Progress in Botany 79

Francisco M. Cánovas
Ulrich Lüttge
Rainer Matyssek
Editors

Progress in Botany

Springer

Progress in Botany

Volume 79

More information about this series at http://www.springer.com/series/124

Francisco M. Cánovas • Ulrich Lüttge •
Rainer Matyssek
Editors

Progress in Botany Vol. 79

Springer

Editors
Francisco M. Cánovas
Depto. Biología Molecular y Bioquímica
Universidad de Málaga
Málaga, Spain

Ulrich Lüttge
Department of Biology
TU Darmstadt
Darmstadt, Germany

Rainer Matyssek
Ecophysiology of Plants, School of Life Sciences Weihenstephan
Technische Universität München LS Ökophysiologie der Pflanzen
Freising, Bayern
Germany

ISSN 0340-4773 ISSN 2197-8492 (electronic)
Progress in Botany
ISBN 978-3-319-71412-7 ISBN 978-3-319-71413-4 (eBook)
https://doi.org/10.1007/978-3-319-71413-4

Library of Congress Control Number: 2017959590

This Springer imprint is published by Springer Nature
The registered company is Springer International Publishing AG
The registered company address is: Gewerbestrasse 11, 6330 Cham, Switzerland

Contents

Plant Water Relations: A Whirlwind of Change 1
John S. Boyer

Molecular Markers for Genetic Diversity 33
Kornel Burg

Advances in Asparagine Metabolism 49
Shrikaar Kambhampati, Ebenezer Ajewole, and Frédéric Marsolais

Redox Control of Autophagy in Photosynthetic Organisms 75
María Esther Pérez-Pérez, Inmaculada Couso, Mónica Domínguez-González, Stéphane D. Lemaire, and José L. Crespo

Senescence-Associated Genes in Response to Abiotic/Biotic Stresses 89
Blanca Velasco-Arroyo, Mercedes Diaz-Mendoza, Maria Estrella Santamaria, Pablo Gonzalez-Melendi, Andrea Gomez-Sanchez, Ana Arnaiz, Manuel Martinez, and Isabel Diaz

Interactions Between Copper Homeostasis and Metabolism in Plants 111
Anna Schulten and Ute Krämer

Interplay of Circadian Rhythms and Light in the Regulation of Photosynthesis-Derived Metabolism 147
Matthew A. Jones

Photosynthesis-Related Functions of Vasculature-Associated Chlorenchymatous Cells 173
Zbigniew Miszalski, Andrzej Kornaś, and Elżbieta Kuźniak

Drought Stress and the Recovery from Xylem Embolism in Woody Plants 197
Andrea Nardini, Tadeja Savi, Patrizia Trifilò, and Maria A. Lo Gullo

A Resource Centric View of Climate and Mast Seeding in Trees 233
Robert B. Allen, Peter Millard, and Sarah J. Richardson

Terrestrial Microalgae: Novel Concepts for Biotechnology and Applications 269
Michael Lakatos and Dorina Strieth

Background Insect Herbivory: Impacts, Patterns and Methodology 313
Mikhail V. Kozlov and Elena L. Zvereva

Erratum to: Interplay of Circadian Rhythms and Light in the Regulation of Photosynthesis-Derived Metabolism 357
Matthew A. Jones

Contributors

Ebenezer Ajewole Department of Biology, University of Western Ontario, London, ON, Canada

London Research and Development Centre, Agriculture and Agri-Food Canada, London, ON, Canada

Robert B. Allen Private Researcher, Lincoln, New Zealand

Ana Arnaiz Centro de Biotecnologia y Genomica de Plantas, Universidad Politecnica de Madrid (UPM), Instituto Nacional de Investigacion y Tecnologia Agraria y Alimentaria (INIA), Madrid, Spain

John S. Boyer Division of Plant Sciences, College of Agriculture, Food and Natural Resources, 52 Agriculture Laboratory, University of Missouri, Columbia, MO, USA

Kornel Burg Austrian Institute of Technology, Vienna, Austria

Inmaculada Couso Instituto de Bioquímica Vegetal y Fotosíntesis, Consejo Superior de Investigaciones Científicas (CSIC)-Universidad de Sevilla, Sevilla, Spain

José L. Crespo Instituto de Bioquímica Vegetal y Fotosíntesis, Consejo Superior de Investigaciones Científicas (CSIC)-Universidad de Sevilla, Sevilla, Spain

Isabel Diaz Centro de Biotecnologia y Genomica de Plantas, Universidad Politecnica de Madrid (UPM), Instituto Nacional de Investigacion y Tecnologia Agraria y Alimentaria (INIA), Madrid, Spain

Mercedes Diaz-Mendoza Centro de Biotecnologia y Genomica de Plantas, Universidad Politecnica de Madrid (UPM), Instituto Nacional de Investigacion y Tecnologia Agraria y Alimentaria (INIA), Madrid, Spain

Mónica Domínguez-González Instituto de Bioquímica Vegetal y Fotosíntesis, Consejo Superior de Investigaciones Científicas (CSIC)-Universidad de Sevilla, Sevilla, Spain

Andrea Gomez-Sanchez Centro de Biotecnologia y Genomica de Plantas, Universidad Politecnica de Madrid (UPM), Instituto Nacional de Investigacion y Tecnologia Agraria y Alimentaria (INIA), Madrid, Spain

Pablo Gonzalez-Melendi Centro de Biotecnologia y Genomica de Plantas, Universidad Politecnica de Madrid (UPM), Instituto Nacional de Investigacion y Tecnologia Agraria y Alimentaria (INIA), Madrid, Spain

Matthew A. Jones School of Biological Sciences, University of Essex, Colchester, UK

Shrikaar Kambhampati Department of Biology, University of Western Ontario, London, ON, Canada

London Research and Development Centre, Agriculture and Agri-Food Canada, London, ON, Canada

Andrzej Kornaś Institute of Biology, Pedagogical University, Kraków, Poland

Mikhail V. Kozlov Section of Ecology, Department of Biology, University of Turku, Turku, Finland

Ute Krämer Molecular Genetics and Physiology of Plants, Ruhr University Bochum, Bochum, Germany

Elżbieta Kuźniak Department of Plant Physiology and Biochemistry, Faculty of Biology and Environmental Protection, University of Lodz, Łódź, Poland

Michael Lakatos Applied Pharmacy, University of Applied Sciences Kaiserslautern, Pirmasens, Germany

Stéphane D. Lemaire Laboratoire de Biologie Moléculaire et Cellulaire des Eucaryotes, Institut de Biologie Physico-Chimique, Sorbonne Universités, UPMC Univ Paris 06, CNRS, UMR8226, Paris, France

Maria A. Lo Gullo Dipartimento di Scienze Chimiche, Biologiche, Farmaceutiche e Ambientali, Università di Messina, Messina, Italy

Frédéric Marsolais Department of Biology, University of Western Ontario, London, ON, Canada

London Research and Development Centre, Agriculture and Agri-Food Canada, London, ON, Canada

Manuel Martinez Centro de Biotecnologia y Genomica de Plantas, Universidad Politecnica de Madrid (UPM), Instituto Nacional de Investigacion y Tecnologia Agraria y Alimentaria (INIA), Madrid, Spain

Peter Millard Landcare Research, Lincoln, New Zealand

Zbigniew Miszalski Institute of Plant Physiology, Polish Academy of Sciences, Kraków, Poland

Małopolska Centre of Biotechnology, Jagiellonian University, Kraków, Poland

Andrea Nardini Dipartimento di Scienze della Vita, Università di Trieste, Trieste, Italy

María Esther Pérez-Pérez Instituto de Bioquímica Vegetal y Fotosíntesis, Consejo Superior de Investigaciones Científicas (CSIC)-Universidad de Sevilla, Sevilla, Spain

Sarah J. Richardson Landcare Research, Lincoln, New Zealand

Maria Estrella Santamaria Centro de Biotecnologia y Genomica de Plantas, Universidad Politecnica de Madrid (UPM), Instituto Nacional de Investigacion y Tecnologia Agraria y Alimentaria (INIA), Madrid, Spain

