

Commentary

Ecology of metal hyperaccumulation

Research into the accumulation of extraordinary levels of metals by plants – hyperaccumulation (Brooks *et al.*, 1977) – has moved from exploration through investigations of mechanisms to exploitation. During the past decade, in addition, the ecological consequences of metal hyperaccumulation began to attract attention. Ecological studies of metal hyperaccumulation have been designed to: provide insight into how and why metal hyperaccumulation has evolved by determining its adaptive value; and to examine how the extraordinary metal concentration of hyperaccumulators impacts species relationships in the habitats in which hyperaccumulators have evolved. In this issue, Hanson *et al.* (pp. 655–662) make important contributions to our understanding of the ecology of Se hyperaccumulation.

Hyperaccumulation – discovery to ecology

Plant bodies contain many elements, some in relatively large amounts, some in trace quantities, and others in between. The familiar lists of plant macro- and micronutrients, derived from centuries of research on plant nutrition, show the importance of various elements to plant growth. Thus, we have extensive data that define the ‘normal’ concentrations for many elements found in plant tissues. Some plants, however, contain unusually large concentrations of certain elements on a dry mass basis. Termed hyperaccumulators because of their extraordinary level of accumulation (Brooks *et al.*, 1977), the threshold that defines hyperaccumulation depends on the element involved. Hyperaccumulation thresholds for the best studied metals are: 10 000 $\mu\text{g g}^{-1}$ of Mn or Zn; 1000 $\mu\text{g g}^{-1}$ of Ni, Cu or Se; and 100 $\mu\text{g g}^{-1}$ of Cd, Cr, Pb or Co (Reeves & Baker, 2000). Other less studied metals, including Al (Jansen *et al.*, 2002) and As (Ma *et al.*, 2001; Meharg, 2002), have hyperaccumulation thresholds of 1000 $\mu\text{g g}^{-1}$.

The unusual elemental composition of hyperaccumulators has attracted considerable scientific interest (Cobbett, 2003; Kraemer, 2003; Macnair, 2003). Some researchers have focused on identifying new species of hyperaccumulators through botanical explorations. For example, research in Cuba (Reeves *et al.*, 1996) has discovered more hyperaccumulators of Ni than have been found in any other location to date. Most hyperaccumulators are found on soils that are unusually rich in metals, such as ultramafic soils, and Cuba contains

a number of ultramafic soil areas (Reeves *et al.*, 1996). Other researchers have explored the physiological mechanisms whereby hyperaccumulators acquire, process, and sequester these normally toxic metals (Salt, 2001). Still others are examining the usefulness of these species for phytoremediation, cleaning up metal contaminated sites (McGrath & Zhao, 2003), or for phytomining, using them to mine metals from high metal soils (Anderson *et al.*, 1999).

During the past decade, a fourth area of interest came to the fore: ecology. Hanson *et al.* have looked at Se. Most plant species contain little Se, usually less than 1.5 $\mu\text{g g}^{-1}$ (Reeves & Baker, 2000). However, plants containing unusually elevated levels of Se have been known from deserts of the United States since at least the 1930s (Rosenfeld & Beath, 1964). Originally attracting attention because of their toxic effects on livestock (Rosenfeld & Beath, 1964), they were later studied by physiologists interested in the mechanisms of Se metabolism (Shrift, 1972). Recently, these plants have received attention for their potential use in phytoremediating Se-contaminated soils (Bañuelos, 2001). Despite this attention, study of the ecology of Se hyperaccumulation has languished until now. As recently reported in *New Phytologist*, Hanson *et al.* (2004) showed that Se hyperaccumulated by *Brassica juncea* was toxic to an invertebrate herbivore and to fungal pathogens. In their current paper, they extend these results to include defensive effects against phloem-feeding aphids. Together, these papers illustrate how studies of hyperaccumulator ecology can illuminate the ecological function and evolutionary value of metal hyperaccumulation.

Functions of metal hyperaccumulation

Hyperaccumulation has been hypothesized to perform several functions in hyperaccumulator species. Tests of these hypotheses seek to demonstrate an adaptive value of hyperaccumulation and to explain how hyperaccumulation may have evolved. As with any discussion of function in an evolutionary context, it must be kept in mind that the current function of a trait may not be that for which the trait originally evolved (the latter being adaptation *sensu stricto*). If the former is the case, then the term exaptation may be applied to the trait (Gould & Vrba, 1982). Separating adaptation from exaptation is extraordinarily difficult and would be greatly aided by study of the phylogeny of hyperaccumulation, which is only just getting under way (Broadley *et al.*, 2001; Mengoni *et al.*, 2003).

An early review (Boyd & Martens, 1992) summarized four postulated benefits of metal hyperaccumulation in plants, which during the last decade have begun to be tested

