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Proceedings of the 8th Plant biomechanics International conference

Hiroyuki Yamamoto, Miyo Terao Morita, Joseph Gril, Miyuki Matsuo

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Proceedings of

The 8th Plant Biomechanics International Conference

第８回　植物バイオメカニクス国際会議　研究発表要旨集

30th November to 4th December, 2015

2015 年 11 月 30 日（月）～12 月 4 日（金）

ES Hall, Nagoya University, JAPAN

名古屋大学　ES 総合館

Hiroyuki YAMAMOTO

Laboratory of Biomaterial Physics, Graduate School of Bioagricultural Science, Nagoya University, Japan

Miyo T. MORITA[1]

Laboratory of Plant Environmental Responses, Graduate School of Bioagricultural Science, Nagoya University, Japan

Joseph GRIL

Laboratory of Mechanics and Civil Engineering (LMGC), CNRS, Univ. Montpellier 2, France

Miyuki MATSUO[2]

Laboratory of Biomaterial Physics, Graduate School of Bioagricultural Science, Nagoya University, Japan

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[1] currently: National Institute for Basic Biology, Okazaki, Japan

[2] currently: Research Institute of Sustainable humanosphere, Kyoto University, Uji, Japan

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Committees

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Dr Bernard THIBAUT* - LMGC, CNRS, Univ. Montpellier II, France
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Dr Simon POPPINGA - Plant Biomechanics Group, Botanical Gardens, Univ. of Freiburg, Germany
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Dr Takahisa HAYASHI - Grad. School of Agriculture, Tokyo Univ. of Agriculture, Japan
Dr Ryo FUNADA - United Grad. School of Agricultural Sci., Tokyo Univ. of Agriculture and Technology, Japan
Dr Ugai WATANABE - Grad. School of Engineering, Chiba Inst. Of Technology, Japan
Dr Naomi NAKAYAMA - Collage of Sci. and Engineering, The Univ. of Edinburgh, Japan

About PBM

Plant Biomechanics and Mechanobiology is an expanding interdisciplinary field, at the interfaces of biology, mechanics, physics and engineering. Over the years, the Plant Biomechanics International Conference has become the central event of this community, as well as a unique place for interdisciplinary exchanges around the amazing features that Plants have evolved to sense, acclimate and adapt to the mechanical challenges they have been submitted to. 8th PBM follows a series of conferences organized every third year since 1994 when the first of these conferences took place in Montpellier, France. Since then, conferences in Reading, UK (1997), Freiburg- Badenweiler, DE (2000), East Lansing, MI, US (2003), Stockholm, SE(2006), Cayenne, French Guyana (2009), Clermont-Ferrand, FR (2012) were organized. We hope that this forthcoming conference in Nagoya will be able to bring you a similar stimulating experience as the ones in the past.

Conference format

The sessions include keynote lectures, invited and contributed papers, and posters with short oral presentations. The official language of the conference is English.

* All oral presentations will be given in English. For the plenary discussions any language is allowed as long as an English speaker is available to help for the translation.

Special sessions

Plant Biomechanics and 500 million history of land plant evolution

The greening of the planet Earth over the past 500 million years has involved vast and intricate patterns of changing phylogenetic and functional diversity. Biomechanics lies at the heart of this diversification, from the colonization of the land and the appearance of stems, roots and leaves to the complex and diverse growth forms and ecosystems we see today. The first aim of this special session is to discuss recent findings on some of the main biomechanical innovations that have characterized land plant evolution culminating in today's complexity and biodiversity; the second objective is to invite both empirical and theoretical discussions on how “biomechanical innovations” that have proved such a success for plants, can be used as bioinspiration for technical applications and biomimetics research.

Chairs: Nick Rowe (FR), Miyo Morita (JP)

Young scientists forum

A young scientists forum will be organized to encourage participation of PhD students, postdocs and young staff.

Session advisors: Kentaro Abe (JP), Naomi Nakayama (UK), Simon Poppinga (DE)

Post-conference tours

Ise Jingu (Ise City) (Bus tour) http://www.isejingu.or.jp/english/

Ise Jingu is one of the oldest Shinto-Shrine that has formally established in AC 685 at the present place, consisting of two sanctuaries. Ise Jingu is located at Ise City, 2 hours by bus from Nagoya. Naiku and Geku. The buildings in each sanctuary are made by traditional wooden structure, using Japanese Cypress (Cupressaceae). Those buildings are reconstructed in each 20 years since AC 690. The building materials are supplied from the man-made forest neighboring to Naiku Sanctuary. We visit sanctuaries and their man-made forest.

Nagoya Historical Culture (Nagoya City) (Walk and subway tours)

http://www.nagoyajo.city.nagoya.jp/13_english

Nagoya is the 4th biggest City in Japan, which has formally started from Atsuta Jingu Shinto-Shrine since AC672. Name of the city "Nagoya" has established since AC 1610, from the construction of Nagoya-Jo Castle and the city at the present place by Tokugawa-Shogunate Family. The Nagoya-Jo Castle was a symbol of Shogunate government in Nagoya, located at the center of city. After the end of Shogunate government, it became one of the most famous historical points in Japan. Unfortunately, Nagoya-Jo Castle was destroyed by the war fire in 1945. After the world war II, Nagoya-Jo Castle was reconstructed and regenerated as a shorgunate museum in 1959. Hommaru Goten-Palace that was used by the load of Nagoya-Jo Castle was also reconstructed in 2014, which shows cultures of Shogun life. We visit Nagoya-Jo Castel and Hommaru Goten-Palace as well as a museum of historical culture.

Wood machinery company and botanical garden (Bus tour)

1. Meinan Machinery Works http://www.meinan.co.jp/ENGLISH

Meinan Machinery Works is a private machinery company where many skillful engineers are working, to produce various types of machines for plywood manufacturing, which is located in Obu-City, 1 hour by bus from Nagoya City. Some of their new products has caused a revolution in plywood industries in Asia, which enabled them to use small-diameter logs (the minimum size is lesser than several cm). We visit them in the morning, and return to Nagoya City to visit Higashiyama Doh-Shokubutsu-en (Zoo and Botanical Garden).

2. Higashiyama Doh-Shokubutsu-en (Zoo and Botanical Garden)

www.higashiyama.city.nagoya.jp/english

One of the biggest Zoo and Botanical Gardens in Japan, which is located close to Nagoya University. They have wild animals and plants from across the globe. We visit in the afternoon after coming back from Meinan Machinery Company.

Sponsors

Japan Society for the Promotion of Science,

Fondation Franco Japonaise Sasakawa,

The Naito Foundation,

The Japan Wood Research Society,

The National Center for Scientific Research,

Jointly hosted by

The Japan Wood Research Society

List of sessions

(* Opening or keynote lectures)

Day 1 (Monday 30th November 2015)

Opening lecture

* Hidetoshi IIDA (Univ. Tokyo, JP)
* Meriem Fournier (AgroParisTech Nancy, FR)

Root

* Lionel Dupuy (The James Hutton Inst., UK)

Growth dynamics & morphogenesis

* Olivier Hamant (ENS Lyon, FR)
* Kazuhiko Nishitani (Univ. Tohoku, JP)

Mechanosensing

Day 2 (Tuesday 1st December 2015)

Kinematics and statics of growth

Mechanics of cells to organs

* Peter Lucas (Univ. Kuwait, KW)

Structural integrity and functions of plants

* Niels Anten (Univ. Wageningen, NL)
* Barbara Lachenbruch (Oregon State Univ., US)

Wood formation and growth strains

* Yoon Soo Kim (Chonnam Nat. Univ., KR)
* Tancrède Alméras (CNRS Montpellier, FR)
* Yan San Huang (Nat. Chung Hsing Univ., TW)

Day 3 (Wednesday 2nd December 2015)

Tools and Cell-wall polymers

Wind and other external loading

*John Moore (Scion Rotorua, NZ)

Internal fluid dynamics

* Melvin Tyree (Northwest A&F University, CN)

Plant motion

* Jiro Sakamoto (Univ. Kanazawa, JP)

Biomimetics

Day 4 (Thursday 3rd December 2015)

Young scientist Forum - Motion & morphogenesis

Young scientist Forum - Wood and other lignified tissues

Plant Biomechanics and 500 million history of land plant evolution

* Jonathan Wilson (Haverford Coll., US)
* Hideyuki Takahashi (Univ. Tohoku, JP)

Day 5 (Friday 4th December 2015)

Technical visits

Program

PBM8 Nagoya 2015 - Status of oral presentations: G = general lecture (45'); K = keynote (30'); L = long (20'); R = regular (15'); Y = medium for YSF (12.5'); S = short (5' + poster)