Tadeja Savi Dipartimento di Scienze della Vita, Università di Trieste, Trieste, Italy

Anna Schulten Molecular Genetics and Physiology of Plants, Ruhr University Bochum, Bochum, Germany

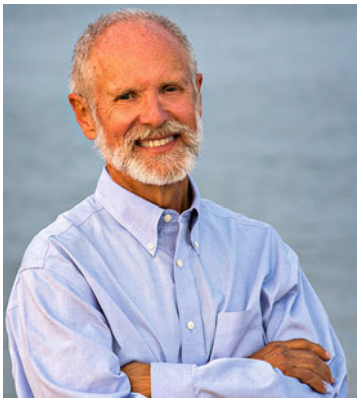
Dorina Strieth University of Kaiserslautern, Kaiserslautern, Germany

Patrizia Trifilò Dipartimento di Scienze Chimiche, Biologiche, Farmaceutiche e Ambientali, Università di Messina, Messina, Italy

Blanca Velasco-Arroyo Centro de Biotecnologia y Genomica de Plantas, Universidad Politecnica de Madrid (UPM), Instituto Nacional de Investigacion y Tecnologia Agraria y Alimentaria (INIA), Madrid, Spain

Elena L. Zvereva Section of Ecology, Department of Biology, University of Turku, Turku, Finland

Curriculum Vitae



John Strickland Boyer College of Agriculture, Food and Natural Resources 302-645-1618
University of Missouri (boyerjs@missouri.edu)

Professional Preparation

1959	A.B. Biology, Swarthmore College
1961	M.S. Botany, Univ. Wisconsin-Madison
1964	Ph.D. Botany, Duke Univ.

Appointments

1964–1965	Visiting Asst. Professor, Duke Univ.
1965–1966	Assistant Scientist, Connecticut Agricultural Experiment Station
1966–1978	Assistant, Associate, and Full Professor, Dept. Botany and Dept. Agronomy, Univ. Illinois at Urbana-Champaign

1978–1984	Plant Physiologist, USDA/ARS, Univ. Illinois at Urbana-Champaign
1984–1987	Prof. Dept Soil and Plant Sci. and Dept. Biochemistry and Biophysics, Texas A&M Univ.
1987–2005	E. I. du Pont Prof. Marine Plant Biochemistry/Biophysics, Coll. Marine Studies and Coll. Agriculture, Univ. Delaware
1998–2002	Program Director, Marine Biology-Biochemistry, Univ. Delaware
2005–present	E. I. du Pont Prof. Biochemistry/Biophysics Emeritus, Univ. Delaware
2014–present	Distinguished Research Professor, Univ. Missouri

Awards

1961–1964	James B. Duke I Fellow, Duke Univ.
1972	Climate Laboratory Fellow, DSIR, Palmerston North, New Zealand
1977	Shull Award, Am. Soc. Plant Physiologists
1983–1984	Senior Scientist, von Humboldt Foundation
1985	Citation Classic, ISI (Boyer. 1970. Plant Physiol 46:233)
1985	Fellow, Crop Science Society of America
1986	Fellow, American Society of Agronomy
1987	Distinguished Lecturer, National Science Council, Taiwan
1987	Walker Lecturer, University of Washington, Seattle
1988	Fellow, Japan Society for Promotion of Science
1988	International Lecturer, Japan Crop Science Society
1989	Tansley Lecturer, Univ. Edinburgh, Univ. Bangor, Univ. Lancaster, UK
1990	Member National Academy of Sciences, USA
1993	Kramer Lecturer, Duke University
1995	Fellow, Research School of Biological Sciences, Austral. Nat. Univ.
1997	Bullard Fellow, Organismal and Evolutionary Biology, Harvard Univ.
1999	Francis Alison Award, University of Delaware
2002	Listed, 250 most highly cited scientists in the plant and animal sciences
2005	Fellow Australian Academy of Science
2007	Fellow American Society of Plant Biologists
2007	Barnes Lifetime Membership Award, Am. Soc. Plant Biologists
2007	Sir Frederick McMaster Research Fellowship, CSIRO, Canberra

Plant Water Relations: A Whirlwind of Change

John S. Boyer

Abstract Water is increasingly recognized as a limiting resource consumed by plants in copious amounts. Its large role in agriculture has awakened interest in how plants use it and how they conserve it. Fortunately the study of plant water relations underwent major changes starting about 50 years ago with thermodynamically based methods for measuring plant water status. The methods allowed conditions to be repeated, biochemical events to be repeatedly detected, and responses to water deficits understood more fully. This was followed by the realization that enzymes were not responding directly to water deficits nor was photosynthesis responding only by closing stomata (which conserved water and limited CO_2 entry) but also by diminishing CO_2 demand. The decrease in demand suggested that photosynthesis might acclimate to water deficits, and tests showed not only that this occurred but also that cellular Mg^{2+} concentrations were a central controller of the acclimation. Osmotic adjustment was discovered in a form that used photosynthetic products to maintain turgor and allowed growth where otherwise none would occur. It was found that water potentials were induced by the growth process itself and were important controllers of growth rates in land plants. In an alga surrounded by water and unaffected by these potentials, the chemistry of pectin determined cell enlargement and is now being explored in land plants most of which contain pectin in their cell walls. Ultimately, it became possible to reverse reproductive failure during a drought by feeding photosynthetic products to the plant, thus identifying biochemical origins of failure and gene targets to enhance tolerance to those environments. In fact, commercial agriculture increasingly sees drought tolerance as an important plant character and it is gratifying that reproductive reversal may be contributing to

Communicated by Rainer Matyssek

J.S. Boyer (✉)
Division of Plant Sciences, College of Agriculture, Food and Natural Resources, 52 Agriculture Laboratory, University of Missouri, Columbia, MO 65211, USA
e-mail: boyerjs@missouri.edu

Progress in Botany (2018) 79: 1–32, DOI 10.1007/124_2017_3,
Published online: 14 April 2017

this effort. Although plant water relations are inherently multigenic and complex, these findings demonstrate benefits of understanding plant water relations that were scarcely imagined 50 years ago.

Contents

1 Introduction 2
2 Nitrogen Metabolism 7
3 Photosynthesis 11
4 Plant Enlargement 14
5 Chemistry of Cell Enlargement 19
6 Reproduction 23
7 Retrospectives 26
References 27

1 Introduction

My brother and I grew up on a beef cattle farm that we worked for our parents as we aimed for university training. At the university, we became amazed at how little we knew about the basic biology of farming. When a chance came to go to graduate school, I took it because the application of biology to farming was so fascinating. Raising enough food for the animals had been difficult, so I focused on plant sciences. Aside from the importance of soils, it seemed that mineral nutrition and water availability were two key factors, and I resolved to learn more about both. A couple of years with Gerry C. Gerloff at University of Wisconsin acquainted me with soils and the genetics of mineral nutrition and then an opportunity to work with water availability led me to the lab of Paul J. Kramer at Duke.

No sooner had I arrived in 1961 than it became apparent that water availability was a tough subject. The availability of soil water was continually changing and the literature was scarce. Those papers that were published were based mostly on the time after water was withheld. This meant the soil type, atmospheric conditions, and plant size controlled the results and could scarcely be repeated. In addition, only a few methods were available to characterize conditions in the plant and soil. Terms like "diffusion pressure deficit" and "osmotic pressure" were opaque. Many hours were spent delving into these concepts with the other students.

Then it occurred to me that using NaCl to vary salinity would combine the aquatic culture methods of mineral nutrition with a study of water availability that would allow steady, repeatable conditions to be achieved. It would then be possible to test the new ideas of Bernstein (1961) that certain plant species grow in saline conditions because they absorb the external salt and use it internally to improve the osmotic potential for water uptake. Bernstein termed this process "osmotic adjustment." I chose cotton for my study because it was moderately salinity tolerant. Would osmotic adjustment allow water uptake but also permit stomata to function normally and photosynthesis to occur when plants adjusted to the salinized medium?