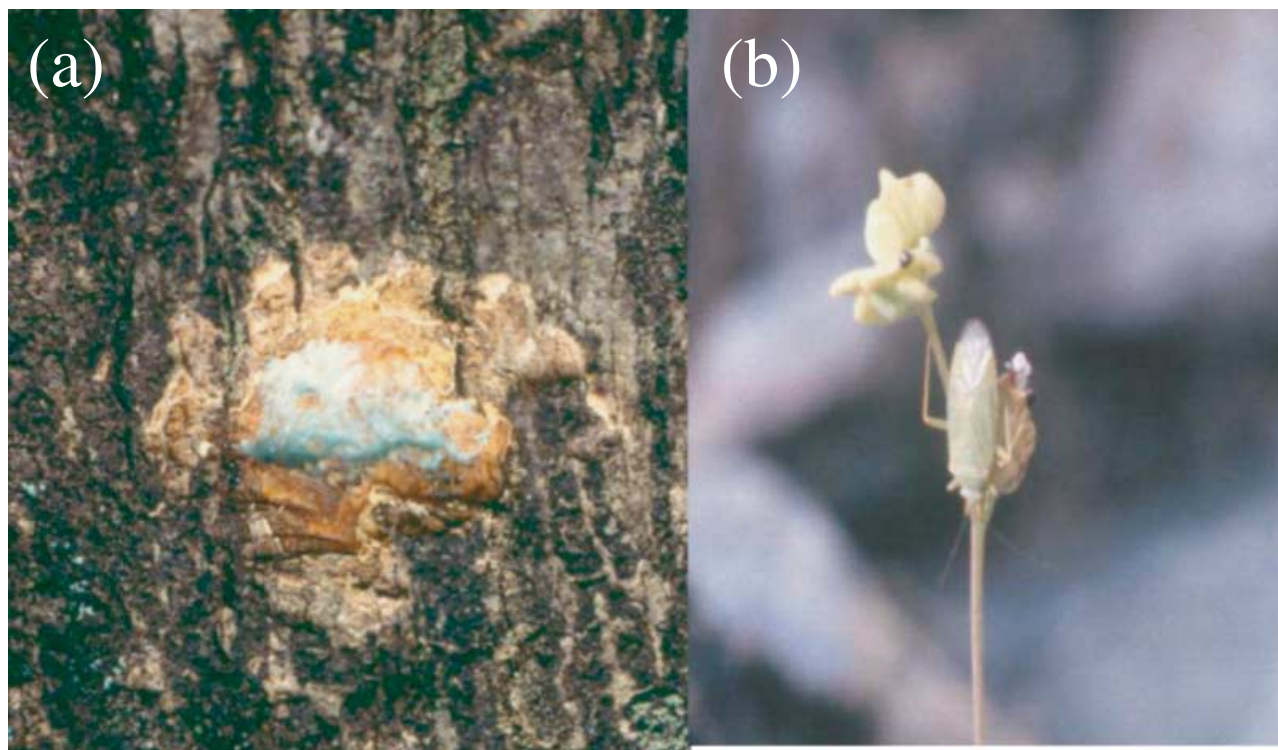


Fig. 1 An Ni hyperaccumulating plant and a high Ni herbivorous insect. (a) the New Caledonia Ni hyperaccumulator *Sebertia acuminata* produces a latex with extraordinarily elevated Ni concentration (up to $260\,000\ \mu\text{g Ni g}^{-1}$ dry mass: Jaffré *et al.*, 1976). This large quantity of Ni gives the latex its bluish cast. (b) The high Ni mirid bug *Melanotrachus boydi* on a flowering stem of the California Ni hyperaccumulator *Streptanthus polygaloides*. This specialist insect, found only on this hyperaccumulator species, was unknown to science prior to insect surveys by Wall & Boyd (2002), illustrating the potential of studies of hyperaccumulator ecology to yield unique discoveries.

experimentally. First, the tolerance/disposal hypothesis suggested that hyperaccumulation is a mechanism that allows sequestration of metals in tissues (tolerance) and, in some cases, elimination of metals from the plant body by the shedding of those high metal tissues (disposal). A recent genetic analysis showing that Zn tolerance and Zn hyperaccumulation are decoupled (Macnair *et al.*, 1999) argues against the tolerance portion of this hypothesis, and there is also little evidence to support the disposal portion (Boyd & Martens, 1998). Second, the interference hypothesis suggests that perennial hyperaccumulator plants enrich the surface soil under their canopies by production of high-metal litter to prevent establishment of less metal tolerant species. This hypothesis, re-named the elemental allelopathy hypothesis by Boyd & Martens (1998), remains untested, although Boyd & Jaffré (2002) showed that surface soil metal levels are elevated under canopies of the long-lived Ni hyperaccumulator tree species *Sebertia acuminata* (Fig. 1a). Third, the drought resistance hypothesis states that hyperaccumulated metal may help hyperaccumulators withstand drought. Evidence bearing on this hypothesis is scarce, but elegant manipulative experiments by Whiting *et al.* (2003) found no evidence that Ni or Zn hyperaccumulator status provided increased drought resistance to the hyperaccumulators *Alyssum murale* and *Thlaspi*

caerulescens. Finally, the defence hypothesis suggests that elevated metal concentrations in plant tissues protect plants from certain herbivores or pathogens. It is this hypothesis that has attracted the most research and about which there is both current debate and continuing exploration.

Hyperaccumulation as an elemental defence

Plant chemical defences (e.g. alkaloids, terpenes, glucosinolates) are generally derived from photosynthate, but hyperaccumulated metals represent a suite of defences, termed elemental defences (Martens & Boyd, 1994), derived from soil minerals. Martens & Boyd (1994) suggested that elemental defences differed from secondary (organic) chemical defences because: their toxic principle is an element taken up from the soil rather than one derived from photosynthate; and they could not be biochemically degraded by the chemical counterdefences of herbivores because of their elemental nature. The vast majority of experimental investigations regarding this hypothesis have focused on hyperaccumulators of Ni or Zn (Boyd, 1998). Defensive effects have been shown in some cases (Boyd & Martens, 1994; Boyd *et al.*, 1994; Pollard & Baker, 1997; Jhee *et al.*, 1999; Boyd *et al.*, 2002) but not others (Boyd *et al.*, 1999; Huitson & Macnair, 2003).

