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P9–JOHN BOYER SYMPOSIUM—REPRODUCTIVE DEVELOPMENT

The programme is a celebration of the work of John Boyer, marking his retirement, and has been organised by Bob Sharp (University of Missouri, Columbia), Bill Davies (University of Lancaster) and Howard Griffiths (University of Cambridge)

P9.1
John Boyer Symposium: 1. Drought tolerance
John Boyer: Doyen of plant water relations

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John Boyer has shaped the field of plant water relations as no other over the last 40 years. His application of isopiestic psychrometry to plants, his discovery of osmotic adjustment and growth-induced gradients in water potential, his elucidation of the processes behind embryo abortion in maize, have been rich sources of inspiration to many others. The most controversial of these discoveries has been that of the large gradients in water potential within growing tissues. Its implications for our understanding of tissue stresses, of the provision of water throughout growing stems, and of the control of elongation rates of constrained cells have been profound. This topic has been controversial because his experiments have uncovered behavior of the tissue water relations that at first sight seem highly counterintuitive. For example, changing the water potential of the xylem feeding the growing tissue can greatly affect elongation rate without affecting the water potential of the epidermal cells which are known to be limiting the elongation rate. Such behavior is now starting to make sense in terms of the subtle interactions between the resistance to radial water flow in growing tissue, the constraints resulting from the necessarily uniform growth rate of all the cells involved, and the large stiffness of the epidermal cell walls.

Keywords: Drought, Growth, Salinity, Water relations, Tissue stress

P9.2
Sorghum and the genetic basis of drought tolerance

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Sorghum bicolor is well adapted to drought prone and nutrient limited environments and contributes significantly to subsistence and commercial agriculture. Sorghum is also considered a genome model for C4 grasses because it has a relatively small genome (800 Mbp), high degree of collinearity with the rice genome, facile genetics, and an extensive germplasm collection. Over the past 5 years, a comprehensive set of genome and genetic resources for sorghum has been developed starting with a high-density integrated genetic and physical map. BAC-FISH analysis revealed that the distal portions of most sorghum chromosomes are euchromatic and gene dense whereas the pericentromeric regions are heterochromatic, gene poor, and have low rates of recombination. Targeted sequencing demonstrated that ~80–90% of the genes in sorghum are collinear with genes in rice. Genes involved in responses to stress have been identified using microarrays containing ~22,000 gene sequences derived from an extensive EST project. This information and phylogenetic analysis of sorghum, rice and maize gene sequences is helping elucidate the biochemical pathways and gene regulatory networks that mediate responses to stress in these grasses. The sorghum genome resources are also being used to map and clone genes that control flowering time, drought tolerance (i.e., stay green), fertility restoration, and nutrient acquisition. We are also exploring the relationship between sorghum germplasm diversity, the selection of beneficial alleles in breeding programs, and adaptation of sorghum to adverse environments. The integrated approach described above was inspired by John Boyer, a pioneer in this challenging field of research.

Keywords: Sorghum, Genomics, Drought tolerance

P9.3
Identification of transcription factors regulating water stress responses

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Abiotic stress, particularly drought, is a complex phenotypic and physiological phenomenon in plants. The identification of transcription factors underlying the complex responses of plants to drought stress can provide a solid basis for improving drought resistance. We have conducted a large-scale, high throughput program to identify transcription factors that regulate important plant properties in *Arabidopsis thaliana*. Over 1700 transcription factors were overexpressed constitutively, and about 10% of these have also been analyzed by insertional mutagenesis. The resulting transgenic lines have been screened through physiological assays to identify transcription factors that control abiotic stress response pathways. For example, overexpression of CBF (DREB) genes can increase tolerance to drought or cold conditions in a number of plants. However, constitutive overexpression of CBF genes can lead to the development of off-phenotypes such as reduced size. We will present a number of transcription factors identified in these screens that appear to confer drought tolerance via distinct mechanisms. Progress in developing these leads into commercial products and elimination of off-phenotypes, will be discussed.

Keywords: Transcription factor, Arabidopsis water stress

P9.4
Control of plant water status and water use efficiency through manipulation of ABA biosynthesis

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Genes encoding four enzymes in the pathway from zeaxanthin to abscisic acid (ABA) have now been reported: zeaxanthin epoxidase (ZEP), 9-*cis*-epoxycarotenoid dioxygenase (NCED), xanthoxin oxidase and ABA aldehyde oxidase. An ABA-deficient mutant has also recently been identified that is unable to produce *cis* or *trans* isomers of neoxanthin. We have previously demonstrated that NCED is a key rate-limiting step in this pathway by chemically inducing *LeNCED1* expression in tobacco and then observing a 10-fold ABA accumulation in leaves (Plant J. 2000, 23:363), and we have partially complemented a null mutation in *LeNCED1* (*notabilis*) to create tomato lines with a very mild ABA deficiency (PCE, 2004, 27:459). Here we will describe physiological effects of high endogenous ABA accumulation in stressed and non-stressed tomato plants constitutively over-expressing *LeNCED1* ("high ABA" plants). In these plants, ABA content in leaves, roots and xylem sap was higher than in wild-type and this led to higher turgor, reduced stomatal conductance and increased water use efficiency. When "high ABA" plants were transferred from high to low vapour pressure deficits they displayed increased guttation and flooding of leaf intercellular air spaces, suggesting an ABA-induced increase in root pressure. "High ABA" plants can be considered as drought avoiding as they conserve soil water under non-stressed conditions. Over-production of ABA had some negative effects on growth on younger plants but little effect was observed in older plants. The effects on root-to-shoot signaling and the tissue-specific manipulation of ABA biosynthesis will be discussed.

Keywords: Abscisic acid, 9-*cis*-Epoxycarotenoid dioxygenase, Water use

P9.5
Drought modifies vegetative storage proteins (VSP) during regrowth of alfalfa exposed to elevated CO_2 and temperature

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The increasing atmospheric CO_2 concentration, as consequence of economic development, generally leads to increase in plant biomass production but very little attention has been paid to the effects of combined factors, such as CO_2, temperature or water availability, during regrowth, which is the usual way to manage forage legumes like alfalfa. Furthermore, the plant production response to elevated CO_2 can be affected by soil water availability. Nitrogen pools in alfalfa taproot, especially vegetative storage proteins (VSP), have been demonstrated to condition new regrowing shoots while non-structural carbohydrates are mainly used for respiration in remaining organs, roots and nodules. The aim of our study was to determine the effect of CO_2 (ambient-around 350 μmol mol^{-1} versus 700 μmol mol^{-1}), temperature (ambient versus ambient +4 °C) and water availability (well irrigated versus partially irrigated) on taproot N accumulation, especially VSP, in nodulated alfalfa before defoliation and after 1 month regrowth. At the end of vegetative normal growth, elevated CO_2 enhanced dry matter production only in plants grown under high temperature and irrigated at field capacity. During this period the taproot VSP concentration was increased by elevated CO_2 and especially under drought. This could improve production during subsequent regrowth period in drought treatments. After 1 month regrowth, drought increased again the VSP levels. It is suggested that the increase in VSP will allow a better plant regrowth during cutting-regrowth cycles due to the close relationship between N reserve availability, particularly VSP, and shoot regrowth which has been observed in alfalfa and other forage legume species.

Keywords: Alfalfa, Regrowth, CO_2, Nitrogen, Vegetative storage proteins, Temperature gradient tunnels

P9.6
Control of maize leaf growth under water deficit and evaporative demand: Genetic and physiological dissections of involved mechanisms

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Leaf growth is affected by both soil water deficit and evaporative demand. We have previously modelled its time course as the superposition of two kinetics with different timescales. A "long term" trend, corresponding to the decrease in elongation rate during successive nights, would be the result of root–shoot

signalling responding to soil water status. A "short term" kinetics would be superimposed onto the latter and would depend on evaporative demand. Accordingly, the time course of maize leaf elongation rate (LER), analysed in more than 1000 leaves of different genotypes showed a slow night-to-night reduction in relation to the decrease in soil water potential. LER was much more reduced during days than during nights, with increasing reductions when evaporative demand increased. Genetic analyses were carried out on the parameters of response curves to soil water status (night-time LER) and evaporative demand (day-time LER). This was carried out in 3 mapping populations, allowing one to determine QTLs of the susceptibility of leaf growth to soil water deficit and to evaporative demand by a common analysis of experiments in different conditions (field, greenhouse and growth chamber). However, several arguments suggest that the model based on the additivity of two independent kinetics might be inadequate. This is based on (i) co-location of QTLs, (ii) the analysis of the behavior of transgenic plants affected on the NCED gene, which under- or over-produce ABA, (iii) in situ measurements of turgor pressure in growing cells of plants subjected either to jumps of evaporative demand or to soil water deficit.

Keywords: Leaf growth, Genetic variability, ABA, Turgor, Water deficit

P9.7
Roles of peroxiredoxins and LEA proteins in drought stress in transformed Arabidopsis and in resurrection plants

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The sustainability and predictability of crop production worldwide are severely restricted by environmental factors, particularly drought. Drought is one of the most difficult challenges currently faced by agriculture today, and it is predicted that Africa is heading for a period of even more prolonged drought, which researchers warn could be the most severe in decades. Drought is a complex stress that has been extensively studied but remains poorly understood. Here, we report on the functions of two defence proteins: a 1-Cys peroxiredoxin (XvPer1) isolated from the resurrection plant *Xerophyta viscosa* and AC3, a late embryogenesis-abundant (LEA)-like protein from *Arabidopsis thaliana*. The former was isolated via its induction during desiccation while the latter was isolated via its ability to confer H_2O_2 tolerance to the oxidative stress sensitive Δ*yap1* yeast mutant. Expression of *AC3* also increased tolerance of Δ*yap1* cells to the pro-oxidants diamide, menadione and *tert*-butyl hydroperoxide. Unlike most LEAs, *AC3* is not seed-specific but is highly expressed in roots and reproductive organs. Constitutive expression of *AC3* was low in leaves but strongly induced by dehydration and oxidative stress treatments. Under dehydration stress, *AC3* was highly induced in leaves of *abi1-1* (ABA-insensitive) and *aba1-1* (ABA-deficient) Arabidopsis mutants. Taken together, these data suggest that AC3 might be involved in protection of roots and reproductive organs under normal conditions and under stress conditions, root-to-shoot signalling induces *AC3* expression mainly by an ABA-independent pathway in leaves protecting the latter from oxidative damage.

Keywords: 1-Cys peroxiredoxin, Late embryogenesis-abundant protein, Hydrogen peroxide, Redox signalling, Drought tolerance

P9.8
Adaptation and acclimation of Rubisco specificity factor to drought: ecological significance.