The answer was yes, stomata could open and close normally and photosynthesis took place even though water availability was limited by the saline conditions (Fig. 1). The leaves had adjusted to the salinity and the stomata followed suit. I had grown the plants in saline conditions from the time of seedling establishment but an oversight was that I neglected to mention this fact in my first paper (Boyer 1965). Papers from other labs sometimes dumped salt on established plants and generally found incomplete adjustment (e.g., Gale et al. 1967). I had also observed this behavior but should have addressed it in my paper to avoid confusion.

Later, I had the chance to revisit osmotic adjustment. Bob Meyer was an early graduate student at University of Illinois and he discovered osmotic adjustment in water-deficient plants (Meyer and Boyer 1972). Because NaCl was absent, the plants could not use salt to adjust osmotically and instead used photosynthetic products. The phenomenon was particularly noticeable in enlarging tissues that accumulated the products of photosynthesis extensively (Meyer and Boyer 1972, 1981; Michelena and Boyer 1982). The accumulation allowed water uptake with the result that turgor was

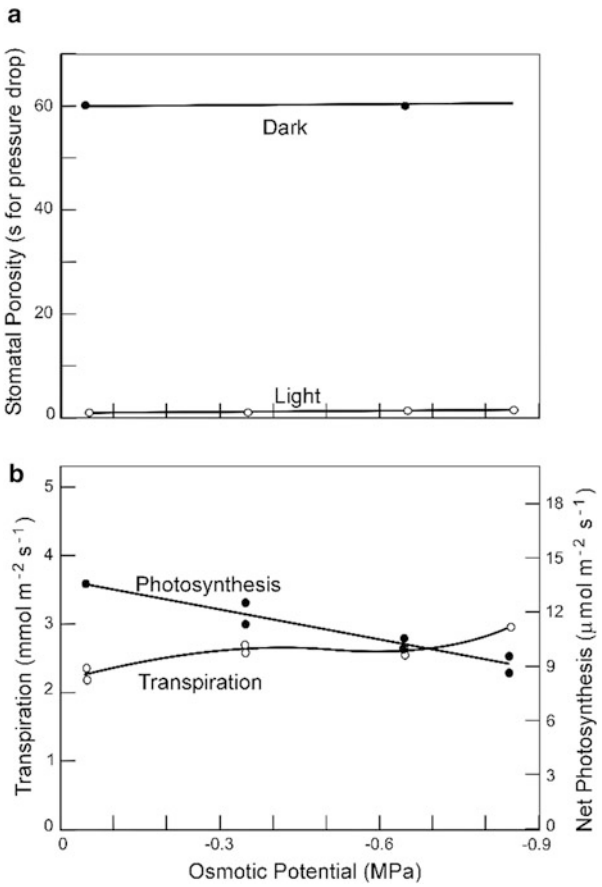


Fig. 1 Stomatal porosity (a), transpiration and net photosynthesis (b) in cotton salinized from the time of seedling establishment. Highest salinity was about 1/3 that of seawater (osmotic potential of −0.85 MPa). Porosity was measured with a porometer that forced air through the leaf (stomata on both sides). Less time indicated more open stomata, which opened fully in light regardless of salinity, and closed in the dark. Transpiration increased as salinity increased, but photosynthesis tended to diminish. Redrawn from Boyer (1965). Copyright American Society of Plant Biologists

nearly fully maintained (Fig. 2). Because turgor had to be above a minimum in order for growth to occur, this helped the cells continue growing. If the adjustment was prevented, turgor fell and growth was more inhibited than when the adjustment occurred. Therefore, osmotic adjustment was more general than Bernstein (1961) ever imagined.

But returning to my graduate work at Duke, Ralph Slatyer visited in 1963 to collaborate with Paul Kramer on a water relations textbook. Ralph was well-known for his pioneering work in micrometeorology and he knew physics and thermodynamics. I

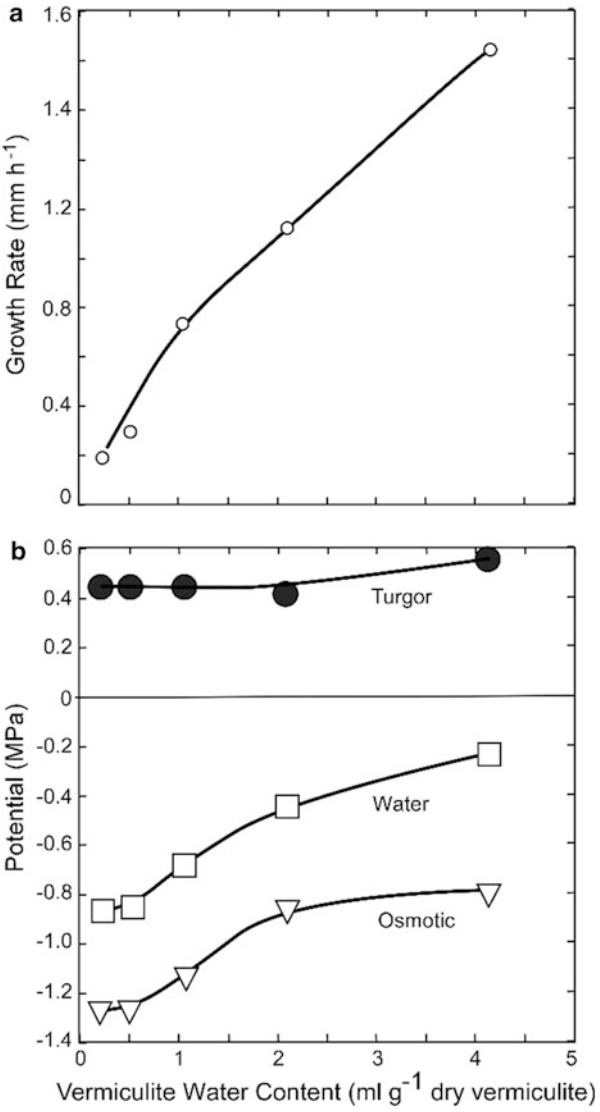


Fig. 2 Growth (**a**) and osmotic adjustment (**b**) in the elongating region of soybean hypocotyls in vermiculite having various water contents. Osmotic potential essentially kept pace with the water potential in the elongating region so that turgor decreased only slightly. No NaCl was present and instead the intact seedlings used photosynthetic products from the cotyledons to adjust the osmotic potential. Note that growth was strongly inhibited by the water deficit (**a**) and indicated that something in addition to turgor (**b**) was inhibiting growth. Redrawn from Meyer and Boyer (1972)

was enthralled. He suggested that we should abandon "diffusion pressure deficits" and relate plant work to thermodynamics using potentials as described by J. Willard Gibbs (1875–1876). In fact, Ralph Slatyer and Sterling Taylor (1960) had recently published a paper suggesting that soil and plant water status could be unified by this approach. I was persuaded and resolved to write in those terms (Boyer 1965). Ralph's influence on the water relations and plant transport fields cannot be overestimated, as is apparent also in the essay of Ulrich Lüttge (2016).

While my cotton paper was in press, Steve Rawlins (1964) published that the vapor pressure method I used to characterize salinity conditions was in error by up to 90%!! I was distraught until it occurred to me that his argument rested on the calibration necessary for the method. Thus, if I used the solution indicated by the calibration, its vapor pressure should be the same as the tissue. It wasn't! Steve was right!

From that experiment, we developed the "isopiestic" method of measuring the water potential meaning equal vapor pressures (Boyer and Knipling 1965; Boyer 1969, 1995; Kramer and Boyer 1995). Vapor methods measure plant water status by placing a tissue sample in a small sealed chamber and letting the liquid evaporate to the air, raising the humidity until no net vapor exchange occurs, that is, until the vapor activity matches the liquid activity in the sample. It is then only necessary to measure the vapor activity to determine the liquid activity. The simplest way is to expose a standard solution to the vapor in the chamber. If it neither evaporates nor condenses, its vapor activity is the same as the vapor in the chamber and thus the liquid in the sample.