In two cases, hyperaccumulation status actually promoted attack: in one case by a pathogen (Turnip Mosaic Virus; Davis & Boyd, 2001) and in another by herbivorous snails (Hanson *et al.*, 2003). These results show that, like other plant defences, elemental defences are not absolute and provide protection against only some of the myriad enemies that plants face in natural situations. The paper by Hanson *et al.*, in this issue, along with earlier work by these authors (Hanson *et al.*, 2003), explores a third element hyperaccumulated by plants and shows evidence of defensive effects against two invertebrate herbivores and two fungal pathogens. Unlike earlier work with Ni hyperaccumulation, which failed to show defensive effects against phloem-feeding aphids (Boyd & Martens, 1999), Hanson *et al.* show that aphids are susceptible to Se-based elemental defences. This illustrates that elements may differ in their defensive outcomes against herbivores with particular feeding strategies. Realization of this point should stimulate additional investigations into the postulated defensive nature of each of the metals hyperaccumulated by plants, using herbivores that represent each of the various modes of herbivory (e.g. folivores, phloem feeders, xylem feeders) as well as examination of specialist and generalist herbivores.

Elemental defence below the hyperaccumulation threshold

Most studies of the defence hypothesis have contrasted herbivore/pathogen response to hyperaccumulating and nonhyperaccumulating plants. However, tantalizing evidence hints that defensive effects of metals may extend to concentrations far below the minima used to define hyperaccumulation (Boyd & Martens, 1998). For example, in this issue Hanson *et al.* show that Se is toxic to aphids at a leaf concentration of $125 \mu\text{g g}^{-1}$ and that a sublethal effect (reduced aphid population growth rate) was observed at only $1.5 \mu\text{g g}^{-1}$ Se. This latter effect occurred for an Se concentration that is at the upper boundary of the normal range of Se in plant tissues (Reeves & Baker, 2000).

This evidence suggests that elemental defences may be effective at levels below those used to define hyperaccumulation. If this is indeed the case, then elemental defences may be more widespread in natural communities than previously suspected (Boyd & Moar, 1999). In an interesting twist, studies of the ecology of metal hyperaccumulation may in turn influence the threshold values used to define hyperaccumulation. In the case of Se, Reeves & Baker (2000) stated that reports of Se toxicity to grazing animals at levels less than $1000 \mu\text{g g}^{-1}$ are one reason for their suggestion that the threshold value for defining Se hyperaccumulation be lowered to $100 \mu\text{g g}^{-1}$.

The possibility of a selective effect of metals at concentrations below the hyperaccumulation level is exciting because these effects may reveal mechanisms whereby metal

hyperaccumulation has evolved. Any ecological benefit of elevated metal concentrations, whether herbivore defence, elemental allelopathy or another, could provide a basis for the progressive evolution of still greater metal concentrations. Thus, studies of hyperaccumulator ecology may help illuminate the evolutionary basis of metal hyperaccumulation.

The trophic ecology of hyperaccumulation

It is important to realize that the question of *why* hyperaccumulation has evolved is separate from questions regarding the *consequences* of this trait for other species in hyperaccumulator habitats. Regardless of why plants hyperaccumulate, elevated metal levels in plant tissue will have consequences for other organisms that share those habitats.

The elemental defence hypothesis predicts that metal hyperaccumulation will negatively affect at least some herbivores/pathogens in a given habitat. However, by various means (Boyd & Martens, 1998), others will be unaffected by elemental defences. A field study of the California Ni hyperaccumulator *Streptanthus polygaloides* showed that mammalian herbivores damaged high-Ni plants, probably because their generalist diet diluted hyperaccumulator foliage with that of low-Ni species (Martens & Boyd, 2002). Specialist herbivores have also been found that evolved in association with hyperaccumulators and feed on these plants without harm. Examples discovered to date include the mirid bug *Melanotrichus boydi* (Fig. 1b) feeding on *S. polygaloides* (Schwartz & Wall, 2001) and the chrysomelid beetle *Chrysolina pardalina* feeding on the South African Ni hyperaccumulator *Berkheya coddii* (Mesjasz-Przybyłowicz & Przybyłowicz, 2001).

Specialist herbivores that consume metal hyperaccumulators without harm are of great scientific interest for three reasons. First, their ability to consume high metal tissues implies they possess adaptations that may give insights into metal tolerance mechanisms in animals (Przybyłowicz *et al.*, 2003). Second, coevolution between hyperaccumulators and metal tolerant herbivores could have accelerated the evolution of metal hyperaccumulation by plants. The stepwise interplay between plant defences and herbivore counterdefences, considered a biochemical 'arms race' (Kareiva, 1999), helps to explain the diversity of plant chemical defences. In a similar fashion, we can hypothesize that coevolution mechanisms may have fostered the extremely elevated metal concentrations that characterize metal hyperaccumulators. Pollard (2000) suggested that our ability to address coevolutionary questions about metal hyperaccumulators and their specialist herbivores constituted a valuable model system. The third reason is that elevated metal levels in adapted herbivores may have consequences for organisms at other trophic levels in the food webs of ultramafic communities. Some hyperaccumulator-specific herbivores have relatively elevated whole body metal concentrations. *Melanotrichus*

boydi (Fig. 1b) averages 780 $\mu\text{g Ni g}^{-1}$, and work in progress in South Africa by our lab has documented an insect herbivore containing an astounding 3500 $\mu\text{g Ni g}^{-1}$. Such elevated metal levels may have consequences for organisms at other trophic levels in food webs that contain hyperaccumulators and their specialist herbivores.

