Commentary

Functional–structural plant modelling

In the last decade, many research teams throughout the world initiated a new approach in plant science by developing computer models of plant functioning and growth. The intention of this approach was to understand the complex interactions between plant architecture and the physical and biological processes that drive the plant development at several spatial and temporal scales, using so-called functional–structural plant models (FSPMs). Explicitly taking into account the spatial distribution of plant organs has multiple consequences: (i) FSPMs are usually associated with 3D plant models where plant architecture is represented as a collection of interconnected plant components, which are distributed in the 3D below- and above-ground space; (ii) FSPMs usually deal with the spatial distribution of both environmental and biological processes; (iii) FSPMs are usually based on scaling up, mostly from the organ to the plant, but also from tissue to organ or from plant to stand; and (iv) to deal with the system complexity owing to the high number of plant constituents, and to deal with the potentially high numbers of interacting processes, FSPMs must develop adequate computational methods.

To discuss these questions, in 1996 the FSPM series of workshops (http://amap.cirad.fr/workshop/FSPM04/index.html) was established to assemble regularly from all over the world scientists who integrate 3D representations of plants, physiological models, environmental models, computer science and mathematics into their approach. The general aim of this community is to understand better, through the use of 3D representations, the importance of taking into account the *spatialisation* of processes in plant functioning and morphogenesis. The FSPM community contributes to unravelling these integrated plant functioning mechanisms and in this issue of *New Phytologist* we feature a set of papers that address the different aspects and questions raised (Godin *et al.*, 2005), so highlighting the current advances and future directions required.

'Plant spatial representations can be made at various levels of detail, ranging from accurate descriptions of each organ to coarse descriptions of branching systems at plant level.'

Nature of plant spatial representation

This question is central to the design of FSPMs. Plant spatial representations can be made at various levels of detail, ranging from accurate descriptions of each organ (leaf, metamer, etc.) to coarse descriptions of branching systems at plant level. Plant organs may be represented as constituents simply distributed in space. This *geometric* representation can be augmented by a description of the physical connections between organs, corresponding to the plant *topological* structure. Geometric structures are useful to model interactions of the plant with its environment. Topological structures are useful to model the circulation of substances or physical quantities within the plant (water, sugars, strains, etc.).

Different methods have been designed to digitise plants of various sizes in 3D or to map their topological structures (Sinoquet & Rivet, 1997) (Godin *et al.*, 1999) (Hanan & Wang, 2004). Using these methods, researchers can build up plant architecture databases that are used for further analysis or modelling. To analyse these complex databases, dedicated tools are necessary. Durand *et al.* (pp. 813–825, this issue) give a very innovative illustration of such analysis tools. They introduce a new Markovian-based stochastic model designed to identify homogeneous zones in tree structures and transitions between these zones. This approach contributes to creating a new generation of tools for automating complex tree architecture analysis in the context of understanding the botanical organisation of plants or building and assessing models.

Plant structure as an interface

The surfaces of plant organs are the location of energy, mass and information exchanges with the environment. From the plant viewpoint, this includes phylloclimate (Chelle, pp. 781–790, this issue), which is the microclimate and the environmental signals sensed by plants at the organ scale, and includes mass and energy fluxes gained or lost by the plant. From the environment point of view, plants act as modifiers of both soil and microclimate variables, as the result of plant presence (e.g. light interception, wind attenuation, disease propagation) and also due to tree functioning (e.g. air humidity due to transpiration, soil drying). Most of the interactions between the plant and the environment only depend on plant geometry, namely the 3D distribution of plant organs (e.g. light interception). In particular, the plant ability for resource capture mainly depends on spatial display by foraging organs. However, plant topology also influences the interactions between the plant and the environment. By using a FSPM, Pearcy *et al.* (pp. 791–800, this issue) show how light-capture

strategies are influenced by topological constraints, namely hydraulics and biomechanics, and how plants regulate biomass investment in order to achieve multiple purpose optimisation. This illustrates the usefulness of FSPMs to understand interactions between multiple processes better.

Plant as a network

The plant structure provides the support for different forms of fluxes (water, sugars) and signals (mechanical constraints, hormones) that control the plant functioning and growth. Models of water transport have been developed in the past decade and rely on the application of Darcy's law to express the relationship between fluxes and water potential in porous media, (e.g. Früh & Kurth, 1999). More recently, the simulation of mechanical stress and strains in plants has been considered by several teams (Jirasek *et al.*, 2000; Alméras *et al.*, 2002; Fourcaud & Lac, 2003) and is now considered to be a tractable issue. The problem of carbon transport and allocation is more complex because the underlying physiological processes are difficult to observe and are not yet well understood. Current modelling approaches use different variants of the concept of source and sink strength, reviewed in this issue by Minchin & Lacointe (pp. 771–779, this issue). The authors suggest that a 'minimal Münch model' can provide a sound and general theoretical framework to model carbon transport, where allocation priorities are an emergent property of the model. Allen *et al.* (pp. 869–880, this issue) illustrate this approach by describing a transport-resistance model based on the integration of similar assumptions for carbohydrate flow and allocation and L-systems for studying the growth of peach trees.

Plant as a developing organism

The growth of the plant continuously modifies the network of components and space occupation, which in turn changes the general balance between organ demand and production. This dynamic feedback between structure and function is probably one key issue in the understanding of plant development which necessitates further theoretical and applied developments. Current work in this area consists of developing mechanistic models that integrate models of physiological processes and descriptive information where knowledge of the underlying mechanisms is lacking. Knowledge is usually expressed at the metamer or growth unit level, and the growth of the entire organism is considered as an emerging property of the locally defined interactions between plant components or between plant components and environmental factors.

Two approaches in this issue were designed to study the effect of environmental factors (here, light) on plant growth. Evers *et al.* (pp. 801–812, this issue) adopted a detailed descriptive approach to model the growth of wheat. The variation of architectural variables throughout time (e.g. leaf dimensions, internode length, phyllochrone and leaf number) was estimated according to field measurements or bibliographic data. Sterck *et al.* (pp. 827–843, this issue) designed a model where each metamer can produce a flush of growth. Flushes are controlled by the product of probabilities, depending on their metamer topological and environmental context. This growth principle is then integrated with computation of light capture, photosynthesis and carbon allocation at each cycle of growth to study the effect of different light environment on tree growth.

Models of plant development can also be used to study the ability of locally specified hypotheses to generate a range of emerging behaviours through complex interaction at plant level. Fournier *et al.* (pp. 881–894, this issue) describes a model of grass leaf that was designed to analyse the likelihood of event synchronisation during plant growth, such as leaf emergence and triggering of leaf elongation. Allen *et al.* developed a model able to integrate various sources of physiological knowledge. Here, emphasis is put on the handling of complex dynamical systems. First, efficient algorithms are described to compute carbohydrate flows within the dynamically changing tree structure. Second, correct qualitative behaviour of the model is shown to emerge from simple quantitative local rules expressing carbon allocation and storage, water supply, light availability and fruit growth.

Finally, the approach of Buck-Sorlin *et al.* (pp. 859–867, this issue) shows the possibility of using growth models to test the integration of new levels of knowledge in a complex system. Their model combines morphogenetic rules of the development of Barley and a description of metabolic regulatory network simulating the biosynthesis of gibberellic acids that control the elongation of internodes.

Challenges and future directions of research

Functional–structural approaches are intended to face several aspects of plant architecture complexity: (i) *complexity of the biological system*, in particular due to the high variability and plasticity of plant growth, and to the multitude of interwoven scales at which physical, ecophysiological and morphogenetic phenomena occur; (ii) *complexity of integrating various sources of knowledge*, possibly at different time scales, into one consistent modelling framework; and (iii) *computer simulation complexity*, which necessitates management of numerous dynamically changing and interacting parts. These questions are currently being discussed within the FSPM research community (Godin *et al.*, 2004), where the following new approaches are emerging.

Integration. To tackle the lack of a modelling framework for developing integration of structure and function, new modelling paradigms are being developed and tested. This is illustrated for instance by the intermediate-level approach (not completely mechanistic and not completely descriptive), based on a systematic flux-based representation of the various phenomena at different scales (Renton *et al.*, pp. 845–857, this issue).