A thermocouple detects whether the standard evaporates (cooler) or condenses (warmer). The approach is to expose a standard to the vapor and then follow it with a second standard closer to the vapor activity in the chamber. This allows extrapolation to the standard neither evaporating nor condensing, i.e., having no net vapor exchange with the sample. Because the extrapolated standard is in equilibrium with liquid in the sample, the liquid activity is identified and no calibration is required. The extrapolated standard is unaffected by the arrangement of the sample in the chamber, the tortuosity of the vapor path through the sample, or any waxes coating the tissue surface. All other measurements are affected by these factors. In addition, the thermocouple can be used to test isothermal conditions around the sample important for isopiestic measurements. This is the power of thermodynamic equilibrium.

The method is similar to the isopiestic method used in physical chemistry to determine the vapor pressure of solutions. The availability of this method for soils and plant tissues and even intact plants has been central to all of our subsequent experiments for my entire career. Not only could it measure tissue water potential but also the osmotic potential (Ehlig 1962) from which turgor pressure could be determined. So much of plant performance depends on these three parameters that the method opened many avenues of investigation for us.

While this was going on, Paul Kramer received a manuscript from Science for review. Written by Scholander et al. (1965), it described a pressure chamber to measure tension on water in the conducting xylem of trees. Paul asked me to review it and I recommended publication. I also suggested to Scholander in the review that, because he had already published that the solute concentrations in the xylem were low,

the tensions measured with the pressure chamber ought to be nearly the same as the water potential. Scholander did not accept my suggestion and published without making the connection between his pressure chamber results and the water potential.

Shortly thereafter, I had an offer from Connecticut Agricultural Experiment Station in New Haven and found myself in Steve Rawlins' old position. For 11 months, I devoted myself to a comparison of the pressure chamber and the isopiestic psychrometer. The comparison was very favorable so that the pressure chamber essentially measured the water potential (Boyer 1966, 1967), which was the first time this had been demonstrated (Fig. 3). It confirmed that pressure (tension during the daytime) was the main xylem component in plants and also indicated that water in soil and plants experiences the same thermodynamics despite being in vastly different systems.

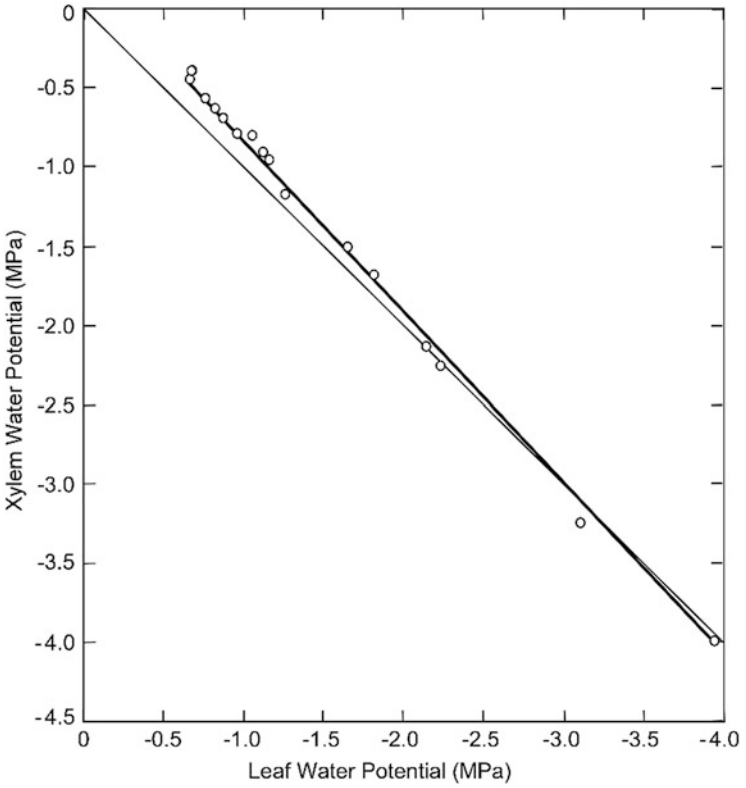


Fig. 3 Xylem water potential measured on branches with a pressure chamber in comparison with water potential measured on leaves of the same branches with an isopiestic thermocouple psychrometer in yew (*Taxus cuspidata*). Xylem water potential was the sum of the pressure chamber result and the osmotic potential of xylem solution that was always dilute. This indicates that water potential in xylem was mostly tension. The *solid line* indicates a 1:1 correspondence between the two methods. Similar results were obtained in several other spp. Redrawn from Boyer (1967). Copyright American Society of Plant Biologists

Gibbs (1875–1876) had originally conceived of chemical potentials as the Gibbs free energy per mole of substance but Slatyer and Taylor (1960) suggested that the concept could be converted to pressure units by dividing Gibbs' chemical potential by the partial volume of a mole of water (partial indicates that other substances can be present and for water the partial volume is 18.05 cm^3 mol^{-1} at 20°C, considered constant in most biological systems). Expressed this way, the chemical potential was called the "water potential" and had units of pressure, i.e., megaPascals where 1 MPa = 10^6 N m^{-2} = 10 bar = 9.87 atm. Compared to pure water that acted as a reference (water potential of zero), forces that contributed to the water potential were pressure (positive or negative), solute (negative because it spread water molecules apart), matrices (negative because solid media like soil or cell walls also spread water apart), and gravitational pull (important over large distances). The isopiestic method and pressure chamber became major ways to measure these forces (Boyer 1995).

A few years later, Hüsken et al. (1978) developed the pressure probe that could measure turgor directly in individual plant cells. In retrospect, it seems remarkable that these three methods and their associated terminology developed so quickly. I think having a thermodynamic basis helped in their development. They made soil and plant water relations accessible to scientists in other disciplines but even more importantly, they allowed experiments to be repeated. In effect, knowing the thermodynamic state of water in plants and soils allowed a scientist to return to that state over and over again. This laid the groundwork for physiological and biochemical investigations and, more recently, molecular genetic work.

Soon (1966) opportunity arose in the form of an offer from the University of Illinois and I took it. The attraction was interaction with exceptional colleagues like Jack Hanson, Dick Hageman, Jim Gerdemann, Larry Vanderhoef, Govindjee, Gregorio Weber, and many others with their students. Through the years, we published together and their students added rich diversity to our efforts. The trade-off was heavy teaching and administrative responsibilities that restricted my research to evenings and weekends. My wife Jean, herself a plant scientist, was fully supportive. I was very grateful and to conserve time, my efforts focused only on nitrogen metabolism, photosynthesis, growth, and reproduction because they seemed particularly relevant to agriculture. The remainder of this essay is devoted to how water relations affect these areas.

2 Nitrogen Metabolism

With the recent application of thermodynamics to water relations, a central question was how plant metabolism responded to water availability. Did enzymes react to changes in turgor pressure? Or water potential? Nitrogen metabolizing enzymes were candidates because nitrogenase activity had recently been detected in vivo in nitrogen fixing species (Hardy et al. 1968; Fishbeck et al. 1973). Also, the synthesis of nitrate reductase had just been shown to respond to the water status of the plant (Morilla et al. 1973).