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Drought conditions strongly influence photosynthetic metabolism in a way that might be extremely important to determine a positive carbon balance in highly stressed environments, such as the Mediterranean. However, plants may have evolved towards more efficient photosynthetic mechanisms, possibly including acclimation and adaptation of photosynthetic enzymatic traits. Because of its central role in photosynthesis, Rubisco is one of these potential traits to be selected under stressful conditions. Plants respond to low water availability by decreasing leaf diffusive conductances, which, however, leads to an increase of the barriers to diffusion of CO_2 to the primary site of carboxylation. We hypothesized that arid environments leading to water stress and, thus, decreased CO_2 availability for photosynthesis, may impose increased selection pressure on Rubisco for improving its specificity factor (τ), a measure of the relative affinity of the enzyme for CO_2 and O_2. To test this hypothesis, τ was measured on purified Rubiscos from 24 Mediterranean species having a variety of ecological, phylogenetic and morphological traits. A high variability in Rubisco τ was found among plants, which was related to environmental pressure factors and not to phylogeny. τ was significantly higher in species inhabiting the most arid areas, and the Rubisco of a xeric species, *Limonium gibertii*, presented the highest τ value hitherto reported among higher plants. This was sequenced and some interesting residues were found to be different to other higher plant Rubiscos but identical to *Galdieria*. Finally, to check whether plants can also acclimate Rubisco kinetic properties to drought, τ was measured in tobacco leaves developed under different drought intensities. The results showed that Rubisco τ does not acclimate to water stress in the short time.

P9.9
Root-ABA1: A QTL influencing L-ABA concentration and root traits in maize

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A previous study conducted on a maize mapping population derived from Os420 × IABO78 identified a quantitative trait locus (QTL) for leaf-abscisic acid concentration (L-ABA) on chromosome 2 (bin2.04) (Tuberosa et al., 1998, T.A.G. 97: 744–755). To characterize more accurately the direct and associated effects of this QTL, sets of near isogenic lines (BDLs) were developed for both parental lines. The isogenization of a QTL in more than one genetic background provides the opportunity for evaluating the QTL in an otherwise hybrid background, an important prerequisite for appropriately testing QTL effects for traits, such as yield, affected by inbreeding depression. These materials were field tested under water-stressed and well-watered condition for 2 years. The QTL confirmed its effect on L-ABA and interestingly, the high L-ABA hybrids showed a significantly lower root lodging (44.6%) compared to the low L-ABA hybrids (66.1%). To further elucidate the effects of the QTL on root architecture, root traits of two pairs of BDLs were measured in plants grown in controlled environment at three water regimes. Root differences among BDLs were not affected by water regimes. Across water regimes, the QTL confirmed its effect on L-ABA and showed a concurrent effect on root angle, branching, number, diameter and dry weight. Based on these results, we speculate that the QTL effects on root traits and L-ABA are likely due to pleiotropy rather than linkage and propose a model in which the primary effect of the QTL is on root architecture and not L-ABA.

Keywords: Maize, ABA, Drought stress, Roots

P9.10
Genotypic differences in rooting patterns and soil water extraction related to drought tolerance in sugar beet

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Drought is the major cause of sugar beet yield losses in the UK and other areas where summer rainfall is less than potential evapotranspiration. In arid regions as well as the UK, irrigation is limited because water is scarce and devoted to more valuable crops. The drought tolerance of sugar beet may be improved by selecting genotypes that are able to extract more soil water than current varieties. Root growth and patterns of water extraction were examined in genotypes that showed contrasting yield responses to drought. Laboratory screens of differential rooting ability through dry vermiculite or sand columns packed to different penetration resistances showed significant genotypic differences, but the ranking of genotypes did not relate well to field performance. In field studies, root activity was inferred from soil water extraction patterns measured using neutron scattering or capacitance probes. Drought was imposed by covering plots with large polythene tunnels. Genotypes differed in the ability to extract water from deep soil layers (110 cm from the surface), which correlated with yield under droughted and irrigated conditions. However, genotypic differences in season-long water use were small, suggesting that genotypes differed in water use efficiency. Field excavation of root systems showed greater root density at 50 cm in droughted compared with irrigated plots, but with few roots below 80 cm. Water extraction by sparse, deep roots was a sensitive indicator of yield potential, but above-ground factors may be more important in controlling per se drought tolerance for sugar beet under these conditions.

Keywords: Drought tolerance, Roots, Sugar beet, *Beta vulgaris*, Water use

P9.11
Embolism repair

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The mechanism by which some plants are able to reverse cavitation and restore water flow through embolized vessels remains poorly understood. Although the process requires energy input and thus the involvement of living cells, it remains unknown how plants are able to increase the hydrostatic pressure within cavitated conduits without raising the pressure of the entire vascular system. Using cotton as our model system, we present data relevant to the driving force for water flow into cavitated conduits. We also address the time scale for such repair based on in-vivo MRI studies in stems of *Acer*. How these data help to distinguish between proposed mechanisms for embolism repair will be discussed.

Keywords: Cavitation, Drought, Embolism repair, MRI, Xylem

P9.12
The effect of the topology of the xylem network on its vulnerability to drought-induced embolism

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The xylem provides a low resistance pathway for water transport from roots to leaves. Its characteristics are related to transport efficiency (i.e. maximum hydraulic conductivity) and safety (risk of embolism) and, therefore, resistance to drought. Both hydraulic conductivity and vulnerability to embolism have classically been attributed to the characteristics of individual conduits or the pit membrane between them. However, this overlooks the fact that the xylem is a network of interconnected conduits with a complex structure. Here we present a model that takes into account system-level properties, such as connectivity, to simulate water transport in the xylem, using the tools of graph theory and assuming Darcy's flow under steady-state conditions. Our results showed that higher connectivity shortens the pathway for water flow, thereby providing lower resistance to sap ascent (increasing maximum hydraulic conductivity). However, according to the air-seeding theory, drought-induced embolism is a neighbourhood process, propagating from conduit to conduit. As a result, higher connectivity increases the likelihood of embolii to spread within the xylem and, therefore, its vulnerability to water stress. Our results also suggest an explanation for the fact that within a plant the longest conduits tend to be most vulnerable to cavitation as a bigger surface increases the number of contacts and, as a consequence, the chance to be in touch with an air-filled neighbour. In conclusion, network properties affected maximum hydraulic conductivity as well as vulnerability to drought-induced embolism, and bring new arguments into the long-standing discussion on the efficiency vs. safety trade-off in the xylem.

Keywords: Xylem, Network, Cavitation, Water transport, Efficiency vs. safety

P9.13
Intra vs. interspecific traits associated with water stress in tree seedlings: Do they match?

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It is well known that species differ regarding their drought tolerance and a number of features have been associated to this differential tolerance. Analogously, it is well-known that individuals of a given species can modify their morphology and physiology in response to drought. However, since evolutionary and ecological selective pressures can significantly differ inter and intraspecific responses to drought might not match. To test this idea we have determined survival, as an estimator of tolerance, and a number of functional variables in a factorial experiment of seedlings of four tree species grown in two conditions, well-watered and water-stressed. We expected that drought tolerance decreased with increasing specific leaf area (SLA), leaf area ratio (LAR), total leaf area and shoot–root ratio, both between and within species. However, expectations were confirmed in only two of these variables (SLA and total leaf area) and the trend was not observed between well-watered and water-stressed individuals within the species. A similar situation was found for physiological variables. We expected that photochemical efficiency, non-photochemical quenching, and carotenoids–chlorophyll ratio increased and total chlorophyll content decreased with drought tolerance. While this was apparent among species, the trend was not observed within species. Our results suggest that drought tolerance is not achieved by a single combination of trait values and that these values differ at different levels of analysis, i.e. in inter vs. intra specific comparisons.

Keywords: Drought tolerance, Functional traits, Woody seedlings

P9.14
Previous advances and future promises in plant water relations research

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A brief overview of water relations research will be presented, with emphasis on how new concepts and methods have contributed to progress. Also highlighted will be a summary of plant responses to water limited environments particularly for plant growth, photosynthesis and reproduction. For growth, it will be shown that hydraulic factors are important for the enlargement of multicellular land plants but obscure certain molecular aspects of the enlargement process. By working with simpler marine plants, some molecular mechanisms have emerged that implicate cell wall constituents themselves in the growth process, linking wall deposition to cell enlargement. In photosynthesis, it is becoming more evident that conclusions depend on the way experiments are conducted, especially when assessing the contribution of stomatal closure in comparison to limitations from photosynthetic biochemistry. It appears that leaf shrinkage associated with water loss contributes to the tightness of stomatal closure and thus the conclusions. And for reproduction, new biochemical imaging methods are implicating sugars not only as necessary substrates but also as signals for gene expression for enzymes controlling the fate of floral development. These areas and many others are accelerating the application of physiological, biochemical, and genomic tools for improving the drought tolerance of crops, and some possibilities for the future will be described.

Keywords: Cell enlargement, Photosynthesis, Reproduction

P9.15
Looking back, looking forward: Reference frames for growth–environment interactions

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The well-known view of the root, looking backward from the tip, provides a convenient reference frame for analyzing root architecture and for understanding the effects of environmental variation on root growth. This co-moving reference frame is mathematically connected to a stationary reference frame with origin in the soil. Nutritional elements are extracted by root cells from soil adjacent to the root. The stationary reference frame can be used with the root elongation rate to characterize root calcium accumulation from layered soils. For growing root particles, the pathlines $Z(t)$ show particle position as distance Z from the soil surface over time, t. These pathlines in a stationary reference frame can be used to discover the history of contacts between tissue elements and soil particles. The $Z(t)$ have been computed from marking experiments used to obtain the particle pathlines $X(t)$ in the conventional co-moving reference frame, where X is the distance from the moving root tip. Particle tracking in time lapse photographic records confirms the computations and reveals that only particles found initially in the apical three millimeters of the maize root move very far relative to neighboring soil particles. Growth analysis is also important for understanding the chemical composition, including the pH, of the rhizosphere around root tips. The co-moving reference frame was used with an environmental frame moving upward with the velocity of the root tip to produce a growth-diffusion model for chemical fields surrounding the moving root tip.

Keywords: Growth, Root, Rhizosphere, Reference frame, Soil

P9.16
An artificial apoplast. Turgor adjustment in sugarbeet and *Suaeda* by alterations in the apoplast water potential in vitro

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We set out to test the hypothesis that turgor pressure regulation in some tissues can be due to osmotic adjustment of the apoplast. To achieve this we first rinsed blocks of tissue in a range of osmotica (both higher and lower than that believed to occur in the intact

apoplast in vivo)—with the aim of bringing the apoplast osmotic pressure to pre-selected levels. The blocks were then dabbed dry and immersed in water-saturated silicone oil—in order to limit the volume of the extracellular space to that of the in vivo apoplast. We then monitored the water potential of this "artificial apoplast" by continuous measurement of the turgor pressure of the cells using a cell pressure probe. Turgor pressures both above and below that measured in the intact plant were initially achieved. In *Suaeda maritima* leaf tissue, both up and down turgor adjustment was observed, with all preparations settling to the same turgor (corresponding to than observed in vivo), with sugarbeet, however, only the down regulation component has been observed. The final pressure, however, also corresponded to that of the intact organ. No such turgor recovery was seen after rinsing the sections with a non-penetrating osmoticum. We propose that this "Artificial Apoplast" system will be a useful tool for studying both apoplast water relations and uptake by individual cell types within complex tissues.