One ecological consequence of the existence of high metal herbivores is that metal may influence the herbivore's interactions with other species, such as predators or pathogens. Organic plant defences sequestered by some tolerant herbivores can chemically defend them against predators (Termonia *et al.*, 2002). We investigated this question using *M. boydi*, with mixed results. Tests involving three arthropod predator species fed either high Ni *M. boydi* or low Ni prey showed no effect in two cases but significantly higher mortality for a thomisid spider when fed *M. boydi* (Boyd & Wall, 2001). Another test, using entomopathogenic bacteria and nematodes as example pathogens, showed no beneficial effect of the high Ni concentration of *M. boydi* on insect mortality rates (Boyd, 2002). Further research with insect herbivores containing greater amounts of Ni or other hyperaccumulated metals should be conducted to determine the generality of these results.

The potential toxicity of high metal insects to predators may also raise environmental concerns about applied uses of hyperaccumulators for phytoremediation or phytomining. For example, toxicological studies have shown that vertebrates can be negatively affected by a diet containing > 500 $\mu\text{g Ni g}^{-1}$ (Outridge & Scheuhammer, 1993), so that consumption of high-Ni insects could harm birds or other animals if large numbers were consumed.

Hyperaccumulators may also affect ecosystem level processes by mobilizing metals into food webs. Hyperaccumulators are generally minor vegetation components in most European and North American habitats, but can be relatively abundant in some locations in New Caledonia, Cuba and South Africa. In these situations, significant quantities of metals may be mobilized into food webs. A pioneering study (Peterson *et al.*, 2003), performed in a Portuguese ultramafic community containing only a single Ni hyperaccumulator species (*Alyssum pintodasilvae*), showed that hyperaccumulators can act as gateways for metals into food webs. Predator assemblages from communities containing the hyperaccumulator showed significantly elevated Ni concentrations compared to those from communities lacking the hyperaccumulator. Other ecosystem level consequences have scarcely been articulated, much less studied. For example, the influence of high metal (hyperaccumulator) litter on decomposer communities and nutrient cycling rates (Boyd & Martens, 1998) is as yet virtually unexplored.

Perspectives

Study of metal hyperaccumulator ecology is in its infancy, yet this area of research promises to reveal exciting biological

phenomena. Research to date has focused mainly on Ni and Zn hyperaccumulation, but the ground-breaking research of Hanson *et al.* has begun to explore the ecology of Se hyperaccumulation. It is clear that much research remains to be conducted: we have multiple functional hypotheses to test regarding multiple hyperaccumulated metals. It is likely that some hypotheses will be validated for one metal but not another and, for a given hypothesis (such as the defence hypothesis), the result may be dependent on the ecological specifics of both the plant species and the herbivores involved. These questions are important because they provide an ecological context for molecular/physiological studies as well as for studies that are exploring the uses of metal hyperaccumulators for phytoremediation/phytomining. Research on hyperaccumulator ecology promises more fully to illuminate a fascinating plant trait and to extend our knowledge of hyperaccumulation across the biological hierarchy from molecules to ecosystems.

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Key words: elemental defence, herbivore defence, metal hyperaccumulation, predation, trophic webs.

Coordinating stomatal and xylem functioning – an evolutionary perspective

Xylem cavitation has been recognized as a threat to water transport since the cohesion-tension theory was first proposed over 100 years ago. John Milburn was probably the first to postulate a link between control of xylem cavitation and stomatal regulation (Milburn, 1973). The concept is simple: it is mal-adaptive for stomata to permit the transpiration stream to dry itself up by cavitation. Avoiding this fate requires coordination between stomatal regulation and the process of cavitation so that the demand for water by the foliage does not exceed the xylem's supply capacity (Box 1). An efficient match of supply and demand has the stomata maximizing CO_2 uptake by pushing the xylem to its carrying capacity (Jones & Sutherland, 1991). Just as a rope will hold the most weight while its fibers are yielding to the verge of mechanical failure, so will the xylem achieve its greatest flow rate when its conduits have cavitated to the verge of complete blockage (Fig. 1). Exploiting this strategy for getting the most out of xylem transport requires rapid stomatal responses to avoid hydraulic failure, and a mechanism for daily reversal of cavitation so that the feat can be repeated. Brodribb & Holbrook (see pp. 663–670 in the issue) are the first to take a comparative and evolutionary perspective on the coordination of stomatal and hydraulic conductances in plants.

'The hallmark of important results is that they expose new avenues of investigation'

Ancestral stomatal regulation – club mosses and ferns

Brodribb & Holbrook compared seedless vascular plants (species of Lycophyta and Pteridophyta) with selected flowering plants from the same seasonal dry forest in Costa Rica. They found dramatic differences in the coordination of stomatal vs hydraulic conductances. The angiosperms closed their stomata at lower (more negative) values of leaf water potential (Ψ) and much higher values of leaf xylem cavitation – sufficient to drop the hydraulic conductance of the leaf by over 50%. This is consistent with a tight coordination between stomatal regulation and hydraulic failure – a riskier, but presumably more efficient, match of supply with demand (Fig. 1, narrow

safety margin, A). By contrast, the seedless vascular plants closed their stomata at relatively high leaf Ψ and at less than 10% drop in hydraulic conductance from cavitation (Fig. 1, broad safety margin, P). This indicates a much more conservative strategy – keeping demand well below levels that would challenge supply. The striking difference between the groups was in their stomatal regulation rather than xylem traits because the comparison groups had similar resistances to cavitation.