Beginning with nitrogen fixing soybean, Chi-Ying Huang found that nitrogenase activity could be detected in the nodules of completely intact plants growing in soil (Huang et al. 1975a). When water was withheld, plant water potential declined and photosynthesis was inhibited in the shoot. In the root, Fig. 4 shows nitrogenase immediately lost activity but could be partly recovered by increasing photosynthesis using high CO_2 (Huang et al. 1975b). At the same time, Janet Sprent in Scotland found that the activity could be partly recovered by increasing the oxygen concentration around the nodules. Although nodules normally shield nitrogenase from oxygen (Avenhaus et al. 2016), the gas is required for the respiratory activity of the cells around the nitrogenase (Pankhurst and Sprent 1976). Water deficits had increased the oxygen barrier so that the concentration in the nodule became too low for the necessary respiration. The ability to reverse the effects of limited water indicated that the flux of carbon skeletons from shoot photosynthesis together

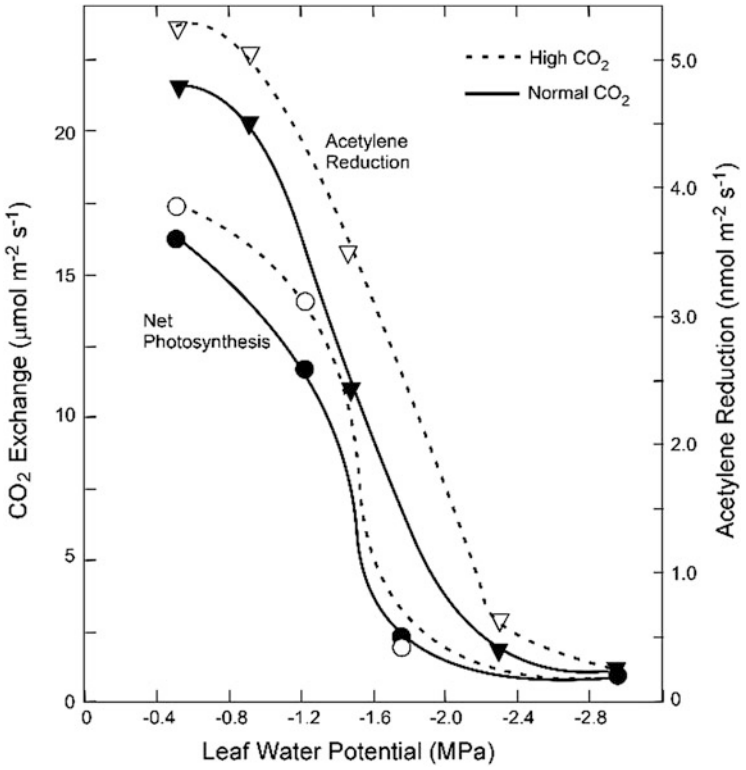


Fig. 4 Net photosynthesis and acetylene reduction at various leaf water potentials in intact soybean plants growing in soil. Normal CO_2 concentration was 300 μmol mol^{-1} and high CO_2 concentration was 600 μmol mol^{-1}. Note that measurements were in situ without disturbing the plants. High CO_2 concentration caused increased photosynthesis in the shoot and thus increased acetylene reduction in the root nodules. Redrawn from Huang et al. (1975b). Copyright American Society of Plant Biologists

with the diffusion of oxygen from the soil controlled the rate of nitrogen fixation in drying soil. In effect, water deficits were affecting these processes through physiological means rather than by altering the enzyme nitrogenase directly. This was the first time that enzyme activity had been monitored in a completely intact plant exposed to limited water and was the closest we ever came to monitoring the biochemistry of whole plants.

Moving on to nitrate reductase, Dale Shaner repeated the work of Morilla et al. (1973) that shoots of maize seedlings lost reductase activity when dehydrated (Shaner and Boyer 1976b). But the controls lost activity too, even if they were kept in water at high humidity in the light (Shaner and Boyer 1976a). Dale was intrigued and tried adding nitrate to the water of the controls. The nitrate prevented the lost activity. Could the flux of nitrate be important for the control shoots?

We imagined that the molecular budget for the reaction would be similar to a mass or energy budget in which INPUT + OUTGO + STORAGE = 0. The INPUT would be the flux of the substrate nitrate (positive). The OUTGO would be the flux of the product nitrite (negative). The STORAGE would be the difference between the two represented by the enzyme reaction. As a sequence of enzymatic events, the budget would then be shown as:

where [*S*] is the substrate concentration in the cytoplasm (nitrate) and [*P*] is the product concentration in the cytoplasm (nitrite). The pools of *S* and *P* would be small because of the small volume of the cytoplasmic compartment. *S* would be quickly controlled by the Nitrate Flux coming from the xylem. Likewise, *P* would be controlled by the Nitrite Flux.

We knew substantial amounts of nitrate were in the vacuoles but this large pool would be sequestered and have little effect on the enzyme in the cytoplasm. Basically because of the small cytoplasmic pool the enzyme activity would appear to be determined by the flux of nitrate when in fact the enzyme responded to the local concentration of nitrate (Shaner and Boyer 1976a). Consequently, *S* (nitrate) served two roles for the enzyme: its flux altered the rate of enzyme synthesis (Morilla et al. 1973) and controlled the enzyme activity (Shaner and Boyer 1976a).

The flux to the cytoplasm diminished as water potentials became low in large part because the soil delivered less nitrate to the root. The flux could be partially recovered by feeding higher concentrations of nitrate to the soil before water was withheld (Shaner and Boyer 1976b). This doubled the flux of nitrate to the leaves during dehydration. Figure 5 shows that the higher flux improved the enzyme activity. Again, the enzyme response was controlled by a physiological process (nitrate flux) rather than the enzyme being directly affected by the water deficit.

There is no doubt that water is required for enzyme activity. Substrate must diffuse to the active site through water, and the reaction at the active site depends on the

motion of enzyme subunits or domains in the aqueous medium. Dehydration restricts this motion. However, the amount of water necessary to restrict enzyme motion is much less than in a typical water limited cell. For example, urea when free in soil decreases its activity below water potentials equivalent to −15 MPa where the water would approach only monomolecular layers (Skujins and McLaren 1967). But nitrogenase and nitrate reductase in cells lost all activity at water potentials equivalent to −1 to −2 MPa in intact plants (Figs. 4 and 5). With this result, we abandoned the hypothesis that low water potentials acted directly on cellular enzymes. We began to emphasize instead the physiological impacts of low water potentials.

After this work was done, others found that light/dark transitions and phosphorylation also alter nitrate reductase activity (e.g., Lillo et al. 2003). Although the water potential had no direct effect, the advent of molecular genetics allowed many additional control and signaling systems to be discovered. It will be exciting to see how these affect this important enzyme for nitrogen entrance into plants.

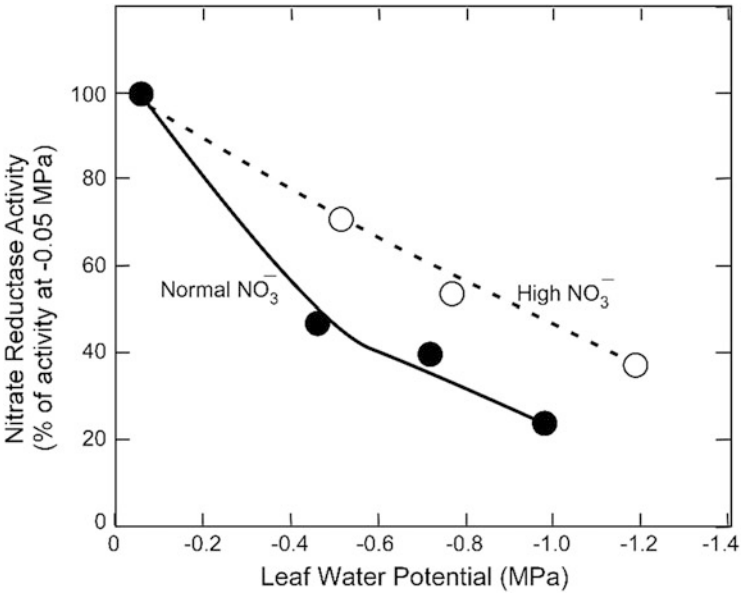


Fig. 5 Effect of nitrate flux on activity of nitrate reductase at various leaf water potentials. Normal NO_3^- was 15 mM, High NO_3^- was 45 mM. Flux of nitrate to the site of nitrate reductase synthesis was increased by feeding the higher concentration of nitrate to soil immediately prior to exposure to low leaf water potentials. Activity is expressed as a percent of the control at leaf water potential of −0.05 MPa. Redrawn from Shaner and Boyer (1976b). Copyright American Society of Plant Biologists

3 Photosynthesis

Plants encountering water deficits lose photosynthetic activity much more than they lose respiratory activity (Fig. 6). In an ecological context, this makes sense because the plant remains alive and potentially able to propagate itself for the next season despite minimal photosynthesis. In view of the previous section on enzyme function, we wondered if photosynthetic enzymes or membranes might be inhibited by water deficits. Boyer and Bowen (1970) found large losses in Photosystem 2 activity during water deficits, and Boyer and Potter (1973) and Potter and Boyer (1973) reported that turgor changes (and thus water potential) did not cause the photosynthesis alterations. Instead the osmotic potential tracked the changes. In other words, water contents were important (because they control the osmotic potential) or the composition of solutes had changed. Once again physiological conditions were more important than direct effects of water deficits on the enzyme in the chloroplast.