Link between models and the real world. Because virtual plants and FSPMs are firstly tools developed to address biological questions, they must show properties and behaviours similar to those of real plants. FSPMs allow scientists to make virtual experiments and measurements, impossible to set in the real world due to time, cost or feasibility constraints. Light partitioning between fruiting units in tree canopies, for example, cannot be measured from light sensors distributed in the canopy, whereas virtual plants allow accurate estimations of light sharing at intra-canopy scales (Allen *et al.*). In order for there to be confidence in the results from the virtual experiments, quality requirements are needed. This should be carefully checked from quantitative assessment. This applies for both plant structure and function. As a result, virtual experiments should presently be used in applications where FSPMs results have been validated from comparison with field or lab measurements, while more interaction between modellers and experimenters should develop in order to ensure close relationships between FSPMs and real plants.

Understanding the effect of genes in the development of plant form. The large number of recent results obtained in both molecular and cellular biology makes it possible to consider a new approach of developmental biology based on modelling at a cellular scale. Today, several teams are building models of meristem development, organ growth and hormone signals, to grasp the role of different parameters in the control of phenomena like phyllotaxy, meristem maintenance and response to environment (for a review, see Prusinkiewicz, 2004). A first step in this direction is illustrated by Buck-Sorlin *et al.*

Design of new languages and formalisms. Several attempts are being made in order to generalise the L-system approach. Classical L-systems only manipulate strings or tree structures. However, at the scale of tissues for instance, structures correspond to complex 2D or 3D objects and cannot be simply represented by strings or trees. In an attempt to generalise L-systems to model the development of discretised surfaces, Prusinkiewicz and colleagues introduced a language, called VV, for rewriting meshes of triangles (representing tissues). They applied this generic system to the problem of modelling the growth of apical meristem and the emergence of phyllotactic patterns (Smith & Prusinkiewicz, 2004). In a similar perspective, Buck-Sorlin *et al.* introduce another extension of L-systems, RGG, that can represent genetic, metabolic and morphological aspects of plant development within the same framework.

Conclusion

The FSPM community is young and multidisciplinary in nature. Its size is increasing at each FSPM conference since 1996 and around 200 participants attended FSPM04 in Montpellier, June 2004. As illustrated by the selection of papers in this special feature of *New Phytologist*, major trends are readily observable: (i) L-systems, for instance, are adopted by a majority of teams as a paradigm to model plant development; (ii) there is a real need for modellers to exchange ideas, formalisms and experience independently of the type of plant (grass, bush or tree) they work on; and (iii) there is a new and growing interest in the FSPM community in modelling the interaction between genes and form. These trends will probably be the basis of major topics of the next FSPM conference, which will be held in New Zealand in November 2007.

Christophe Godin[1] and Hervé Sinoquet[2]*

[1]INRIA, UMR AMAP, TA40/PSII, Boulevard de la Lironde, 34398 Montpellier Cedex 5, France; [2]UMR PIAF, INRA-Université Blaise Pascal, Site de Crouelle, 234, avenue du Brezet, 63100 Clermont-Ferrand, France (*Author for correspondence: tel +33 4 73 62 43 61; fax +33 4 73 62 44 54; email sinoquet@clermont.inra.fr)

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Key words: complexity, computer models, FSPM, integration, plant architecture, virtual experiments, virtual plants.

Fructans and freezing tolerance

Fructans are linear or branched polymers of fructose that occur in about 15% of flowering plant species, including many which are cultivated commercially – they are synthesized from sucrose in the vacuole, where they are stored as reserve nonstructural carbohydrates. However, their role is wider than just storage. For example, fructan metabolism may impact on the tolerance of plants to drought and frost, and may aid in the defense against infection by fructan-producing pathogens. In this issue (pp. 917–932), Van den Ende *et al.* report exciting new findings pertaining to the physiological role(s) of fructan. These authors employed a diverse experimental approach that led to the cloning and biochemical characterization of two 6-kestose exohydrolase (6-KEH) isozymes from cold-hardened wheat crown tissue. The 6-KEHs are novel among enzymes of fructan metabolism because of their high specificity for a single substrate, 6-kestose, and their localization in the apoplastic fluid of fructan-containing sink tissues. The thorough examination of fructan metabolism by Van den Ende *et al.* provides perhaps the best evidence to date that fructan degradation in the apoplast functions to stabilize cell membranes exposed to freezing temperature.

'6-kestose exohydrolase degradation of apoplastic 6-kestose to sucrose and fructose may be a mechanism to protect membranes from freezing'

Synthesis and degradation

Determining the metabolic pathway(s) of fructan synthesis/degradation proved to be a painstaking endeavor owing to the unusual kinetic properties of the enzymes. Nearly 30 years elapsed before the conceptual model for fructan synthesis proposed by Edelman & Jefford (1968) was shown to be correct (reviewed in Vijn & Smeekens, 1999). The enzymes sucrose:sucrose 1-fructosyl transferase (1-SST) and fructan:fructan 1-fructosyl transferase (1-FFT) synthesize inulin, the most basic fructan. Both 1-SST and 1-FFT are nonspecific with regard to substrate utilization, and the activity is essentially nonsaturable and dependent on both enzyme and substrate concentration (Koops & Jonker, 1996). Subsequently, the enzymes that catalyze the formation of more complex fructans have been isolated and have been shown to have similar properties to 1-SST and 1-FFT.

Compared with fructan synthesis, relatively little is known about the degradation and subsequent mobilization of fructans from the vacuole (Vijn & Smeekens, 1999). Fructan exohydolase (FEH) enzymes have been localized in vacuoles and catalyze the removal of terminal fructose residues. In addition to degrading fructans before mobilization out of the vacuole, vacuolar FEHs are thought to function as trimming enzymes during fructan synthesis (Van den Ende *et al.*).

Beyond storage

Vijn & Smeekens (1999) discussed the long-held suspicions of researchers that fructans have physiological roles

not directly associated with the role as a storage form of carbohydrate. As predicted by Vijn & Smeekens (1999), the advent of molecular genetics has been a great benefit to researchers concerned with fructan metabolism. Some surprising results have opened avenues of research not previously considered. Livingston & Henson (1998) reported the presence of fructans and FEH activity in the apoplastic fluid of second-phase cold-hardened crown tissue of oat, leading to the suggestion that fructan metabolism may contribute to the mechanism of freezing tolerance. Van den Ende *et al.* confirm and greatly extend this research. The two 6-kestose exohydrolase enzymes cloned from a cDNA library prepared from cold-hardened wheat crown tissue were rigorously characterized at the molecular and biochemical levels. The absolute specificity of 6-KEH for 6-kestose provides unequivocal evidence that the enzymes are indeed 6-KEHs, enzymes never before detected in plants. The activity of the 6-KEHs was predominantly found in the apoplastic fluid of the crown tissue, consistent with the array of fructan substrates found in the apoplasic fluid. Furthermore, both 6-KEHs have N-terminal sequences consistent with secretion from the cell. Although the evidence for localization of the 6-KEHs in the apoplastic fluid is not totally definitive, when considered with the novel properties of 6-KEH (especially the extreme specificity for a single substrate) and the expression of 6-KEH in sink tissues that must tolerate freezing temperature, it is logical to conclude that 6-KEH functions to degrade apoplastic 6-kestose to sucrose and fructose as a mechanism to protect membranes from freezing. Alternatively, the authors note that 6-KEH may function to inhibit infection by fructan-producing pathogens, a role that has been proposed for FEH enzymes that occur in plants such as sugar beet that do not synthesize fructans (Van den Ende *et al.*, 2003, 2004).

Transport to the apoplast

Although only briefly discussed by Van den Ende *et al.*, there remains a relevant unresolved question pertaining to fructan metabolism. Specifically, how are fructans, which are synthesized and stored in the vacuole, transported to the apoplast? The authors speculate that fructan transport may occur by a vesicle-mediated mechanism (exocytosis) as described by Echeverria (2000). New results of E. Etxeberria (aka E. Echeverria, pers. comm.) indicate that a similar mechanism exists for import of solutes from the apoplast to cellular compartments (endocytosis). Such a mechanism of transport is attractive because multiple solutes of varying sizes could be transported with no requirement for specific membrane carriers. It seems essential that the mechanism(s) of fructan transport from the vacuole be resolved in order obtain a complete picture of the physiological roles of fructan in plants.