The implication is that the ancestral state is a conservative pattern of stomatal regulation which keeps xylem pressures from rarely if ever causing cavitation. As the authors point out, such large safety margins from hydraulic failure would compensate for sluggish stomatal responses to vapor pressure deficit, changing plant conductance, and soil moisture. Walking the tightrope between maximizing gas exchange and avoiding hydraulic failure requires rapid stomatal responses to these factors. Furthermore, large safety margins in seedless vascular plants would be necessary in the absence of efficient cavitation repair mechanisms. If cavitation is forever, then its prevention becomes much more imperative. Many seedless vascular plants do show root pressure and thus have the potential to repair cavitation when soil is wet and transpiration is minimal (Sperry, 1983). However, xylem refilling has

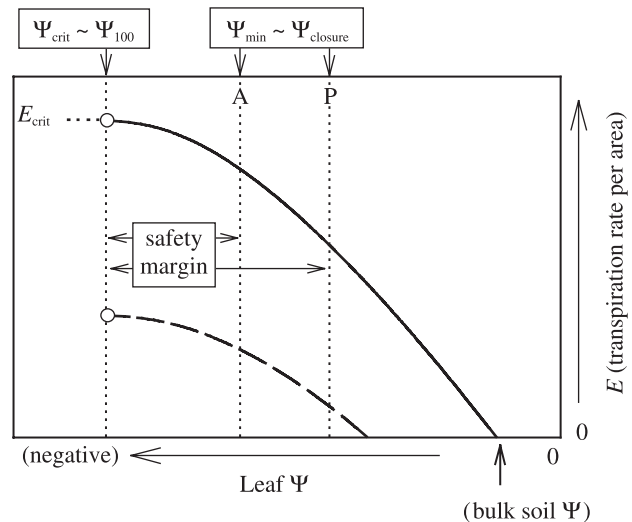


Fig. 1 Theoretical trajectory of steady-state transpiration rate (E) vs leaf water potential (Ψ ; see Box 1). Loss of hydraulic conductance with negative Ψ leads to a maximum sustainable $E = E_{crit}$ which is associated with a Ψ on the verge of complete loss of hydraulic conductance (Ψ_{crit}). If cavitation is limiting, Ψ_{crit} will approximate the Ψ causing 100% loss of hydraulic conductance (Ψ_{100}). The safety margin from hydraulic failure is the difference between the minimum regulated leaf Ψ (Ψ_{min}) and Ψ_{crit} . Brodribb & Holbrook estimate Ψ_{min} from the Ψ associated with complete stomatal closure in detached leaves ($\Psi_{closure}$) and found larger safety margins for seedless vascular plants (P) than in angiosperms (A) from the same habitat. Modified from Sperry *et al.* (2002).

Box 1 Theory of hydraulic limitations

If one could make a plant transpire at any rate E , the trajectory of E vs leaf water potential (Ψ) should look something like Fig. 1 (solid curve; Sperry *et al.*, 2002). When E is zero, the leaf Ψ would equal the bulk soil Ψ (ignoring gravitational effects). As E is increased the Ψ will drop disproportionately because the hydraulic conductance of the flow path declines with increasingly negative Ψ . There are two well understood reasons for this decline: cavitation in the xylem, and soil drying in the rhizosphere between bulk soil and the root surface. Although there may be additional changes in hydraulic conductance with E , such as variable aquaporin activity in root or leaf membranes (Henzler *et al.*, 1999), or variable KCl concentration in xylem sap (Zwieniecki *et al.*, 2001), the Ψ -dependence of these factors is not well characterized, as opposed to the inevitable physical processes of rhizosphere drying and xylem cavitation.

The E vs Ψ trajectory cannot go to infinity, but has a maximum steady-state value of E : E_{crit} (Fig. 1, open symbols) with an associated Ψ_{crit} . Any higher steady-state rate of E is impossible, because the drop in pressure drives the remaining hydraulic conductance in the bulk soil-leaf pathway to zero, breaking apart the hydraulic continuum. The critical values of E and Ψ describe a physical boundary to gas exchange with respect to soil and plant hydraulics. Transpiration and plant Ψ must be regulated to stay within these physical limits or else canopy desiccation will occur. A drier soil will have a lower Ψ intercept and a flatter E vs Ψ trajectory with a lower E_{crit} (Figure 1, dashed curve). Maximizing gas exchange while avoiding hydraulic failure means operating on the edge of dysfunction, and requires rapid stomatal control of E to prevent it from exceeding E_{crit} .

Under many situations, xylem cavitation is more limiting than rhizosphere drying. In these cases, Ψ_{crit} approximates the Ψ causing 100% loss of hydraulic conductance (Fig. 1, $\Psi_{crit} \approx \Psi_{100}$). Brodribb & Holbrook estimate safety factors from hydraulic failure by measuring the leaf Ψ in detached leaves at full stomatal closure. This Ψ represents the lowest leaf Ψ permitted by stomatal regulation (vertical $\Psi_{min} \approx \Psi_{closure}$ line). The difference ($\Psi_{min} - \Psi_{crit}$) gives the safety margin in terms of leaf Ψ (Fig. 1, safety margin). Seedless vascular plants had broader safety margins than angiosperms from the same habitat, as shown diagrammatically in Fig. 1. The difference was primarily due to a less negative $\Psi_{closure}$ in the seedless vascular plants (Fig. 1P) vs angiosperms (Fig. 1A). The vulnerabilities to cavitation (and hence Ψ_{crit}) did not differ systematically between the two groups. Note that Brodribb & Holbrook report safety margins slightly differently – as ($\Psi_{closure} - \Psi_{50}$), where Ψ_{50} is the leaf Ψ at 50% loss of conductivity.

not been demonstrated in these basal vascular plants, and it is unknown whether they can reverse cavitation under transpirational conditions as seems to occur in many flowering plants (Bucci *et al.*, 2003).