If water was withheld from sunflower leaves and chloroplasts were isolated from them, further tests showed less activity for Photosystems 1 and 2 and photophosphorylation compared to control tissue supplied with water (Keck and Boyer 1974). Apparently, withholding water exposed the chloroplasts to conditions that limited their activity even when they were isolated and assayed in aqueous media without a water deficit. This was surprising, so we tested if there were chloroplast changes in

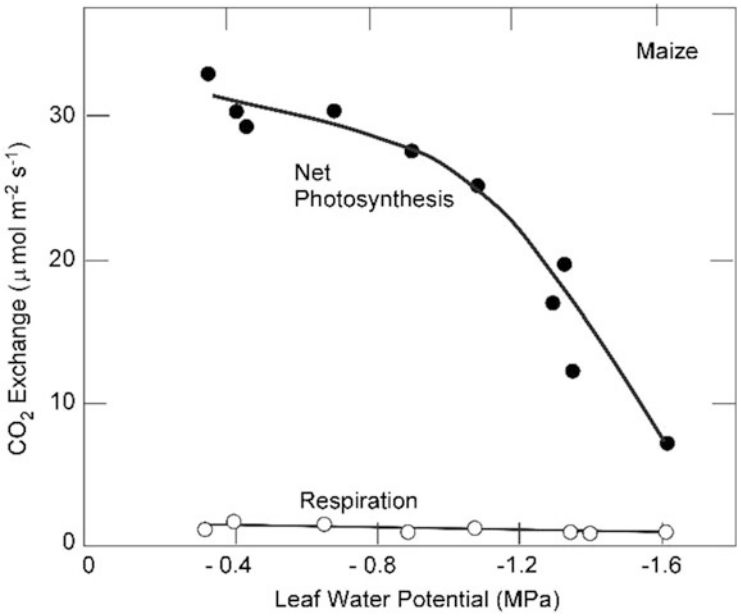


Fig. 6 Net photosynthesis and respiration in maize at various leaf water potentials. Activities were measured in the entire shoot of intact plants grown in soil for 4–5 weeks. Redrawn from Boyer (1970). Copyright American Society of Plant Biologists

intact plants and found lower quantum yields (Mohanty and Boyer 1976; Matthews and Boyer 1984) and altered membrane structure when chloroplasts were viewed with an electron microscope (Fellows and Boyer 1976). Chloroplasts were obviously losing activity in the intact leaves.

Further work in sunflower eliminated photoinhibition as a reason for the loss at least in sunflower (Sharp and Boyer 1987) and indicated that photosynthesis could acclimate to prolonged exposure to low water potentials (Matthews and Boyer 1984). Rao et al. (1987) implicated changes in Mg^{2+} which is largely free in the cytoplasm and chloroplasts despite its structural presence in chlorophyll (Portis and Heldt 1976; Portis 1981). Inorganic ions concentrate as water is lost from cells but photophosphorylation (Younis et al. 1979, 1983) and protein synthesis (Rubin et al. 1979) require Mg^{2+} to be in a narrow concentration range for maximum activity. Our current hypothesis is that water deficits cause Mg^{2+} to concentrate outside of this range (Rao et al. 1987). It would be useful to test further if Mg^{2+} homeostasis confers drought tolerance in plants.

Photosynthesis requires CO_2 as a substrate and land plants inevitably trade water for CO_2 because the CO_2 must diffuse into leaves and dissolve in water wetting the surfaces of the leaf cells. In fact, leaves usually lose water at least 100 times faster than CO_2 enters, and the gateways for this exchange are the stomatal pores. Stomata open by accumulating osmotica in the guard cells, particularly potassium (Fischer 1968; Fischer and Hsiao 1968; Mansfield and Jones 1971). During a water deficit, stomata close and conserve water. Ehret and Boyer (1979) found that the closure was caused by potassium loss from the guard cells. Figure 7 shows the loss and indicates that it is reversed when water is resupplied to the leaf. Therefore, two events seemed important for photosynthesis during a water deficit: stomata closed and restricted water loss and CO_2 entry but also photosynthetic metabolism appeared to diminish and possibly demand less CO_2.

In order to determine which process was more important, we measured the concentration of CO_2 inside leaves (c_i). If stomata closed during a water deficit, photosynthesis might be starved for CO_2 and c_i would decrease. On the other hand, if demand diminished, c_i would increase or at least remain constant. The c_i was thus a key for determining the limitation of photosynthesis during a water deficit. In sunflower, Lauer and Boyer (1992) used a method pioneered by Sharkey et al. (1982) to measure c_i directly and avoid possible errors in calculations of c_i (see below). The c_i increased and indicated that photosynthesis was demanding less CO_2 than the stomata were allowing in.

We also developed a gas exchange system to detect photosynthesis at CO_2 concentrations as high as 50,000 μmol mol^{-1} (5% CO_2). This allowed us to force CO_2 into the leaf despite stomatal closure (Graan and Boyer 1990). We developed gentle ways to peel the epidermis to determine photosynthesis in the complete absence of closed stomata (Tang et al. 2002). These methods confirmed the c_i findings that photosynthetic metabolism was more directly limiting than the restricted entry of CO_2 into sunflower leaves.

It would be helpful to extend this type of work to various species because the c_i balance is likely to be a key response to dehydrating environments. A major difficulty

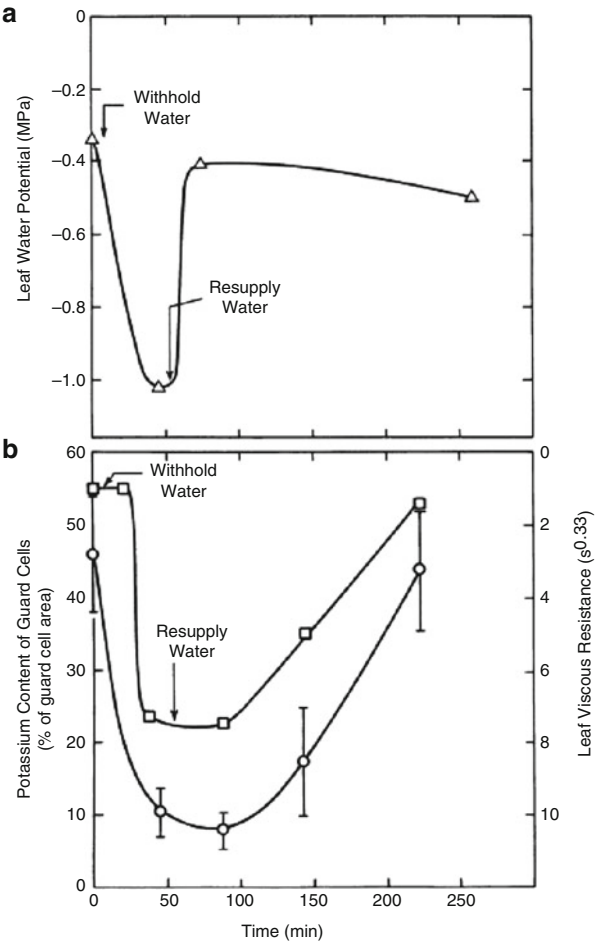


Fig. 7 Leaf water potentials (**a**), potassium content of stomatal guard cells (**b**, *squares*), and leaf viscous resistance to air flow through the leaf (**b**, *circles*) at various times after withholding water, then resupplying it in sunflower. The leaves have stomata on both surfaces, so viscous flow of air through the leaf indicates the degree of stomatal opening. When the viscous flow becomes higher, stomata are opening. From Ehret and Boyer (1979)

is knowing c_i accurately because it is usually calculated instead of being directly measured. In order to determine c_i directly, we incorporated into our gas exchange apparatus a cup that could be sealed to the abaxial (lower) epidermis of a leaf (Boyer and Kawamitsu 2011). The c_i equilibrated with the air in the cup and gave a measure of c_i similar to that pioneered by Sharkey et al. (1982). We compared the measured c_i with the calculated one described by Moss and Rawlins (1963) and their later variants (von Caemmerer and Farquhar 1981; Boyer and Kawamitsu 2011):

$$c_i = c_a - 1.6\frac{A_s}{E_s}(w_i - w_a) \quad (1)$$

where c_a is the CO_2 concentration in the bulk air outside of the leaf (mol mol^{-1}), A_s and E_s are the rate of CO_2 assimilation and transpiration through stomata, respectively (mol m^{-2} s^{-1}), w_i and w_a are the water vapor concentrations inside and outside of the leaf (mol mol^{-1}), and 1.6 is the ratio of diffusivities for water vapor and CO_2 in air.

