

Editorial

New Phytologist performance – times are a-changing

The performance of a journal emerges from the nexus of author, reader and publisher. The author looks for the most relevant and highly ranking journal, the reader searches for topics across different journals, while the publisher aims to provide a service to the community and to maximize the publication and dissemination of high-quality research in a cost-effective manner. Rapid expansion of electronic publishing, from article submission to article downloads, has changed the nature of publishing hugely over the last 5 to 10 yr, realizing the potential for greater global authorship and readership. In 2007, for example, there were just short of one million downloads of *New Phytologist* articles: an order of magnitude increase since 2002. The number of countries submitting articles to *New Phytologist* has also increased, showing a growth of 20% between 2004 and 2007 (Fig. 1; Woodward & Ingram, 2004).

Authors seek rapid publication, a metric that is determined by the activities of the *New Phytologist* Central Office in Lancaster (UK), our Editors, Advisors and Referees, and the publishing speed of Wiley–Blackwell, our publisher. Our time from article receipt to first editorial decision has decreased from 44 days in 2002 to 28 days in 2008, reflecting the enhanced efficiency of our editorial activities. Authors may now see their article published online in just less than 2 months after acceptance. As the processing times become faster so too does the rate of manuscript submissions, which have grown markedly since 2001 (Fig. 2). Although the journal has increased in size by over 40% (just short of 250 pages per year) over the same period, and is committed to a further increase in 2009, this growth has been, by necessity, less than the growth in submissions. This means that the rejection rate has gone up. On the plus side, this has probably contributed to our increased ISI Impact Factor (Fig. 3; Thomson Corporation, 2008), bringing benefits to authors and to the journal.

The editorial workload continues to grow with the increased rate of article submissions. The New Phytologist Trust, a not-for-profit organization that owns the journal, has taken on more staff in the Central Office and has increased the size of the editorial board in order to maintain the standard of

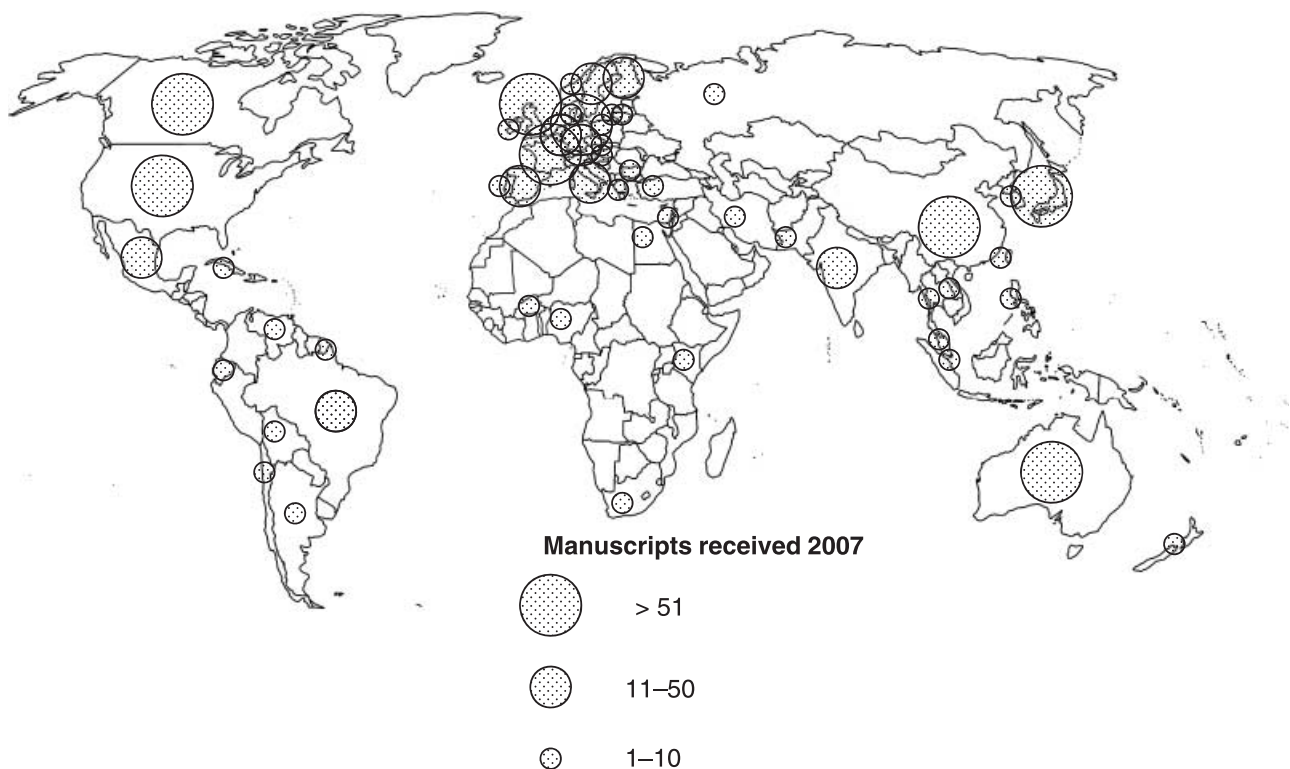


Fig. 1 Manuscript submissions to *New Phytologist* in 2007.

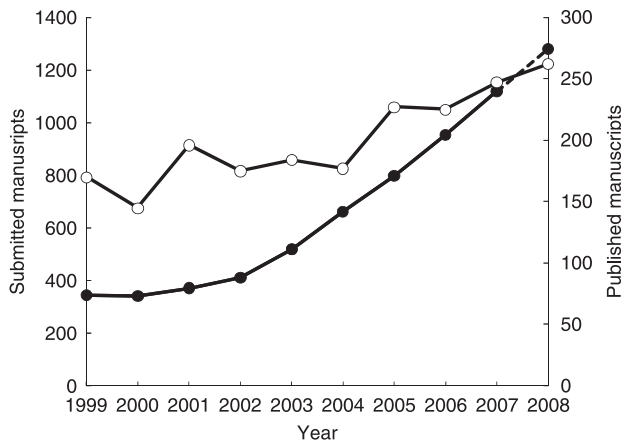


Fig. 2 Regular (article) manuscripts submitted to (closed circles) and published (open circles) by *New Phytologist*. The number of submitted manuscripts for 2008 is an estimated value based on the numbers received up to the end of October 2008.