Gymnosperms

These conclusions are consistent with observations from many gymnosperms, strengthening the notion that large safety factors from hydraulic failure may be an ancestral condition. To mention a few examples, *Pinus taeda*, *P. ponderosa* and *P. edulis* all close their stomata at approx. -2 MPa, while their stem xylem does not even begin to cavitate until -3 MPa or below (Linton *et al.*, 1998; Hacke *et al.*, 2000; Hubbard *et al.*, 2001). Like seedless vascular plants, under well-watered conditions, these conifers maintain enormous safety margins from cavitation. A difference is that these trees have considerably greater resistance to cavitation than the seedless vascular plants studied by Brodribb & Holbrook, which were completely cavitared by -3 MPa. The quantum leap in cavitation resistance may be related to the evolution of torus-margo pit membranes in gymnosperms, which appears to afford much lower flow resistance for the same protection from cavitation as the homogenous type of pit membrane in other groups (Hacke *et al.*, 2004). It should be noted that while large safety factors accompany well-watered conditions, drought does cause significant root cavitation which can severely limit gas exchange in conifers (Linton *et al.*, 1998; Hacke *et al.*, 2000).

Evolution of xylem and specialized stomatal physiology

Brodribb & Holbrook's study makes one realize that the evolution of more specialized stomatal physiology is just as important as the much better-documented trends in xylem evolution. The evolution of vessels, in particular, is often cited as important for the tremendous success of angiosperms. However, vessels have also evolved in Permian Gigantopterids (Li *et al.*, 1996), ferns (Carlquist & Schneider, 1997), and Gnetophytes (Doyle, 1998), yet these groups never achieved the dominance of angiosperms. Furthermore, some early angiosperm lineages appear to have actually lost vessels (Feild *et al.*, 2002). Finally, vessel-bearing wood is not necessarily more efficient on a conductivity per area basis, as Brodribb & Holbrook show in their comparison of ferns vs angiosperms. Vessels by themselves may be only half the story. It seems possible that the additional evolution of rapid stomatal response mechanisms could provide a crucial competitive advantage by allowing better optimization of the water-for-carbon trade-off. Physiologists tend to neglect the basal vascular plants, but studying the comparative physiology of these organisms is important for a better understanding of macro-evolutionary trends in plants.

Perspectives

Brodribb & Holbrook provide a valuable 'snapshot' assay of safety factors from excessive cavitation across diverse growth

forms, using the leaf Ψ at stomatal closure in detached leaves as a proxy for minimum leaf Ψ (Box 1). In following up on this work it would be useful to determine the *in situ* safety factors throughout the active growing period and also to discriminate between the importance of phylogenetic background vs growth form. The seedless vascular plants could be conservative in stomatal regulation because of their basal phylogenetic position, or because their growth form is more compatible with a water-miser strategy where conserving water is more important than maximizing gas exchange. A comparison of palms and tree ferns in similar habitats, for example, would minimize differences in growth form and circumstances that could select for different stomatal regimes independently of phylogenetic background. The hallmark of important results is that they expose new avenues of investigation.

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Key words: xylem cavitation, stomatal regulation, water transport, hydraulic architecture, seedless vascular plant physiology.

Letters

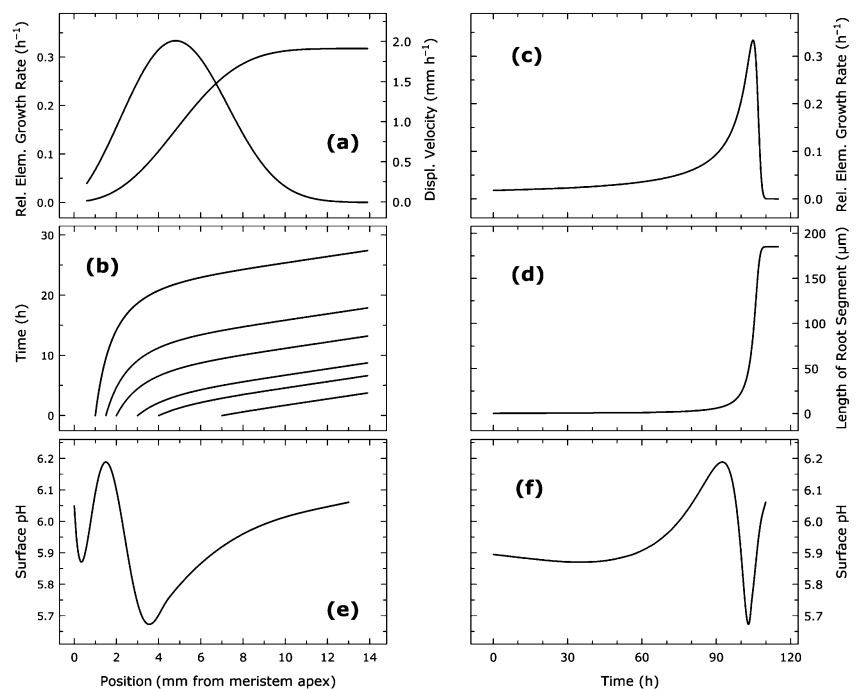
Growth rate gradients and extracellular pH in roots: how to control an explosion