Perspectives

In a more general sense, the experimental approach of Van den Ende *et al.* provides new information at several levels, from the gene to the intact tissue. Indeed, at least two reports could have been derived from the wealth of data reported in this paper, leading to a more impressive resumé but a much less satisfying story for the scientific community. It can be expected that breakthroughs, such as the discovery of 6-KEH reported in this issue, will soon be forthcoming, leading to new avenues of research. Advances in understanding fructan metabolism may provide new strategies to bioengineer stress tolerance in commercially cultivated plant species and may also impact exploitation of the commercial utility of fructans that has been hampered by the difficulties in obtaining homogeneous complex fructans.

Steven J. Crafts-Brandner

United States Department of Agriculture, Agricultural Research Service, Western Cotton Research Laboratory, 4135 East Broadway Road, Phoenix, AZ 85040, USA
(tel +1 602 437 0121; fax +1 602 437 1274;
email scraftsbrandner@wcrl.ars.usda.gov)

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Key words: apoplast, freezing tolerance, fructan, 6-ketose exohydrolase, solute transport, vacuole.

New Phytologist

Letters

Leaf-level light compensation points in shade-tolerant woody seedlings

Photosynthetic traits such as respiration rate and light compensation point (LCP) likely play an important role in determining a plant's tolerance to low light levels, which can dictate long-term partitioning of the light supply among species and successional patterns (Givnish, 1988; Pacala *et al.*, 1994). Walters & Reich (1999) reviewed patterns of leaf-level LCPs and associated leaf parameters in seedlings of tree species. They reported differences in mass-based dark respiration rates ($R_{d,mass}$) and specific leaf area (SLA), but no differences in area-based dark respiration rates ($R_{d,area}$), quantum yield (QY) or LCP among species that are considered shade-tolerant, shade-intolerant or intermediate in their tolerance of shade. Although other aspects of plant ecophysiology are certainly important for shade tolerance, is it true that leaf LCPs are unimportant?

Shade tolerance: questions

There are, in fact, several reasons to question the truth of this assertion. First, for a given plant, shade leaves are considered to have lower LCPs than sun leaves (Ellsworth & Reich, 1993). If phenotypic and genotypic plasticity operate in the same direction, then shade-tolerant plants would have lower LCPs compared with shade-intolerant species. Second, shade-tolerant species are thought to intercept a higher fraction of the incident light, i.e. species that are tolerant of shade cast deeper shade (Horn, 1971; Canham *et al.*, 1994). For this pattern to exist, the leaves of shade-tolerant plants at the bottom of the canopy would have to have lower LCPs than leaves of shade-intolerant species because mature leaves likely need to be energetically self-sufficient (Lacointe *et al.*, 2004; Sprugel *et al.*, 1991). Third, individual studies that compare shade-intolerant and shade-tolerant species under similar growth conditions often show that shade-tolerant species have lower LCPs than shade-intolerant species (e.g. Lusk, 2002). Finally, data for $R_{d,area}$ and LCPs were log-normally distributed, and should have been log-transformed to meet the requirements of ANOVA (Sokal & Rohlf, 1994).

Patterns of photosynthetic traits

In order to investigate this, we started with data on $R_{d,area}$, QY and LCP from appendix 1 of Walters & Reich (1999), which included the leaves of 67 species of broadleaf evergreen trees and 27 species of temperate deciduous trees that were grown under low light (< 4% full sun) and/or slightly higher light levels (4–12% full sun). Using the same criteria as Walters & Reich, we obtained data on photosynthetic characteristics of seedlings of woody species published since 1998 (see Appendix). These included 22 new species from six publications (Einig *et al.*, 1999; Valladares *et al.*, 2000; Hattenschwiler, 2001; Lusk & Del Pozo, 2002; Lusk, 2002; Muraoka *et al.*, 2003) for a total data set of 115 species. Following Walters & Reich, no needleleaf evergreens were included.

Species means were recalculated for each of the two light levels (< 4% and 4–12% full sun). Also following Walters & Reich, species were categorized with respect to shade tolerance based on published survival data in shade by original authors and/or previously published observations of tolerance. Species with ambiguous classification (e.g. *Acer pseudoplatanus* of Hattenschwiler, 2001) were not included in this analysis. Data from Einig *et al.* (1999) were taken from leaves positioned at intermediate height on the seedlings. Hattenschwiler (2001) data were averages of means at 1.3 and 3.4% full sun for ambient CO_2. Only shade plants were used from Lusk (2002), because it was uncertain whether the high-light category exceeded 12% full sun. For Muraoka *et al.* (2003), we averaged data for seedlings in deciduous–coniferous and deciduous forests. We removed one strong outlier from the analysis. *Fraxinus americana*, an intermediate shade-tolerance species that was reported to have an LCP of 1.1 μmol m^{-2} s^{-1} (Bazzaz & Carlson, 1982), but a calculated LCP ($R_{d,area}$/QY) of 11.3. All analyses were performed in JMP 5.0.1 (SAS, Cary, NC).

When examining the relationships among photosynthetic characteristics, species with lower area-based dark respiration rates and/or higher QY had lower LCPs. Both $R_{d,area}$ and QY were significant predictors of LCP ($\log \text{LCP} = 1.73 + 0.84 \log R_{d,area} - 11.25\ \text{QY}$; $r^2 = 0.61$, $P < 0.001$ for each coefficient) with significant bivariate relationships using a Model II regression (Fig. 1).

Differences in $R_{d,area}$, QY and LCP among categorical factors that include species classification (broadleaf evergreen and deciduous), light levels and shade-tolerance were tested using two separate full-factorial ANOVAs. Because the light levels, shade-tolerance categories and leaf habit contrasts represent

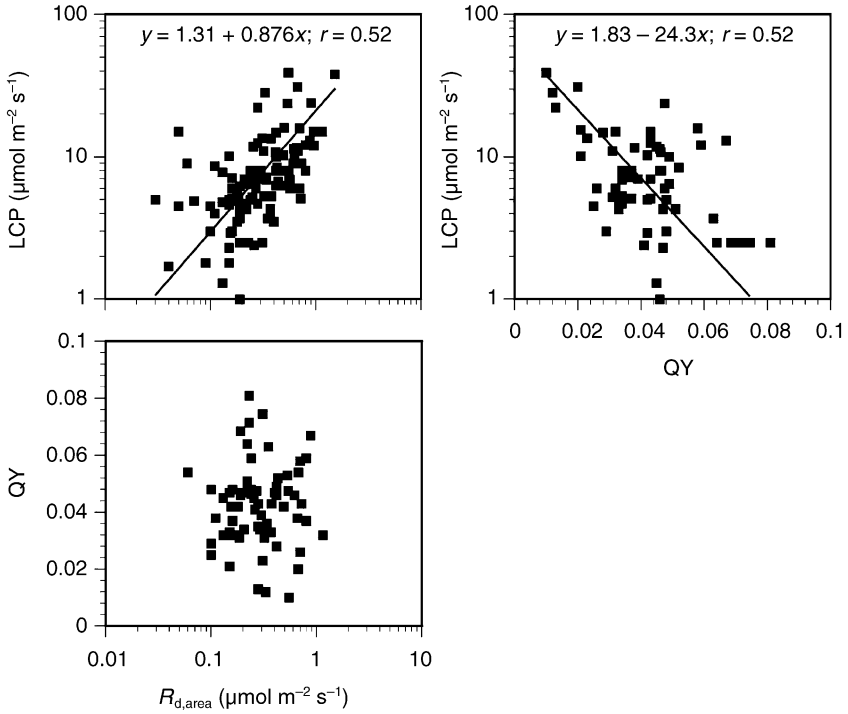

Fig. 1 Bivariate relationships among $R_{d,area}$, QY and LCP. Lines represent Model II regression best fit. LCP and $R_{d,area}$ were log-transformed before regressions.

post hoc categories, nonsignificant predictors ($P > 0.05$) were removed serially.