nb	status	Speaker	country	institution & city	Full abstract title	Author list	session
Monday 30 November 2015							
08:00-08:45		**Registration and reception**					
08:45-10:30							
Welcoming address							
1	G	Hidetoshi Iida	JP	Tokyo Gakugei U.	Molecular aspects of mechanosensitive channels in plants	H. Iida	Opening lecture
2	G	Bruno Moulia	FR	LERFoB Inra Nancy	Reverse Plant Biomechanics: from wood technology to plant mechanobiology, the example of reaction-wood and tropisms	B. Moulia, M. Fournier	Opening lecture
10:30-11:00		**Coffee break**					
11:00-12:40							
3	K	Lionel Dupuy	UK	James Hutton Inst.	Physical factors affecting root growth and interactions with the soil environment	L. Dupuy, A.G. Bengough	Root
4	R	Jie Yan	CN	Chongqing U.	Modeling of helix morphology of Arabidopsis primary root extending through agar medium with low mechanical strength	J. Yan, B.C. Wang, X.Y. Yang	Root
5	R	Rosemary Dyson	UK	U. Birmingham	Multiscale modelling of plant root growth	R. Dyson, J. Chakraborty	Root
6	S	Michitaka Notaguchi	JP	Nagoya U.	Development of on-chip physical characterization method for root growth	H. Hida, K.a Ozoe, I. Kanno, T. Higashiyama, M. Notaguchi	Root
7	S	Youra Hwang	KR	Seoul Nat. U.	Cell wall-associated ROOT HAIR SPECIFIC 10, a pro-rich receptor-like kinase, is a negative modulator in *Arabidopsis* root hair growth	Y. Hwang, M. Park, H. Lee, H.T. Cho	Root
8	S	Nathan Hervieux	FR	LRDP ENS Lyon	A mechanical feedback channels sepal growth and shape in *Arabidopsis*	N. Hervieux, A.L. Routier-Kierzkowska, M. Dumond, S. Tsugawa, C.B. Li, T. Komatsuzaki, R. Smith, A.H.K. Roeder, A. Boudaoud, O.	Growth dynamics & morphogenesis
9	S	Sarah Robinson	CH	U. Bern	Quantification of the mechanical properties of growing Arabidopsis hypocotyls	S. Robinson, M. Huflejt, P. Saxena, C. Kuhlemeier	Growth dynamics & morphogenesis
10	S	Sandra Natonik	PL	U. Silesia Katowice	Heterogeneity *vs* symplasticity of leaf epidermis growth in transgenic arabidopsis exhibiting perturbed cell cycle and differentiation	S. Natonik, J. Elsner, D. Kwiatkowska	Growth dynamics & morphogenesis
11	S	Ugai Watanabe	JP	Chiba IT	Variation of gene expression of tubulin isotypes in the tree cambium	U. Watanabe, H. Abe, N. Futamura, K. Shinohara, R. Funada, S. Nakaba, Y. Yamagishi	Growth dynamics & morphogenesis
12	S	Daeeun Ki	KR	Seoul Nat. U.	Functional conservation of the M3 phosphorylation site in *Arabidopsis* long PINs for their polarity and biological roles	D. Ki, H.T. Cho	Growth dynamics & morphogenesis
13	S	Hyodong Lee	KR	Seoul Nat. U.	Expansin-mediated cell wall modification system to understand the mechanism from cell wall dynamics to cellular processes	H. Lee, H.T. Cho	Growth dynamics & morphogenesis
12:40-14:00		**Poster session and lunch break**					
14:00-15:30							
14	K	Olivier Hamant	FR	LRDP ENS Lyon	Mechanical conflicts in growth heterogeneity	O. Hamant	Growth dynamics & morphogenesis
15	R	Ibrahim Cheddadi	FR	VirtualPlant Inria Montpellier	Coupling physiology and mechanics in multicellular models of plant growth	I. Cheddadi, V. Baldazzi, N. Bertin, M. Génard, C. Godin	Growth dynamics & morphogenesis
16	R	Mariya Ptashnyk	UK	U. Dundee	Multiscale modelling of plant cell wall biomechanics: interactions between mechanical properties and chemical processes	M. Ptashnyk, B. Seguin	Growth dynamics & morphogenesis
17	R	Hirofumi Wada	JP	Kyoto U.	Mechanical basis for the helical pattern in twisting mutants of	H. Wada	Growth dynamics & morphogenesis
18	R	Matilde Dumond	FR	LRDP ENS Lyon	The role of memory-less tissue mechanics in the robustness of morphogenesis in plants	M. Dumond, O. Hamant, A. Boudaoud	Growth dynamics & morphogenesis
15:30-16:00		**Coffee break**					
16:00-17:25							
19	K	Kazuhiko Nishitani	JP	Tohoku U.	Actions of XTH family of enzymes in construction and remodeling of the plant cell wall	H. Kuki, N. Shinohara, R. Yokoyama, T. Higaki, S. Hasezawa, N. Sunagawa, K. Igarashi, S. Tamura, M. Ueda, K. Nishitani	Growth dynamics & morphogenesis
20	R	Firas BouDaher	UK	U. Cambridge	Stop and Go: cell wall pectin controls growth magnitude in the elongating hypocotyl	F. Bou Daher, M. Aita, Y.J. Chen, S.A. Braybrook	Growth dynamics & morphogenesis
21	S	Bruno Moulia	FR	PIAF Inra Clermont	Quantification and comparison of phototropic and gravitropic sensitivities of three hardwood species by original isotropic light devices and model-assisted phenotyping	C. Coutand, B. Adam, S. Ploquin, D. Lopez, B. Moulia	Mechanosensing
22	S	Valérie Legué	FR	PIAF Inra Clermont	Cytosolic calcium increases in response to a change in the positioning of gravisensors: results from space experiments	V. Pereda-Loth, J. Gérard, F. Bizet, V. Legué	Mechanosensing
23	S	Nathalie Leblanc-Fournier	FR	PIAF Inra Clermont	Involvement of a wall associated kinase after gravistimulation in poplar stems	K. Tocquard, N. Brunel, J. Franchel, N. Fournier-Leblanc, J.S. Venisse, P. Roeckel-	Mechanosensing
24	S	Aki Nakamura	JP	Tokyo Gakugei U.	Identification of proteins that potentially interact with Ca^{2+}-permeable mechanosensitive channels MCA1 and MCA2 in Arabidopsis	A. Nakamura, H. Iida	Mechanosensing
25	S	Gilles Pilate	FR	AGPF Inra Orléans	Integration of transcriptomic and proteomics approaches in characterizing short-term gravi-perception signaling networks in poplar wood	N. Richet, M. Mauriat, M.C. Lesage-Descauses, O. Rogier, F. Laurans, S. Huguet, S. Balzergue, N. Lapalu, G. Pilate, C. Coutand, C. Plomion,	Mechanosensing
26	S	Masatoshi Taniguchi	JP	Nagoya U.	DGE1, DGE2 and DTL genes are involved in gravity signaling in gravity sensing cells of *Arabidopsis*	M. Taniguchi, K. Baba, A. Yuasa, M. Tasaka, M.T. Morita	Mechanosensing
27	S	Hideto Hiraide	JP	Nagoya U.	A laccase gene is involved in compression wood lignification of *Chamaecyparis obtusa*	H. Hiraide, M. Yoshida, S. Sato, K. Ihara, Matsuo, H. Yamamoto	Mechanosensing
28	S	Pauline Durand-Smet1	FR	LMSC U. Paris 7	Quantitative evaluation of feedback mechanisms between cell shape and cytoskeleton organization	P. Durand-Smet, A. Sampathkumar, E. Meyerowitz	Mechanosensing
17:25-18:00		**Poster session and coffee break**					
18:00-19:30							
29	R	Haruko Ueda	JP	Kyoto U.	An actin-myosin XI cytoskeleton determines plant posture by regulating organ straightening	H. Ueda, T. Shimada, K. Tamura, M.T. Morita, I. Hara-Nishimura	Mechanosensing
30	R	Geoffroy Guena	FR	IUSTI Marseille	A universal mechanism for hydraulic signals generation in natural and artificial branches	J-F. Louf, G. Guena, E. Badel, Y. Forterre	Mechanosensing
31	R	Yasmine Meroz	US	Harvard U.	A minimal model for plant tropisms as a noisy sensorimotor system	Y. Meroz, R. Bastien , L. Mahadevan	Mechanosensing
32	R	Olivier Pouliquen	FR	IUSTI Marseille	On the role of gravity in shoot gravisensing	H. Chauvet, O. Pouliquen, Y. Forterre, V. Legué, B. Moulia	Mechanosensing
33	R	V Bonnesoeur	FR	LERFoB Inra Nancy	Tree acclimation to wind in a dense beech forest to investigate how mechanical cues are filtered and modify radial growth.	V. Bonnesoeur, T. Constant, M. Fournier, B. Moulia	Mechanosensing
34	R	Nathalie Leblanc-Fournier	FR	PIAF Inra Clermont	Secondary growth regulation by strains induced by wind: from stem structure to gene expression	E. Badel, N. Leblanc-Fournier, J. Franchel, M. Decourteix, C. Coutand, B. Moulia	Mechanosensing

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nb	status	Speaker	country	institution & city	Full abstract title	Author list	session
Tuesday 1 December 2015							
09:00-10:30							
35	R	Eran Sharon	IL	Hebrew U. Jerusalem	The intermittent nature of leaf growth fields	E. Sharon, S. Armon	Kinematics and statics of growth
36	R	Tobias Baskin	US	U. Massachusetts	On the stability of the growth pattern in the root	T.I. Baskin	
37	R	Felix Hartmann	FR	LERFoB Inra Nancy	Control of the kinematics of wood formation by a morphogenetic gradient	F.P. Hartmann, C.B.K. Rathgeber, M. Fournier, B. Moulia	
38	R	Dmitry Suslov	RU	St. Petersburg State U.	Cell wall basis of rhythmical growth changes in *Arabidopsis* hypocotyls	A. Ivakov, A. Flis, F. Apelt, M. Fünfgeld, F. Kragler, U. Scherer, M. Stitt, K. Vissenberg, S. Persson, D. Suslov	
39	R	Julien Derr	FR	LMSC U. Paris 7	Interplay between nutation and leaf development with or without gravity	J. Derr, L. Laffond, M. Rivière, A. Peaucelle, S. Douady	
40	R	Dorota Kwiatkowska	PL	U. Silesia Katowice	Stress distribution in the cell wall – why does the innermost wall layer buckle after stress removal?	D. Kwiatkowska, M. Lipowczan, D. Borowska-Wykręt, S. Natonik	
10:30-11:00			**Coffee break**				
11:00-12:40							Mechanics of cells to organs
41	K	Peter Lucas	KU	Kuwait U.	Tooth wear of mammals and plant foods	P. W. Lucas, A. van Casteren, A. G. Henry, K. Al-Fadhalah, A. S. Almusallam, S. Michael, L. A. Thai, A. Shekeban, J. Watzke, S. Philip, B. W. Wright, D. S. Strait, A. G. Atkins	
42	R	Anja Geitmann	CA	U. Montreal	The mechanics of invasive cellular growth	A. Sanati Nezhad, M. Ghanbari, C.G. Agudelo, M. Packirisamy, A. Geitmann	
43	R	Hugh Woolfenden	UK	John Innes C. Norwich	Inference of material properties from guard cell dynamics	H.C. Woolfenden, M. Kopischke, S. Robatzek, R.J. Morris	
44	S	Suhaiza Hanim Hanipah	MY	U. Putra Malaysia,	Micromechanics of oil palm fibres with silica bodies	S.H. Hanipah , F.N. Omar , L.X. Xiang , M.A.P. Mohammed, A.S. Baharuddin , J. Abdullah	
45	S	Marcin Lipowczan	PL	U. Silesia Katowice	Surface growth quantification of puzzle-shaped epidermal cells with subcellular resolution	M. Lipowczan, J. Elsner, D. Kwiatkowska	
46	S	Mathieu Rivière	FR	LMSC U. Paris 7	Morphogenetic motions: a tool to study growth in leaves	M. Rivière, J. Derr, A. Peaucelle, S. Douady	
47	S	Michal Sahaf	IL	Hebrew U. Jerusalem	Stress-induced changes in the mechanical properties of leaves	M. Sahaf, E. Sharon	
48	S	Vaclav Sebera	CZ	Mendel U. Brno	Analysis of strain transfer from xylem to bark with use of DIC	V. Sebera, J. Kunecký, L. Praus, J. Tippner, D. Ševčík, . Horáček, Š. Surňák	
49	S	Kiyosada Kawai	JP	Kyoto U.	Division of labor in leaf functions among vein orders: an example of *Fagaceae*	K. Kawai , N. Okada	
50	S	Frédéric Mabille	FR	IATE Montpellier	Chemical treatment for the degradation of the mechanical properties of miscanthus stems to facilitate fragmentation	F. Mabille, A. Sadoudi, C. Barron	
51	S	Christophe Baley	FR	LIMatB Lorient	Influence of genetic pool on the stem microstructure and the lodging stability of flax	C. Baley, A. Bourmaud	
12:40-14:00			**Poster session and lunch break**				
14:00-15:30							Structural integrity and functions of plants
52	K	Niels Anten	NL	Wageningen U.	Strong or flexible: some ecological aspects of plant resistance to mechanical stress.	N. Anten, S. Puijalon, F. Sterck, Y. Onoda	
53	R	Thierry Fourcaud	FR	AMAP Montpellier	Modelling tree anchorage and estimating different contributing factors	M. Yang, P. Défossez, F. Danjon, T. Fourcaud	
54	R	Jan Tippner	CZ	Mendel U. Brno	Marker-tracking analysis of tree trunk deflection and root plate movement during pulling test	J. Tippner, V. Sebera, L. Praus, D. Ševčík, J. Čepela, M. Brabec	
55	K	Barbara Lachenbruch	US	Oregon State U.	It's cool but what's it for? Multifunctionality and interpretation of function	B. Lachenbruch	
15:30-16:00			**Coffee break**				
16:00-17:25							
56	R	Alain Bourmaud	FR	LIMatB Lorient	Impact of the seeding rate on flax stem stability and fiber mechanical properties	A. Bourmaud, C. Baley	
57	S	Douglas Cook	AE	New York U. Abu Dhabi	Structural role of the leaf sheath	M. Julias, D. Robertson, D. Cook	
58	S	Margaret Julias	AE	New York U. Abu Dhabi	Forensic analysis of stalk failure in maize	M. Julias, D. Robertson, B.W. Gardunia, D. Cook	
59	S	Delphine Jullien	FR	LMGC U. Montpellier	Numerical study of the stress distribution in bending trees: analysis of the effects of shear stress and maturation stress on tree resistance	D. Jullien, T. Almeras	
60	K	Yoon Soo Kim	KR	Chonnam Nat. U.	Heterogenity of cell wall in relation to wood quality	F. Xu, Z. Ji, X. Zhang, Y.S. Kim	Wood formation and growth strains
61	S	Takahisa Hayashi	JP	Tokyo U. Agric.	Occurrence of xyloglucan in poplars for wind and earthquake	R. Kaida, E. Obataya, M. Yoshida, F. Ishiguri, J. Tanabe, T. Taniguchi, M. Kurita, K. Baba, T.	
62	S	Nicholas Davies	NZ	U. Canterbury	Screening eucalyptus for growth strain	N. Davies, M. Sharma, C. Altaner, L. Apiolaza	
63	S	Kana Yamashita	JP	FFPRI Tsukuba	Gradient of residual stress within logs affecting deformation of sawn timber	K. Yamashita, T. Fujiwara, Y. Ikami, Y. Matsumura, H. Kato, M. Matsuo, H. Yamamoto	
64	S	Sandrine Bardet	FR	LMGC U. Montpellier	Modelling Hygrothermal recovery of wood in relation with locked-in strains during tree life	S. Bardet, J. Gril	
65	S	Thiery Constant	FR	LERFoB Inra Nancy	Retrospective analysis over ten years of the static loading of beech tree crowns based on allometric relationships between branch dimensions and biomass distribution	T. Constant, J.B. Morisset	
17:25-18:00			**Poster session and coffee break**				
18:00-19:30							
66	K	Tancrede Almeras	FR	LMGC U. Montpellier	The motor function of wood: mechanisms underlying the generation of maturation stress during cell-wall formation	T. Almeras	
67	R	Bruno Clair	GF	EcoFoG Kourou	Modifications in the cell wall during the development of tension wood in a G-layer and a non-G-layer species	B. Clair, S.S. Chang, J.R. Roussel, F. Quignard, J. Beauchêne, T. Alméras	
68	R	Gilles Pilate	FR	AGPF Inra Orléans	Evaluation of non-cellulosic polysaccharide distribution in differentiating and mature poplar tension wood fibres: abundance of rhamnogalacturonan I, presence of acetylated glucomannan and absence of xyloglucan in the G-layer	F.T.P. Guedes, F. Laurans, B. Quemener, A. Secerovic, C. Assor, N. Boizot, J. Vigouroux, M.C. Lesage-Descauses, J.C. Leplé, A. Déjardin, G. Pilate	
69	K	Yan San Huang	TW	Nat. Chung Hsing U.	Biomechanical aspect of stem movement and the growth strains in monocotyledonous giant bamboo	Huang YS, Lee CM, Hung LF, Hsu LF, Chang HT and Kuo-Huang LL	