P9.17
Root growth maintenance under water deficits: Region-specific responses of the cell wall proteome

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Kinematic studies have revealed different responses of cell elongation to water deficit in distinct regions of the growth zone of the maize primary root. In the apical 3 mm, elongation is maintained at well-watered rates under severe water deficit; the 3–7 mm region exhibits maximum elongation in well-watered roots but progressive inhibition of elongation in roots under water deficit; in the 7–12 mm region, elongation decelerates in well-watered roots and is completely inhibited under water deficit. Previous work demonstrated that cell wall proteins (CWP) may play important roles in enhancing cell wall extensibility in the apical region under water deficit, and thus maintaining cell elongation despite reduced turgor pressure. We are using a proteomics approach to gain a comprehensive understanding of how the abundance of specific CWP changes in association with the differential growth responses to water deficit in the different regions. As the first step, we extracted water-soluble and loosely ionically-bound CWP using a vacuum infiltration–centrifugation technique, and examined protein profiles using 2D gel electrophoresis and mass spectrometry. The results reveal major changes in protein profiles between well-watered and water-stressed roots. For example, in the apical 3 mm, 34 protein spots were unique to well-watered roots, and 5 were unique to water-stressed roots. Of 58 spots common to both treatments, 19 were up-regulated and 10 were down-regulated in the water-stressed roots. Protein identifications and functional analysis of the stress-induced changes will be presented, and integration of the results with microarray analysis of CWP gene expression will also be discussed.

Keywords: Roots, Growth, Water deficits, Cell walls, Proteins

P9.18
Contrasting turgor relations in cells of dicot leaves and fleshy fruits

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Grape, *Vitis vinifera*, berries exhibit a double sigmoid growth pattern and undergo several physiological changes at veraison, the onset ripening. Field experiments showed that, in addition to inhibiting berry growth, there are effects of vine water status on fruit composition, increased color and tannin, that arise independent of the resultant differences in fruit size. The cell-pressure probe was utilized to examine turgor (P) of cells in the mesocarp during berry development and in response to plant water deficits. Cell P at predawn was on the order of 2.5 bars pre-veraison, and was reduced by an order of magnitude to 0.25 bars post-veraison. Thus, the onset of ripening involves a loss of P. When water was withheld from potted vines, predawn water potential declined about 10 bars in 12 days, and cell P decreased with vine water status before veraison, but was insensitive to similar decreases in vine water status after veraison. Rewatering of stressed plants resulted in the recovery of cell P before, but not after veraison. The insensitivity of P to changes in vine water status post-veraison is consistent with field observations that the concentration of color and tannins in both grapes and resultant wines are increased more by pre-veraison water deficits than post-veraison water deficits. Sensory analysis of wines indicate that water deficits decrease veggy aroma and flavor, and that pre-veraison water deficits result in wines distinguishable from those made after post-veraison water deficits. Implications for winegrape production and wine quality will be discussed.

P9.19
A quantitative biophysical comparison of algal and fungal cell growth

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A quantitative understanding of expansive growth of algal, fungal, and plant cells can play an important role in future developments in crop sciences and biotechnology. The research conducted in our Bioengineering Laboratory has focused on the development of *quantitative biophysical equations* for expansive growth of these cells, i.e. cells with walls. Differences in magnitudes and behavior of inclusive *biophysical variables* determined for different cells can indicate differences in the functions and behavior of interrelated cell organelles. Furthermore, a comparison of the magnitudes and behavior of inclusive biophysical variables determined for different cells can help identify which biophysical variables and interrelated organelles are involved in growth and growth regulation. In this presentation, a comparison of the magnitude and behavior of relevant biophysical variables for algal cells (internodes of *Chara corallina*) and fungal cells (sporangiophores of *Phycomyces blakesleeanus*) is presented. This comparison can provide insight into the similarities and differences of these two different biological systems (Ortega 2004).

Keywords: Biophysical variables, *Chara*, *Phycomyces*
Reference: Ortega, J.K.E. (2004) A quantitative biophysical perspective of expansive growth for cells with walls. In *Recent Research Development in Biophysics*. (Ed: S.G. Pandalai) Vol. 3, Part II, pp. 297–324 (Transworld Research Network: Kerala, India) ISBN: 81-7895-130-4.

P9.20
Growth activity of pectins in *Chara corallina*

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Pectins are natural structural components of plant cell walls. Pectin effects on cell growth were investigated using living internode cells of *Chara corallina* with natural turgor pressure (*P*) or primary cell walls isolated from the internodes and exposed to the same "artificial" *P*. When *P* was high enough to cause live cells or isolated walls to grow, their rate of extension accelerated dramatically when external citrus pectin was added to the normal growth medium. Pectins of various degrees of polymerization and gelling ability displayed similar growth activity. When the walls were analyzed for cation content before and after the tests, wall-bound Ca and Mg had been captured by the external pectins. The acceleration of growth was reversed by removing the pectin and restoring the normal Ca^{2+}-containing medium. The chelator EGTA caused responses similar to pectin. Adding excess Ca^{2+} quenched the effects of pectin and EGTA. Living cells eventually lost *P* and the ability to grow in the prolonged presence of external pectin. Isolated walls showed similar inability to hold *P* when most Ca was removed from the walls by EGTA. It was concluded that the primary load-bearing bonds controlling the rate of wall extension in *Chara* were wall pectins cross-bound by Ca and Mg. The external pectin chelated Ca and Mg from the wall pectin, thereby softening the wall while Ca^{2+} from the medium hardened the wall. It is suggested that new pectins normally contributed by the cytoplasm would have a similar effect.
Keywords: Cell walls, Chelators, Calcium, Turgor pressure

P9.22
Enhancing growth and yielding of crops in drying soil using rhizobacteria that disrupt stress perception

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Certain bacteria occurring on the root surface and containing the enzyme ACC deaminase degrade the ethylene precursor ACC, thus decreasing root ACC concentrations and root ethylene evolution, stimulating root growth. We evaluated the physiological responses of pea (*Pisum sativum*) to one such bacterium (*Variovorax paradoxus* 5C-2) at two levels of soil moisture. Generally, bacterial effects were more pronounced and more consistent (in replicated experiments) under controlled soil drying. In short-term pot experiments (up to 21 days after sowing), bacterial inoculation stimulated root growth (length, biomass and proliferation at the base of the pot) by up to 25%. Positive effects of bacteria on shoot biomass, leaf area and plant transpiration were also observed. Plant responses to bacteria were qualitatively similar to irrigation with a chemical inhibitor of ethylene biosynthesis, suggesting that the observed bacterial effects were mediated by altering plant ethylene status. Growing plants with the wild-type bacterium and a mutant in which ACC deaminase activity had been decreased, provided further evidence that the bacterial effects were ethylene-mediated, since the positive bacterial effects were attenuated by inoculation with the mutant. Long-term experiments determined seed number, yield and nitrogen status and also monitored bacterial root colonisation during the crop life cycle. After soil inoculation with a bacterial suspension of 10^7 cells mL^{-1}, the bacteria maintained a population density about several millions cells per g fresh roots until seed set. In the 2 weeks prior to seed set, bacterial inoculation increased leaf water potential of droughted plants by circa 0.1 MPa. Plants grown with the bacterium showed increased seed yield (25–41%), seed number and seed nitrogen accumulation. Bacterial inoculation also restored nodulation of droughted plants to the levels of well watered uninoculated plants. We discuss the opportunities for the use of rhizobacteria to disrupt plant signalling processes and enhance crop yield in dryland environments.

P9.23
Elevated CO_2 does not stimulate C_4 photosynthesis directly, but impacts water relations and indirectly enhances carbon gain during drought stress in maize (*Zea mays*) grown under free-air CO_2 enrichment (FACE)

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The potential for, and mechanism of, $[CO_2]$ effects on C_4 plants has received considerable interest but remains poorly understood. In 2002 and 2004, a rainfed-field experiment utilizing FACE technology was undertaken, in the U.S. Corn Belt, to determine the effects of elevated $[CO_2]$ on *Zea mays*. FACE allows experimental treatments to be imposed on an undisturbed soil–plant–atmosphere continuum without the effects of experimental enclosures on plant microclimate. Each year, crop performance was compared at ambient $[CO_2]$ (~370 ppm) and the elevated $[CO_2]$ (~550 ppm) predicted for 2050, within a fully replicated design. The diurnal course of gas exchange of upper canopy leaves was measured across the growing season of 2002. This was repeated in 2004 along with analysis of carbon and nitrogen metabolism, water relations, growth and yield. This tested if elevated $[CO_2]$ would directly: (1) stimulate C_4 photosynthesis, and (2) reduce stomatal conductance and, therefore, crop water use. The experiments also tested if altered water relations under elevated $[CO_2]$ could feedback to enhance carbon gain during water stress. 2004 was unusual climatically in that at no time in the growing season was there any soil water deficit. In this year, there was no $[CO_2]$ effect

on photosynthesis, carbon metabolism, growth or yield. Nevertheless, elevated [CO_2] reduced stomatal conductance, crop evapotranspiration and soil moisture depletion. 2002 was a "typical" year in which plants experienced episodic water stress. During these dry periods, photosynthesis was greater under elevated [CO_2]. We conclude, elevated CO_2 can only indirectly enhance carbon gain during drought.

Keywords: Climate change, Drought, SoyFACE, Transpiration, Water use

P9.24
The relationship between photosynthesis and respiration in crop leaves using $^{13}C/^{12}C$ isotope labelling

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The origin of the carbon atoms in the CO_2 respired by wheat leaves during the night has been studied using $^{13}C/^{12}C$ isotopes as tracers. The stable isotope labelling was achieved using an IRGA open system composed of a LI-6400 open gas exchange system and a Gas Chromatography–Combustion–Isotope Ratio Mass Spectrometer (GC–C–IRMS, Finnigan), similar as previously described (Nogués et al., 2004). In our study, we took advantage of the difference in ^{13}C abundance between atmospheric CO_2 ($\delta^{13}C = -10‰$) and commercially available (^{12}C-enriched) CO_2 ($\delta^{13}C = -40‰$). The ^{13}C abundance in the CO_2 used for labelling is close to the values found in the nature, thereby allowing us to calculate proportions of 'new' (i.e. recently fixed) carbon in CO_2 subsequently respired in the dark. Preliminary results show that the CO_2 evolved in the dark after the labelling period was ^{12}C-enriched, and the labelled carbon abundance in the respired CO_2 progressively decreased during the night. Interestedly, the carbon recently assimilated by photosynthesis during the labelling accounts for less than 50% of the carbon in the CO_2 lost during the night. Similar proportions were obtained in another cultivated plant (*Phaseolus vulgaris*, Nogués et al., 2004), whereas lower percentages were found in a native Mediterranean plant (*Chamaerops humilis*) and in an alpine plant (*Ranunculus glacialis*), i.e. 7% and 5% of the carbon in the CO_2 respired was labelled, respectively.