LCPs were lower for shade-tolerant species than shade-intolerant species and species intermediate in their tolerance for shade ($P < 0.001$, Table 1). Shade-tolerant species have positive carbon balance at light levels approximately 3 μmol m^{-2} s^{-1} less than intermediate or intolerant species (least squares means LSMs = 5.3 vs 8.1, 9.0 μmol m^{-2} s^{-1}, respectively). Combining the intolerant and intermediate species into a single category strengthens the significance of difference between tolerant and other species for $R_{d,area}$ and LCP (Table 1). The lower LCPs of shade-tolerant species compared with intermediate and intolerant species was associated with lower $R_{d,area}$ ($P = 0.06$ for three categories, $P = 0.01$ with two), but no difference in quantum yield (Table 1).

Table 1 Backwards elimination ANOVA results for species-averaged $R_{d,area}$, QY and LCP

	log $R_{d,area}$	QY	log LCP
Light	0.52	0.009	
Habit		0.08	
Shade tolerance	0.06		< 0.001
Shade tolerant	**0.25**[a]		**5.32**[a]
Intermediate	**0.33**[a]		**8.10**[b]
Shade intolerant	**0.35**[a]		**9.04**[b]
Light*Habit		0.04	
Light*ST	0.03		
Light	0.18	0.009	
Habit		0.08	
Shade tolerance	0.01		< 0.001
Shade tolerant	**0.25**[a]		**5.32**[a]
Other	**0.34**[b]		**8.61**[b]
Light*Habit		0.04	
Light*ST	0.01		

Note that the table also includes *P*-values for categorical contrasts (light level and evergreen vs deciduous leaf habit) and least squares means (LSM) for shade-tolerance categories. Superscript letters refer to Tukey's HSD comparisons ($P = 0.05$) among LSMs (bold text) of shade-tolerance categories for a given factor. Units for $R_{d,area}$ and LCP are μmol m^{-2} s^{-1}. Neither the interaction between leaf habit and shade tolerance nor the three-way interactions was significant in either model.

Relevance of lower LCPs

There are three major consequences to shade-tolerant species having lower values of $R_{d,area}$ and LCP. First, net carbon balance in low light should be greater for tolerant than for less-tolerant species, and hence contribute (along with whole-plant characteristics) to the success of tolerant species in shade. Light levels in the understory of forests can be near the LCPs of seedlings for the greater part of the day. Ellsworth & Reich (1992) measured 10-min means of photosynthetic photon flux density over the course of a growing season in the understory of an *Acer saccharum* forest. Over 90% of the time light levels were below 20 μmol m^{-2} s^{-1}. The low LCP and $R_{d,area}$ of shade-tolerant species should allow them both to conserve carbon better than less tolerant species and to gain carbon better, having net photosynthesis at lower light levels.

Second, the trade-off between low respiration rates and high maximum photosynthetic rates among species was

thought to reflect differential selection for conservation of carbon in shaded habitats vs the ability to photosynthesize at high rates in high-light environments (e.g. Givnish, 1988). With shade-tolerant species having lower LCPs, this trade-off should be extended such that plants can either have leaves that photosynthesize at low light levels or photosynthesize at high rates at high light levels. Hence, there is an additional dimension to the evolutionary trade-off between success in shade and sun habitats. Third, shade-tolerant trees should be able to reduce light levels to lower levels than shade-intolerant species by building canopies with a greater leaf area index that intercept a greater fraction of total incoming radiation. Combined with differences in carbon balance at low light, this could serve as a mechanism by which shade-tolerant species can prevent the establishment of seedlings of shade-intolerant species.

Joseph M. Craine[1]* and Peter B. Reich[2]

[1]Environmental Studies, Dartmouth College, Hanover, NH 03755, USA; [2]Department of Forest Resources, University of Minnesota, Saint Paul, MN 55108, USA (*Author for correspondence: tel +1 603 646 2721; fax +1 603 646 1682; email crain010@umn.edu)

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Key words: light compensation point, low light, photosynthetic traits, respiration rate, shade tolerance.

Appendix

Presented below are data published on photosynthetic characteristics since Walters & Reich (1999). Categorical data include leaf habit (deciduous or evergreen), the shade tolerance of the species and the light levels under which the seedlings were grown (low (< 4% full sun) or medium (4–12% full sun). All species were from temperate habitats. Reported data include $R_{d,area}$ (μmol m^{-2} s^{-1}), QY (unitless) and LCP (μmol m^{-2} s^{-1}).

Leaf habit	Shade tolerance	Light	Genus	Species	$R_{d,area}$	QY	LCP	Study
Deciduous	Tolerant	Medium	*Acer*	*distylum*	0.19	0.0685	2.5	Muraoka *et al.* (2003)
Deciduous	Intolerant	Medium	*Acer*	*rufinerve*	0.22	0.064	2.5	Muraoka *et al.* (2003)
Evergreen	Tolerant	Low	*Aextoxicon*	*punctatum*	0.19		3.7	Lusk (2002)
Evergreen	Tolerant	Medium	*Aextoxicon*	*punctatum*	0.24		7	Lusk and Del Pozo (2002)
Evergreen	Tolerant	Medium	*Amomyrtus*	*luma*	0.45		8	Lusk and Del Pozo (2002)
Evergreen	Intermediate	Low	*Aristotelia*	*chilensis*	0.44		6.7	Lusk (2002)
Evergreen	Intolerant	Medium	*Aristotelia*	*chilensis*	0.73		9	Lusk and Del Pozo (2002)
Evergreen	Intolerant	Medium	*Auracaria*	*angustifolia*	1.52		38	Einig *et al.* (1999)
Evergreen	Intolerant	Medium	*Caldcluvia*	*paniculata*	0.61		9	Lusk and Del Pozo (2002)
Evergreen	Tolerant	Medium	*Dasyphyllum*	*diacanthoides*	0.55		8	Lusk and Del Pozo (2002)
Evergreen	Intermediate	Medium	*Drimys*	*winteri*	0.64		9	Lusk and Del Pozo (2002)
Evergreen	Intolerant	Low	*Embothrium*	*coccineum*	0.57		6.9	Lusk (2002)
Evergreen	Intolerant	Low	*Eucryphia*	*cordifolia*	0.42		6.3	Lusk (2002)
Evergreen	Intolerant	Medium	*Eucryphia*	*cordifolia*	0.68		11	Lusk and Del Pozo (2002)
Evergreen	Tolerant	Low	*Gevuina*	*avellana*	0.49		6.3	Lusk (2002)
Evergreen	Tolerant	Low	*Laurelia*	*philippiana*	0.37		3.8	Lusk (2002)
Evergreen	Tolerant	Medium	*Laurelia*	*philippiana*	0.56		7	Lusk and Del Pozo (2002)
Evergreen	Tolerant	Low	*Luma*	*apiculata*	0.38		5.3	Lusk (2002)
Evergreen	Tolerant	Low	*Myrceugenia*	*planipes*	0.4		3.5	Lusk (2002)
Evergreen	Tolerant	Medium	*Myrceugenia*	*planipes*	0.57		6	Lusk and Del Pozo (2002)
Evergreen	Intolerant	Medium	*Nothofagus*	*dombeyi*	0.95		15	Lusk and Del Pozo (2002)
Evergreen	Intolerant	Medium	*Nothofagus*	*nitida*	0.96		12	Lusk and Del Pozo (2002)
Evergreen	Intolerant	Low	*Quercus*	*coccifera*	0.55	0.01	39	Valladares *et al.* (2000)
Deciduous	Tolerant	Medium	*Quercus*	*crispula*	0.31	0.0745	2.5	Muraoka *et al.* (2003)
Evergreen	Intolerant	Low	*Quercus*	*ilex*	0.67	0.02	31	Valladares *et al.* (2000)
Deciduous	Intermediate	Low	*Quercus*	*robur*	0.28		4.7	Hattenschwiler (2001)
Evergreen	Intolerant	Medium	*Weinmannia*	*trichosperma*	0.63		10	Lusk and Del Pozo (2002)

Meeting report

Advances in forest tree genomics

Forest Trees Workshop, Plant and Animal Genome XIII Conference, San Diego, CA, USA, January 2005

What's up in forest tree genomics?