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nb	status	Speaker	country	institution & city	Full abstract title	Author list	session
Wednesday 2 December 2015							
09:00-10:30							
70	R	Mahmoud Fakih	FR	AMAP Montpellier	Analysis of the mechanical interaction between a growing root and a granular substrate using the Discrete Element Method	M. Fakih, J.Y. Delenne, F. Radjai, T. Fourcaud	Tools and Cell-wall polymers
71	R	Frédéric Mabille	FR	IATE Montpellier	Mapping of the structure of a corn kernel by three complementary experimental techniques	F. Mabille, A. Sadoudi, T. Ruiz, R. Sidiboulenouar, C. Coillot	
72	R	Iris Brémaud	FR	LMGC U. Montpellier	Use of vibrational properties to categorize the different types of wood within trees - as compared to interspecific diversity	I. Brémaud, . Cabrolier	
73	R	Brigitte Chabbert	FR	FARE Inra Reims	Nanocharacterization of chemical and mechanical properties of plant cell walls and lignocellulosic bioinspired assemblies	B. Chabbert, L. Muraille, B. Bercu, V. Aguié-Béghin, M. Molinari	
74	R	Lennart Salmén	SE	Innventia Stockholm	Role of lignin in the structure of wood fibres	L. Salmén	
75	R	Karol Kulasinski	CH	EMPA Zurich	Water sorption in S2 layer: role of the crystalline-amorphous interface	K. Kulasinski, R. Guyer, D. Derome, J. Carmeliet	
10:30-11:00			**Coffee break**				
11:00-12:30							
76	S	Merve Özparpucu	CH	IFB Zürich	Micromechanical and structural characterization of genetically modified poplar	M. Özparpucu, M. Rüggeberg, I. Burgert	
77	S	Olivier Arnould	FR	LMGC U. Montpellier	Experimental characterization of hydrogel swelling under plant cell wall environment	C. Assor, F. Quignard, T. Almeras, O. Arnould	
78	S	Daniel Robertson	AE	New York U. Abu Dhabi	Nondestructive predictors of stalk and stem strength	D. Robertson, M. Julias, S.Y. Lee, D. Cook	
79	K	John Moore	NZ	SCION Rotorua	Impacts of wind loading on tree form and wood properties	J. Moore	Wind & other
80	R	Barry Gardiner	FR	ISPA Inra	Plant wind damage	B. Gardiner, B. Moulia, P. Berry	
81	R	Dominique Derome	CH	EMPA Zurich	Integrating trees and vegetation in urban microclimate simulations: a multiscale approach	D. Derome, T. Defraeye, J. Allegrini, J. Carmeliet	
82	S	Toby Jackson	UK	U. Oxford	Modelling trees response to wind forcing using terrestrial LiDAR data	T. Jackson, P. Raumonen, A. Shenkin, Y. Malhi	
~~83~~	~~S~~	~~Natalya Kizilova~~	~~UA~~	~~Kharkov Nat. U.~~	~~Evolutionary optimization of fluid transportation systems in plant leaves~~	~~N. Kizilova~~	Internal fluid dynamics
84	S	Hanna Rademaker	DK	T.U. Denmark, Copenhagen	Diffusion and bulk flow of sugar and water in leaves I: experiments	H. Rademaker, K.E. Villumsen, K.H. Jensen, H.J. Martens, A. Schulz, T. Bohr	
12:35-14:00			**Poster session and lunch break**				
14:00-15:30							
85	R	Tomas Bohr	DK	T.U. Denmark, Copenhagen	Diffusion and bulk flow of sugar and water in leaves II: theory	T. Bohr, H. Rademaker, K.E. Villumsen, K.H. Jensen, H.J. Martens, J. Dölger, J. Liesche, A. Schulz	
86	K	Mel Tyree	CN	Northwest A&F U.	An overview of tensile water in living and dead cells: The role of negative pressure in water transport and in the water balance of leaves.	M. Tyree, D. Yang, Y. Ding	
87	K	Jiro Sakamoto	JP	Kanazawa U.	Seeding strategy of the autochore plant and bursting mechanism of the fruits	J. Sakamoto, Y. Endo, K. Ito	Plant motion
88	R	Hidetoshi Kobayashi	JP	Osaka U.	Geometry in closing motion of leaflet of sensitive plant (*Mimosa pudica*)	H. Kobayashi, K. Horikawa, N. Tominaga, T. Yamauchi	
15:30-16:00			**Coffee break**				
16:00-17:30							
89	S	Mitsuhiro Aida	JP	Nara IST	Carpel closure by protodermal tissue adhesion in *Arabidopsis thaliana*	R. Tsujino, A. Yokoi, H. Ichikawa, M. Iwano, S. Takayama, M. Aida	
90	S	Koji Ito	JP	Kanazawa U.	Seed ejection simulation of autochore plant fruit in case of *Japonica*	K. Ito, J. Sakamoto	
91	S	Denis Terwagne	BE	Fac. Sciences Brussels	Curving the fold, an actuation mechanism for plant reconfiguration	J. Segers, D. Terwagne	
92	R	Markus Rüggeberg	CH	IFB Zürich	Smart wooden actuators	M.Rüggeberg, C.Vailati, M. Hassani, F.Wittel, I.Burgert	Biomimetics
93	R	Antoine Le Duigou	FR	LIMatB Lorient	Pine cone as a source of inspiration for moisture induced self-shaping flax/PP biocomposite	A. Le Duigou, M. Castro	
94	R	Olga Speck	DE	U. Freiburg	Wound reactions of herbaceous plants	O. Speck, S. Anandan, C. Paul-Victor, A. Cegna, K. Schmauder, A. Rudolph, T. Speck	
95	R	Thomas Speck	DE	U. Freiburg	Bio-inspiration by plants for construction technology and architecture: a short overview of recent work in the CRC 141	T. Speck	
96	S	Goerg Bauer	DE	U. Freiburg	Energy dissipation in plants – from puncture resistant seed coats to impact resistant tree barks	G. Bauer, S. Schmier, M. Thielen, T. Speck	
97	S	Toshiko Furukawa	JP	Tokyo Metropolitan U.	A model of plant and microbe symbiosis: ricardian approach	T.Furukawa, T. Iimura	
98	S	Marc Thielen	DE	U. Freiburg	How the pomelo peel (*Citrus maxima*) absorbs impact energy by distributing stresses	M. Thielen, T. Speck	
17:30-18:00			**Poster session and coffee break**				
18:30-21:00			**Banquet**				