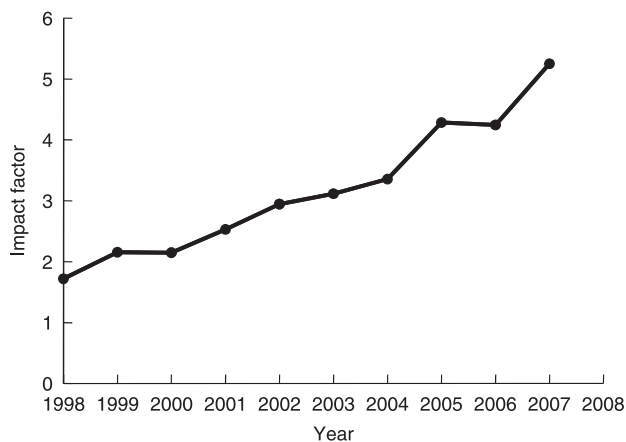


Fig. 3 *New Phytologist* ISI Impact Factor (Thomson Corporation, 2008).

service offered to our authors. The number of editors has increased by just over 40% since 2002, in keeping with the increase in journal size. We are pleased to announce that we have recently appointed André Kessler (Cornell University, Ithaca, NY, USA) and Anne Osbourn (John Innes Centre, Norwich, UK) as Editors. Both André and Anne study the interactions between plants and other organisms, but whereas André's research interests focus on the ecological consequences of plants' induced responses to herbivores, Anne's work has an emphasis on natural products and plant defence at molecular and biochemical levels.

We are continually seeking ways to manage the increased flow of submissions to the journal while maintaining the quality of service to authors. To achieve this we have made changes to our Author guidelines for 2009. *New Phytologist* submissions should still contain timely research that provides new insights into the broad principles of plant science. The original research should address clear hypotheses or questions and offer new insights on topics of interest to a broad cross-section

of our readers. Authors are now asked to answer the following questions in their cover letter:

- 1 What hypotheses or questions does this work address?
- 2 How does this work advance our current understanding of plant science?
- 3 Why is this work important and timely?

Where an Editor concludes that a submission falls outside the scope of the journal, or where the answers to the questions above are not convincing, we reserve the right to return the manuscript without review but make every effort to do this within 7 days so that authors can move forward without delay. Authors are, of course, welcome to submit a presubmission enquiry to the Managing Editor (see Author guidelines for procedure). After submission, all papers are checked to ensure that the information is in order and that the paper is complete and formatted according to *New Phytologist* style. From 2009 these checks will also include a word count, and any original research paper that exceeds 6500 words will be returned, except in exceptional circumstances, without review. The word limit refers only to the main body of the text (i.e. Introduction, Materials and Methods, Results, Discussion, Acknowledgements). We hope that our efforts to maximize the number of published papers will be matched by the Authors' commitment to write as concisely as possible.

The performance of *New Phytologist* has continued to improve over recent years, as is evident from the numbers presented here regarding submissions, processing times, increasing Impact Factor and the rapid publication of high-quality research. Yet, these exciting times bring the inevitable need for a few changes and modifications to our system to allow us to progress accordingly. We hope very much that you, as readers, authors and reviewers, will continue to support the journal in 2009 and we look forward to your future participation.

F. Ian Woodward

Editor-in-Chief

Holly Slater

Managing Editor

Ian J. Alexander

Chair of the New Phytologist Trust

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Key words: Author guidelines, electronic publishing, global authorship, Impact Factor, New Phytologist Trust, processing times, word limit.

Commentary

The dilemma of controlling heavy metal accumulation in plants

Previously it was believed that some nutrients, and especially toxic trace compounds, were accumulated passively by plants from the soil solution, largely through transpiration, entering the xylem via entirely apoplastic pathways in the root. We now know that one key process determining the accumulation of nutrients in the shoot is their loading into the root xylem through transporter proteins in the plasma membrane of the adjacent cells (Hamburger *et al.*, 2002; Takano *et al.*, 2002). Two articles in this issue of *New Phytologist* – those of Wong & Cobbett (pp. 71–78) and Wong *et al.* (pp. 79–88) – examine two metal pumps in the P-type ATPase superfamily (HMA2 and HMA4) that are known to be responsible for the loading of zinc ions (Zn^{2+}) into the xylem of *Arabidopsis thaliana* (Hussain *et al.*, 2004; Williams & Mills, 2005). Wong & Cobbett demonstrate that these metal pumps are also of primary importance for the accumulation of the toxic heavy metal cadmium (Cd) in the shoot. This is an important finding because Cd accumulation in crops such as wheat that are grown on soils containing Cd derived from the bedrock, mineral fertilizers or sewage sludge applications, has long been a major concern (Grant *et al.*, 2008). Wong *et al.* report a first *in planta* structure–function analysis of one of these proteins, *AtHMA2*. This study highlights the importance of moving beyond a heterologous or *in vitro* system for such an analysis, and provides important clues for future applications that aim to abolish root-to-shoot Cd transport while conserving transport of the essential micronutrient zinc (Zn).

‘Consequently, even mild Zn deficiency may cause significant reductions in crop yields.’