Keywords: Photosynthesis, Respiration, Carbon isotope labelling

Nogués S., Tcherkez G., Cornic G., Ghashgaie J. (2004) Respiratory carbon metabolism following illumination in intact French bean leaves using $^{13}C/^{12}C$ isotope labelling. *Plant Physiology* 136: 3245–3254.

P9.25
Limitation to photosynthesis of *Dendranthema indicum* during water stress

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The degree of plant drought tolerance is related to ability withstand adverse conditions. Potted chrysanthemums (*Dendranthema indicum* cv. 'Surf') plants were subjected to three water stress treatments to determine alternation of their acclimation potential. The water treatments were as follows: 1—water supply at the 40% level of the daily consumption of the control plants, 2—cyclic treatment (plants were watered every second day as control plants), 3—overwatered plants. The net photosynthesis rate (P_n), stomatal conductance (g_s), transpiration rate (E) and intracellular CO_2 (Ci) were measured on fully expanded single leaves of each plant with an infrared gas analyzer CIRAS-1 at constant conditions (PPFD 500 $\mu mol.m^{-2}.s^{-1}$, leaf temperature 25 °C, VPD 15 mbar and ambient CO_2 concentration of 400 ppm). When the water content of the substrate decreased as a result of transpiration and soil surface evaporation measured characteristics (P_n, g_s, E, Ci) decreased in the drought-stressed plants compared to the controls (except of overwatered plants). Highly significant differences was observed between P_n of control plants and plants stressed on 40% water consumption of control plants, between P_n of control plant and cyclic plants and also between P_n within subvariants of cyclic plants. Following the drying cycle, efficient and fast recovery of the cyclic stress plants was discovered. Significance difference was observed between P_n of control plants and over watered plants and none significant difference was observed between P_n of cyclic treated plants after watering and plants stressed on 40% water consumption of control plants.

Keywords: Ornamental plant, Photosynthesis rate, Stomatal conductance, Transpiration, Water stress

P9.26
Effect of heterogeneous soil moisture content on canopy gas exchange

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Plant canopies generally exhibit a heterogeneous spatial distribution. Heterogeneity can be caused by the occurrence of several species in a plant community or, within a monospecific community, when plants develop in a patchy pattern. This is the case when there is a heterogeneous soil moisture distribution. Most research on the response of plants to soil water has focused on the response of single leaves or single plants. However, few studies have evaluated the effect of the interaction between neighbouring plants, and of microclimate on gas exchange. We created a heterogeneous surface using 25 soil-filled perspex boxes ($0.5 \times 0.5 \times 0.25$ m), planted with dwarf sunflowers. Nine of these boxes were precision weighing lysimeters, yielding accurate estimates of evapotranspiration. Differential amounts of irrigation water were applied during the growing season. To evaluate the interaction between high-irrigation (HI) and low-irrigation (LI) patches, two SVAT (Soil Vegetation Atmosphere Transfer) model approaches were used: a tile model that did not allow for interaction between patches, and an interactive model which did. Evapotranspiration measured for HI lysimeters was up to 4 times that of LI ones, and often well above available energy (net radiation minus soil heat flux). This indicated the occurrence of micro-advection, causing an enhancement of transpiration for the HI patches and a depression for the LI patches. Furthermore, the interactive model yielded realistic back-

calculated values of surface temperature and stomatal conductance. The effect of this micro-scale advection on canopy photosynthesis was also evaluated using a mechanistic photosynthesis model.
Keywords: Canopy gas exchange, Interactive model, Surface temperature, Soil water stress

P9.27
Interactive roles for light, reactive oxygen species and leaf water status in signalling responses for gene expression during photo-oxidative stress

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When *Arabidopsis* leaves are exposed to photo-oxidative stresses, rapid expression of a gene encoding a cytosolic ascorbate peroxidase (*APX2*) occurs in bundle sheath cells of the vascular tissue. This gene expression appeared to be associated with decreases in leaf water content, increases in abscisic acid (ABA) and production of hydrogen peroxide in the bundle sheath chloroplasts. The light requirement for *APX2* expression involves two components; a DCMU-sensitive component associated with photosynthetic electron transport and a DCMU-insensitive component, which has a low photon fluence threshold and characteristics of phytochrome A. Reduction of tissue water potential by increasing leaf transpiration or osmotic shock resulted in increases in ABA content due to de novo synthesis and *APX2* expression. A large number (ca. 40) of other stress-related genes were found to be co-expressed with *APX2* in drought-stressed leaves and osmotically challenged petioles. Consequently, it appears that a complex signalling cascade, involving responses to low light through phytochrome A, high light through production of hydrogen peroxide via a Mehler reaction and increased ABA content as a result of synthesis of ABA in response to decreases in leaf water potential, is implicated in the regulation of gene expression in response to photo-oxidative stress.
Keywords: Abscisic acid, Ascorbate peroxidase, Hydrogen peroxide, Phytochrome

P9.28
Role of ETR1 in ethylene and hydrogen peroxide signalling in guard cells

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Hydrogen peroxide (H_2O_2) is an essential signal mediating several plant growth and developmental processes including responses to environmental stresses such as water deficit. Guard cells that surround stomatal pores on the surface of leaves are highly specialised cells that swell and shrink to mediate gas exchange between the plant and the environment. The mechanisms mediating stomatal movements are highly complex, and involve cross-talk between various signalling molecules. H_2O_2 is now recognised as a key signal mediating stomatal closure, although the mechanisms by which H_2O_2 mediates this process are not clear. Here we provide evidence that identifies the ethylene receptor ETR1 as a mediator of this process. Genetic and physiological data demonstrate that a functional ETR1, and a Cys residue in the N-terminal region of ETR1 in particular, are essential for H_2O_2 signalling leading to stomatal closure. These data identify a previously uncharacterised role for ETR1, indicating multiple functions for this protein, including mediating cross-talk between ethylene and H_2O_2 signalling in guard cells leading to stomatal closure.
Keywords: Hydrogen peroxide, Ethylene, Guard cells, ETR1, Signalling

P9.29
Responses to extensive annual drought in a Mediterranean pine forest; mechanisms and implications from the leaf to the canopy

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A flux-tower research site and leaf scale physiology measurements in a *Pinus halepensis* forest located in a Mediterranean–semi-arid climatic transition zone are used to investigate ecosystem response to drought. Average annual rainfall is ~280 mm, with and aridity factor of about 0.18, yet forest productivity is on the order of that observed in temperate zones. A high degree of stomatal regulation maintains hydraulic conductivity through the drought period, but limits CO_2 fixation to the early morning and late afternoon. This increases the reliance on pigment mediated process to reduce the energy load on the needles under non-CO_2 assimilating conditions. Through the reduction in chlorophyll content and an up regulation of the xanthophyll cycle, damage to the photosystems is avoided. By avoiding the drought, but maintaining the integrity of the hydraulic pathway and photosynthetic apparatus, the trees are able to respond quickly to an alleviation of the drought conditions at daily and seasonal time scales, recover quickly from occasional high VPD weather events and optimise assimilation potential in the more favourable periods. The high energy load on the canopy during the summer combined with the low latent heat fluxes result in monthly average Bowen ratios of more than 16, probably the highest recorded above a vegetated surface.
Keywords: Ecosystem productivity, Stomatal conductance, Energy dissipation

P9.30
Stable isotopes as integrators of exposure and habitat preference in epiphytic bromeliads

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Epiphytic bromeliads are subject to intermittent precipitation inputs, such as rainfall and fog, which can be exploited over

varying timescales, depending on seasonality, growth form (use of tank and/or trichome), and exposure within the forest canopy. By comparing leaf-water and organic material 18O signals, as well as δ13C composition, along an altitudinal and latitudinal gradient between Panama and Mexico, we relate the habitat preference of C3 and CAM bromeliads to exposure, and seasonality of precipitation inputs. We examine the 18O isotopic relationship between precipitation inputs, leaf water and variation along the monocot leaves, and the analysis of water vapour to distinguish isotopic enrichment due to evaporation and transpiration. We will show how epiphytes act as markers of changing environmental conditions and could be used throughout the neotropics to characterise environmental gradients and changing climatic conditions.
Keywords: Stable isotopes, Epiphytes, Crassulacean acid metabolism

P9.31
Physiological mechanisms limiting kernel set in drought-stressed maize

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Despite many decades of selection for improved performance under dry conditions, kernel formation in maize remains quite vulnerable to drought during pollination. Greater yields have been obtained by selecting under severe drought conditions, and are associated with a shorter anthesis-silking interval, fewer spikelets formed per ear and significantly faster spikelet and ear growth during pollination. All three phenotypic indicators reflect the current priorities for vegetative growth over reproductive growth and apical (tassel) development over lateral branch (rachis) development. These genetically-determined priorities ultimately lead to irreversible disruption of pistillate flower function and early zygotic development under drought conditions. Early studies in John Boyer's laboratory implicated the importance of carbohydrate supply and ovary water relations in ovary development and kernel formation under drought. Subsequent studies confirmed the fundamental importance of current assimilate supply to the ovaries for limiting floral and zygote abortion, determined that sucrose transport from leaves to ovaries was the critical path for assimilate delivery, and identified loss of cell wall and vacuolar invertase activities as critical metabolic lesions that led to abortion of droughted ovaries. These seminal discoveries have inspired numerous investigations in academia and the private sector to define the molecular basis for loss of invertase activity and associated regulatory genes. This presentation will explore the current linkages between assimilate supply and ovary abortion exposed by these studies, and consider other potential mechanisms that might limit kernel set in drought-stressed maize.
Keywords: Reproductive development, Carbohydrate metabolism, Sugar signaling

P9.32
Improving harvest index is an effective way to increase crop water use efficiency