Genomics can be defined as the development and application of genome-wide experimental approaches to assess gene structure and function, which include DNA sequencing, gene mapping and gene expression profiling. Forestry entered the genomic era in the early 1990s, with the introduction of forward (genetic mapping for quantitative trait loci (QTL) detection, reviewed by Cervera *et al.*, 2000; Sewel & Neale, 2000) and reverse (knockout and overexpression; MacKay *et al.*, 2004) genetics approaches. In the late 1990s, the establishment of functional genomics tools in many forest tree research laboratories has enabled the simultaneous analysis of thousands of transcripts or proteins, providing an opportunity to understand protein function, gene regulation and eventually how these long-lived organisms are assembled.

In 2004, this discipline reached an unprecedented level with the public release of the complete genome sequence of *Populus trichocarpa* (http://genome.jgi-psf.org/Poptr1/Poptr1.home.html; Tuskan *et al.*, 2004). The Forest Trees Workshop, begun in 1993 and organized within the Plant and Animal Genome meeting, has been an excellent forum for reviewing the current state of knowledge and discussing new directions of research in this community. In January 2002, this workshop also became the annual meeting of the Forest Genomics working party of the International Union of Forestry Research Organizations (IUFRO; http://www.iufro.org/science/divisions/division-2/) and is complemented by the biannual Tree Biotech meeting (http://www.iufro.up.ac.za/) which focuses on the molecular biology, genetics and biotechnology of trees as well as more basic aspects of growth, development and environmental and biological forest tree interactions. Here we focus on the 2005 Forest Trees workshop, where many advances were reported and discussed, covering aspects of tree genomes, environment–genome interactions, genomics of wood formation, molecular ecology and leveraging model systems.

'The need for improved EST resources is also argued by the evidently large number (approximately 75%) of genes predicted from the poplar genome sequence that are not supported by EST sequence information'

Which genes really matter for forest trees adaptation?

Growth, development and survivorship of long-lived organisms such as forest trees are continuously challenged by biotic and abiotic stresses. Accelerated climatic changes and the biotic environmental challenges resulting (e.g. the rapid colonization of previously hostile niches by pests and pathogens) suggest that if these long-lived, late-reproducing, immobile organisms are to survive in their current physical distributions, they will need to respond via both phenotypic plasticity and selection-driven changes on allele frequencies. An immediate question, now partly testable, is whether the present structures and levels of genetic diversity in existing forest tree species/populations is sufficient to allow adaptation to these future conditions. Addressing this question will help underpin actions to preserve adaptability and possibly avoid major losses, especially in managed forests. Quantitative transcriptomics and proteomics allow remarkable variation in gene/protein expression to be discovered in many forest tree species, thus making available 'expressional candidate genes' for many traits of ecological interest (e.g. cold and drought tolerance, disease resistance and phenology). Gene activation tagging and reverse genetics approaches also allow exploration of gene expression–phenotype correlation (but stop well short of demonstrating causation). The second question, concerning whether observed expressional/physiological variation matters (i.e. whether it affects the function and fitness of organisms in natural populations), is the next frontier. Here we report on advances in forest tree genomics and especially on how functional genomics combined with association genetics promises major new insights into forest tree adaptation.

Functional genomics in forest trees

Systematic and genome-wide approaches are now being applied for gene discovery and analyses of gene function by using complementary methods. Work in hardwood trees (angiosperms) including *Populus* and *Eucalyptus* is now poised to benefit directly from the entirely sequenced, assembled and annotated *Populus* genome sequence. On the other hand, work on the very distantly related commercial softwoods (gymnosperms) must rely much more heavily on expressed sequence tag (EST) sequence information and comparative genomic approaches, because the whole genome sequence is not expected to be available in the foreseeable future.

From EST sequencing to gene expression profiling

The large-scale sequencing and analysis of ESTs remains a fundamental part of genomics research in most forest tree species. Results were presented for loblolly pine (J. Dean, University of Georgia at Athens, USA), maritime pine (J. Paiva, IBET/INRA, France) and white spruce (J. MacKay, Laval University, Canada). Whereas many EST sequencing projects carried out in forest trees focus on wood formation and secondary xylem, more and more projects have involved a broader diversity of tissues and a growing interest in the response to abiotic stresses (including drought stress and frost tolerance). In the loblolly pine ADEPT project (J. Dean; http://dendrome.ucdavis.edu/adept/), cDNA libraries were made from seedlings during drought stress and the recovery from drought. For each condition, separate libraries were made from three different genotypes and the comparative analysis of their ESTs identified several putative drought-responsive genes. Furthermore, the transcript profiles of one of the genotypes strongly suggested that it responded more strongly to the stress. If further analysis shows that this genotype displays a greater tolerance to drought, its differentially expressed genes may represent *expressional candidate genes* for drought response or tolerance. Gene discovery

projects are also sequencing cDNAs from both 5′ and 3′ ends as means to augment the sequence information and improve the annotation of genes, while providing sequences that offer highly robust assemblies. The need for more complete information has been highlighted by the recent analyses of pine genes, which show that the majority of contigged sequences which have no sequence similarity to other genomes are indeed very short and that a large majority of sequences above 1 kb in length give strong matches to *Arabidopsis* in particular (Kirst *et al.*, 2003; Pavy *et al.*, 2005). The need for improved EST resources is also argued by the evidently large number (approximately 75%) of genes predicted from the poplar genome sequence that are not supported by EST sequence information (S. DiFazio, Oak Ridge National Laboratory, USA).

Gene sequences identified through large-scale EST sequencing, using targeted cDNA discovery methods such as suppressive subtractive hybridization (SSH), or based upon the poplar genome sequence, are now being used in several laboratories to characterize gene families and develop microarrays for comprehensive gene expression profiling. Several groups are focusing on transcription factors, which are thought to play regulatory roles in primary and secondary xylem formation in conifers, poplar and *Arabidopsis*. Systematic prospecting of pine xylem ESTs has identified several sequences from diverse families of well-characterized plant transcription factors, including AP2, MYBs, HDzip, KNOX, LIM-domain, MADS and others (S. Rui, North Carolina State University, USA). Analysis of their transcript abundance by quantitative polymerase chain reaction (PCR) indicated that many of the sequences are somewhat ubiquitous and a minority are preferentially expressed in secondary xylem tissues. A detailed analysis of the KNOX-1 family in conifers revealed that gene evolution and the resulting family structure is clearly distinct from that of angiosperms (Guillet-Claude *et al.*, 2004). Conifers KNOX-1 genes form a single rapidly evolving cluster and are found in only one of the three large clades observed in Angiosperms. The report suggests that extrapolation of the biological role of genes in this family based upon sequence similarities alone may be inadequate, and it argues in favor of reverse genetic studies using gain-of-function and loss-of-function approaches. Unfortunately, there have been few reverse genetic experiments owing to the lack of simple transformation and regeneration methods. During the meeting it was reported that several transgenic spruce lines misexpressing conifer transcription factors are now being analyzed to help to delineate more clearly the function and biological role of genes belonging to multimember families (J. MacKay).

The discovery of a large number of micro-RNAs from poplar xylem was also reported, and the potential implications of this important class of regulatory molecules in trees were discussed (V. Chiang, North Carolina State University, USA). Although many studies in *Arabidopsis* have linked micro-RNAs to the regulation of plant development through their action on transcripts of homeotic transcription factor, it was reported that the putative targets of nearly 50% of the poplar micro-RNAs isolated in this study are structural proteins and enzymes. Transcripts encoding cellulose synthase, mananne biosynthesis enzymes, and enzymes implicated in cell wall phenylpropanoid and flavonoid biosynthesis are thought to represent novel targets for micro-RNAs. This intriguing finding may suggest a specialization of micro-RNAs in secondary xylem of trees.