The role of HMA2 and HMA4 proteins in root–shoot partitioning of Zn and Cd

Our current model of metal homeostasis includes several critical processes that are thought to govern the accumulation

of micronutrients, such as Zn, and toxic trace elements, such as Cd, that have no known function in higher plants (Clemens *et al.*, 2002). To begin with, the often poorly soluble transition metal cations are mobilized in the soil through acidification of the rhizosphere and the release of organic chelators by the root. Then, transition metal ions or chelates are taken up into the root symplasm through transport proteins in the plasma membrane of root cells. Inside the root, metals are believed to move symplastically from cell-to-cell for passage through the endodermal cell layer, where the casparian band blocks apoplastic movement of solutes inwards into the vasculature. Along this pathway, immobilization and storage, primarily inside the vacuoles of root cells, can influence the amount of metals accumulated in the shoot (Arrivault *et al.*, 2006). For transport into the shoot, metals have to be loaded into the apoplastic xylem, where metal precipitation or binding of free metal cations to cell walls can slow down their movement into the shoot with the transpiration stream. Inside the shoot, cellular uptake of metals, distribution between cells and sequestration into storage sites are further processes necessary for metal accumulation. To date there is still little experimental evidence concerning the relative importance of each of these processes and the proteins mediating them. This is where Wong & Cobbett make some interesting contributions.

After entering a plant cell, Cd activates the synthesis of low-molecular-weight Cd ligands (named phytochelatin), of the chemical structure $(\gamma\text{-EC})_n\text{G}$, by the enzyme phytochelatin synthase 1 (*AtPCS1*) in the cytoplasm (Clemens, 2006). This promotes the immobilization of Cd in the root (Wong & Cobbett, 2008), at least partly by generating the substrate for the transport of a Cd–phytochelatin complex into root vacuoles. Root-to-shoot Cd translocation is increased by approx. 10% over the first 4 d of exposure to a nontoxic Cd concentration in a *cad1* (= *pcs1*) mutant that cannot synthesize phytochelatin, when compared with wild-type plants. Wong & Cobbett show, however, that the loading of Cd from the root symplasm into the xylem has a much larger effect on Cd partitioning between roots and shoots. In the *hma2/hma4* double mutant lacking both transport systems, Cd translocation over the first 4 d of exposure is reduced to as little as approx. 3% of that in wild-type plants. This demonstrates a strict requirement for these two HMA proteins, and thus for xylem loading of Cd, in shoot Cd accumulation. Previously it was shown that the shoot of the *hma2/hma4* double mutant displays severe Zn-deficiency symptoms when grown on a normal soil, which demonstrates that HMA2 and HMA4 are also required for root-to-shoot Zn translocation on normal soils (Hussain *et al.*, 2004; Fig. 1).

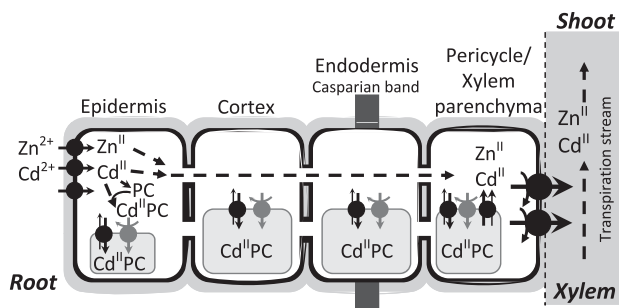


Fig. 1 Model of the role of HMA2 and HMA4 in the root of *Arabidopsis thaliana*. Zinc (Zn) and cadmium (Cd) are probably transported into the root symplast as free cations through various plasma membrane transporters. Inside the root symplast both metals are likely to be present in chelated form. Cadmium triggers the synthesis of phytochelatin (PC). Whether this occurs in a specific cell layer is unknown. The Cd–PC complexes are probably formed in the cytoplasm. Both Cd²⁺ cations and Cd–PC complexes have been proposed to be transported into root vacuoles by proton–Cd²⁺ antiport (e.g. via CAX2, Hirschi *et al.*, 2000) and possibly through ATP-binding cassette transporters, respectively. The ability to form Cd–PC complexes is associated with increased immobilization of Cd in roots (Wong & Cobbett, this issue of *New Phytologist*; pp. 71–78), but it is unknown whether this occurs in a specific cell layer. There is evidence for Cd²⁺ re-export from vacuoles into the cytoplasm by NRAMP3 in the vasculature (Thomine *et al.*, 2000, 2003). Inside the vasculature, HMA2 and HMA4 (large icons) are the principal transporters contributing to xylem loading of Zn and Cd. Note that Zn can also be transported into and stored in root vacuoles (data not shown).

HMA2 and *HMA4* are not fully redundant: *HMA4* makes a larger contribution to basic Cd tolerance than *HMA2*, although their contributions are minor compared with the major role of *CAD1* in basic Cd tolerance. Furthermore, a contribution of *HMA2* to root-to-shoot Cd transport is apparent only in the *hma4* mutant background (Wong & Cobbett, 2008). By contrast, *HMA4* of *Arabidopsis halleri* appears to have a major role in naturally selected Cd hypertolerance in this species (Hanikenne *et al.*, 2008). This difference deserves further attention.

The trace elements Zn and Cd in agriculture

Zinc is an essential micronutrient, and nutritional deficiencies of Zn are widespread, affecting at least 25%, and possibly up to 40%, of the world's population (Maret & Sandstead, 2006). The bio-fortification of crops with Zn is thus an active topic of current research (Palmgren *et al.*, 2008). In addition, large areas of agricultural soils contain suboptimal available Zn concentrations. In plants, Zn deficiency causes stunting, chlorosis and defects in pollen fertility and embryo development (Krämer & Clemens, 2006; Broadley *et al.*, 2007). Zn status is not only important for crop quality. This is highlighted by Wong *et al.* who show that a C-terminally truncated form of HMA2, which displays partial cellular mislocalization and probably possesses reduced transport activity *in planta*, is able

to rescue the shoot growth and pollen fertility defects of the *hma2/hma4* double mutant, but not the defect in embryo development, which could only be fully restored by additional application of Zn. Consequently, even mild Zn deficiency may cause significant reductions in crop yields. Increasing the Zn efficiency of crops is thus a desirable breeding goal in agriculture.

