s by a factor of four, which made it the second-most economic species when water was scarce.

Conclusions

Our data showed that species in which g_s and A_{max} were strongly related to soil water availability (reflected by stress duration and RWC$_{soil}$) were able to survive longer in conditions of intense, progressive drought combined with extreme elevated temperatures. In contrast, species in which g_s and A_{max} likewise declined but responded relatively more to the daily fluctuations in irradiance, temperature, and VPD during the heat wave died sooner, probably because they had consumed more water during the first days of the stress during which they showed high peaks in g_s and A_{max}. Maintenance of photosynthetic capacity, demonstrated

by a high average value of F_v/F_m and a low value of C_i during the first 10 days of stress, was also associated with long survival. The most surprising result of our experiment, however, was the positive relationship between leaf area per plant and survival time and the lack of an effect of SLA. These results suggest that survival of extreme climatic events may rely on specific mechanisms that have received little attention in ecological stress research thus far. More explicit studies, separating the influences of the local environment of the leaf and the root signals involved in reducing stomatal conductance during severe stress, are needed to further the development of a mechanistic understanding of responses to heat and drought extremes. We suggest that further research should as well test whether our findings apply also to other grass species and to other functional types.

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