As plant growth is restricted to defined zones of growing organs, the methodologically consistent analysis of spatial growth rate patterns in organ growth zones is prerequisite for studies into the regulation of cell growth *in situ* (Silk, 1984). The elongation zones at the tips of growing roots are classical objects of kinematic growth analysis, which aims at the quantitative description of growth rate gradients (Erickson & Sax, 1956). Root growth zones were generally believed to be characterized by growth rates that change gradually along bell-shaped growth gradients (Erickson & Sax, 1956; Sharp *et al.*, 1988; Beemster & Baskin, 1998; Peters & Felle, 1999; Walter *et al.*, 2003). However, recent kinematic growth analyses of dramatically increased spatio-temporal resolution indicated that root growth zones consist of two distinct domains with different but constant growth rates (van der Weele *et al.*, 2003). In this biphasic model of the root growth zone, cells elongate at a low rate as long as they are located close to the meristem, and then suddenly, not gradually, switch into

a rapid growth mode. I here suggest that previous results on the correlation of growth and cell wall pH can be combined with the biphasic growth zone model to form a plausible hypothesis on the role of pH-mediated growth mechanisms in root cell development.

To many researchers, the common bell-shaped growth profiles seemed to suggest that cells undergo a smooth, gradual development of growth rates as they traverse the growth zone. This idea belies the dramatic dynamics of the process, as I will show using data obtained previously by a classical approach (Peters & Felle, 1999). In that study, we had combined kinematic growth analysis with high-resolution measurements of surface pH in maize (*Zea mays* L.) primary roots, focussing on the correlation of growth rates and extracellular pH in individual plants. To create a generalized growth model, I chose the 11 (out of 16) datasets from marking experiments in roots growing under what we had defined as standard conditions (root medium: KNO₃, NaCl, CaCl₂ at 1 mM each; 0.5 mM MES pH 6.75; 22°C), that had survived rigorous tests for analytical consistency (Peters & Felle, 1999). The datasets were pooled, and a modified Weibull equation (Peters *et al.*, 2001) was fitted. From the resulting mean profile of relative elemental growth rate (REGR) along the root tip (Fig. 1a), the profile of displacement velocities (DV; the integral of the REGR profile; Fig. 1a), and

Fig. 1 Kinematic characterization of elongation growth and surface pH in maize primary roots. (a) Profiles of relative elemental growth rate (REGR) and displacement velocity (DV) along the growing root tip. (b) Trajectories of root elements in a plot of time vs position. (c) Time-course of relative elemental growth rate of a root element located at 0.2 mm above the root apex at time 0. (d) Time-course of elongation of a root segment with a length of 0.25 μm at time 0, when the distal margin of the segment is located at 0.2 mm above the root apex. (e) Profile of surface pH along the growing root tip at a bulk medium pH of 6.75. (f) Time-course of surface pH of a root element located at 0.2 mm above the root apex at time 0.



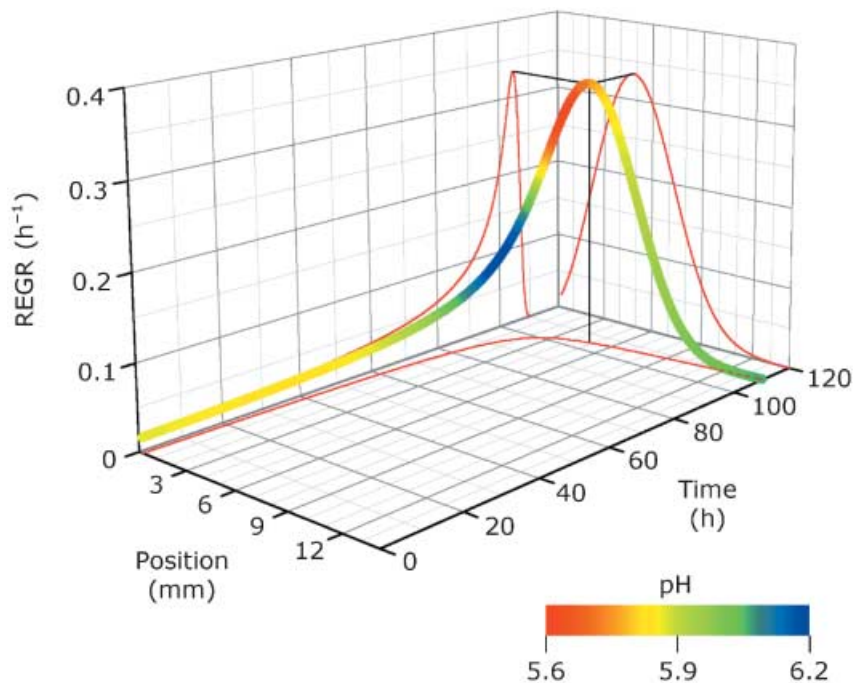


Fig. 2 Trajectory (coloured bold line) of a root element showing the relation of the parameters time, position on the root, relative elemental growth rate (REGR), and surface pH (colour-coded) in a growing maize root tip. The element considered is located at 0.2 mm above the root apex at time 0. The projections (red lines) of the '4D'-trajectory onto the three planes defined by the three cartesian axes correspond to the REGR profile (Fig. 1a), the REGR time-course (Fig. 1c), and the trajectory in the position/time plane (Fig. 1b), respectively.

trajectory functions (integrals of the reciprocal DV profile; Fig. 1b) were computed (for a summary of the mathematical basis, see Peters & Bernstein, 1997). The results were in line with classical reports (Erickson & Sax, 1956).