Cadmium is highly toxic at low concentrations and is known to be a carcinogen in humans. Plants can accumulate significant quantities of Cd, even from soils containing low concentrations of Cd, which may not cause toxicity symptoms in plants, but are above the concentrations considered to be safe in human food (Grant *et al.*, 2008). In plants, Cd is accumulated because it can act as a substrate for some membrane transporters of iron (Fe²⁺) and calcium (Ca²⁺) ions, and Zn²⁺. Wong & Cobbett showed that the Zn pumps HMA2 and HMA4 are necessary for Cd accumulation in shoots of *Arabidopsis thaliana*. This suggests that by inactivating the orthologues of HMA2 and HMA4 in crop plants, shoot Cd accumulation can be substantially reduced. However, this approach is not feasible because the resulting plants would suffer from Zn deficiency in above-ground organs. It is thus desirable to identify mutant alleles of *HMA2* or *HMA4* which encode a pump that can still transport Zn at high rates but that cannot transport cadmium. By conducting an *in planta* functional analysis of deletion and substitution mutants of the cytoplasmic N-terminal and C-terminal domains of HMA2, both of which contain metal-binding motifs, Wong *et al.* begin to approach this aim.

Dissecting HMA2 protein function

Wong *et al.* determined whether expression of the mutant *HMA2* cDNAs under the control of the endogenous *HMA2* promoter was able to complement various phenotypes of the *hma2/hma4* double mutant to an extent similar to that observed in the *hma4* single mutant, which contains a wild-type *HMA2* gene. Compared with *in vitro* assays using the protein produced in a heterologous system (Eren *et al.*, 2006, 2007), this approach had the advantage of using the unknown *in vivo* substrate of HMA2. The substrate of HMA2 is likely to be either a metal chelate or metal bound to a metallochaperone protein, which upon interaction with HMA2 may release the metal cation for subsequent transport. In addition, this approach tested HMA2 function in the context of possible, as-yet-unidentified, protein modifications and interacting regulatory proteins. Importantly, Wong *et al.* also analyzed whether the mutant proteins were made and where they localized in the cells – this is indispensable information for any conclusions concerning the functionality of mutant proteins in metal transport. In this manner, Wong *et al.* found that the N-terminal cytoplasmic domain and the metal-binding site therein is essential for HMA2-mediated transport of both Zn and Cd *in planta*. Previous work

indicated that these mutants possess about 50% residual transport activity *in vitro* (Eren *et al.*, 2007). Furthermore, Wong *et al.* found that the cytoplasmic C-terminus, which contains three Zn-binding sites and has been speculated to have a regulatory role (Eren *et al.*, 2006), is not essential for HMA2 function in either Zn or Cd transport *in planta*. In continuation of this work it would be interesting to know whether the C-terminus is important when plants are moved from normal to high-Zn substrates. Taken together, these studies suggest that future mutagenesis approaches aiming to separate Zn and Cd transport functions should primarily target residues in the central portion of the HMA2 protein containing the eight transmembrane helices.

In summary, the two publications by Wong *et al.* and Wong & Cobbett in this issue of *New Phytologist* are elegant, well-designed and insightful contributions to a topic of major current research interest.

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Key words: zinc, cadmium, heavy metal ATPase, CPx-ATPase, xylem loading, phytoremediation, biofortification, metal accumulation.

Precipitation variability and primary productivity in water-limited ecosystems: how plants 'leverage' precipitation to 'finance' growth

In strongly water-limited ecosystems, water to support primary productivity and other aspects of ecosystem function is, by definition, in short supply. It is widely recognized that water limitation partly reflects low total quantities of rainfall on an annual or a growing season basis. However, annual precipitation quantities represent one level in a hierarchy of temporal scales of precipitation variability (Greenland, 1999). It is becoming increasingly recognized that variation in the seasonality of precipitation, the timing between precipitation events and the

quantity of rainfall per event can be as important as annual quantity for net primary productivity and other ecosystem processes (Knapp *et al.*, 2002, 2008). This topic is particularly important in light of continuing climate change. Increases in both total rainfall amounts and in the frequency of extreme events have been documented and are likely to continue during the 21st century (Groisman *et al.*, 2005, Christensen & Hewitson, 2007, Groisman & Knight, 2008). Studies on primary productivity/precipitation relationships often focus on total above-ground net primary productivity (ANPP) because of its coupling with biogeochemical cycles and with the atmosphere. In this issue of *New Phytologist*, Robertson *et al.* (pp. 230–242) make an important contribution by going beyond total ANPP to examine the effects of interannual and seasonal variation in precipitation inputs on the productivity of three dominant plant species in a desert grassland in the southwestern USA. Their results reveal the complexity of species responses to precipitation variability, reflecting both feedback from precipitation variability on other growth-limiting resources and the life-history adaptations of these species.

‘... to understand how precipitation patterns influences the water limitation of ecosystem productivity one needs to understand the responses at the species and functional group levels.’

Robertson *et al.* studied a sotol grassland ecosystem in the Chihuahuan desert in southwestern Texas, USA. Their study site receives approx. 365 mm of mean annual rainfall, mostly in the summer and in the fall. They chose three dominant perennial plant species for study: a shrub, *Dasyliirion leiophyllum* (‘sotol’); a succulent, *Opuntia phaeacantha* (‘brown spine prickly pear’); and a grass, *Bouteloua curtipendula* (‘sideoats grama’). Several traits make these species likely to differ in response to variation in seasonality and annual amount of precipitation. The shrub *Dasyliirion* has an expansive root system (with both dense fibrous roots at the surface and well-developed deep roots), a woody caudex that can store water and reaches peak biomass during summer. The succulent, *Opuntia*, has a shallow root system (mostly 10–30 cm deep), stores water in its fleshy pads and reaches peak biomass in spring to early summer. The grass, *Bouteloua*, is a shallow, fibrous-rooted bunchgrass reaching peak biomass in mid to late summer.