Wood formation in forest trees is characterized by its remarkable phenotypic plasticity, especially in conifers where the morphology and properties of xylem cells change significantly across the growth season, with the age of the cambial meristem, and in response to environmental cues. Macroarray analyses were used in maritime pine to uncover differential gene expression related to the shift between early wood and late wood (formed within a single growing season), juvenile and mature wood (formed at different developmental stages), and in compression wood (formed in leaning or bent trees; J. Paiva). Microarrays developed with cDNAs isolated from subtractive libraries are being used to investigate the acquisition of frost tolerance in scots pine (*Pinus sylvestris*) and European beech (*Fagus sylvatica*; P. Balk, ATO, the Netherlands). Frost tolerance and cold resistance are phenomena that vary widely across plant species. This study has identified several gene transcripts related to osmotic stress in particular, which appear to show similar responses in the angiosperm and gymnosperm trees during the acquisition of frost tolerance. Gene sequences (including dehydrins, ABA responsive genes and PR proteins) were up-regulated, whereas tubulin, certain membrane-intrinsic proteins and expansins (among others) were down-regulated. Finally, a major outcome of the poplar genome sequence is the development of oligonucleotide arrays (Y. Sun, North Carolina State University, USA). The high level of specificity of oligo arrays is being used to delineate systematically between members of multigenic families of transcription factors and cell wall associated proteins, in order to determine which family members are specifically expressed in secondary vascular tissues.

Proteomics

Proteomics methods have yet to gain widespread application to forest trees. Protein profiling using one- and two-dimensional gel electrophoresis has been applied in several studies in the last decade (e.g. Plomion *et al.*, 2000). However, few studies have reported advances based upon recent technological developments enabling the identification of proteins at higher throughputs, using much smaller quantities of protein and at much lower cost. D. Lippert (University of British Columbia, Canada) presented a study in which protein profiles of somatic embryos of white spruce were analyzed over a developmental sequence using two-dimensional gel

electrophoresis and proteins identification by liquid chromatography - mass spectrometry (LC-MS) analyses (Lippert *et al.*, 2005). Similar methods are now being applied to characterize protein profiles in response to herbivory by insects and are helping to differentiate between the wounding response and the response to weevil infestation in spruce. The rate of identification of proteins in both studies was 60–70%. It was shown that the identification of a majority of proteins was made possible with information derived from EST sequence data. Identical results were recently reported in pine (Gion *et al.*, 2005). These findings argue in favor of developing proteomic studies in conjunction with EST sequencing and transcript profiling research.

Bioinformatics

The growing need to process, analyze and integrate data in functional genomics is leading to the development of more and more advanced and accessible bioinformatic tools. The flow of data and information between different genomic technologies is at the very heart of several experiments. For example, processed EST sequences must be analyzed to identify and annotate unigene sets, used to design and manufacture microarrays, and for the discovery of single nucleotide polymorphisms (SNPs) (Le Dantec *et al.*, 2004). The MAGIC database package (L. Pratt, University of Georgia at Athens, USA) was designed to accommodate this central need for integration. It is a portable package that was designed for use by biologists with minimal informatic expertise (http://www.fungen.org/Laboratory/Bioinformatics.htm). The database is currently used in conjunction with EST sequencing and microarray manufacture in loblolly pine (J. Dean); it also supports microarray databasing.

Beyond candidate genes

By bringing together ecologists, molecular biologists and population geneticists, a new research area is being developed in the forestry community to identify the genes responsible of forest tree adaptation. The approach seeks to identify functionally important genes from the study of nucleotide diversity patterns and to test these nucleotide polymorphisms for associations with the phenotypic variation in adaptive traits (Neale & Savolainen, 2004). Once a set of candidate (structural or regulatory) genes is determined, mutations of adaptive significance (i.e. that natural selection has favored) can then be identified, based on the within and between species nucleotide diversity pattern analysis (Kreitman, 2000). To determine whether or not observed variation has adaptive significance, scientists are describing the following.

- The number (SNPs and insertion/deletion events (INDELs)), nature (silent vs nonsynonymous) and genomic location (coding vs noncoding) of nucleotide polymorphisms (Brown *et al.*, 2004; Pot *et al.*, 2005).
- The level and the structure of diversity (at the nucleotide and haplotype level). Diversity is one of the key elements to preserve the adaptive potential and the capacity of organisms to adapt to new environmental conditions. It is therefore of main importance to quantify the level of diversity in genes putatively involved in forest tree adaptation. The structure of diversity (i.e. how the genetic diversity is distributed within and among populations) is also an important feature to be considered.
- The extent of linkage disequilibrium (LD), i.e. the tendency of alleles to be inherited together, is also an important parameter, both for understanding the genealogical history of populations, and for predicting the efficiency of association studies in natural populations (Goldstein & Weale, 2001).
- The extent to which nuclear diversity patterns are the result of selection. There are a number of methods used to search for 'molecular signatures' of natural selection (Ford, 2002). They generally use neutrality as the null hypothesis, and they are based on site/allele frequency distributions or ratios of synonymous vs nonsynonymous polymorphisms within and between species.

The validation of putatively important SNPs (or haplotypes) is carried out in a third step, wherein nucleotide polymorphisms are tested for association with the phenotypic variation of adaptive traits in natural populations (Cardon & Bell, 2001). Given the low LD window frequently observed in these largely outbred and highly polymorphic species (in poplar: Ingvarsson, 2005; in pine: Brown *et al.*, 2004; in spruce: presented by E. De Paoli, University of Udine, Italy), it is suggested that this association strategy should lead to the identification of functional variation to a scale that could not be reached by classical QTL mapping experiments.

Christophe Plomion[1]*, Tom Richardson[2] and John MacKay[3]

[1]UMR BioGeco, INRA Equipe de Génétique, 69 Route d'Arcachon, 33612 Cestas Cédex, France; [2]New Zealand Forest Research, Private Bag 3020, Rotorua, New Zealand; [3]Centre de Recherche en Biologie Forestière, Université Laval, Québec, Canada G1K 7P4
(*Author for correspondence: tel +33 5 57 12 28 38; fax +33 5 57 12 28 81; email plomion@pierroton.inra.fr)

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Key words: adaptation, candidate genes, forest trees, functional genomics

Cell walls: the boundaries of plant development

The role of the extracellular matrix in the control of plant development: the 13th *New Phytologist* Symposium, London, UK, January 2005

The extracellular matrix, or cell wall, plays a diversity of important roles in higher plants. Besides providing skeletal support to the plant as a whole and providing a mechanical counterbalance to turgor pressure in plant cells, the wall is also key to many aspects of growth regulation and cell-to-cell interactions and signalling. From this point of view, it is not surprising that the extracellular matrix is also of consequence to many areas of plant development, and this formed the topic of the 13th *New Phytologist* Symposium. The Symposium brought together biochemists, physiologists, cell biologists and geneticists working on different experimental systems to focus on this topical issue – highlights from which are discussed in this report. The benefits of bringing different disciplines together was no more evident than at this meeting, with many stimulating discussions and debate taking place, from which we can look forward to exciting developments.

'The cell wall is in essence a composite material built on a framework of cellulose microfibrils which have a greater tensile strength than steel'

Growth and expansion

Growth and expansion can be considered as distinct concepts in cell biological terms. Cell growth can be defined as the process by which the machinery of the cell is replicated, and this is intimately tied to the cell cycle or the process of endoreduplication. In the normal cell cycle, chromosomes are replicated before cell division, whilst endoreduplication involves chromosomal replication in the absence of division, resulting in polyploid cells; this process is common in plants. Both events lead to a doubling of nuclear material providing the potential increased production of ribosomal and other cell components, the difference being that in the normal cell cycle this material is partitioned into a new cell. In *Arabidopsis*, ploidy levels vary from 2C in meristematic and stomatal guard cells, for example, up to 24C in trichome cells. Plant cells can expand massively

during their development, and some increase their volumes by 100 000 times or more. The final size that can be attained by plant cells appears closely tied to their ploidy number, with higher levels of ploidy leading to bigger cells (Sugimoto-Shirasu & Roberts, 2003). Whilst growth involves increases in the active components of the cell, that is to say the cytoplasm and its components, expansion can be thought of as an increase in cell volume, and in plants this can be dominated by vacuolar expansion.

Keith Roberts (John Innes Centre, Norwich, UK) described eight *Arabidopsis* mutants united in having smaller sizes than wild type in specific cell types and all of them carrying lesions in one of 4 proteins that make up an enzyme complex known as DNA topoisomerase 6 (Sugimoto-Shirasu *et al.*, 2002). Crucially in all these mutants the lesions lead to the inability to reach a ploidy greater than 8C resulting in some cases in smaller trichomes, in some hairless roots, and in others shorter hypocotyls. Topoisomerase 6 is involved in disentangling replicated chromosomes by cutting and rejoining DNA at entangled points and this activity appears essential for progressing through the endocycle from 8C to 16C. Analysis of these mutants suggests a fairly linear relationship between ploidy levels, nuclear volume and cytoplasmic volume. Ultimately, this relationship extends to overall cell size and hence vacuolar volume although the relationship of ploidy level to cell volume does not appear strictly linear (Sugimoto-Shirasu & Roberts, 2003).