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99	L	Simon Poppinga	DE	U. Freiburg	New insights into the passive nastic motions of pine cone scales and false indusia in ferns	S. Poppinga, T. Speck	Young scientist Forum - Motion & morphogenesis
###	Y	Holger Bohn	DE	U. Freiburg	A passionate climber: functional morphology and biomechanics of the adhesive tendrils in *Passiflora discophora*	H.F. Bohn, F. Günter, S. Schmier, S. Fink, T. Speck	
###	Y	Valeria Hernández-	MX	U. Nat. Autónoma de	Biophysical dynamic module for the polarization of auxin efflux carriers PIN-FORMED (PIN)	V. Hernández-Hernández, N. Nakayama, A. Garay1, R. Barrio, C. Villarreal, E. R Alvarez-	
###	Y	Alexis Peaucelle	FR	LMSC U. Paris 7	Cellular asymmetry in wall elasticity, triggers growth symmetry breaking and cell expansion rate in the *Arabidopsis* hypocotyl	A. Peaucelle, R. Wightman, H. Höfte	
###	Y	Pauline Durand-Smet2	US	CalTech, California	Mechanics of single plant cells	P. Durand-Smet, N. Chastrette, A. Guiroy, A. Richert, A. Berne-Dedieu, M. Bendahmane, J.M. Frachisse, O. Hamant, A. Boudaoud, , A.	
###	Y	Darshil Shah	UK	U. Cambridge	Probing the role of xylan in irreversible deformation of stems of *Arabidopsis thaliana*	D. Shah, T. Reynolds, M. Busse-Wicher, L. Yu, P. Dupree, M. Ramage	
###	Y	Anne-Lise Routier-Kierzkowska	DE	MPI Cologne	Making the mechanics visible: micro-indentation on a confocal microscope	A.L. Routier-Kierzkowska, A. Weber, A. Sapala, T. Ruetti, G. Mosca, R.S. Smith	
10:00-11:00			**Coffee break**				
11:00-12:40							
###	Y	Marie Capron	JP	Nagoya U.	Mechanical characterization of developing tension wood fibre wall by atomic force microscopy	M. Capron, M. Ramonda, F. Laurans, B. Clair, T. Almeras, O. Arnould	Young scientist Forum - Wood and other lignified tissues
###	Y	Barbara Ghislain	GF	EcoFoG Kourou	Does anatomical diversity of tension wood reflect diversity of mechanisms to generate stress?	B. Ghislain, J. Engel, B. Clair	
###	Y	Douglas Cook	AE	New York U. Abu Dhabi	The influence of tissue and morphology on stalk bending stresses	D. Cook, D. Robertson, S.Y. Lee, G. Von Forell	
###	Y	Linnea Hesse	DE	U. Freiburg	Using MRI for analyzing the anatomy and biomechanics of monocotyledons	T. Masselter, L. Hesse, N. Spengler, J.G. Korvink, T. Speck	
###	Y	Pierre Cabrolier	FR	LMGC U. Montpellier	From biomechanics to material behavior of *Buxus sempervirens* L. An angiosperm forming compression wood	P. Cabrolier, I. Brémaud	
###	Y	Miyuki Matsuo	JP	Nagoya U.	Relationships between growth stress and hygrothermal recovery of compression wood	M. Matsuo, K. Nomi, Y. Suzuki, N. Yamashita, G. Niimi, M. Yoshida, H. Yamamoto	
###	Y	Estelle Noyer	FR	LERFoB Inra Nancy	Are old beech poles able to react to heavy thinning? And how does growth response affects pole biomechanics?	E. Noyer, J. Dlouha, C. Collet, M. Fournier	
###	Y	Kazuki Nanko	JP	FFPRI Tsukuba	Simulation of tree deformation of Japanese black pine caused by temporally varied wind	K. Nanko, S. Suzuki, H. Noguchi, H. Hagino, A. Ogura, Y. Ishida, H. Matsumoto, H. Takimoto, T. Sakamoto	
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14:00-15:30							Plant Biomechanics and 500 million history of land plant evolution
###	K	Jonathan Wilson	US	Haverford Coll.	Physiology and biomechanics of extinct plants	J. Wilson	
###	L	John P Moore	ZA	I. Wine Biotech Stellenbosch	Arabinose-rich polymers as an evolutionary strategy to plasticize resurrection plant cell walls against desiccation: a case study of the woody angiosperm *Myrothamnus flabellifolia*	J.P. Moore, E. Nguema-Ona, M. Vicré-Gibouin, I. Sørensen, W.G.T. Willats, A.Driouich, J. Farrant	
###	L	Yusuke Onoda	JP	Kyoto U.	A novel method quantifying stiffness of epidermis and mesophyll layers reveals leaves have a highly efficient sandwich structure	Y. Onoda, F. Schieving, N.P.R. Anten	
###	L	Masato Yoshida	JP	Nagoya U.	Diversity of gymnosperm reaction wood anatomy and growth stress	M. Yoshida, T. Shirai, M. Matsuo, Lahjie AM	
15:30-16:00			**Coffee break**				
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###	K	Hideyuki Takahashi	JP	Tohoku U.	Root tropisms for plant adaptation to terrestrial environment: gravitropism *vs*. hydrotropism	H. Takahashi	
###	L	Rivka Elbaum	IL	Hebrew U. Jerusalem	Hygroscopically coiling cells in a grass – *Stipa capensis*	Y. Abraham, S. Kumar, Y. Silberberg, R. Elbaum	
###	L	Derek Gray	CA	McGill U.,	Vascular structure of leaf petioles	D.G. Gray	
###	L	Nick Rowe	FR	AMAP Montpellier	Evolution of herbs and woodiness	N. Rowe	
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Molecular aspects of mechanosensitive channels in plants

Hidetoshi Iida

Department of Biology, Tokyo Gakugei University, Japan
iida@u-gakugei.ac.jp

Most people don't know how sensitive to mechanical stimulation every plant is except for *Mimosa pudica* and the Venus' flytrap. This is because leaves and stems of most plants do not move following perception of mechanical stimuli such as touch. As experimental methodologies have advanced, however, plant scientists now know that plants are very sensitive to mechanical stimulation. This understanding is partly due to a monumental work with Arabidopsis seedlings expressing the Ca^{2+}-sensitive photoprotein aequorin performed by Knight *et al*. (1991) a quarter-century ago. The seedlings emitted light immediately after touch stimulation, suggesting that they sensed touch and responded to it by increasing the cytoplasmic Ca^{2+} concentration, leading us to have an idea that a Ca^{2+}-permeable mechanosensitive (MS) channel is a sensor of touch.

MS channels are found in all the kingdoms. Between them, MS channels in plants are less understood from the molecular viewpoint. In contrast, bacterial MS channels such as MscS and MscL are well studied and thus have been serving as good models to explain the molecular mechanism of mechano-sensing. An important point of the mechanism is that interaction between the surface of MS channel molecules and lipid bilayers is a basis for the determination of the open and closed states of the channels. In other words, MscS and MscL do not need other cellular components to open and close. Studies of animal and fungal transient receptor potential (TRP) channels are also well performed and have added another point of view on the mechanism: TRP channels are regulated to open not only by interaction with lipid bilayers but also that with cellular components, including the cytoskeletons and integrins. These findings have given a mechanistic insight into plant MS channels that are not well studied so far at the molecular level.

To date, three different families of plant MS channels are identified and characterized to some extent. Those include MscS-like (MSL) channels (Haswell, 2007), MCA channels (Nakagawa et al., 2007) and a recently identified OSCA1 channel (Yuan et al., 2014). Although all the families are interesting in terms of osmoregulation, only MCA channels are studied at the molecular level (Nakano *et al*., 2011; Shigematsu *et al*., 2014). MCA1 was first identified as an Arabidopsis cDNA capable of complementing the conditional lethality and Ca^{2+} influx deficiency of the yeast *mid1* mutant, thus being named after the *mid1*-complementing activity 1 (Nakagawa et al., 2007). MCA1 has a paralog, MCA2, and both share 73% identity in amino acid sequence and have three motifs such as EF hand-like, coiled-coil and plaq8 motifs. Both proteins form homotetramers and have one transmembrane segment near the extracellular N-terminus with a cytoplasmic C-terminus. The structural features are supported by cryo-electron microscopy followed by a single particle analysis showing that MCA2 have a pear-shaped structure with a narrow putative transmembrane region and a large cytoplasmic region. The three motifs should function in the cytoplasm. The EF hand-like and coiled-coil motifs are suggested to regulate the activity of MCA1 and MCA2 differentially with Ca^{2+} uptake assays using a yeast expression system. Deletion of the transmembrane segment and the substitution of Asn for Asp in this segment impair the Ca^{2+} influx activity of both channels, indicating that this segment is crucial for Ca^{2+} permeation. A patch-clamp analysis using *Xenopus* oocytes has shown that MCA1 has a single-channel

activity of approximately 34 pS (Furuichi et al., 2012). Based on these structural characteristics, I will also discuss a physiological role of MCA1 and MCA2.

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Reverse Plant Biomechanics: from wood technology to plant mechanobiology, the example of reaction-wood and tropisms

Moulia B[1,2], Fournier M[3,4]

[1] INRA, UMR 547 PIAF, F-63100 Clermont Ferrand Cedex 01, France
[2] Clermont Université, Université Blaise Pascal, UMR 547 PIAF, BP 10448, F-63000 Clermont-Ferrand, France
[3] INRA, UMR 1092 Laboratoire d'Etude des ressources Forêt Bois (LERFoB), Centre INRA de Nancy-Lorraine, 54280 Champenoux, France
[4] AgroParisTech, UMR 1092, Laboratoire d'Etude des ressources Forêt Bois (LERFoB), ENGREF, 14 rue Girardet, 54000 Nancy, France
bruno.moulia@clermont.inra.fr

Physical factors affecting root growth and interactions with the soil environment

Dupuy L. [1] and Bengough A.G. [1,2]
[1] The James Hutton Institute, Dundee, Scotland UK
[2] University of Dundee, Dundee, Scotland UK
Lionel.Dupuy@hutton.ac.uk

Background

Roots overcome considerable pressure from the surrounding soil environment to elongate or initiate new root primordia. Mechanical impedance varies both temporally and spatially in field soils, depending on soil bulk density, soil matric potential, and the arrangement of continuous macropores. It is frequently the most important abiotic stress limiting root elongation at the scale of the root tip, and we need to understand how roots respond to gradients in soil strength and their ability to bend and grow around stronger regions of soil and small stones. Unfortunately, knowledge and techniques to measure and understand root responses to mechanical impedance are limited.

Results

This talk will present various study cases that illustrate how the kinematics of root elongation is influenced by external mechanical forces and how, in turns, root growth kinematics influence interactions between the root and the soil environment. We also present new approaches to understand root and soil physical interactions, including new micro-mechanical sensors, 3D imaging and image analysis, transparent soils and the development of modelling frameworks (Figure 1).

Conclusion

The mechanics of root growth is central to the establishment of plants and their root systems. The research presented here has application in agronomy, for example, to optimise crop resource use efficiency, but also for the bio-engineering of soils that are reinforced by plant roots.