Plots containing these species were subjected to precipitation treatments representing expected future precipitation patterns for the region: natural precipitation plus either supplemental winter precipitation or supplemental summer precipitation, or both. Additional control plots received only natural precipitation. These treatments were applied for 5 yr, which is itself no mean feat given the ruggedness and isolation of these ecosystems. Above-ground net primary productivity for each species was estimated several times each year, as were as soil NO_3^- and NH_4^+ , and several other parameters.

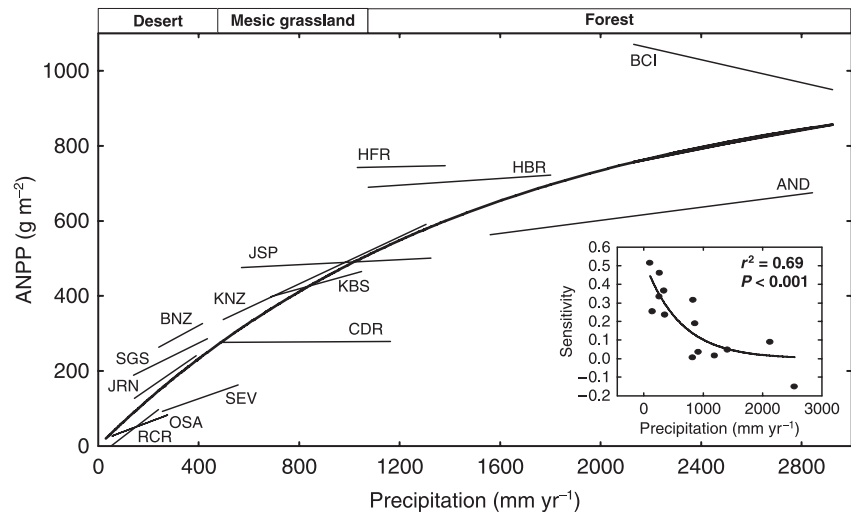
Desert grasslands would be expected to be more sensitive to interannual variation in precipitation compared with more mesic grasslands or forests (Fig. 1; Huxman *et al.*, 2004). Indeed, for all these species, Robertson *et al.* found statistically significant ($P \leq 0.10$) differences among years in ANPP. But surprisingly, there was no correlation between ANPP and annual precipitation for *Dasyliirion* or *Opuntia*, and ANPP actually decreased with increasing annual precipitation for *Bouteloua*. As these results imply, the water-supplementation treatments also had no effects on species ANPP except in *Opuntia*, where summer + winter supplementation increased ANPP over that of control plots.

Feedbacks from precipitation on resource availability

Control of ANPP for these species was not consistently the annual quantity or seasonality of precipitation inputs. This result seemed to be counterintuitive because seasonal and interannual variability is often viewed as the most relevant temporal scale of variability for net primary productivity and species changes (Schwinning & Sala, 2004). Precipitation variability also has feedbacks on other plant resources, especially nitrogen (N) availability. The microbial processes regulating nutrient availability are especially sensitive to short-term variation in soil moisture as a result of varying event patterns (Schwinning & Sala, 2004). This is because, as soils dry and rewet between rain events, the balance between mineralization and immobilization of N is altered, and soils undergoing cyclical wet–dry periods often have higher N-mineralization rates than continuously moist soils (Austin *et al.*, 2004). Thus, precipitation variability not only affects the size of the pool of water in the soil, it also affects the size of the available N pool. Further complicating matters, these two pools do not vary in synch, sometimes peaking together, sometimes peaking apart (Seastedt & Knapp, 1993), creating nonlinear dynamics in temporal trends in ANPP.

Robertson *et al.* next analyzed how precipitation pattern (number of events, event size and event interval), temperature and soil variables may have influenced each species’ ANPP in wet, average and dry years. They found some important feedbacks among precipitation variability, N availability and species ANPP. For example, control of *Dasyliirion* ANPP appeared to oscillate between N availability in wet years and

Fig. 1 Sensitivity of total above-ground net primary productivity (ANPP) to interannual variation in total annual precipitation from 14 sites with long-term ANPP records. The overall regression of ANPP vs precipitation for all sites and years combined was highly significant ($P < 0.001$). Individual sites (noted by three-letter identifiers) varied in their sensitivity to between-year variation in precipitation, with desert sites showing much higher sensitivity to precipitation than those receiving greater annual precipitation totals (inset). Site abbreviations and other details in Huxman *et al.* 2004.



water availability in dry years, with one becoming progressively limiting as the other increased; essentially the Progressive Nitrogen Limitation hypothesis (Luo *et al.*, 2004) applied to water and N instead of to CO₂ and N.

In contrast, for *Bouteloua*, the analysis suggested feedbacks among precipitation amount, pattern and N availability. In wetter-than-average years, the ANPP increased with higher soil N and longer times between precipitation events. This may reflect enhanced N mineralization from greater soil moisture variability (Austin *et al.*, 2004). Thus, *Bouteloua* also appeared to exhibit temporally shifting controls on productivity, but in this case, the feedbacks were between precipitation pattern and N, rather than between precipitation amount and N, as for *Dasyllirion*.

For *Opuntia*, the analysis suggested that ANPP was primarily regulated by precipitation seasonality and pattern rather than by N availability or other soil attributes. For example, in dry years and in years with average precipitation, the ANPP of *Opuntia* increased with small, more frequent, precipitation events and winter supplementation, and decreased in some years when long interpulse periods probably led to drying of *Opuntia*'s main rooting zone.

Resource feedbacks and plant adaptations to water-limited environments: leveraging scarce capital to support growth

Clearly then, the role of precipitation amounts and patterns and feedbacks with N availability differed for these three species, and the ability to acquire moisture differed according to each species' life-history attributes. To illustrate, consider an analogy from current world economic affairs, where a lack of available credit threatens to lower human economic productivity (recalling that both 'economy' and 'ecology' derive from the same root, the Greek *oikos*, 'house'). We can heuristically

consider the precipitation captured in the soil as 'capital', and the plants as borrowing that capital, for it is eventually returned. Plants thus become 'leveraged' to 'finance' growth. As with the current financial situation, plant 'capital' can become scarce, sometimes with little warning! The question, from the plant's perspective, is how to deal with imperfectly matched 'capital' availability, relative to when the plant most needs it. Just like businesses, plants cope with this mismatch in different ways, with some having many sources of credit to draw on, some able to maintain large capital reserves and some living with chronic cash-flow problems.