Hierarchies of control

There are many control points in biological systems, and in line with the words of a Bob Dylan song 'They may call you Doctor or they may call you Chief, but you're gonna have to serve somebody' there are hierarchies of control involved in plant growth. Thus, although transcriptional programmes and hormonal pathways may instigate and mitigate growth, and although part of this control may be through the influence of cell ploidy and the cell cycle, in the end, if a plant cell is going to get bigger it has to increase its volume and this can only be achieved through cell expansion. Cell expansion in plants is dependent on turgor pressure, which provides the driving force for this process, and water uptake leading to cell expansion is dependent on the difference in water potential across the plasmamembrane that is generated by the presence of abundant solutes on the cellular side. The difference in osmotic potential across the cytoplasmic membrane is balanced by the generation of turgor pressure, which is contained by the strong cell wall that supports the cytoplasmic membrane. In a nongrowing situation, the wall is functionally rigid, but for a cell to grow there needs to be a relaxation event in the cell wall, which releases the counterbalance to turgor pressure, leading to a lowering of the cellular water potential. This lowering of cellular water potential then leads to a net movement of water into the cell, causing cell expansion. In theory, cell expansion can be regulated either through turgor pressure or changes in wall mechanical properties. In practice, in all but conditions of severe drought, growth is controlled through the properties of the wall (Cosgrove, 1993).

The plant cell wall is an outstanding material that combines impressive strength with remarkable extensibility. Plant cells typically generate around 0.5 MPa of turgor pressure, but in specialised cells such as stomatal guard cells these pressures can reach 5 MPa (50 times atmospheric pressure) and this is sustained by the counteraction of the relatively thin cell wall. During cell growth, not only must the wall be strong enough to bear the load imposed by turgor, but also be capable of undergoing controlled extension at rates that can exceed 10% h^{-1}. One of the major goals of cell wall research is to understand how these characteristics are achieved.

The cell wall is in essence a composite material built on a framework of cellulose microfibrils. These microfibrils, which have a greater tensile strength than steel, are embedded in a matrix of other polymers. These are predominantly polysaccharides and fall into two general classes: hemicelluloses and pectins. Hemicelluloses can associate with cellulose microfibrils through extensive surface hydrogen bonding and are also sometimes called crosslinking glycans, as it is believed that they may form tethers between individual microfibrils to form a cohesive whole. Proteins (such as expansins) that induce wall extension generally do so by destabilising this cellulose–hemicellulose network (Cosgrove, 2000; Li *et al.*, 2003).

Coextensive with this is another network formed by pectins, acidic polysaccharides dominated by the presence of galacturonic acid residues. The presence of carboxyl groups is a key feature of pectins, which associate with one another through electrostatic interactions. Many of the potential carboxyls in pectic polymers are masked by the presence of methyl esters in the nascent chains, and subsequent de-esterification modulates the properties of pectin in the wall. Keith Roberts described how examination of a wide range of *Arabidopsis* mutants exhibiting impaired hypocotyl elongation, using infrared spectroscopy, revealed that the pectins in all showed higher levels of unesterified carboxyls. The suggestion is that increased carboxyl groups lead to tighter pectin interactions in the wall and that this may be instrumental in limiting wall extension during growth. In support of this hypothesis, it was reported at the meeting that overexpression of an *Arabidopsis* pectin methylesterase gene on an inducible promoter led to dwarfing.

Hypocotyl expansion

Hypocotyl expansion has proved one of the most powerful model systems for examining the role of cell walls in growth, and considerable knowledge has been gained from the

characterisation of mutants with impaired hypocotyls growth. Whilst a great deal has been achieved through the use of molecular genetics, Herman Höfte (INRA Versaille, France) showed the continuing importance of gaining a clear understanding of normal developmental events through careful observation. As part of the process involved in screening for plants with impaired hypocotyls growth, the Höfte group has meticulously examined the patterns of growth in wild-type *Arabidopsis* hypocotyls. Previous work of theirs has shown that hypocotyl elongation almost exclusively arises from the expansion of pre-existing cells laid down during *Arabidopsis* embryo development (Gendreau *et al.*, 1996), as has been seen in other plants. The patterns of cell expansion in hypocotyls has also been characterised during growth both in the light and in the dark. In the dark, hypocotyl cells go through three phases of cell expansion. Initially, cells go through a phase of slow steady expansion; they then progress into a period of rapid elongation growth followed by a period in which growth slows and comes to a halt. The switch to rapid growth progresses almost as a wave of expansion, starting with cells at the base of the hypocotyl and spreading upwards towards the more apical cells. Recently, the Höfte group have described how cell walls in the slowly growing population are thickened early during germination and how, once the cells enter the phase of rapid elongation, the walls become progressively thinner, indicating that there are distinct differences in growth in these two phases (Refregier *et al.*, 2004). Strikingly, the role of cellulose synthesis and deposition also appears quite distinct in these phases.

It is generally held, and logical, that cell wall extension cannot proceed without the synthesis of new cell wall material. This is obvious for long-term growth, where if the two are not coupled, the wall might become too thin to function properly. In line with this, interference in cellulose biosynthesis generally leads to the inhibition of cell expansion, as has been seen with cellulose synthase mutants and with the use of chemical inhibitors. Strikingly, it has been found that whilst treatment with the cellulose synthesis inhibitor isoxaben inhibits the slow phase of etiolated hypocotyl cell expansion, this treatment has no apparent impact on growth (although it did inhibit cellulose production) during the subsequent period of rapid cell expansion (Refregier *et al.*, 2004), indicating that these two phases of growth were quite different in nature and that wall extension in the period of rapid elongation appears to be independent of cell wall synthesis. This suggests that wall integrity during this phase of growth may simply be maintained by rearrangements of existing polymers. In this context, it is fascinating to note that the orientation of microfibrils in these walls appears to undergo major changes during expansion, moving from a radial to an axial orientation during expansion. Because cellulose microfibrils are very long structures, such reorientation suggests that the wall must be in a partially fluid state during this phase of growth to allow their movement.

Division or expansion: which leads in differential growth?

The role of differential growth in the development of form in plants is an obvious one. For example, in the shoot apical meristem, leaf primordia first arise as small bulges on the meristematic flanks as the result of more rapid expansion of tissues in these areas. Whilst there has been extensive characterisation of the various transcriptional regulators that determine meristematic patterning, the actual mechanisms by which differential growth arise have seen less work. Andrew Fleming (University of Sheffield, UK) has been examining whether differential growth in development is led by cell division or by cell expansion. Clearly both components are involved in the process, but which is the leading role? To this end, tools have been deployed to manipulate cell division (localised overexpression of cell cycle regulatory genes) or cell expansion (localised ectopic expansin expression). The fields of growth on the flanks of a meristem are tiny (tens of microns), and to allow localised transgene induction with this level of spatial resolution, a tetracycline-inducible system has been deployed in combination with the application of tetracycline-loaded Sephadex beads. It was shown that localised induction of cyclin expression could induce localised increases of cell proliferation on the flanks of tobacco vegetative meristems, but that this had no developmental consequence in terms of the appearance of new primordia (Wyrykowska & Fleming, 2003). In contrast, locally induced expansin expression led to the appearance of ectopic leaf primordia, and eventually fully formed leaves, clearly demonstrating that locally induced tissue expansion appeared to be sufficient to initiate the entire process of leaf formation on the meristematic flanks (Pien *et al.*, 2001). Similarly, locally induced expansin expression on the margins of primordial led to preferential enlargement of that margin in the mature leaf, whilst a similarly induced increase in local cell division eventually led to a reduction in the final size of the induced margin.

In the course of carefully studying leaf morphology, Andrew Fleming concluded that cells at the outer margin of the leaf lamina may play a key role in determining leaf growth and shape. Such cells are immensely long in the mature leaf and are characterised by the presence of particularly thick cell walls. It was shown that lesion in an *Arabidopsis* margin cell-specific gene (identified in an enhancer trap screen) led to a loss in cell identity for the margin cells, and that in their absence leaf development was severely impaired. Mutants in the yet-to-be-identified gene have small dense and dark-coloured leaves, and the locus has been named *HEPATICA* due to the liverwort-like appearance of the mutant plants.