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Water use efficiency (WUE), if defined as the biomass accumulation over water consumed, may be a highly inherited characteristics of a specific genotype. In practice, WUE can also be enhanced by less irrigation, particularly via stomatal regulation. However, such enhancement is largely a trade-off between lower biomass production and higher WUE. We have presented a case here that WUE may be enhanced through an improved harvest index. Harvest index has been shown as a variable factor in field crop production, especially in cases where whole plant senescence of rice and wheat is unfavourably delayed. Such delayed senescence can delay the remobilisation of pre-stored carbon reserves in the straw and results in lower harvest index. A controlled soil drying, i.e. moderate drying such that overnight rehydration of plants is still possible, should enhance whole plant senescence and therefore improve the remobilisation of pre-stored carbon reserve. The gains from the improved harvest may outweigh any possible biomass loss due to shortened photosynthetic period in grain filling, such as the cases with high N nutrition, lodging-resistant cultivars that stay green for too long, and hybrid cultivars with excessive heterosis. The harvest index and WUE can be substantially improved in these cases.
Keywords: Water use efficiency, Harvest index, Grain filling, Soil drying, Cereal crops

P9.33
Male reproductive failure in water-stressed wheat is associated with selective down regulation of anther invertases

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Water deficit during male meiosis in wheat (*Triticum aestivum* L) causes pollen sterility and reduces yield. With a view to identifying the internal trigger for this failure, we found that water stress specifically impairs the activities of vacuolar and cell-wall invertases in anthers prior to the arrest of pollen development. The enzymes are affected only when water deficit occurs around meiosis. Three cDNAs homologous to cell-wall (*Ivr1*, *Ivr3*) and vacuolar (*Ivr5*) invertases were isolated from an anther cDNA library. RNA gel-blot analysis using floral organs of well-watered plants revealed that these genes were expressed preferentially, though not exclusively, in anthers. Semi-quantitative RT-PCR demonstrated that transitory water deficit during meiosis selectively down-regulated the transcription of two of the three genes – one encoding the vacuolar (*Ivr5*) and the other a cell-wall (*Ivr1*) isoform – without affecting the *Ivr3* message. Their expression did not recover upon resumption of watering. Another homologue of *Ivr1* was also down regulated but only during the post-stress period. Effects of stress on invertase transcripts were consistent with those on the developmental profiles of the corresponding enzyme activities. In situ hybridization revealed that the stress-sensitive genes, unlike an insensitive one, were expressed within the microspores. No evidence for an invertase inhibitor under stress was found. Together the results show that the decline in invertase activity is probably regulated at the transcriptional level in a gene- and cell-specific manner. Prior physiological evidence suggests that these effects are probably remotely controlled by a signal(s) from outside the reproductive structures.

Keywords: Pollen sterility, Water stress, Invertase, Gene expression, Carbohydrate metabolism

P9.34
Quantifying genetic complexity and examining physiological processes associated with kernel abortion in maize

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Kernel set in maize is highly correlated with yield when abiotic stresses occur at flowering. One mechanism associated with decreased kernel set is tip kernel abortion (TKA), a selective abortion of grain at distal spikelet positions. Genetic complexity of TKA was quantified by conducting quantitative trait loci (QTL) analysis from a phenotypic assessment of >900 F2-derived top-cross progeny. Heritability of kernel and ear traits measured in these studies exceeded 0.7 and were suitable for QTL mapping. Multiple QTL were detected per trait and some showed consistency across environments. Phenotypic tails ($n=30$ per tail) were identified from the progeny screen and exhibited a 25-fold mean difference in TKA. Genotypes from these tails were selected for further physiological study, including experiments to assess the relationship between TKA and floral behavior, the effect of kernel developmental gradients on TKA and gene expression and metabolite profiles in aborting and non-aborting tip kernels. Results suggest that (1) the study of complex traits can be aided by trait dissection, (2) examination of more heritable component traits allows for the development of context-rich model systems and 3) combining the use of research tools, such as native variation, gene expression and metabolite profiling, presents an opportunity to conduct open-ended discovery research to identify genes and pathways associated with complex traits in maize.

P9.35
Post-phloem delivery of carboxyfluorescein and glucose to young maize ovaries

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Phloem-mobile carboxyfluorescein diacetate was fed into a well in the stem of well-watered maize (*Zea mays* L.) around the day of pollination in order to explore the phloem path to the young ovary. After 24 h, carboxyfluorescein (CF) was found in the pedicel tissues of the ovary but not the nucellus above it. The CF had spread from the phloem to the surrounding pedicel tissues. Briefly rinsing the cut, live sections removed about 18% of the dye. Another 12% was gradually washed out of tissues surrounding the phloem, i.e., the apoplast. An additional 56% was held inside cells right next to the sieve elements, likely the companion cells and the phloem parenchyma cells, but not in the sieve elements themselves as identified by analine blue stain. This CF was in the post-phloem symplast. A final 14% was released by grinding the sections. In contrast to CF, glucose measured with Resorufin fluorescence accumulated in pedicel tissues outside of the phloem and was not present in the post-phloem symplast. The apoplast glucose was washed out of the section like CF. The glucose was generated from sucrose mostly by acid invertase bound to the cell walls in the pedicel tissues, thus indicating that sucrose had been released to the apoplast where it was hydrolysed. The exit of CF and sucrose from phloem termini to the apoplast indicates that a non-specific efflux of some phloem contents takes place at the phloem termini.

Keywords: Glucose, Sucrose, Apoplast, Symplast

P9.37
Young Scientist Award
Organized cell death in Arabidopsis root cap

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Although organelle breakdown during root cap Programmed Cell Death (PCD) has attracted the attention of many investigators, detailed experimental records have not been previously made. In particular nuclei in root cap cells have never been demonstrated showing chromatin condensation, a hallmark of PCD. We are reporting for the first time that in addition to cytoplasmic condensation and DNA fragmentation, the root cap cells show chromatin condensation. We are also reporting that the ER under its three forms is the first organelle to be degraded while the disruption of the nucleus occurs last. Like other member of the Brassicaceae family, *Arabidopsis thaliana* does not produce border cells (detached living cells) and its root cap detached cells are dead. To obtain an insight in the regulation of this process we have isolated Arabidopsis mutants with altered cell death in the root cap. Mutants isolated so far keep their nuclei inside the detached cells, therefore at least this step of cell death is genetically controlled. Progress on mutant characterisation will be reported. Contrary to the mega-autophagosome PCD, where the rupture of the vacuole is reported to cause the lysis of the cell contents, during Arabidopsis root cap cell death we showed the vacuoles to be intact in the detached cells, where other organelles, such as ER, mitochondria, were already degraded.

P9.41
Collapse of water-stress emboli in the tracheids of *Platycladus orientalis* L.

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Tangential wood sections of *Platycladus orientalis* L. were observed under a microscope. When a dehydrated segment was rehydrated, following the initial contract of the gas in the tracheids, the bubbles in them began to shrink. There were some spherical bubbles out of the tracheids and between coverslip and slide, and the bigger ones were getting larger and the smaller ones were contracting gradually during the collapse of the bubbles in the tracheids. Sometimes, after the smaller bubbles vanished the bigger

bubbles out of the tracheids still got larger and the bubbles in tracheids still contracted. By calculation, the total amount of gas in all bubbles at the beginning of the collapse of the bubbles in the tracheids, including all spherical bubbles out of the tracheids and the bubbles in the tracheids, was almost the same as the amount of the gas in all bubbles at the mid time and at the end. We suggest that as the gas pressure in the tracheids was higher than that of bigger bubbles all the gas in the tracheids might move into the spherical bigger bubbles out of the tracheids. We further suggest that for intact xylem there might be some regions with pressures lower than that of the cavitated conduits. As long as the gas in cavitated conduits is connected with the gas in the places of lower pressures, all the gas in the cavitated conduits might pass into the places of lower pressures, inducing the embolism of the conduits to repair.
Keywords: Bubble contract, Embolism repair, Gas moving, Tracheid

P9.42
Analysis of maize silk growth during water stress

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Drought tolerance in maize is associated to the maintenance of a short anthesis-to-silking interval (ASI) under water stress (Bruce et al., 2002). Because the rate of silk elongation is largely involved in ASI determination, we analysed its response to drought in an attempt to identify involved processes. Maize plants were subjected to different temperature, air humidity and soil water availability levels in a growth chamber, and silk elongation was continuously recorded with a non-destructive method. Silk elongation rate responded linearly to temperature and could therefore be expressed on a thermal time basis. In all studied cases (day/night, high/low air humidity, high/low soil water content, re-watering, bagged ear) silk growth changes paralleled ear leaf water potential changes, irrespective of air humidity surrounding the silks or current photosynthesis. Although photosynthesis and silk dry weight were significantly affected by water depletion, these results indicated that silk growth rate was at least partly controlled by plant water status. Because osmotic adjustment seems to be limited in silks (Westgate and Boyer, 1985), silk elongation could be driven by turgor in the growing zone.
Keywords: Maize, Drought, Silk growth, Water status, Carbon
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P9.43
Drought in a high rainfall environment?

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The Burren, is an area of open Karst limestone with an annual rainfall >1500 mm distributed relatively evenly throughout the year. It has recently been suggested that despite prevailing oceanic conditions in this area, water availability may be of importance with regard to plant distribution, due to the nature of the substrate. In order to determine the effect of water availability on the physiology of plants growing here, a number of parameters relating to plant physiology were measured over the period May 2004–October 2004 in association with continuous measurements of substrate volumetric water content (VWC). Regular measurements (fortnightly) of shoot water potential, gas exchange and chlorophyll fluorescence parameters were determined for *Teucrium scorodonia*, one of the most abundant plants occurring on the open limestone. These data indicate that plants growing on the pavement do experience water deficits, with decreased shoot water potential, stomatal conductance and photosynthesis in response to decreased substrate VWC. Measurements of chlorophyll fluorescence, however, did not indicate photo-damage associated with decreased substrate VWC. Typically, April–July inclusive is the period during which plants might experience water deficits in the Burren. July 2004 was, however, an unusually wet month, relative to 30-year averages. The subsequent increase in substrate VWC was correlated with increased shoot water potential, but was not reflected in a proportional recovery of stomatal conductance. This lag in the stomatal response to increased substrate VWC may indicate that stomatal conductance is modulated by factors other than shoot water potential.
Keywords: Karst, Water availability, Photosynthesis, Fluorescence

P9.44
Carbon isotope discrimination (Delta) and water use efficiency in sugar beet

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Improving yield while conserving water inputs requires improvement water use efficiency (WUE), which can be measured indirectly by Delta. However, WUE has not been studied extensively in sugar beet. The objectives of this study are (1) to evaluate genotypic variation for Delta, WUE and dry matter accumulation in sugar beet under well-watered and water-limited conditions; (2) to test the relationship of Delta with WUE, yield and secondary traits; (3) to identify possible surrogate measures of Delta. Six sugar beet genotypes were evaluated in the field. Drought stress was imposed by using large polythene covers. There was significant genotypic variation for root yield, total dry matter (TDM), specific leaf weight (SLW), succulence index, Delta and WUE among genotypes. Delta variation in leaf samples was significantly correlated with the variation in root samples ($r=0.90$, $p<0.05$). Also, there was a highly significant positive correlation ($r=0.77$, $p<0.01$) between Delta in leaves in well-watered and water-limited conditions. Leaf thickness indicated by SLW was significantly correlated with Delta ($r=-0.78$, $p<0.01$). The expected negative relationship between Delta and WUE was observed, although it was not significant. Poor correlation between Delta and yield indicates opportunities to simultaneously improve both yield and Delta through hybridization. The results show that leaf samples analysed for Delta under well-watered treatment could sufficiently differentiate sugar beet genotypes for Delta, which has a potential to be used as a selection tool for breeding WUE in sugar beet. However, the establishment of this relationship needs further work with more genotypes.