The three species in the study of Robertson *et al.* represent these three ways of coping with temporal variability in the availability of water. *Dasyllirion* exemplified the 'many sources of credit' approach. Its combination of both deep and shallow roots made *Dasyllirion* ANPP more dependent on overall levels of resource availability than on variation in precipitation pattern. The succulent, *Opuntia*, fits the second approach. *Opuntia*'s shallow root system made it more subject to 'capital' scarcity and therefore its growth was sensitive to the seasonality and pattern of precipitation. However, water storage in its fleshy pads made *Opuntia* relatively free of the influences of interannual variation in precipitation and variation in N availability. *Bouteloua*, then, suffers from cash-flow problems. A shallow root system and lack of capacity to store any 'capital' reserves made it live close to the edge in terms of access to moisture. Like a business with chronic cash-flow problems, *Bouteloua* may fail to produce at all if 'capital' becomes chronically unavailable.

The most important message of the article by Robertson *et al.* is that to understand how precipitation patterns influences the water limitation of ecosystem productivity, one needs to understand the responses at the species and functional group levels. This will help to reveal which precipitation patterns may promote species stability and which may lead to species

replacement or even cause community turnover to a fundamentally different assemblage. It is also crucial that future research addresses how temperature, CO₂, or edaphic factors may modify plant responses to precipitation variability. As precipitation is coupled with the processes controlling N availability, ecosystem responses to climate change will depend on how the strength of that coupling varies under future precipitation regimes.

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Meetings

Towards physiological sculpture of plants

19th New Phytologist Symposium: Physiological sculpture of plants, Mt Hood, Oregon, USA, September 2008

Seventy delegates gathered in an inspirational location at the foot of Oregon's highest mountain, a spectacular volcanic peak, for an intensive meeting on the translation of fundamental discoveries in plant science (<http://www.newphytologist.org/physiological/>). The subject 'physiological sculpture' was intended to connote the intentional, informed and innovative science-based design of plant functions to meet humanitarian, economic and environmental goals.

'... The historically major role of the public sector in the development of new technology for "minor" crops, and for the developing world, is not keeping pace.'

The meeting took an unusual approach. Rather than delving deeply into a single discipline or problem, the meeting brought together a wide variety of scientific leaders and broad thinkers to consider what kinds of breakthrough advances in crop improvement could be on the horizon. The meeting

focused on science and technology, not on social constraints to innovation. It also focused on what we could do with the rapidly growing knowledge of plant genomics and molecular physiology, not on the transfer of genes over long phylogenetic distances, to create new products and traits. The organizing committee consisted of Richard Jorgensen (University of Arizona, USA), Dick Flavell (Ceres Inc., CA, USA), Harry Klee (University of Florida, USA), Richard Amasino (University of Wisconsin-Madison, USA), Steve Strauss (University of Oregon, USA) and Holly Slater (*New Phytologist*). Several important observations and questions emerged from the meeting.

Where is the physiological beef?

Several delegates commented that despite the name of the conference, the participation by whole-organism crop physiologists was limited, yet much needed. This reflects a lack of connection from genetic variation all the way up to crop performance, something that systems biology approaches hope to remedy in the coming years. Other important causes of the physiology gap are the dearth of trained physiologists who can conduct research that spans molecular to ecological scales; too little open, published research on the physiology of biotech crops (transgenic and otherwise) to help to understand why and how they perform better; and the growing decline of public funding for translational genomics and biotechnology research that goes beyond *Arabidopsis* and beyond the laboratory/glasshouse. This is in no small part a result of the lack of an effective science-to-commercial pipeline; tremendous regulatory costs and marketing issues have impeded public sector scientists from developing and registering transgenic crops for commercial use. As this area develops, high-quality science that bridges the gap between the molecular and the ecophysiological/applied aspects of research will find a home in this journal. Such studies have long been an area of strong interest to *New Phytologist*, evidenced by recent reviews that have explored the scaling-up of functional genomics, the translation of genomic data for tree breeding, the applications of systems biology, and metagenomics (Wullschlegel *et al.*, 2007; Fuller *et al.*, 2008; Grattapaglia & Kirst, 2008; Martin & Selosse, 2008; Sheehy *et al.*, 2008).

Long live forward genetics

Where the goal is physiological sculpture, the tendency is to tinker with the tools (i.e. plant processes and genes) that one knows well, rather than to explore new processes and to identify new levers to control them. However, forward genetics continues to be invaluable, even in the days of fast sequencing, as we do not know what genes affect what traits, and modification of transcription alone often misses important sources of variation. Scott Tingey (DuPont Corporation, DE, USA) described a case where intensive transgenic efforts to control fatty acid composition from a known regulatory gene

failed until a forward genetics approach was employed, even though it focused on the same gene (Zheng *et al.*, 2008). The problem was that the focus of the transgenic strategy was on the modification of transcription, but the useful genetic variation was found to be manifested by a post-transcriptional mechanism. As pointed out by Tingey, as well as by John Willis (Duke University, NC, USA) and Daniel Rokhsar (Department of Energy/JGI/UC-Berkeley, USA) when discussing studies of phylogenetically novel and wild plants, the decreasing cost of genomic information allowed by rapid sequencing technologies is making forward genetics feasible in virtually any plant species. A greater degree of phylogenetic diversity in forward genetics efforts is certain to identify novel genes and metabolic circuits useful for crop genetic engineering. Some of the species being studied, like the highly outbreeding and ecologically versatile forest trees such as poplar, provide powerful means for gene identification owing to their very low linkage disequilibrium. In species with large genomes such as in polyploids, association approaches appear to be capable of highly precise gene identification because of the strong bias towards recombination in euchromatic areas.