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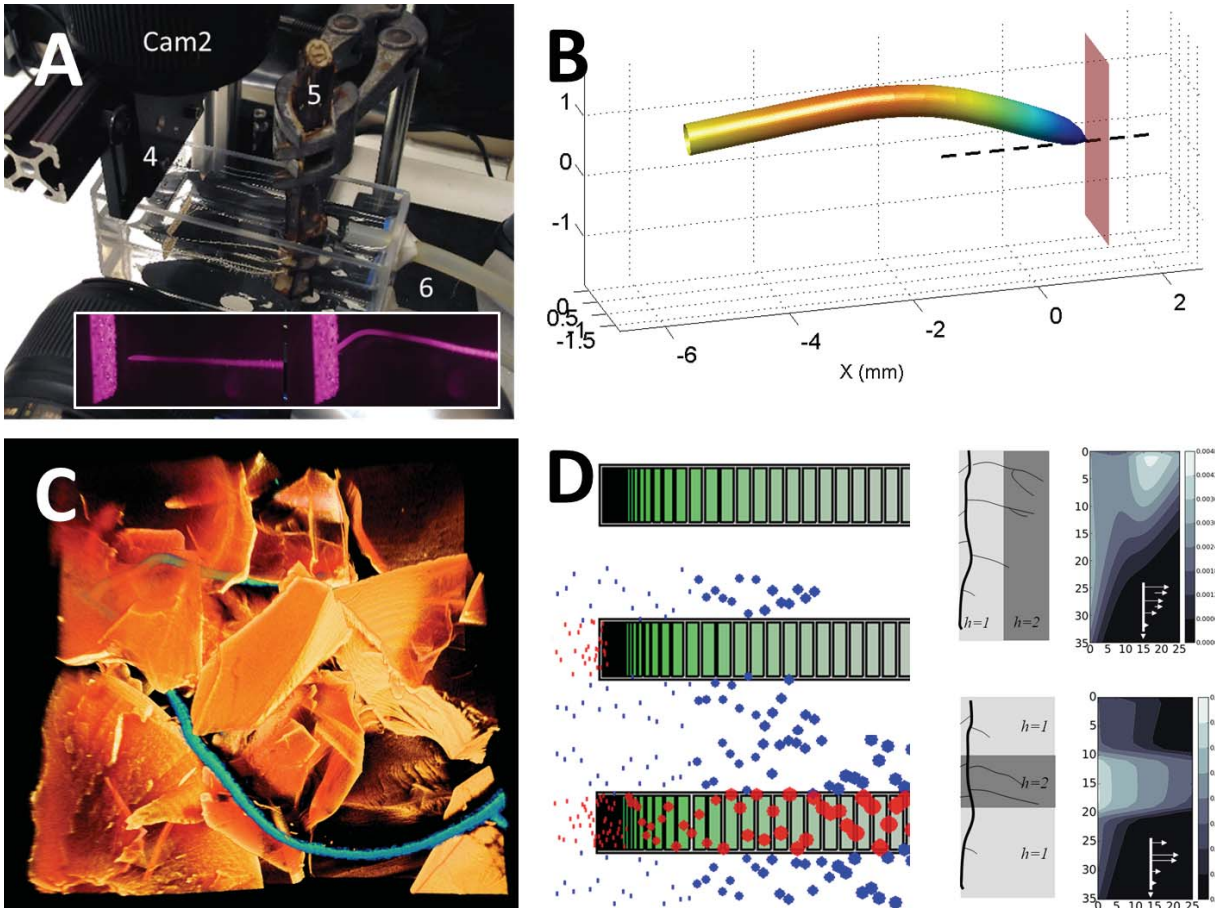

Figure 1: New systems to understand root soil physical interactions. A) combined opto-mechanical sensor and 3D imaging to characterise root growth responses to obstacles and B) reconstruction algorithms are used to determine live stress and strain distribution in root tissue [1]. C) Transparent soils are also combined to 3D microscopy techniques such as Confocal Laser Scanning Microscopy to look root trajectories in granular media and interaction with soil particles [2]. D) Knowledge is then used to develop models to look at how the physics of root growth affects the surrounding soil environment both at the single root and whole root architecture level ([3, 4]).

Modeling of helix morphology of Arabidopsis primary root extending through agar medium with low mechanical strength

Jie Yan, Bochu Wang*, Xingyan Yang

Key Laboratory of Biorheological Science and Technology, Ministry of Education, College of Bioengineering, Chongqing University, Chongqing 400030, China
Corresponding author e-mail: wangbc2000@126.com
Jie Yan: yanjie185@sina.com

Introduction

To explore the mechanisms of root growth behavior, numerous studies reported Arabidopsis root waving on hard agar (1.2%-1.5%) surface. However, few literature reports employed physical laws to investigate roots growth, activity during Arabidopsis roots development. We observed growth behavior of Arabidopsis roots inside 0.5%-1.2% agar media and measured Young's modulus of each agar medium. Arabidopsis primary roots exhibited helix behavior when penetrating 0.5%-0.9% agar media. Based on reasonable biophysical interpretation of root system, root helix morphology was considered as a standard cylindrical helix. Using this model, time dependent mechanical forces on root tip were analyzed. Differences were found in root length, helix diameter and auxin distribution in root tips in agar media with different Young's modulus. The internal force obtained in this model showed variability and it is possible to calculate its size and direction when the accurate mechanical characteristics of medium and root growth velocity were obtained. Root growth force F is essential for the circles of helical roots; friction from surrounding media impacts the property parameters of helix. Taken together, root growth force and external mechanical forces modulate root phenotypes in Arabidopsis. We demonstrated that direct contact between gravity and friction is not sufficient to promote emergence of waving, circumnutation and helix. The present article provides a new look at root growth forces in root behaviors.

Mechanical modeling for helix root inside agar

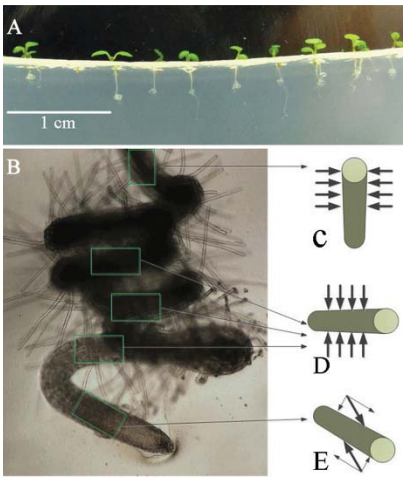

Fig. 1 : the helical root of *Arabidopsis* 7 days after seeding inside 0.5% agar medium and three modes of root reaction from the agar gel aside from friction, which are elastic, viscoelastic, and plastic forces. Black arrows indicate the forces acting on the root. These forces are balanced and negligible.

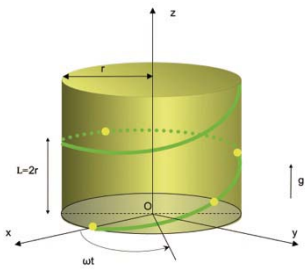

Fig. 2 : A cylindrical helix in the polar coordinates system which is simplified from the architecture of Arabidopsis primary root when growing through 0.5% agar medium..

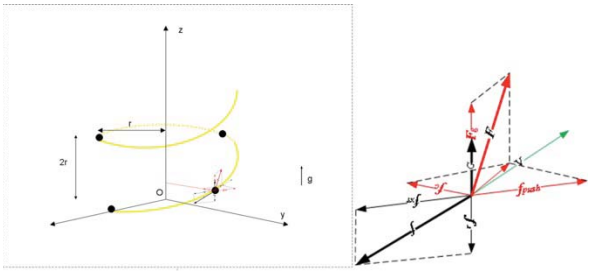

Fig. 3 : Take any point of the cylindrical helix for force analysis in the polar coordinates.

The suitability of model verified by test results

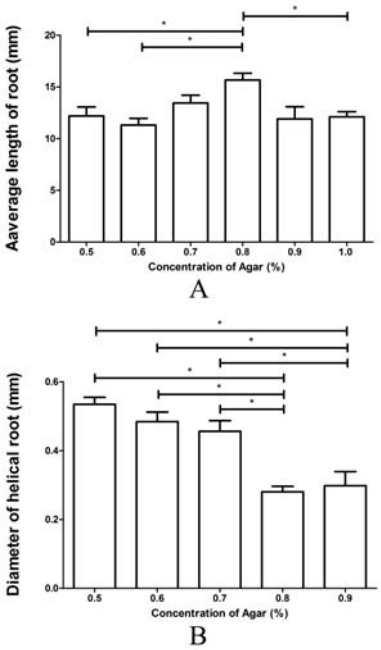

Fig. 3 : Average length and helix diameter of 9-day-old roots living in agar media with different concentration.

	concentrations of agar medium (w/v)							
	0.5%	0.6%	0.7%	0.8%	0.9%	1.0%	1.1%	1.2%
The Young's modulus (MPa)	1.219 ±0.103	2.968 ±0.498	3.711 ±0.581	5.316 ±0.530	6.303 ±0.517	8.272 ±0.440	9.378 ±0.548	10.584 ±0.129
% Plant seen helical roots (n = 90)	100%	100%	90.0%	45.8%	34.0%	21.6%	15.6%	6.7%

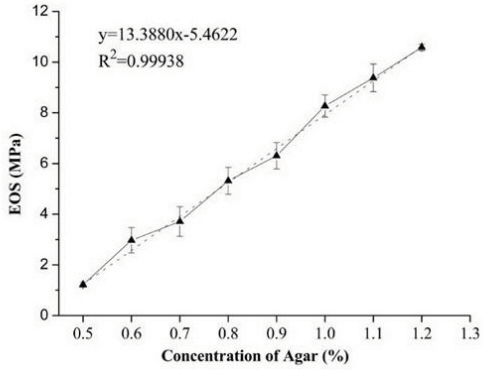

Fig. 4 : The Young's modulus of agar media with different concentration and the percentage of helix growth behaviors in Arabidopsis roots in these media.

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Multiscale Modelling of Plant Root Growth

Dr Rosemary Dyson[1], Dr Jeevanjyoti Chakraborty[1]

[1]School of Mathematics, University of Birmingham, Birmingham, UK
r.j.dyson@bham.ac.uk

From a mechanical perspective, a key determinant of plant growth is the plant cell wall, an ordered network of cellulose microfibrils and hemicellulose crosslinkers embedded within a pectin matrix. The arrangement, and dynamic rearrangement, of these components leads to emergent anisotropic mechanical properties which govern the directional growth of individual plant cells and hence the tissues these cells combine to form. Growth is therefore inherently a multiscale process, with the microstructure determining the mechanical properties of a cell wall segment, which in turn governs the expansion of individual cells and hence the whole tissue. However, this resultant tissue-level growth can also produce active rearrangement of the cell wall microstructure, which will again alter the macroscale mechanical properties. Understanding this interplay is essential to determining the mechanical origins of three-dimensional macroscale plant root behaviour, for example twisting and tropic responses.

We will discuss current work incorporating these macroscale effects on the microscale structure into mathematical models of plant root growth. We will focus in particular on the effects of the current macroscale state of the tissue on the deposition, dispersion and reorientation of cellulose microfibrils, and how this manifests as changes to the evolving cell wall mechanical properties. This in turn determines the directional growth of cells and tissues to produce observable changes in plant phenotype.

Development of On-chip Physical Characterization Method for Root Growth

Hirotaka Hida[1], Katsuya Ozoe[1], Isaku Kanno[1],
Tetsuya Higashiyama[2,3,4], and Michitaka Notaguchi[2,3]

[1]Department of Mechanical Engineering, Kobe University, Japan
[2]JST, ERATO, Higashiyama Live-Holonics Project, Nagoya University, Japan
[3]Graduate School of Science, Nagoya University, Japan
[4]Institute of Transformative Bio-Molecules (WPI-ITbM), Nagoya University, Japan

hida@mech.kobe-u.ac.jp
notaguchi.michitaka@b.mbox.nagoya-u.ac.jp

Abstract
Plant roots grow in soil to take up water and nutrients and physically support plant body. During a lifetime of plant, the roots are affected by various environmental conditions in soil, in a heterogeneous manner. Roots interact with various soil components (sands, stones and etc.), and possibly adopt their characters to increase their fitness to the surrounding conditions. To date, root systems have been characterized by their visible aspects, such as length, density and entire root topology, but not by mechanical force driven by growing roots. PDMS (Polydimethylsiloxane) microfluidic systems [1, 2] are good to quantitatively analyze root physiological aspects, but they have been specialized for chemical/biochemical analyses. Since root mechanical force is another potential factor that determines the performance of plant root system, we decided to develop a new method for physical characterization of roots. Here, we report an on-chip assay method to measure the mechanical force exerted by growing root tip. We developed a silicon microchannel device integrated with force displacement sensor. We overcame technical difficulties, precise alignment of physical sensors to a small root tip and mechanical unreliability of PDMS-based sensor, and successfully measured the driving force of growing root tips in three plant species including a model organism *Arabidopsis thaliana*.