Keywords: Sugar beet, Drought, Delta, WUE

P9.45
Effects of Ca^{2+} on the UV-MALDI-TOF Mass Spectrometry analysis of cell wall oligosaccharides isolated from the red seaweed *Nothogenia fastigiata* (Nemaliales)

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Three xylan fractions, isolated from the red seaweed *Nothogenia fastigiata* (Nemaliales) were analyzed by ultraviolet matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (UV-MALDI-TOFMS). UV-MALDI-TOFMS was carried out in the linear and reflectron modes, and as routine in the positive- and negative-ion modes. Of the several matrices tested *nor*-harmane was the only effective one giving good spectra in the positive-ion mode. The number-average molar masses of two of the fractions, calculated from the distribution profiles, were lower than those determined previously by ^{1}H NMR analysis, suggesting a decrease in the ionization efficiency with the molecular weight; weight-average molar mass and polydispersity index were also determined. As the xylans retained small but significant quantities of calcium salts, the influence of added Ca^{2+} as $CaCl_2$ on UV-MALDI-MS was investigated. The simultaneous addition of sodium chloride and calcium chloride was also analyzed. Addition of sodium chloride did not change the distribution profile of the native sample showing that the inhibitory effect is due to Ca^{2+} and not to Cl^-. Addition of calcium chloride with 1:1 analyte–salt molar ratio gave spectra with less efficient desorption/ionization of oligomers; the signals of these oligomers were completely suppressed when the addition of the salt became massive (1:100 analyte–salt molar ratio).

P9.46
Tulip flower stalk elongation and sugar metabolisms in relation to the water status

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Tulip plants require low temperature to initiate the stalk elongation and flowering. However, if tulip bulbs are stored at high temperature, their stalk elongation will be inhibited. When cell solutions extracted from bulbs stored at low temperature or high temperature were analyzed with the psychrometer, it was found that cells of bulbs stored at low temperature contained much more osmotically active solutes than those stored at high temperature. Because starch is stored in scales of tulip bulbs, it is thought that most osmotically active solutes may be derived from results of starch degradation in cells of bulb scales at low temperature. We attempted mass spectrometry (MS) of cell solutions extracted directly from bulb scales to compare with results obtained with the psychrometry.
Cell solutions extracted from tulip bulbs during vernalization treatments were analyzed with UV-MALDI-MS and ESI-TOF-MS. When accumulation of osmotically active solutes was detected with the psychrometer in scale cells of bulbs stored at low temperature, MS analyses revealed distinctive signals of oligosaccharides derived from starch degradation. Also, distinctive polypetides were detected in the cell solution of the scales with UV-MALDI-TOF-MS when α-cyano-4-hydroxy-cinnamic acid was used as a MALDI matrix.

P9.48
Genetic analysis of the response of maize leaf growth to water deficit in populations which segragate for anthesis-silking interval under drought

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We aimed to quantify the genetic variability of the response of leaf growth to water deficit in tropical maize lines, and to test whether the responses of leaves and silks to water deficit have common genetic determinisms. This was carried out in the P1xP2 mapping population of CIMMYT in which the tolerant parent maintains synchrony of male and female flowering (ASI) under water deficit. Leaf growth and its response to water deficit were analysed by applying a method which combines genetic and ecophysiological modelling. The elongation rate of sixth leaves was recorded automatically in 120 RILs together with environmental conditions in a greenhouse experiment during a drying scenario, and in a growth chamber experiment in which contrasting evaporative demands were imposed to well-watered plants. Response curves of leaf elongation rate to temperature, evaporative demand and soil water status were identified individually for each RIL. An appreciable genetic variability was observed for each response. QTLs of maximum growth under non-stressing conditions and QTLs of the slope of the response to soil water potential were detected. QTLs of the responses of leaf growth colocalised with QTLs of ASI under drought determined by CIMMYT in several field experiments. Common mechanisms of growth maintenance may therefore exist in both leaves and silks, suggesting that the responses of "source" and "sink" to water deficit may be genetically linked. If confirmed these results will provide a novel approach for pyramiding favourable alleles and direct the breeding of genotypes that provide stable grain yield in drought prone environments in maize.
Keywords: Leaf growth, Anthesis-silking interval, QTL, Water deficit

P9.49
Transcripts analysis of backcrossed-derived lines (BDLs) differing for a QTL controlling leaf ABA in maize

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Abscisic acid (ABA) plays a pivotal role in modulating the adaptive response of plants to abiotic stresses. In the mapping population derived from Os420 x IABO78, one major Quantitative trait loci on bin 2.04 accounted for 32% of the total variation for L-ABA (Tuberosa et al. 1998 T.A.G. 97: 744–755). At this QTL, Os420 and IABO78 carry the alleles increasing (+/+) and decreasing (−/−) L-ABA, respectively. To better evaluate the QTL effect, a set of near isogenic lines (BDLs) have been derived for both parental lines. The objective of this study was to monitor expression changes in leaves of two pairs of BDLs (IABO78 (+/+) and (−/−); Os420 (+/+) and (−/−)) near anthesis and subjected to slow naturally-occurring drying conditions in soil. For this purpose, we used the rice whole genome GeneChip® in collaboration with Syngenta. Our results confirmed the differences between (+/+) and (−/−) BDLs as to L-ABA and the analysis of the expression profiles allowed us to identify 121 probes differentially expressed (2-fold difference) between the lines in response to drought stress, most of them already reported as stress-modulated. QTL are in large part a manifestation of DNA sequence polymorphism that cause altered gene expression or encode for a dysfunctional protein. Genes differentially expressed between genotypes carrying opposite QTL alleles can be evaluated as candidate genes. Among these, to reinforce their possible role of candidates, the differentially stress modulated transcripts mapping at the rice chromosome segment syntenic to the maize chromosome region carrying the major ABA-QTL, were identified.

Keywords: Maize, Microarray, ABA, Drought stress

P9.50
Comparative effects of nitrogen sources on growth and physiological response of sugar beet plants exposed to salt stress

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The effect of different levels of salinity (0, 50, 150 and 250 mol m^{-3} $NaCl+CaCl_2$ in 5:1 molar ratio) and three various nitrogen sources (NO_3^-, NH_4^+ and NO_3^-/NH_4^+, 20 mM) on growth and physiology of sugar beet plants (*Beta vulgaris* L.) was studied. Plants were grown in sand culture for 6 weeks in controlled environment. Growth and photosynthesis both were affected by saline water and different nitrogen sources. NH_4^+-fed plants significantly had less shoot dry weight and leaf area than other nitrogen sources at non-saline conditions. However, there was no significant differences between nitrogen treatments at saline conditions. Root dry weight was significantly decreased by salinity, but root dry weight of NH_4^+-fed plants was less affected by salt stress than other nitrogen treatments. NO_3^--fed plants had greater photosynthesis than plants irrigated with NH_4^+, but there was not observed a significant difference in stomatal conductance (g_s) between different nitrogen sources at all levels of salinity. Major ions in shoot tissue were also influenced by nitrogen sources and salinity. There was no significant differences in Na^+ content of the shoot between various nitrogen treatments at non-saline condition but under salt stress NO_3^--fed plants significantly had higher sodium and potassium concentration than NH_4^+-fed plants. Leaf nitrogen content was increased by salinity under all three nitrogen sources. However, NH_4^+-fed plants had higher nitrogen contents than other nitrogen treatments. Salinity and nitrogen sources significantly affected carbon isetope discrimination (Δ). At non-saline condition NO_3^--fed plants had the highest Δ while at the high level of salinity (250 mM), NH_4^+-fed plants had the highest Δ.

P9.52
CFDA as a tracer to image carbohydrate transport to the developing kernels of maize grown under drought or shade

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Plant reproduction depends on a supply of photosynthetic products, and the early stages often respond to inadequate supplies by arresting development irreversibly, that is, by aborting the reproductive structures. Several days after pollination, abortion occurs less frequently and instead the reproductive structures develop to smaller sizes. In maize, decreases in photosynthesis caused by inadequate water result in ovary abortion early in ovary/embryo development but similar conditions two weeks later result in decreased kernel size. In some respects, these effects resemble abortion caused by insufficient light. Reproductive development in maize (*Zea mays* L.) under drought conditions has been extensively studied due to its economic importance. However, the basics are not well understood. We subjected maize to low light intensities and low water potentials (ψ_w) that cause failure in seed development. We observed the transport of phloem-mobile dye (carboxyfluorescein diacetate, CFDA) from infusion site (stem) to the ovaries and its location in the ovaries in comparison to starch and safranin. Analyses showed that both low light intensity and low water potential reduced the transport of carboxyfluorescein to ovaries, but the transport was recovered close to the control transport when plants were rewatered and light intensity was increased to the level of control plants. Images of maize ovaries showed that carboxyfluorescein was transported in the phloem and was located in the same structures as starch. Our results confirm the earlier suggestions that ovary abortion results at least partly due to severely impaired sugar influx.