Combating menaces

Several researchers discussed new options for improving disease resistance, which were based on increasingly detailed knowledge of plant and pest sequences, and were often facilitated by transgenic technologies. Deborah Delmer (Rockefeller Foundation, NY, USA) discussed the many possible uses of RNA interference (RNAi) to confer resistance to pests of the developing world. In addition to conferring resistance to viruses (Waterhouse *et al.*, 1998), RNAi has been recently reported to protect against nematodes and insects (Huang *et al.*, 2006; Baum *et al.*, 2007; Mao *et al.*, 2007). Brian Staskawicz (UC-Berkeley, CA, USA) discussed how our increasing knowledge of plant-pathogen interactions is guiding successful predictive resistance strategies. He illustrated how transfer of resistance (R) genes from pepper to tomato, a close relative, could provide resistance to major diseases such as bacterial spot of tomato (Tai *et al.*, 1999). Similar opportunities exist for gene transfer in other taxa. This includes Solanaceous crops such as potato, in which genes for resistance to late blight and other major diseases are known, as well as in monocots (Zhao *et al.*, 2005). Staskawicz also discussed how the mode of action of various pathogen virulence 'effector' proteins reveals critical pathogen virulence strategies. Effector proteins are secreted into host cells by pathogenic bacteria, modifying host targets to render cells more susceptible. In resistant genotypes, cognate host R genes surveil effector-mediated host target changes and reroute host pathways from a susceptible to a resistant response (for reviews, see Chisholm *et al.*, 2006; Jones & Dangl, 2006). By cataloguing the 'constellation' of pathogen effectors in naturally infected tomato plants in the field, Staskawicz is identifying conserved,

diagnostic effectors that can guide the design of resistance strategies. Based on this expanded catalogue of R-gene \times effector-gene pairs, appropriate resistance genes could potentially be chosen that confer strong and stable resistance to all major races of a pathogen. Tzvi Tzfira (University of Michigan, MI, USA) described zinc finger nucleases (ZFN), a powerful emerging technology that has a multitude of potential applications (Zeevi *et al.*, 2008). Tzfira described how ZFNs could be used as sources of resistance (e.g. Takenaka *et al.*, 2007) by designing them to target highly conserved regions in DNA plant virus genomes.

Transcription factors – use them well, use them with care

Because they control many genes and often entire pathways, transcription factor genes have been considered to be ‘low-hanging genomic fruit’, and thus several companies have focused on them in their genomics projects. Oliver Ratcliffe (Mendel Biotechnology, CA, USA) discussed Mendel Biotechnology’s identification of a transcription factor that promotes drought tolerance in *Arabidopsis* and has been demonstrated to provide drought tolerance in maize (Nelson *et al.*, 2007). The *AtNF-YB1* transcription factor gene appears to provide strong improvement of drought tolerance in maize in multiple years and locations. This is a significant advance given that approx. 70% of the world’s fresh water is used in agriculture, and it has been predicted that water will become increasingly limited in the future as a result of population growth, increased living standards and climate change. Tom Adams (Monsanto, MO, USA) described a large evaluation program at Monsanto that validated *YB1* and several other genes, including multiple transcription factors, as being useful for further development. In their ongoing studies of four commodity crops (cotton, corn, canola, soy) and 31 traits, they tested 2500 constructs in 2008. This included 330 000 field plots at 180 locations in 26 states and six countries. Thus, there is an important need to be able to screen for such genes much more efficiently, especially if these approaches are to be used in the developing world and for ‘minor’ crops such as fruits, vegetables and trees. One important consideration is that strong overexpression and suppression often results in extensive pleiotropic effects: Mike Thomashow (Michigan State University, USA) discussed how the promising *CBF* transcription factor (Cook *et al.*, 2004), which provides cold-tolerance and drought-tolerance, also stunted plants when overexpressed. More careful and subtle expression methods will be needed if genes of potential value as sculpting tools are not to be overlooked in large-scale screens and early field evaluations.

Phenotype, phenotype, phenotype

The growing disparity between the cost and speed of DNA sequencing vs that of obtaining high-quality phenotypes

was continuously highlighted by speakers as being a major impediment to functional genomics and physiological sculpture. In contrast to the extensive field approach discussed above, Pierre Lejeune (CropDesign NV, Ghent, Belgium) described a fully automated glasshouse system for evaluating new genes in transgenic rice. Because the essential unit is a potted plant that can be treated with a variety of stressors and is analyzed from top to bottom via image-analysis software (including imaging of roots through clear-bottomed pots), it could, in theory, be applied to any plant species. He reported that approximately half of the genes identified as causing a useful phenotypic change in the glasshouse evaluation also showed a similar phenotype in the field, suggesting that the great precision of the system is worth the considerable investment. Unfortunately, such systems are largely unavailable to developing-world and public sector crop scientists. Another shortcoming of most phenotyping platforms is that the only phenotypes measured are morphological, creating a huge gap between gene sequence and whole-plant physiology. DNA sequences generally do not allow prediction of protein accumulation or post-translational modifications, or address epigenetic modifications or metabolite pools. In addition to better whole-plant morphology, high-throughput methods for the rapid measurement of these molecular phenotypes would help to tie the effects of genes to crop physiology – an endeavor that is certain to require complex quantitative integration tools, now often referred to as part of ‘systems biology’.

Drowning in data

Rebecca Doerge (Purdue University, IN, USA), in discussing the growing challenges of reaching statistically sound conclusions from exponentially growing genomic data sets, indicated that the sizes of data sets are at and above the capacity of statistical theory and available software to analyze them adequately. She also emphasized the importance of precise and relevant phenotyping for statistical evaluations. Her comments provided an important contrast to those of Susan McCouch (Cornell University, NY, USA) and others who suggested that we may be in a ‘post-hypothesis’ phase of genetic biology, meaning that we may soon have sufficient data that we can simply mine it to answer any important question, obviating the need to set up specific experiments to test specific hypotheses. As discussed by Daniel Rokhsar (Department of Energy/JGI/UC-Berkeley, CA, USA), Steve Goff (University of Arizona, AZ, USA) and others, the speed, intelligence and broad accessibility of quantitative and informatic tools continue to be major impediments to high-quality genome annotation, cross-referencing of genomic information among species and translation. As explained by Goff, the United States National Science Foundation-funded ‘iPlant Collaborative’ seeks to provide new tools to overcome some of these serious problems of integration and inference (iplantcollaborative.org).