Notice that calculated c_i is determined mostly from the ratio A_s/E_s because c_a and w_a are atmospheric properties, w_i is determined from leaf temperature, and 1.6 is a constant. In effect, the equation uses water loss as a tracer for CO_2 entry into the leaf. This has the great advantage that the calculation can be made from standard gas exchange measurements without otherwise disturbing the leaf. But as stomata close, E_s and A_s are increasingly determined by cuticle properties, not stomata. Cuticle properties are not considered in the Moss/Rawlins relation.

We began to investigate how much the cuticle altered c_i. The investigation showed cuticle to be 20–40 times more conductive for water vapor than for CO_2 (Boyer et al. 1997; Boyer 2015a). Consequently, by using water as a tracer for CO_2 (Eq. 1), the cuticle overestimated the amount of CO_2 entering the leaf (Boyer 2015b). As a result, calculated c_i were too high especially when stomata closed and cuticle transport dominated the gas exchange of the leaf. At night of course, this reversed and calculated c_i became too low (Hanson et al. 2016). If calculated c_i was corrected for these cuticle effects, the calculations came closer to c_i measured directly (Boyer 2015b). This indicated that calculations of c_i need to include cuticle properties.

Ideally, it would be best to have a simple method of incorporating cuticle properties into the calculation. This would preserve the advantage that the leaf is undisturbed. But for now this remains in the future.

4 Plant Enlargement

I arrived at University of Illinois with only my isopiestic psychrometer and soon developed a new system to measure the water potential of whole leaves on intact plants (Boyer 1968). The intact leaves gave the same water potentials as samples (disks) taken from the same leaves. But no matter how much water was supplied, the potentials never were higher than −0.2 MPa. I began to think that the psychrometer was in error and decided to wait for up to 30 h to allow time for the water potential to change. During this extended measurement, the potentials remained stable. However, the leaves absorbed water slowly throughout this time and when the leaves were removed from the psychrometer, they were larger. The leaves had grown. Even recently "mature" leaves grew.

Considering how little water enters the leaf as it grows, the potential of −0.2 MPa was surprising. Water enters leaves much more rapidly for transpiration than for growth (often by at least 100-fold) but leaf water potentials are only slightly more negative (say −0.4 MPa). Why were water potentials associated with growth so close to those for transpiration when water was moving so much faster for transpiration? In other words, if water potential differences drive water uptake, why were they so similar for vastly different uptakes?

After monitoring the rapid water movement in individual cells isolated from the leaves, we began to think that perhaps water flowed along two leaf paths. Forces for the movement would differ for the two paths, at least in sunflower leaves. Water for growth had to reach all the cells including the epidermis. Water for transpiration might bypass many of those cells and evaporate early, deep in the leaf (Boyer 1977). If so, water uptake for transpiration might encounter a low resistance and the water potential differences driving the flow could be small. On the other hand, water uptake for growth might encounter a high resistance because water would have to traverse many tissues to reach the epidermises. Water potential differences for growth would then be large.

These concepts forced us to envision water potentials associated with the growth process itself (when transpiration was not occurring), and we termed them "growth-induced water potentials" because: (1) they were not present in mature tissues and (2) they were induced by the growth process (Molz and Boyer 1978; Cavalieri and Boyer 1982; Boyer 1985). Growth induced these potentials because the walls were softened (loosened) and yielded to the turgor pressure (Boyer 2001). The turgor was held lower than it otherwise would be. As a result, the cell compartment was expanded to a larger size.

The water potential of the cell was transmitted to the apoplast as a tension and could be measured with a pressure chamber illustrated in Fig. 8. The tension moved water into the growing cells and would have a size that depended on the anatomy of the growing organ. Basically, the water would move from the vascular system through intervening small cells to the ultimate cells on the surface. Consequently, Mark Westgate found that the growth-induced water potentials differed depending on which organ was measured in the maize plant because the organs differed in size and vascular supply (Westgate and Boyer 1985b), as seen in Fig. 9. John Passioura similarly found that the anatomy of the tissue contributed to the development of growth-induced water potentials in soybean hypocotyls (Passioura and Boyer 2003), and Wendy Silk and her colleagues elegantly showed the effect in maize roots of various sizes (Wiegers et al. 2009).

In fact, Hiroshi Nonami in our lab conceptualized these potentials in three dimensions as growth-induced "fields" (Fig. 10). Using a pressure probe both to measure the turgor and sample osmotic potentials in individual cells, he reported a growth-induced water potential field in three dimensions for the growing region of soybean hypocotyls (Nonami and Boyer 1993). The field was negligible in the mature basal tissues of the same hypocotyls. Moreover, it could be reversed in only a few cells next to the xylem and cause rapid responses of growth rates (Nonami and Boyer 1990; Tang and Boyer 2003). Figure 11 shows this kind of reversal (red) and indicates that the field is in the wrong direction for extracting water from the xylem. Consequently, rapid changes in xylem water status have immediate effects on growth rates for the whole organ (Nonami and Boyer 1990; Maruyama and Boyer 1994; Passioura and Boyer 2003; Tang and Boyer 2003).

Theoretically, growth-induced water potentials should allow water to be extracted from nearby mature tissue because the potential in the growing region would be lower than in the mature region. Rainer Matyssek et al. (1991b) illustrated this effect by

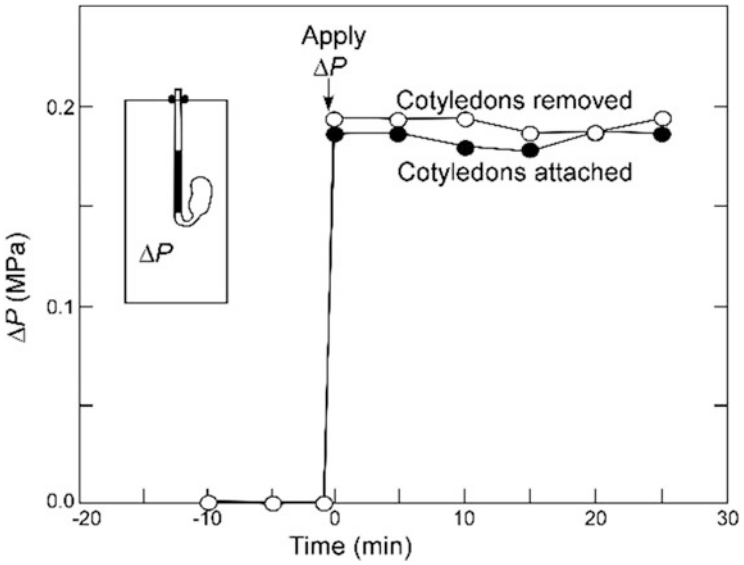


Fig. 8 Measuring growth-induced water potential with a pressure chamber in soybean hypocotyls. In this example, the hypocotyl had been growing rapidly before the roots were excised for the pressure chamber measurement. The growth-induced water potential was determined as the balancing pressure necessary to keep xylem solution at the cut surface (0.2 MPa). This result indicated that the potential existed mostly as a tension (−0.2 MPa) in the apoplast of the elongating region (*black area*). Results are shown for plants with cotyledons but were the same if the cotyledons were removed and thus could not be attributed to cotyledons. The tension measured this way disappeared when growth was fully prevented by pressurizing the hypocotyl of the intact seedling before roots were removed. This illustrated that the growth process itself had caused the growth-induced water potential. Redrawn from Boyer (2001)

excising various plant tissues, as shown in Fig. 12. Only when no mature tissue was attached to the elongating region did growth cease. This showed unequivocally that water in the growing region had a lower potential than in the mature region. The water moved from mature to growing tissue because of the potential difference between the two tissues (Matyssek et al. 1991a, b).