Trajectory functions describe the position of organ elements on the organ as a function of time. They are the fulcrum of kinematic growth analysis, as they allow the transformation of developmental patterns on the organ into time-courses of development. As an example, Fig. 1(c) shows the time-course of REGR experienced by a root element located 0.2 mm above the root apex at time 0. Similarly, the elongation of a root segment can be computed as the difference between the trajectories of the segment's margins. For instance, a section that is only 0.25 μm long when located at 0.2 mm above the root apex will measure 185 μm (the approximate length of adult parenchymal cells in these roots) when it exits the growth zone 110 h later (Fig. 1d). This time-course demonstrates that growing cells in the roots studied here virtually 'explode'; they actually gain 90% of their final volume within less than 10 h just before growth terminates (Fig. 1d). Bell-shaped growth profiles (Fig. 1a) are misleading conceptually when they are taken to imply that cells go smoothly through what Sachs (1887) termed the 'grand period of growth', consisting of symmetric phases of accelerating and decelerating expansion. While zones of cell division and cell enlargement are not neatly separated in root tips (Green, 1976), the time-courses of REGR and element elongation (Fig. 1c,d) suggest that young cells remain in a meristem-related state of slow expansion for a considerable time, until they enter a distinct condition in which they grow explosively for a short period.

Despite disagreement about details, it is generally accepted that the physiological status of cells that are about to leave the meristematic zone changes dramatically (Ishikawa & Evans, 1995; Baluška *et al.*, 1996). The biphasic model of the growth zone (van der Weele *et al.*, 2003) as well as the above considerations support this view. Can we pinpoint a growth-related physiological phenomenon that might serve as an indicator of the transition from slow to explosive growth?

Growing maize root tips establish a complex longitudinal pattern of extracellular pH consisting of two acid bands separated by a more neutral trench (Felle, 1998). Similar profiles have been found in another grass, *Phleum pratense* L. (Monshausen *et al.*, 1996), and in the dicot *Medicago sativa* (H. Felle, pers. comm.). I pooled root surface pH data that we had determined with pH-sensitive microelectrodes (Peters & Felle, 1999) in the same roots as used for the above growth analysis, and fitted a composite polynomial function (5th order between position 0 and 4.3 mm, 3rd order between 4.3 and 13 mm) to obtain a mean pH profile (Fig. 1e). The dynamics of extracellular pH regulation in cells traversing the growth zone becomes more evident in the corresponding time-course (Fig. 1f).

The data discussed combine into the '4D-trajectory' presented in Fig. 2. The path of a root element in a space defined by REGR, position on the root, and time is shown. Extracellular pH, the fourth parameter, is represented by the colour of the element's path. The path's projections onto the three planes spread out by the axes represent the REGR profile, the trajectory function, and the REGR time-course, respectively (red traces in Fig. 2; compare Fig. 1a–c).

Among the wealth of information condensed into this graph, one fact should be stressed: while there is no correlation of extracellular acidity and growth rate distal of the pH maximum at about 2 mm above the apex, the near perfectly parallel development of acidity and growth in the region proximal of the pH maximum suggests the operation of an acid-growth mechanism in that part of the growth zone. This proximal portion corresponds quite well to the phase of explosive growth. Thus, the growth explosion is preceded by a pH maximum; it seems as if the transient pH peak marked the transition of the cells into an acid growth-competent state, in which they burst into their adult size within a short period of time.

The physiologically most important consequence of the study of van der Weele *et al.* (2003) is that growing root cells switch between two distinct growth modes, rather than shifting gradually along a continuous growth gradient. Notably, the phenomenon of explosive growth is pronounced even more dramatically in such a bimodal growth profile than in the continuous one discussed above. If, as argued by van der Weele *et al.* (2003), the instantaneous growth gradient in maize roots is a biphasic 'step stool' profile, the continuous growth profile shown in Fig. 1(a) will have to be interpreted as a smoothing artifact. Averaging data from several roots is not responsible for this artifact, as no individual root showed a 'step stool' REGR profile (see original results from individual roots in Peters & Felle, 1999). Moreover, a methodological test (analogous to that in Peters & Felle, 1999, Fig. 2; see also Peters & Bernstein, 1997) based on a biphasic model profile proved that a *stable* 'step stool' profile would have been detected by our method (not shown). Therefore I conclude that the smoothing artifact is due to fluctuations of the position of the growth rate transition and/or to variations of the growth rate during the measuring interval (as suggested by van der Weele *et al.*, 2003). Noteworthy, a parameter that is strictly correlated with the growth rate in a fluctuating bimodal growth zone will still correlate with growth after smoothing both this parameter and the growth rate over time. Growth rate fluctuations on the time scale of our previous experiments (Peters & Felle, 1999) therefore do not invalidate the general conclusion drawn here. Thus, the hypothesis of a transition of root cells into an acid growth-competent state at the beginning of explosive growth remains plausible in the context of a biphasic growth zone.

More than this, the hypothesis actually links the biphasic growth zone model with current notions of the pH-dependent cell expansion mediated by cell-wall loosening enzymes of the expansin family (Cosgrove, 1999; Wu & Cosgrove, 2000), as it suggests that the abrupt growth rate transition is due to the onset of an acid-growth mechanism. Unfortunately, available information on the distribution of the expression and activity of expansins along root growth zones (e.g. Wu & Cosgrove, 2000; Lee *et al.*, 2003) is far too coarse to allow

unambiguous conclusions to be drawn. The mapping of expression and activity patterns at a drastically increased resolution will be required to integrate molecular results fully into a physiologically meaningful picture.

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Key words: acid growth theory, expansin, kinematic growth analysis, relative elemental growth rate, root growth zone.



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