Keywords: Carboxyfluorescein, Maize, Shade, Water deficiency

P9.53
Changes in stomatal conductance of *Melilotus officinalis* L. implied by synergy of drought and elevated Se concentration

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In the last two decades drought and elevated toxic metal concentration phenomena are gaining the interest of the scientific world. Nevertheless, relatively little ecophysiological research has been conducted to determine the responses of stomatal conductance to the interaction between water stress and elevated Se concentration. A pot experiment was conducted in order to evaluate the effects of the implied synergy of drought and Se uptake on stomatal responses of *Melilotus officinalis*. The effects of two different Se concentrations (0 mg Se/lt irrigation water, 3 mg Se/lt irrigation water) and two water regimes (well watered–water stressed) applied to seedlings of *M. officinalis* were detected by measuring changes in water potential, relative water content, stomatal conductance, transpiration and Se tissue concentration.
The findings of this experiment suggest that Se uptake by *M. officinalis*, which is considered a secondary Se accumulator as it can concentrate up to 200 μg Se/g d.w. in its tissues can affect the water relations of *M. officinalis* causing a small reduction of water potential and relative water content as well as a statistically significant reduction of transpiration and stomatal conductance.
Keywords: Stomatal conductance, Drought, Selenium

P9.54
Expansins and the control of maize leaf expansion: quantitative analysis of expansion and gene expression combining several sources of variability

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Expansins are a large class of cell wall proteins with clear loosening capacities but their role as mediator of the control of organ growth rate in vivo is still lacking. In order to find whether and which expansins mRNA best correlate with expansion, we used a combination of 3D kinematic analysis and quantitative transcript abundance estimation in the elongating region at the base of maize leaves.
A survey of genomic databases revealed the presence of 43 putative expansins in maize and a family wide RT-PCR analysis showed that 14 of them were expressed in the leaf elongation zone. Six of these expansins were chosen for their expression pattern resembling the elongation pattern. Robustness of correlation between expansins expression and elongation was evaluated using real-time PCR and several sources of variability (i) spatial variability as expression was measured in 1cm long segments in accelerating decelerating and non growing regions, (ii) genotypes with long or short elongation zone, (iii) development as young leaves, fully expanding leaves and leaves with decreasing elongation were included, (iv) three temperatures and (v) two levels of water deficit. Expansion was measured in 3D based on kinematic analysis of elongation and on thickness and width deposition using continuity equation. Gradients of total RNA per volume were taken into account in order to allow an estimation of RNA abundance per unit volume of growing tissue. Correlation analysis identified one of the expansins that was strongly associated with expansion whatever the source of variation.
Keywords: Leaf expansion, Temperature, Water deficit, Spatial analysis, Expansins

P9.56
A phenotyping set up for the analysis of the genetic variablity of the response of leaf growth to water deficit

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A set-up has been developed to precisely measure the response of the growth rate of monocot leaves to environmental conditions, for several hundreds of plants (e.g., 120 genotypes with 3 repetitions). The set up consists of (i) a series of balances, connected to a computer, which measure every 15 min the soil water status imposed to each plant. (ii) A set of environmental sensors connected to a data logger which measure meristem temperature, leaf to air VPD and irradiance with the same time step. (iii) Rotating displacement transducers which allow measurement of leaf elongation rate of 300 plants with a 15-min resolution. The main set up is placed in a greenhouse for measurements at a weekly time scale, while a companion equipment is placed in a growth chamber for analysing the effects of controlled short-term changes in temperature or VPD. The data analysis takes advantage of the fact that leaf elongation rate remains constant, in the absence of stress, for two phyllochrons (7–10 days). Each combination of environmental condition and elongation rate can therefore be considered as an individual data point. Response curves to temperature, evaporative demand and soil water status can be established for each genotype in different experiments. They are reproducible between experiments and common to growth chamber and greenhouse experiments, thereby characterising each genotype. Their parameters can be analysed genetically (mapping populations, association genetics). The combination of the effects of different environmental conditions on leaf elongation rate can be studied for establishing models of leaf elongation.
Keywords: Phenotyping, High throughput, Leaf growth, Temperature, Water deficit

P9.57
Physiology, genetics and marker-assisted selection of drought tolerance QTL in pearl millet

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Pearl millet [*Pennisetum glaucum* (L.) R. Br.] is the staple cereal of the hottest, driest areas of the tropics and subtropics. Drought stress is a regular occurrence in these regions, making breeding for drought tolerance one of the most essential objectives in pearl millet breeding programmes. To facilitate such breeding, we have mapped several QTLs that contribute to increased drought tolerance in pearl millet in 2 separate populations (Yadav et al., 2002, 2004). In particular we identified a region on linkage group 2 in both populations associated with grain yield and terminal drought tolerance. Physiological and agronomic processes associated with these QTLs were also characterised. These QTLs were then transferred into the genetic background of the respective drought sensitive parent using marker-assisted back crossing and

the effects of the individual QTL re-assessed in a range of water environments and genetic backgrounds. These experiments validated the major QTL on LG2. The application of the identified QTL in breeding for increased drought tolerance will be discussed.
Keywords: Drought tolerance, QTL, Marker-assisted breeding, Grain yield
Yadav R.S., Hash C.T., Cavan G.P., Bidinger F.R., Howarth C.J. (2002) Quantitative trait loci associated with traits determining grain and stover yield in pearl millet under terminal drought stress conditions. *Theor Appl Genet* 104: 67-83.
Yadav R.S., Hash C.T., Bidinger F.R., Devos K.M., Howarth C.J. (2004) Genomic regions associated with grain yield and aspects of post-flowering drought tolerance in pearl millet across stress environments and testers. *Euphytica* 136: 265–277.

P9.58
Functioning under drought stress of stay-green sorghum developed by marker-assisted selection

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The best characterized component of post-flowering drought tolerance in sorghum is the stay-green trait. A glasshouse study was conducted to evaluate the performance of sorghum genotype RSG 03123 under post-flowering drought stress compared with its stay-green donor parent B35 and senescent recurrent parent R16. RSG 03123 was developed at ICRISAT using marker-assisted selection to introgress several stay-green QTL from B35 into the highly senescent breeding line R16. Green leaf area (GLA) and %GLA were intermediate between B35 and R16 for the duration of the experiment. The RSG plants also had higher chlorophyll contents due to delayed leaf senescence, with more leaves alive than R16 at final harvest. Photosynthesis, leaf stomatal conductance, transpiration and transpiration use efficiency were higher and maintained for a longer period in the RSG plants as compared to R16 under drought. The amounts of basal stem sucrose and total soluble carbohydrates in the RSG plants were higher, while fructose and glucose were lower compared to R16 under either well-watered or drought-stressed conditions. These plants also had higher leaf nitrogen contents both at flowering and at final harvest compared to R16. Grain yield per panicle in RSG was comparable to B35 but higher than in R16. These findings suggest that the stay-green QTL from B35 retained function when introgressed into a senescent background and resulted in yield being maintained in the RSG plants during drought stress by delaying leaf senescence and making photosynthates available for grain-filling.
Keywords: Drought, Marker-assisted selection, Stay-green, Photosynthesis, Senescence

P9.59
Understanding controls on genotypic differences in water use efficiency in droughted coconut

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Genotypic variation in drought response of mature coconut trees was examined by measuring leaf photosynthetic characteristics and leaf $\delta^{18}O$ and $\delta^{13}C$. Palms approximately 15 years old from 40 different varieties or accessions including local and exotic dwarf and tall genotypes were analysed growing in a research station trial in north-western Sri Lanka. Genotypes differed substantially in their rates of photosynthesis and stomatal conductance after a 77-day drought, partly because some did not reach low leaf water potentials. There was a very close agreement between $\delta^{13}C$ derived estimates of water use efficiency and those from gas exchange for leaves of 15 genotypes at the end of the drought, probably because the leaves had developed in a prior drought period. In a later year, there was substantial variation in both $\delta^{18}O$ and $\delta^{13}C$ between the full set of 40 genotypes (range of 2.1‰, and 2.7‰, respectively). When leaves developed in wet and dry periods were sampled together, mean $\delta^{13}C$ was slightly higher for the 'dry' leaves (mean increase 0.7‰) and mean $\delta^{18}O$ lower (1.2‰). However, these averages conceal many differences in genotype response to dry conditions. For example, 11 genotypes showed no difference in $\delta^{18}O$ and 13 no difference in $\delta^{13}C$, but only 4 of these null-change genotypes were common. Genotype ranking showed some consistency between 'wet' and 'dry' leaves for $\delta^{13}C$, but not for $\delta^{18}O$. There was a positive relationship between $\delta^{18}O$ and $\delta^{13}C$ ($R^2=0.44$) for 'wet' leaves, but not for 'dry' leaves.
Keywords: Drought tolerance, Water use efficiency, Isotope, Discrimination

P9.60
Maintaining yield under drought stress by different mechanisms in a range of genetic sources of stay-green sorghum

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Sorghum plants that retain green leaf area for longer under post-flowering drought are referred to as *stay-green*. Compared with senescent plants, they also continue to fill their grain for longer when water is limiting. A range of stay-green sorghum cultivars (B35, QL41, KS19, QL12, E36-1) were compared with a senescent cultivar (R16) under well-watered (WW) and post-flowering drought stress (DS) conditions in a glasshouse pot experiment. B35, QL41, KS19 and QL12 retained higher chlorophyll contents while E36-1 plants had lower contents under both DS and WW conditions compared to R16. Under DS, loss of green leaf area began earlier in E36-1 and R16 compared to B35, QL41, KS19 and QL12. The Fv/Fm ratio and Φ_{PSII} also were significantly reduced in the DS plants with greater reduction in E36-1 and R16 plants under DS. CO_2 assimilation rate, stomatal conductance and transpiration rate were significantly reduced in drought-stressed R16 and E36-1 plants. Protein and nitrogen were however retained to a greater extent in the stay-greens compared to R16 under DS. Grain yield was also maintained in the stay-greens under DS. The maintenance of yield in the drought-stressed E36-1 plants was probably due to remobilisation of carbohydrates from the stem to the head for grain-filling, which suggest the stay-greens may maintain yields under drought stress by different mechanisms.
Keywords: Drought, Stay-green, Photosynthesis, Carbohydrates, Grain yield

P9.61
Nitrogen nutrition in grasses under soil water deficit: Possible involvement of a direct response of root absorption capacities to water status

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In grasslands, among other mineral deficiencies, water scarcity most often also induces a reduction of the nitrogen status. Under certain conditions, the drought-induced nitrogen deficiency may even account for most of the effects of water deficit. Although identified for long, qualitatively, the interaction with nitrogen still remains difficult to take into account in a quantitative analysis of the sward physiology under water deficits. It is generally accepted that water movements in soils reduce mineral nitrogen availability. The paper deals with a possible direct physiological response of the root absorption capacity at low soil water potential. To determine the importance of the latter process, a split root experiment on three grass species grown in nutrient solutions was made. When half of roots of the plants were grown in low osmotic pressure solution without nitrogen and the other half in high ^{15}N-labelled nitrogen ($NO3^{-}NH4^{+}$) and high osmotic pressure solution (PEG 6000, 0.6 MPa) for 8 days, *Festuca arundinacea* and *Lolium multiflorum* both exhibited a reduction of their nitrogen status, whereas the nitrogen status of *Dactylis glomerata* remained unchanged. In the latter case, in addition to higher resistance of N uptake to water deficits, the maintenance also was brought about by a change in allocation of nitrogen to leaves. These inter-specific differences were consistent with field observations, so far explained only by different architectures of the root systems. This suggests that the sensitivity of the root N absorption to the solution water potential is important.