If one considers that growth occurs while transpiration occurs, the competition between the two processes becomes important. For example, a typical grass leaf has the growing region at the base and the more mature blade exposed to the atmosphere. Water moves into the growing region but also through the growing region to be lost in the exposed blade by transpiration. The two processes were investigated first by Mark Westgate who showed that the growth-induced water potential had to be lower than the water potential in the xylem in order for water to enter the base of the leaf for growth (Westgate and Boyer 1984). As water moved through the growing region to the exposed blade, it followed a shallow gradient in water potentials along the xylem. An-ching Tang explored how this gradient related to the anatomy of the maize leaf and found that the protoxylem passed right through the growing region and

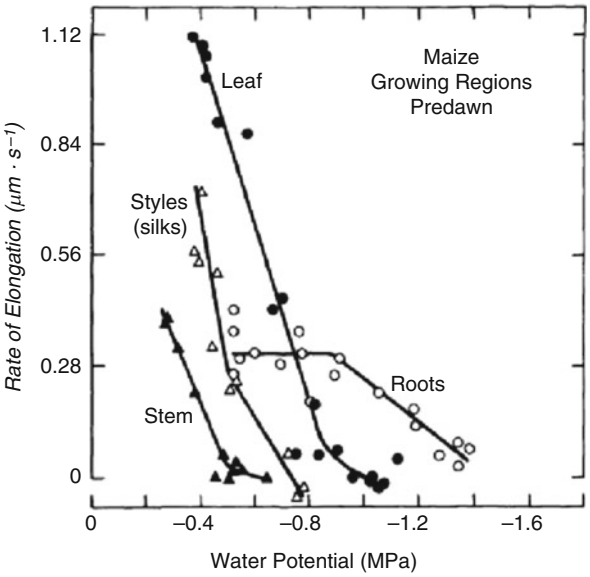


Fig. 9 Growth-induced water potentials in growing regions of various organs of the maize plant. As shown, the growth-induced potentials were −0.3 MPa (Stem), −0.4 MPa (Styles and Leaf), and −0.55 MPa (Roots) during rapid growth. By contrast in adjacent mature tissues the potentials were close to those in the soil (−0.07 to −1.7 MPa, not shown here for clarity but details are in Westgate and Boyer (1985b)). When water was withheld from the soil, growth-induced water potentials became lower and growth ceased at potentials of −0.6 MPa (Stem), −0.75 MPa (Styles), and −1.0 MPa (Leaf). Roots continued growing slowly at −1.4 MPa. All potentials were measured predawn when transpiration was essentially zero. From Westgate and Boyer (1985b)

transported water to the mature blade (Tang and Boyer 2002). The growing region had to extract water from this protoxylem and did so by forming a growth-induced water potential field around each xylem vessel. The field extracted small amounts of water for the growth process while the bulk made its way to the exposed leaf for transpiration.

Growth-induced water potentials are all around us even though we may not notice them. Matyssek et al. (1991a, b) had found that a growing region could extract water from nearby basal mature tissues, so this explains why potatoes can sprout in storage even though no external water is present. The growth-induced water potential of the sprout moves water from the surrounding mature potato tissues into the growing cells. It is only necessary for the cell walls of the bud to become more extensible and prevent the turgor from rising as much as in a mature cell, nicely shown by Sachio Maruyama (Maruyama and Boyer 1994). Despite the fact that water transport for growth is slow compared to transpiration, growth-induced water potentials explain everyday phenomena such as how greens become crisp in the cold or how leaves grow on a tree previously cut down and having no external water supply.

Cosgrove and Cleland (1983) and Cosgrove et al. (1984) viewed these processes differently. At first, Cosgrove and Cleland (1983) obtained solute from growing tissue

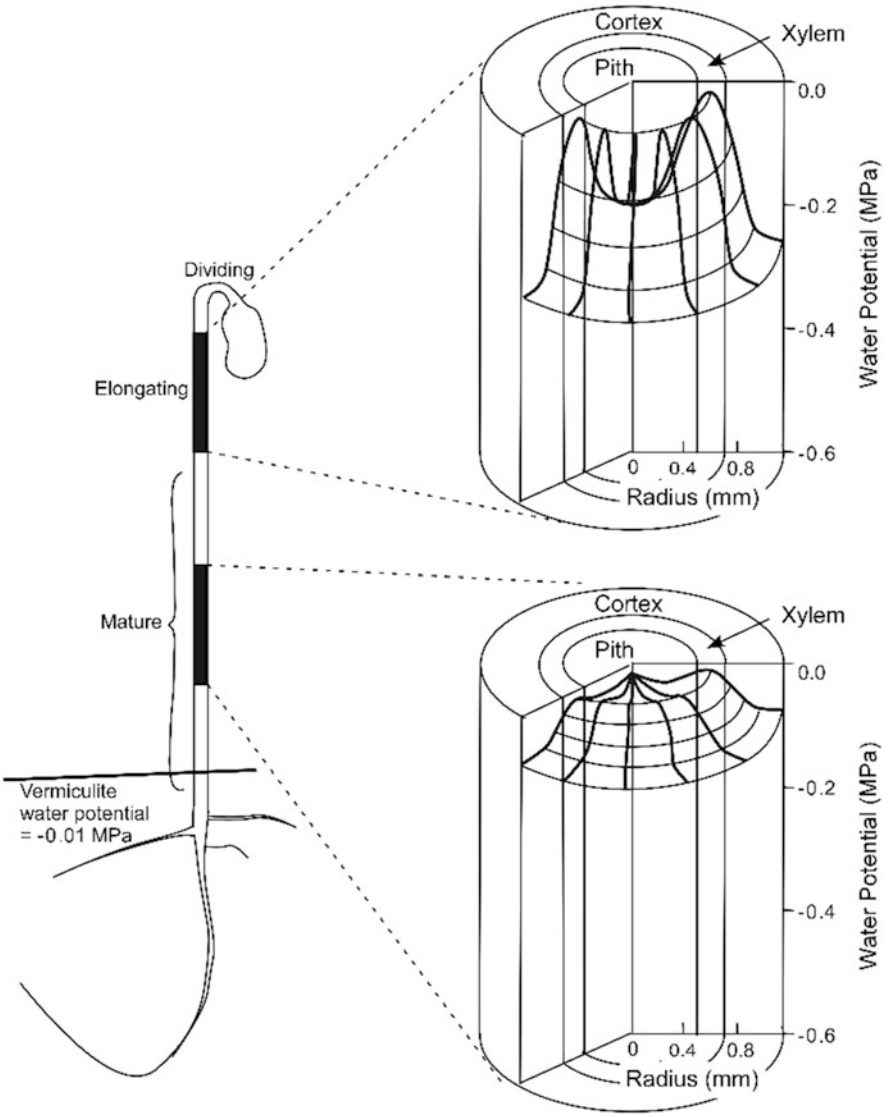


Fig. 10 Growth-induced water potential field in elongating region compared with water potential field in mature region of the same plants. The hypocotyls of these plants were elongating at about 1.5 mm h^{-1}. Fields show the highest potential in xylem and lower potentials in pith and cortex. Note that growth-induced one is sizable in comparison with that in the mature zone. Fields were directly measured in intact plants with microcapillary of a pressure probe. Redrawn from Nonami and Boyer (1993). Copyright American Society of Plant Biologists

and thought it came from the apoplast and thus kept turgor low in the growing cells. However, we succeeded in obtaining apoplast solution from completely intact seedlings and it contained little solute (Nonami and Boyer 1987). Subsequently, Cosgrove et al. (1984) considered our measurements to be artifacts of tissue excision that