Be green, really

As the world faces the growing challenges of greenhouse-gas-mediated climate change, reduced availability of fresh water, and trade-offs between food and energy production, there will be a need for expanded and more rigorous life cycle scale evaluations of the uses of plant biotechnologies to improve the environmental footprint from agriculture. Steven Savage (Cirrus Partners, CA, USA) discussed the likelihood of increasing the market incentives to reduce net greenhouse gas emissions in agriculture and forestry, and to sequester additional carbon in agricultural soils. He cited five broad ways that biotechnologies could contribute towards achieving that end result: yield improvement, as appears to have already occurred with pest-resistant transgenic crops; soil carbon sequestration, as has occurred on a large scale using the low-till systems facilitated by herbicide-resistant soy; fertilizer optimization, through the use of crops engineered for more efficient fertilizer use (demonstrated at the research scale); adaptability, for example with crops such as the maize with improved drought tolerance discussed earlier; and sculpting soil microbial populations or root physiology to reduce nitrous oxide production. A theme that re-occurred several other times in the meeting was the inadequate degree of study of the molecular physiology of roots and of the metagenomics of root ecosystems, given their importance for adaptation and environmental mitigation. Rob Horsch (Gates Foundation, WA, USA) described how C4 rice, if achieved, would both increase rice yields and exhibit dramatically improved water-usage efficiency (Hibberd *et al.*, 2008) – suggesting that major breakthroughs of economic and environmental consequence are feasible with sufficient research. However, Steve Savage also cited several risks to society of not investing, not recognizing, or otherwise not allowing these benefits to be achieved.

Chris Somerville (Carnegie Institute of Plant Research, CA, USA) gave a broad view of biological energy systems. He discussed a number of approaches to reduce the net greenhouse gas emissions from the many different bioenergy options being studied in the large Energy Biosciences Institute that he directs (www.energybiosciencesinstitute.org). On the list of systems to avoid were those that support consumption of meat over plant sources of protein, and those that promote the use of annual crops for biological energy production over those of perennial lignocellulosic energy crops. For example, GreenWood Resources (OR, USA), a meeting sponsor, has produced very high yields in some of their trials in perennial coppice systems in poplar (Fig. 1). Somerville suggested a broad survey of species, with special emphasis on perennial grasses such as *Miscanthus* that have high water-use efficiency and require little or no nitrogen fertilizer. Some of these species are able to remobilize nutrients into the roots before harvest, and may contain nitrogen-fixing symbionts (Christian *et al.*, 2008). He also discussed the problem of potential invasiveness of new species and varieties of perennials that are grown



Fig. 1 Rapid growth of a perennial biofuels crop in GreenWood Resources' hybrid poplar nursery at Los Angeles, Chile. Shown is five months coppice growth from 2-yr-old stools (i.e. annual biomass harvest results in a proliferation of sprouts from 25-cm-high stumps, which are then reharvested in repeated 2-yr cycles). Annual yield, averaged across a group of elite clones, approximates 30 dry metric tons per hectare. Hybrid poplar is considered a premier feedstock for Chile's developing renewable biofuels industry. Photo courtesy of Carlos Sierra Silvia, GreenWood Resources, USA.

on a large scale, and stressed that candidate energy species need to be carefully evaluated in an attempt to prevent problems. He noted that nonnative species which are not interfering with native species may be preferable to native species in order to avert gene-flow issues. However, others considered that the hazard caused by the much larger degree of ecogenetic novelty from nonnative species should suggest just the opposite preference.

Sculpt forward

The large number of senior scientists, many of whom have been directly involved with public and private breeding efforts, had no shortage of examples and ideas for progress in fundamental science and its translation. There was no shortage of direct linkages made between science and the pressing needs of society as a result of population growth, resource depletion and the desire for economic development. However, a dark recurring theme was that – in stark contrast to the extraordinary progress being made by the large companies on the major commodity crops – the public sector is falling further and further behind in translation. The historically major role of the public sector in the development of new technology for 'minor' crops, and for the developing world, is not keeping pace. This is a result of declining investment, increasing intellectual property-right hurdles and the resulting lack of institutional capacity and will to deal with complex genomic technologies in translation, especially transgenic ones. Bob Goldberg (UC-Los Angeles, CA, USA), in an animated

lecture, emphasized the extraordinary diversity of transgenic innovations that have already been produced, but that they are a tiny fraction of those on the market – largely a result of the huge social obstacles that prevent commercialization of any but blockbuster traits in the major commercial species. Organizations such as the Public Intellectual Property Resource for Agriculture (www.pipra.org) are trying to help, but need large increases of funding, strong institutional collaboration to support and release useful products, and much more participation from the scientific community.

Surprisingly, however, the method of sculpture *per se* barely arose as a scientific issue at the meeting. Both marker-informed conventional and transgenic methods could be useful for nearly all trait targets. The choice of which to use would depend on a range of species, trait, environment, economic, humanitarian and efficiency issues that are idiosyncratic to each application. All would have benefits and potential drawbacks. This contrasts with the extreme polarization in society about the use of transgenic methods. There appears to be an almost complete avoidance of most transgenic methods in breeding outside the major commodity crops in a handful of countries. This is a result of the added regulatory and intellectual property costs, as well as the marketplace and brand-risk issues they bring. Although the meeting did not focus on social issues, it would appear, from the lectures and discussions, that the large majority of attendees implicitly agreed that given the pressing challenges facing humanity and the global environment, all tools should be brought to the table. The extreme attention being paid by society to the method vs the ends is at best penny-wise, and most clearly pound-foolish.

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