Method
The developed microsystem for characterizing root growth consists of a silicon microchannel integrated a force displacement sensor and covers made of PDMS elastomer, which is transparent and biocompatible material (Fig. 1). We fabricated the silicon microchannel device by micro-electro-mechanical systems (MEMS) techniques, photolithography and deep reactive ion (D-RIE) etching processes. The method for measuring the mechanical force by growing root tip is as follows. First, a seed was sown at the seed pocket. Then, the root grew along the microchannel and attained to the V-shaped trap integrated with the force displacement sensor. Even after the root tip was reached to the sensor, the cell developmental processes, division, expansion and differentiation, can continue. As a result, driving forces by grown roots were detected by observing the sensor deformation under an optical microscope. To demonstrate availability of the developed assay method, we measured three plant species, *Arabidopsis thaliana, Capsella rubella* and *Nicotiana benthamiana*. The microchannel was filled with 1% (W/V) agarose gel containing half-strength Murashige and Skoog (MS) medium. After the dormancy breaking process, the plants were grown in growth chamber at 22 ± 2°C, continuous light conditions.

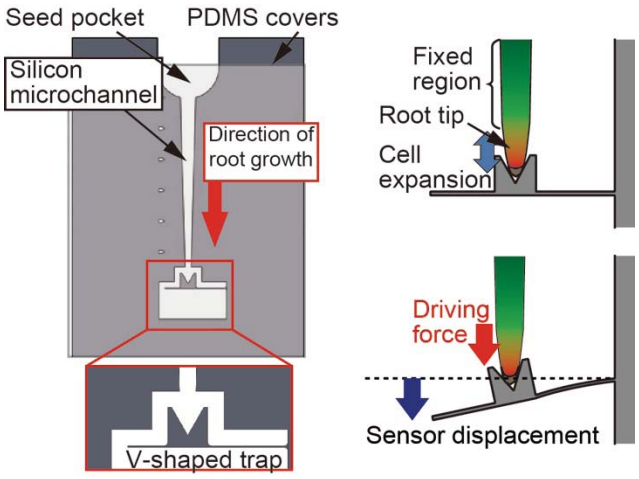

Fig. 1: Illustrations of microsystem for characterizing root (left) and detecting mechanism of mechanical force by growing root tip (right).

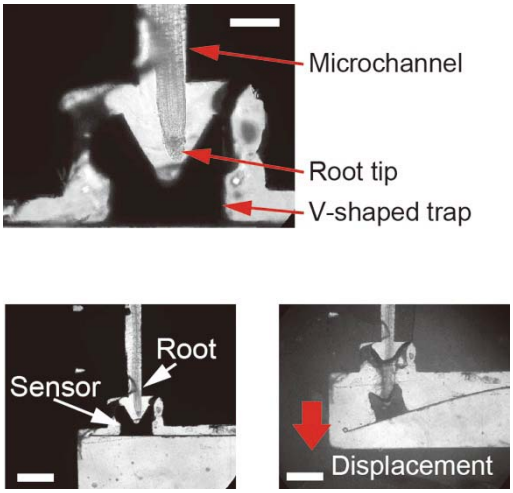

Fig. 2: Microscope images of the root tip on microsystem (upper, Bar: 200 μm) and measurement of mecahical fouce by growing root tip (lower, Bar: 500 μm).

Results

Transparent PDSM covers allowed us to observe growing roots in the device (Fig. 2). The driving force exceted by a growing root tip was quantitatively calculated by relationship between the mechanical stiffness of the sensor and measured sensor displacement (Fig. 2), and ranged from 1 to 4 mN for tested three plant species.

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Cell Wall-Associated ROOT HAIR SPECIFIC 10, A Pro-rich Receptor-like Kinase, Is a Negative Modulator in *Arabidopsis* Root Hair Growth

Youra Hwang[1], Minho Park, Hyodong Lee[1], and Hyung-Taeg Cho[1]

[1] Department of Biological Sciences, Seoul National University, Seoul, Korea
youra.h90@gmail.com, htcho@snu.ac.kr

Introduction

A root hair develops in a stepwise manner through fate determination, initiation, bulge formation, and tip growth (Grierson and Schiefelbein, 2002 and 2008). And cell growth should be modulated so as to maintain a proper size. In a previous study, we identified a *cis*-element (RHE for Root Hair-specific cis-Element) that directs root hair cell-specific gene expression and demonstrated that RHE is structurally and functionally conserved in angiosperms (Kim et al., 2006). In an effort to find more root hair-specific genes in the *Arabidopsis* genome, which include the RHE motif in their promoter region, we acquired 19 root hair-specific (*RHS*) genes (Won et al., 2009). *RHS* genes include the morphogenetic genes such as cell-wall dynamics, kinases, and signaling-related genes, and their loss of function or overexpression altered root hair elongation and polarity (Won et al., 2009). Among these *RHS* genes, here, we report a Pro-rich receptor-like kinase, ROOT HAIR SPECIFIC 10 (RHS10), which defines the root hair length of *Arabidopsis thaliana* by negatively regulating hair growth (Won et al., 2009).

Receptor-like kinases (RLKs), localizing to the plasma membrane (PM), are thought to mediate extracellular signals to the cytoplasm and the nucleus. The *Arabidopsis* genome includes more than 600 members of RLKs which are classified into 46 subfamilies (Shiu and Bleekcker, 2003). Although some subfamilies belonging to such as Leu-rich repeat (LRR) RLKs have been relatively well characterized, the molecular and biological functions of most RLKs have remained to be studied. At least four RLK subfamilies have been implicated in cell wall-associated functions; wall-associated kinases (WAKs), lectin receptor kinases (LecRKs), THESEUS1 (THE1), and PERKs (Humphrey et al., 2007).

The PERK family kinase was first identified in *Brassica napus* (*BnPERK1*; Silva and Goring, 2001), and the *Arabidopsis* genome includes 15 PERK homologs (Nakhamchik et al., 2004). A few studies have been conducted for the biological function of PERKs. Antisense-mediated suppression of PERK1-related genes caused diverse phenotypic effects such as loss of apical dominance, increased branching, and floral organ defects, and overexpression of BnPERK1 increased lifespan, lateral shoots, and seed sets in *Arabidopsis* (Haffani et al., 2006). Inhibition of *Arabidopsis* root cell elongation was observed in *perk4*, *perk13*, and triple *perk8/9/10* mutants (Humphrey et al., 2007; Bai et al., 2009a). Recent studies by Bai et al. (2009a and 2009b) showed that *Arabidopsis* PERK4 is required for ABA-mediated gene regulation, Ca^{2+}-channel opening, and inhibition of root growth and seed germination. *Arabidopsis IGI1*/*PERK12* has been implicated in branching and growth of the shoot (Hwang et al., 2010).

Although a few studies aforementioned have demonstrated the basic aspects of PERK's function, further questions remain to be answered. In this study, we have characterized these molecular and biological functions of RHS10/PERK13 during root hair growth.

RHS10 function is conserved in Angiosperms

RHS10 modulates the duration of root hair growth rather than the growth rate. As poplar and rice RHS10 orthologs also showed the root hair-inhibitory function, this receptor kinase-mediated function seems to be conserved in angiosperms.

Minimal Pro residues of the extracellular domain are sufficient for the RHS10 function
RHS10 showed strong association with the cell wall most likely through its extracellular Pro-rich domain (ECD). The deletion analysis of ECD demonstrated that minimal arabinogalactan protein motifs are required for RHS10-mediated root hair inhibition.

RHS10 mediates cell wall-associated signals by regulating RNA catabolism and ROS accumulation
RHS10 suppressed the accumulation of reactive oxygen species (ROS) in the root which is necessary for root hair growth. A ribonuclease (RNS2) was identified to physically interact with the RHS10 kinase domain. Root hair-specific *RNS2* overexpression significantly decreased RNA levels and root hair growth whereas *RNS2* loss increased them. Consistently, *RHS10* overexpression decreased and *RHS10* loss increased RNA levels in the hair-growing root region. Our results suggest that RHS10 mediates cell wall-associated signals to maintain proper root hair length at least by regulating RNA catabolism and ROS accumulation.

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A mechanical feedback channels sepal growth and shape in Arabidopsis

Nathan Hervieux[1], Anne-Lise Routier-Kierzkowska[2], Mathilde Dumond[1], Satoru Tsugawa[3], Chun-Biu Li[3], Tamiki Komatsuzaki[3], Richard Smith[2], Adrienne H. K Roeder[4] , Arezki Boudaoud[1] , Olivier Hamant [1]

[1] Laboratoire de Reproduction de développement des plantes, INRA, CNRS, ENS Lyon, UCB Lyon 1, Université de Lyon, France

[2] Department of Comparative Development and Genetics, Max Planck Institute for Plant Breeding Research, Cologne, Germany

[3] Research Institute for Electronic Science, Hokkaido, Japan

[4]The Weill Institute for Cell and Molecular Biology, Cornell University, USA
nathan.hervieux@ens-lyon.fr

Many organs and organisms have remarkably consistent shapes, yet at the cellular level, cell growth and shape can be highly variable. We propose that organ-scale signals, i.e. morphogen gradients or force fields, may coordinate cell behavior, involving multiple feedback loops to yield such reproducible shapes. Because of the recent progress in live-imaging techniques, micromechanics and modeling, the relation between cellular noise, cell-cell communication and global shape is now amenable to analysis. We chose to work on the abaxial sepal, as it displays consistent shapes and is easily accessible for live imaging. We focus our analysis on cortical microtubules: as they guide cellulose deposition, the main load bearing component in plant cell walls, we can infer the mechanical anisotropy of cell walls in the sepal, and relate it to the shape and growth of the sepal. Using this framework, we identified a supracellular alignment of microtubules at the tip of the sepal. While this local pattern does not simply correlate with growth, it instead matches predicted tensile forces directions in this domain (Fig. 1). Using micromechanical approaches, we confirm that microtubules in the sepal can align along maximal tension directions. We propose that this response triggers a wave of growth arrest from the tip of the sepal and thus restricts the expansion of the sepal. More locally, we also analyzed the contribution of mechanical conflicts between adjacent cells that grow at different rates and we identified a contribution of growth heterogeneity in the final sepal shape, again via the microtubule response to mechanical forces. Altogether, this provides a scenario in which a mechanical feedback on microtubules channels the final shape of the sepal.