Signalling: wax, shine and fiddleheads

Development in plants is coordinated through cell-to-cell communication and such signalling may be either symplastically or apoplastically transmitted. Evidence that cell wall components might serve as important developmental signals began to emerge in the 1990s. Paul McCabe (University College Dublin, Ireland) described work he carried out in Roger Pennell's laboratory (University College London) in which it was found that cells bearing a specific cell wall epitope produced a soluble signal necessary for suspension cells to form embryos. In this work, they showed that cells recognised by JIM8, a monoclonal antibody that binds to an arabinogalactan protein, produced a soluble signal that was sufficient to induce cells that did not carry this epitope to form embryos (McCabe *et al.*, 1997). Current attempts to purify the soluble signal were described.

Many developmental signals are propagated through the apoplast and therefore must pass through cell walls. The role of the cell wall in signalling processes was highlighted in talks on stomatal development and on mutants that exhibit postgenital organ fusion. Julie Gray (University of Sheffield, UK) presented a talk on the *Arabidopsis HIC* gene, which when mutated leads to increased guard cell proliferation at high CO_2 levels (Gray *et al.*, 2000). The gene itself encodes a putative beta ketoacyl CoA synthase thought to be involved in producing very long chain fatty acids that play a role in cuticular waxes. Mutants in two other genes involved in wax biosynthesis, *CER1* (a decarbonylase) and *CER6* (a fatty acid elongase), exhibit greater numbers of stomatal pores (Aarts *et al.*, 1995; Fiebig *et al.*, 2000). These observations indicate a role for cuticular waxes in the control of stomatal proliferation during leaf development. A model was suggested whereby the cuticle controls the diffusion of a diffusible signal from guard cells which inhibits the development of stomatal pairs in close proximity.

There are a number of well-characterised systems where normal signalling across the cell wall and cuticle is involved, such as pollen–pistil interactions and carpel fusion, and there are a number of *Arabidopsis* mutants where ectopic organ fusion occurs, presumably because of altered signalling functions. One of the best characterised of these mutants is in a gene called *FIDDLEHEAD*, which Robert Pruitt's group, at Purdue University (West Lafayette, IN, USA), have shown to encodes a lipid biosynthetic enzyme (Pruitt *et al.*, 2000) thought to be involved in epidermal wax biosynthesis. Organs at the shoot apex generally become fused in these plants, leading to the characteristic fiddlehead appearance of the plants. In addition, a number of other mutants showing ectopic organ fusion have been identified that carry lesions in genes thought to be involved in cuticular wax biosynthesis (Tanaka *et al.*, 2001; Wellesen *et al.*, 2001). It was suggested that these mutants all indicate that an intact cuticle is essential to maintain an inert, nonresponsive interface at the epidermal surface.

A more familiar role of the cuticle is in providing a waterproof and protective covering to the plant, roles fulfilled by cutins and waxes. Andy Pereira's group (Plant Research International, Wageningen, the Netherlands) have been studying the SHINE transcription factors of *Arabidopsis*, which were identified in an activation tagging screen by the shiny appearance of leaves of plants carrying a gain of function mutation in one of these genes. The shiny appearance of the leaves resulted from the increased deposition of waxes on the surface of the cuticle, with wax quantities having increased up to six-fold compared to wild type. Cloning of the gene identified it as one of a clade of three closely related AP2/EREBP transcription factors. Subsequent overexpression of the other two genes resulted in similar waxy phenotypes (Aharoni *et al.*, 2004). Another group also independently showed that overexpression of these transcription factors led to a waxy phenotype (Broun *et al.*, 2004).

At first glance, this appears as a relatively straightforward story. The regulation of cuticular wax biosynthesis is poorly understood, and this looks as if the transcriptional regulators have been overexpressed leading to increased wax formation. If so, this represents an excellent opportunity to study key components of this process. The story is, however, somewhat more complex. Logic would predict that because waxes form a hydrophobic barrier at the surface of leaves, this structure would be less permeable in the shine mutant than in wild type. In fact, it was shown that excised mutant leaves lose water more rapidly than wild type and also that chlorophyll was more readily extracted from the mutant than from wild type leaves, indicating enhanced permeability. Some clues to this apparent paradox came from comparative examination of wax components in mutant and wild type, which revealed alterations in composition as well as quantity of waxes in the mutants. This indicates that the overexpression of the *SHINE* genes does not increase all aspects of wax production proportionally and it may be that this change in wax composition is in part responsible for the increased permeability of the epidermis in these plants. Promoter/GUS reporter experiments showed that the *SHN1* gene is normally predominantly expressed in areas of cell separation (abscission and dehiscence zones for example) and it was suggested that the transcription factor was involved in sealing the surfaces of such zones to make them less susceptible to pathogen ingress and water loss. Microarray analysis of the mutant plants were reported to reveal the induction of a number of transcripts of genes normally associated with abscission, suggesting that the SHN transcription factors may have a more general role in these processes beyond sealing the surfaces with cuticular waxes.

It was shown that the mutant plants showed a greater resistance to severe droughting than do wild type plants. In these experiments, water was withheld until the plants became

wilted, at which time they were rewatered and examined for how well they recovered. The greater resistance of the SHN1 overexpressor mutants seems paradoxical, given that they showed more rapid water loss from excised leaves. However, the plants exhibited a number of phenotypic abnormalities, including reduced stomatal abundance, and it was suggested that this accounted for the greater apparent drought resistance.

A central theme running throughout this section of the meeting was the accumulating evidence that the cuticle has important roles in aspects of the control of cell fate. Both *HIC* and *FDH* encode members of the same gene family that encode putative beta ketoacyl CoA synthases that form part of the complex believed to be responsible for the synthesis of the very long chain fatty acid components of the cuticle. Along similar lines, SHN is a transcriptional activator involved in the control of wax biosynthesis, and shn overexpressors with abnormal cuticle composition exhibit developmental phenotypes such as reduced stomatal density.

Cellulosic cell walls are not land plant-exclusive

The possession of a cellulosic cell wall is not exclusive to land plants, and is a common feature among many algae, even the distantly related brown algae. The zygotes of the brown alga *Fucus* have provided a productive model for studying the development of polarity in plant cells (Brownlee *et al.*, 2001). Polarity is established in the free-living single-celled zygotes of this species following fertilisation, with one pole giving rise to the thallus and the other to a rhizoid that eventually forms the holdfast. There then follows an asymmetric division, producing a smaller basal cell that gives rise to all rhizoid tissues and a larger apical cell that will give rise to the thallus. Before cell division occurs, the cell produces an outgrowth at the basal pole. Colin Brownlees' group (Marine Biological Association, Plymouth, UK) have contributed greatly to this field of research, especially through the use of advanced optical methods leading to a sophisticated view of molecular mechanisms underlying the development of polarity and the generation of polarised growth in these zygotes. It was described how polarity could be determined by a range of different stimuli. Polarisation is initiated by perception of these stimuli and this is then translated to intracellular asymmetries that appear to be set up by changes in local levels of reactive oxygen species and calcium (Coelho *et al.*, 2002). Outgrowth of the cell at the rhizoid pole appears to be established by the anchoring of actin microfibrils to the cytoplasmic membrane, as well as anchorage of the membrane to the wall. This presumably then establishes sites of preferential deposition of new wall material, and indeed the site of rhizoid outgrowth appears to be characterised by the presence of sulphated fucan polysaccharides (Shaw & Quatrano, 1996).

The cell wall is central to the development of macroscopic form in plants and provides the medium through which cell-to-cell and organ-to-organ communication is mediated. The importance of this structure in a whole range of processes in plants is gradually emerging, and this role is nowhere greater than in the control of growth and development, as was made clear from the range of talks presented at this lively meeting.

Simon McQueen-Mason

CNAP, Biology Department, University of York,
PO Box 373, York, YO10 5YW, UK
(tel +44 (0)1904 328775; fax +44 (0)1904 328786;
email sjmm1@york.ac.uk)

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