Keywords: Water potential, Nitrogen uptake, *Dactylis, Festuca*, *Lolium*

P9.62
Photosynthesis of C_3, C_4 and CAM plants at low leaf water potential

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Photosynthesis at low leaf water potential (Ψ_w) induced by the stepwise addition of NaCl and water stress were determined with some C_3 (rice, mangrove), C_4 (sugarcane), and CAM (pineapple) plants grown under glasshouse conditions. We mainly focussed on the determination of leaf Ψ_w at which gas exchange rate stopped for its application to irrigation programs and to understand the behavior of stomata and other components limiting the photosynthesis. Photosynthesis and leaf Ψ_w were measured by a handmade gas exchange system with IRGA and an Isopiestic Psychrometer (Boyer, 1995), respectively. Among rice plants, there were no changes in wild rice photosynthesis at a Ψ_w value of -1.5 MPa due to its high salt tolerance system compared with the cultivars. Among mangrove species, leaf photosynthesis was maintained at a Ψ_w value of -2.4 MPa, and water use efficiency increased with decreases in the Ψ_w. Under submerged conditions, mangrove leaves uniquely performed the gas exchange between leaf and seawater. Photosynthetic O_2 evolution was increased when NaCl concentration in the medium was increased, indicating halophilic behavior. Among C_4 sugarcane species, there were no decreases in photosynthesis in wild type (*Saccharum spontaneum*) and Tainan (*S.* spp.) due to the higher PEP-Case activities overcoming the inhibited stomatal conductance. In the pineapple plants, a CAM plants, gas exchange rate and CO_2 balance decreased up to -1.5 MPa, however, this value was the lowest leaf Ψ_w due to the lower cuticle conductance. These results clearly establish the existence of species variation, rather than variations in photosynthetic type, in the limitation of photosynthesis in higher plants.

Keywords: C_3, C_4, and CAM plants, Leaf water potential, Photosynthesis

P9.63
Reconciling the incompatible: water limitation in a high rainfall environment

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Absolute drought, in meteorological terms, is a very rare event in areas subjected to a low evaporative demand and a high annual rainfall that lacks seasonality. Despite this field measurements have indicated a low mean substrate volumetric water content, (~20% for 2004) in a Karst area called the Burren in western Ireland where the annual rainfall is ~1600 mm per year. Both an herbaceous (*Teucrium scorodonia*) and woody (*Corylus avellana*) species were found to experience water-driven and seasonally independent cyclical variations in performance that were broadly correlated with variations in stomatal conductance (G_s). During an extended period of particularly low substrate moisture contents (SMC) from May to June 2004, the maximum photosynthetic rate (A_{max}) declined to near zero due to almost complete stomatal closure. This was correlated with some of the lowest values for shoot water potential. Subsequent recovery of photosynthesis was delayed for ~1 month despite the immediate recovery of SMC after rainfall. This study shows that although meteorological drought may be rare, water availability can have a significant impact on the performance of Burren plants due to the low water-holding capacity of the limestone substrate.

P9.64
HvPIP1;6, a barley plasma membrane intrinsic protein particularly expressed in growing leaf tissues of barley

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The role of aquaporins in water transport in growing leaf tissues was studied in the developing leaf three of barley. A cDNA clone was isolated with high homology to plasma membrane intrinsic proteins, PIPs. The clone was termed HvPIP1;6. Expression of HvPIP1;6 along the developing leaf three was highest in the elongation zone and almost absent in non-growing tissues. Even within the elongation zone, faster-grower regions showed higher expression

than slower-growing ones. Changes in expression of HvPIP1;6 in response to salinity and source-reduction were in agreement with predictions of changes in tissue hydraulic conductance based on previous cell-biophysical analyses (cell-pressure probe). Expression of HvPIP1;6 in *Xenopus laevis* oocytes increased osmotic water permeability, P_f, four- to six-fold. Water channel activity was inhibited by pre-incubation of oocytes with 50 μM $HgCl_2$ and increased following incubation with the phosphatase inhibitor okadaic acid or the plant hormone abscisic acid. Plasma membrane preparations were analysed by Western blot using an antibody that recognised barley PIP1s. Levels of PIP1s were highest in the growth zone and adjacent (enclosed by sheaths of older leaves) leaf region, and lowest in the emerged portion of the growing blade. The role of HvPIP1;6 in barley leaf growth and development is discussed, in particular the question whether hydraulic properties of cells and tissues limit leaf elongation velocity in grasses.

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P9.65
In situ staining of sugars in *Sporobolus stapfianus*, a desiccation-tolerant plant accumulating sucrose during water stress. An informative technique for sugar localization in plant tissues using tetrazolium and "coupling" enzymes

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The build up of carbohydrates during dehydration stress is thought to be a very important mechanism for the acquisition of desiccation tolerance. *Sporobolus stapfianus* (a C4 plant), similar to other desiccation tolerant plants, is shown to accumulate sucrose during water stress. As photosynthesis rapidly declines during water stress, starch has been hypothesised to be a major carbon source for sucrose accumulation.

Despite the importance of sucrose accumulation in the acquisition of desiccation tolerance, little is known about the concurrent breakdown of starch. Moreover, nothing is known about the cellular localization of either the sucrose or hexose pools inside the leaf.

The present study proposes an original, simple and selective method for in situ localization of sucrose and glucose. The detection of sucrose and glucose in the leaf is based on coupled enzymatic reactions (sucrose phosphorylase, EC 2.4.1.7+PGM, EC 5.4.2.2+G6PD, EC 1.1.1.49 for sucrose detection; HK EC 2.7.1.1+G6PD, EC 1.1.1.49 for glucose) leading to the formation of NADH with the subsequent reduction of iodonitrotetrazolium (INT) giving the red-colored insoluble INT formazan.

The present study shows that there is an increase in sucrose content until 47% relative water content and that starch breakdown only accounts for 1/3 of the required carbon for sucrose accumulation. The staining for glucose shows intensive coloration of the vascular bundles at the beginning of water stress. Sucrose staining shows uniform coloration of all leaf compartments: bundle sheet cells, mesophyll cells and epidermal cells.

Keywords: Cytochemistry, Resurrection plant, Sucrose phosphorylase, Starch

P9.66
Ammonium metabolism during dehydration stress in the "resurrection" plant *Sporobolus stapfianus*. A comparison between desiccation-sensitive older leaves and desiccation-tolerant younger leaves

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Desiccation-tolerant plants ("resurrection" plants) represent a unique model for the study of metabolism in response to dehydration stress. During desiccation stress, many protective mechanisms interact leading to the acquisition of desiccation tolerance. *Sporobolus stapfianus*, similar to other resurrection plants, undergoes general nitrogen reorganization during dehydration stress (proteolysis, increase in free amino acid content and protective proteins, chlorophyll loss, upregulation/maintenance of important enzymes). Nitrogen metabolism is therefore strongly involved in the desiccation process. To date nitrogen metabolism has never been investigated in "resurrection" plants. Since the metabolism of ammonia is integral to nitrogen metabolism, the present study will focus on ammonium metabolism during desiccation stress.

Interestingly, in *Sporobolus stapfianus*, the younger leaves are desiccation-tolerant (DT) whereas the older leaves are not able to acquire desiccation tolerance during dehydration stress (desiccation-sensitive, DS) and do not survive rehydration. In making possible the comparison between DT and DS leaf material, *Sporobolus stapfianus* is an ideal model to understand the metabolism of desiccation tolerance.

This study will analyse the differences in DS and DT ammonium metabolism speculating about the links between leaf aging and desiccation tolerance. Attention will be focused on the activity of the various enzymes involved in ammonium metabolism, including GDH (EC 1.4.1.2) aminating and deaminating activity, and GS (EC 6.3.1.2) activity.

Keywords: Glutamate dehydrogenase, Glutamine synthetase, Desiccation stress, Leaf aging

P9.68
Magnitude and regulation of high nighttime stomatal conductance and transpiration in C3 plants: Do these processes interact with water and nutrient relations?

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C3 and C4 plants are expected to close their stomata at night when there is no photosynthetic carbon gain. However, an experimental survey in western US habitats and a survey of the literature demonstrate that nighttime stomatal conductance (g) and transpiration (E) can be substantial in a diverse range of plant species. Additionally, there are genetically based differences within and among closely related species in nighttime g and E that may be associated with habitat resource availability. This nighttime water loss may interact with water and nutrient relations. We have demonstrated for several species that leaf-level nighttime g and E (measured with LI-6400) respond to soil water availability and

VPD, but not directly to soil N availability. However, nighttime E may still affect plant nutrition. The Barber–Cushman model was used to show that increasing water flux through the plant (as with high nighttime E) could increase nitrate supply to plant roots. This potential is being explored empirically in a growth chamber study with *Arabidopsis thaliana*, where nighttime relative humidity was manipulated to suppress or allow nighttime E in plants with sufficient or limited N supply. Preliminary results indicate that N-limited plants grown under high nighttime relative humidity (~90%) showed decreased plant fitness relative to plants in which nighttime E was not suppressed (~55% RH), indicating that under non-water-limiting conditions, nighttime E benefited N-limited plants. Taken as a whole, there is growing evidence that in C3 plants, high nighttime g and E interact with plant water and nutrient relations.

Keywords: Stomatal conductance, Transpiration, Water potential, Nutrients, Drought

P9.69
Multi-scale spatial variation in hydraulic lift by the desert halophyte, *Sarcobatus vermiculatus:* Implications for resource acquisition from dry soils

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Passive water movement through roots from moist to dry soils, i.e., hydraulic lift, can be important for plant water use, nutrient acquisition, and competition/facilitation. Although hydraulic lift is known from many species and habitats, little is known about how it varies across species ranges. We documented variation in hydraulic lift by the desert halophytic shrub, *Sarcobatus vermiculatus*, at three spatial scales: landscape, shrub-island versus interspace, and depth. Hydraulic lift varied among sites across the landscape. It was most prevalent at a low salinity site with deep groundwater (9.4 m), but of lower magnitude at more saline sites with shallower groundwater. At the low salinity site, shrub-island soils had substantial net increases in Ψ_{soil} during week–month long periods in the early part of the growing season, concomitant with self-irrigated root growth into dry soils, as documented with mini-rhizotrons. The source of this root-system-transported water was both moist interspace soils and moist deep soil layers. Wetting up of otherwise dry shrub-island soils is likely essential for nutrient mineralization and acquisition from trapped litter, making hydraulic lift an important driver of landscape-scale biogeochemical cycles in these saline basins. In addition, hydraulic lift buffered spatial variation in water availability among sites, depths, depth to groundwater, and for plants with different root distributions, such that plant $\Psi_{predawn}$ and Ψ_{midday} differed little across the landscape. Multi-scale variation in hydraulic lift needs to be considered when predicting effects of hydraulic lift by a community dominant on stand water balance and nutrient cycling.

Keywords: Desert, Hydraulic redistribution, Roots, Soil moisture, Spatial heterogeneity