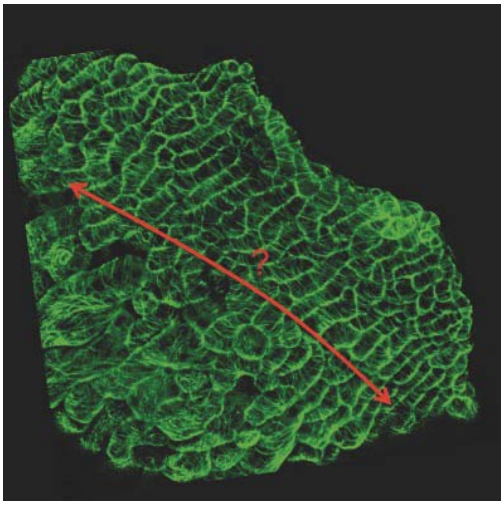

Fig. 1 : Predicted tensile force direction based on microtubules orientation

Quantification of the mechanical properties of growing Arabidopsis hypocotyls

Sarah Robinson[1], Michal Huflejt[1], Prashant Saxena[2], Cris Kuhlemeier[1]

[1]University of Bern, Switzerland
[2]University of Lausanne, Switzerland
sarah.robinson@ips.unibe.ch

Whether is it regulated by changing environmental conditions, pathogen attack or the progression of development; growth is a fundamentally physical process. By developing better investigative tools we can improve our understanding of how this complex network of regulators is integrated with the physical parameters that underpin growth. To this end we developed ACME, an automated confocal micro extensometer, which enables simultaneous measurement of mechanical properties and visualisation with confocal microscopy. Using ACME we have investigated the material properties of growing tissue in response to hormones as well as in mutants with altered growth rates. We are able to make measurements with cellular resolution and in contrast to other popular indentation methods we can measure the mechanical properties of the tissue in the direction of the principal growth. In addition to measuring the mechanical properties that might underlie growth, we have investigated the possibility of feedback onto gene expression by applying stress or strain while imaging known molecular markers. By combining ACME with finite element modelling and mutant analysis we are beginning to better understand the regulation of the physical parameters controlling growth.

Heterogeneity *vs* symplasticity of leaf epidermis growth in transgenic arabidopsis exhibiting perturbed cell cycle and differentiation

Sandra Natonik, Joanna Elsner, Dorota Kwiatkowska

Department of Biophysics and Morphogenesis of Plants, University of Silesia in Katowice, Poland
sandra.natonik@us.edu.pl

Introduction

Complex pattern of cell expansion, divisions, and differentiation plays a key role in leaf epidermis morphogenesis. Leaf epidermis growth is heterogeneous at organ and cellular levels, i.e. within individual pavement cells (Elsner et al., 2012). In terms of growth variation it is natural to discretize walls of an individual cell into segments contacting with different neighbors. Since growth of adjacent wall segments of neighboring cells is somehow coordinated, despite the overall heterogeneity leaf epidermis growth is symplastic. Here we aim at characterization of epidermis growth in an extreme case of a transgenic *Arabidopsis thaliana* line in which cell differentiation and divisions are disturbed due to dominant negative mutation in *CDKA* (Gaamouche et al., 2010).

Material and methods

We quantify cell growth in the young leaf epidermis in transgenic *A. thaliana* (*CDKA;1.N146*, a negative dominant allele of A-type cyclin dependent kinase, and Col-0 as wild type) using sequential replicas combined with scanning electron microscopy, and in vivo confocal microscopy imaging. Cell growth and geometry parameters are quantified with the aid of MorphoGraphX software (Barbier de Reuille et al., 2015) and original Matlab codes (Elsner et al., 2012).

Results and Discussion

Most striking feature of the mutant leaf epidermis are local discontinuities that are randomly spaced and surrounded by unaffected epidermis patches. Sites with disturbed continuity have different appearance and origin (Borowska-Wykręt et al., 2013): some are places where a cell is “missing”, others are narrow cracks between adjacent pavement cells. Discontinuities of both types are eventually “closed” but their occurrence is recorded in atypical cell wall patterns (Fig. 1).

Both growth and cell sizes in the mutant leaf epidermis are much more variable than in the wild type (Fig. 2). We postulate that due to the local discontinuities the symplastic growth may be disturbed, and using in vivo imaging and quantitative analysis we address two questions: (i) is symplastic character of growth affected in epidermal cells of the transgenic line; (ii) in what way the discontinuities become closed. We also examine the formation of wavy anticlinal walls in cells with disturbed contacts with neighbors.

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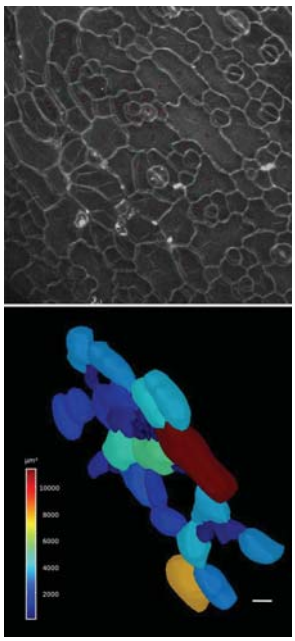

Fig. 1 : Mutant leaf epidermis in which the discontinuity has be "closed" but can be recognized by atypical cell pattern (upper panel). Lower panel shows 3D reconstruction of cells labelled by red dots in the confocal image.

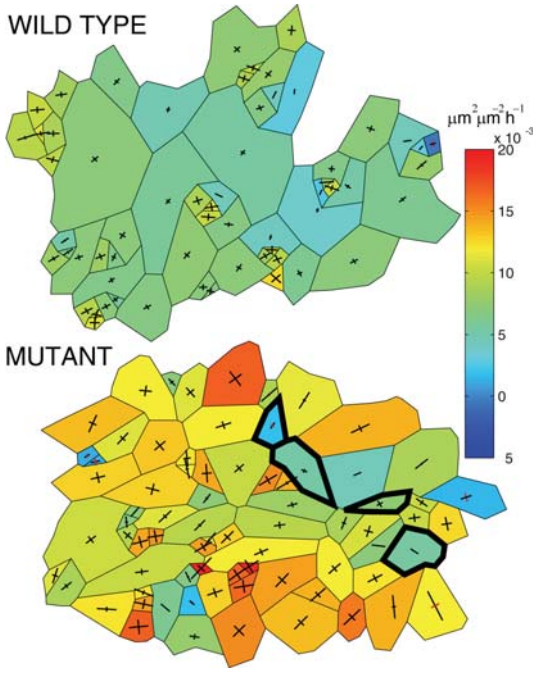

Fig. 2 : Colormaps representing areal growth rates and principal growth directions (crosses) for analogous epidermis fragments of WT and mutant leaves. Thick outlines in latter point to cells that are lost during the next time interval, for which growth was computed.

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Variation of Gene Expression of Tubulin Isotypes in the Tree Cambium

Ugai Watanabe[1], Hisashi Abe[2], Norihiro Futamura[2], Kenji Shinohara[3], Ryo Funada[4], Satoshi Nakaba[4], Yusuke Yamagishi[4]

[1]Faculty of Engineering, Chiba Institute of Technology, Japan,
[2]Forestry and Forest Products Research Institute, Japan, [3]RIKEN, Japan,
[4]Institute of Agriculture, Tokyo University of Agriculture and Technology, Japan
ugai.watanabe@it-chiba.ac.jp

Introduction

Cellulose is one of the main chemical components in the plant cell walls and exists in the form of paracrystalline assemblies, called microfibrils. High elasticity of cellulose microfibrils greatly contributes to the physical properties of wood as well as the mechanical support of trees. The orientation of cellulose microfibrils varies among the wood cell wall layers (Abe and Funada, 2005). In the primary cell walls of tracheids in coniferous wood, the cellulose microfibrils are not well-ordered and their orientation changes as the differentiation proceeds. The orientation of cellulose microfibrils gradually rotates within the outer layer of the secondary wall (S1 layer). The similar change of the orientation can be observed in the inner layer of the secondary wall (S3 layer). The cellulose microfibrils deposited in the middle layer of the secondary wall (S2 layer) are arranged in a steep Z-helix. The mechanical properties of wood cell wall strongly relate to the layered structure and micrifibril orientations. Especially, the orientation of microfibrils in the S2 layer that makes up the majority of the secondary wall almost directly determines the mechanical constants of the whole cell wall (Watanabe and Norimoto, 2000).

Cortical microtubules are believed to regulate the deposition of cellulose microfibril in plant cells (Baskin, 2001). In many types of plant cells, the orientation of cortical microtubules can be observed to parallel that of cellulose microfibrils (Baskin, 2000). The cortical microtubule array gradually changes in the cambium of coniferous wood as the cell wall formation proceeds (Furusawa, et.al., 1998). From these facts, it is considered that the cambium cells in trees continuously change the orientation of cortical microtubules during the cell wall formation, probably with repeating the polymerization and depolymerization. However, a molecular mechanism for this phenomenon is still unclear. The interaction of cortical microtubule with plasma membrane and other molecules associated with tubulins during the secondary wall formation may be essential for this mechanism. For analyzing this interaction, structural changes of cortical microtubules during the cell wall formation should be investigated at the initial stage. In this study, local expression of genes encoding tubulin isotypes in the cambium of *Cryptomeria japonica* was analyzed by laser microdissection (LMD) and digital PCR, and the influence of their expression levels on the structure and

function of cortical microtubule was discussed.

Materials and Methods

Genes of tubulin isotypes expressed in the cambium cells were searched in a *C. japonica* cDNA library constructed in the Forestry and Forest Products Research Institute, and their full-length sequences were analyzed.

Young trees of *C. japonica* were grown in an artificial light room to collect small blocks containing the cambium. The conditions of time and temperature were 14h and 25 ºC for light period, and 10h and 20 ºC for dark period, respectively. The blocks collected were immediately fixed in liquid Freon 22. Radial sections 20 μm thick were cut from the fixed blocks under -20 ºC using a cryo-microtome (CM3050S, Leica Microsystems), followed by freeze drying overnight. Three regions, namely, cell division region, cell expansion region and secondary wall formation region were set in the cambium zone to collect the cells locally by LMD system (LMD7000, Leica Microsystems). Total RNA contained in the collected materials was extracted using the Hgih Pure RNA Tissue Kit (Roche Diagnostics GmbH). After the reverse transcription of total RNA, the copy numbers of the genes encoding the tubulin isotypes were determined by digital PCR system (QX200, Bio-Rad Laboratories). The copy number of 18S rRNA (accession No. D85304) was determined as a standard at the same time.

Results and Discussion

From the *C. japonica* cDNA library, five clones encoding α-tubulin and four clones encoding β-tubulin were identified. Analyzing the translated amino acid sequences elucidated that two β-tubulin clones encoded the same protein each other. The cambium cells could be collected separately from the cell division, cell expansion and secondary wall formation regions by LMD. The digital PCR could determine the copy numbers of targeted genes expressed locally in the cambium, which was impossible in a traditional real-time PCR analysis.

Figure 1 shows the comparison of copy numbers of tubulin genes. The gene expression level of α- and β-tubulins varied within the cambium zone of *C. japonica*. The gene expression ratio of the α-tubulin isotypes differed for each region. The expression level of clone No. CFFL011 increased in both cell expansion and secondary wall formation regions. The gene expression ratio of the β-tubulin isotypes also differed for each region. The expression level of CLFL050 increased in both cell expansion and secondary wall formation regions, which was the most remarkable change among the *C. japonica* tubulin genes examined. In *Eucalyptus grandis*, the orientation of cellulose microfibrils in the S2 layer of fiber is correlated with the gene expression of a β-tubulin (Spokevicius, et.al., 2007). From the results of this experiment, it is considered that in *C. japonica* the β-tubulin encoded by clone No. CLFL050 is involved with the control of cellulose microfibril orientation during

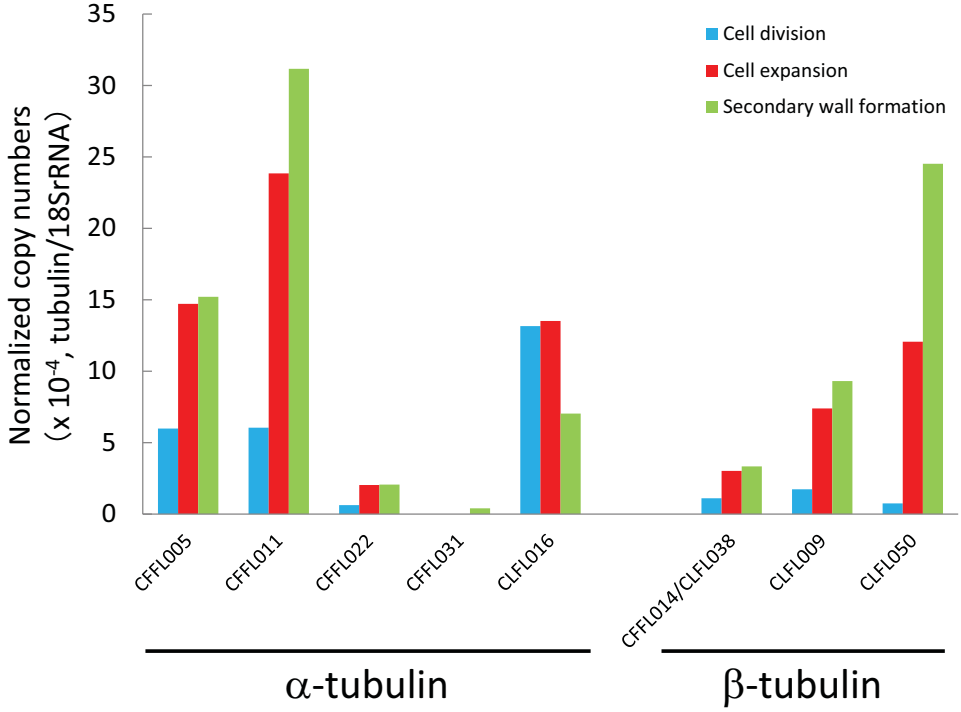

Fig.1. Expression of genes encoding tubulin isotypes in the cambium of *C. japonica*.

xylogenesis.

Figure 2 shows the multiple alignment of translated amino acid sequences of *C. japonica* β-tubulins. A high homology was observed among these four sequences, but there were characteristic changes in the sequence encoded by CLFL050 that was highly expressed in cell expansion and secondary formation regions. Especially, this β-tubulin lacked a part of amino acid sequences at the carboxyl-terminus compared with the other three sequences. The carboxyl-terminal tail of β-tubulin positions at the surface of microtubules with α-helix that precedes. This α-helix is known to be a binding site, for example, for microtubule-associated proteins. Post-translational modifications occur at the carboxyl-terminal tails of tubulins, which affects an interaction between microtubule and other proteins and microtubule depolymerization (Sirajuddin, et.al., 2014). The gene expression ratio among tubulin isotypes is considered to relate to the variation of surface structure of cortical microtubules. This structural variation may affect the functions in the rotation of cortical array during the cell wall formation and the consequent deposition of cellulose microfibrils. Further analysis of the structure and function of cortical microtubules in tree cambium cells may bring the elucidation of the nature of xylogenesis in plants.

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CFFL014  MREILHIQGGQCGNQIGSKFWEVVCEEHGIDPTGRYSGS---SDLQLERVNVYYNEASCG
CLFL038  MREILHIQGGQCGNQIGSKFWEVVCEEHGIDPTGRYSGS---SDLQLERVNVYYNEASCG
CLFL009  MREILHIQGGQCGNQIGAKFWEVVCDEHGIDPTGRYHGT---SDLQLERVNVYYNEASCG
CLFL050  MREILHVQGGQCGNQIGAKFWEVICDEHGIDPTGRYRGNESDSNLQLERINVYYNEASCG
         ******:**********:*****:*:********** *   *:*****:**********

CFFL014  RFVPRAVLMDLEPGTMDSVRTGPYGQVFRPDNFVFGQSGAGNNWAKGHYTEGAELIDAVL
CLFL038  RFVPRAVLMDLEPGTMDSVRTGPYGQVFRPDNFVFGQSGAGNNWAKGHYTEGAELIDAVL
CLFL009  RFVPRAVLMDLEPGTMDSVRTGPYGQIFRPDNFVFGQSGAGNNWAKGHYTEGAELIDAVL
CLFL050  RFVPRAVLMDLEPGTMDSVRSGPYGQIFRPDNFVFGQSGAGNNWAKGHYTEGAELIDSVL
         ********************:*****:******************************:**

CFFL014  DVVRKEAENCDCLQGFQVCHSLGGGTGSGMGTLLISKIREEYPDRMMLTFSVFPSPKVSD
CLFL038  DVVRKEAENCDCLQGFQVCHSLGGGTGSGMGTLLISKIREEYPDRMMLTFSVFPSPKVSD
CLFL009  DVVRKEAENCDCLQGFQVCHSLGGGTGSGMGTLLISKIREEYPDRMMLTFSVFPSPKVSD
CLFL050  DVVRKEAENCDCLQGFQVCHSLGGGTGSGMGTLLISKIREEYPDRMMLTFSVFPSPKVSD
         ************************************************************

CFFL014  TVVEPYNATLSVHQLVENADECMVLDNEALYDICFRTLKLTTPSFGDLNHLISATMSGVT
CLFL038  TVVEPYNATLSVHQLVENADECMVLDNEALYDICFRTLKLTTPSFGDLNHLISATMSGVT
CLFL009  TVVEPYNATLSVHQLVENADECMVLDNEALYDICFRTLKLTTPSFGDLNHLISATMSGVT
CLFL050  TVVEPYNATLSVHQLVENADECMVLDNEALYDICFRTLKLTTPSFGDLNHLISATMSGVT
         ************************************************************

CFFL014  CCLRFPGQLNSDLRKLAVNLIPFPRLHFFMVGFAPLTSRGSQQYRALTVPELTQQMWDAK
CLFL038  CCLRFPGQLNSDLRKLAVNLIPFPRLHFFMVGFAPLTSRGSQQYRALTVPELTQQMWDAK
CLFL009  CCLRFPGQLNSDLRKLAVNLIPFPRLHFFMVGFAPLTSRGSQQYRALTVPELTQQMWDSK
CLFL050  CCLRFPGQLNSDLRKLAVNLIPFPRLHFFMVGFAPLTSRGSQQYIALTVPELTQQMWDAK
         ******************************************* *************:*

CFFL014  NMMCAADPRHGRYLTASAMFRGKMSTKEVDEQMINVQNKNSSYFVEWIPNNVKSSVCDIP
CLFL038  NMMCAADPRHGRYLTASAMFRGKMSTKEVDEQMINVQNKNSSYFVEWIPNNVKSSVCDIP
CLFL009  NMMCAADPRHGRYLTASAMFRGKMSTKEVDEQMMNVQNKNSSYFVEWIPNNVKSSACDIP
CLFL050  NMMCAADPRHGRYLTASAMFRGKMSTKEVDEQMINVQNKNSSYFVEWIPNNVKSSVCDIP
         *********************************:********************.****

CFFL014  PRGLSMASTFIGNSTSIQEMFRRVSEQFTAMFRRKAFLHWYTGEGMDEMEFTEAESNMND
CLFL038  PRGLSMASTFIGNSTSIQEMFRRVSEQFTAMFRRKAFLHWYTGEGMDEMEFTEAESNMND
CLFL009  PTGLKMASTFIGNSTSIQEMFRRVSEQFTAMFRRKAFLHWYTGEGMDEMEFTEAESNMND
CLFL050  PTGLKMASTFVGNSTSIQEMFRRVSEQFTAMFRRKAFLHWYTGEGMDEMEFTEAESNMND
         * **.*****:**************************************************

CFFL014  LVSEYQQYQDATADEEADFDDEE-
CLFL038  LVSEYQQYQDATADEEADFDDEE-
CLFL009  LVSEYMQYQDATADEEEEYDDEEE
CLFL050  LVSEYQQYQDATA-----------
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Fig. 2. Multiple alignment of translated amino acid sequences of *C. japonica* β-tubulins.

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Functional conservation of the M3 phosphorylation site in Arabidopsis long PINs for their polarity and biological roles

Daeeun Ki[1] and Hyung-Taeg Cho[1]

[1]Department of Biological Sciences, Seoul National University, Seoul, Korea
kidaeeun55@gmail.com, htcho@snu.ac.kr

Introduction

Asymmetrically localized PIN-FORMED (PIN) auxin efflux carriers play an important role in regulating directional intercellular auxin movement and thus generating local auxin gradients. The polar localization of PINs is tightly controlled by phosphorylation in the central hydrophilic loop (HL) of PINs. The importance of M3 phosphorylation site, which locates in evolutionarily conserved RKSNASRRSF(/L) and TPRPSNL motifs of the HL, has been reported for PIN3.

Conserved role of M3 phosphorylation site for PIN polarity and biological function

We analyzed the conserved role of the M3 phosphorylation site of PIN1 and PIN7. Phosphorylation-defective mutations of five phosphorylatable residues (Ser/Thr to Ala or Gly) in the M3 site of PIN1-HL changed its subcellular polarity and caused defects in PIN1-mediated biological functions such as floral organ formation, root growth and leaf phyllotaxy. The M3 phosphorylation-defective mutants of PIN7 impaired its proper subcellular trafficking in root columella cells, which then disrupted normal root gravitropism. Our data indicate that the M3 phosphorylation site is functionally conserved among long PINs and plays important roles for their subcellular trafficking, auxin distribution, and auxin-mediated developmental processes.

Expansin-mediated cell wall modification system to understand the mechanism from cell wall dynamics to cellular processes

Hyodong Lee[1], Hyung-Taeg Cho[1]

Department of Biological Sciences, Seoul National University, Seoul, Korea
lbdt87@naver.com, htcho@snu.ac.kr

Introduction

A plant cell is surrounded by the cell wall which consists of cellulose microfibrils, matrix polysaccharides and minor proteins and play both mechanical support and restraint for the cell. For a plant cell to proceed division, expansion, and differentiation, intimate interactions between the cell wall and the protoplast would be required. Diverse cell wall-associated transmembrane kinases are indicative of the presence of the interface for these interactions. Mechanical changes in the cell wall, via this interface, would cause diverse downstream cytoplasmic or nuclear events for the cellular processes. Among many cell wall-modifying proteins, expansins are unique in that they reassemble the cell wall without apparent hydrolytic activity and cause cell expansion.

Analyzing downstream events of mechanical cell wall changes caused by expansin-mediated cell-wall modification

We have adopted this expansin-mediated cell-wall modification to identify the events downstream of mechanical cell wall changes. In this study, we have expressed several types of expansin proteins by the glucocorticoid-inducible system in Arabidopsis seedlings and analyzed the time-dependent transcriptome changes. This analysis would give a starting clue to understand the mechanism from cell-wall dynamics to cellular events.

Mechanical conflicts in growth heterogeneity

Hamant O

Laboratoire de Reproduction de développement des plantes, INRA, CNRS, ENS Lyon, UCB Lyon 1, Université de Lyon, France
olivier.hamant@ens-lyon.fr

Coupling physiology and mechanics in multicellular models of plant growth

Ibrahim Cheddadi[1,2], Valentina Baldazzi[2], Nadia Bertin[2], Michel Génard[2], Christophe Godin[1]

[1]INRIA project-team Virtual Plants, joint with CIRAD and INRA, Montpellier, France
[2]INRA unit PSH, Avignon, France

ibrahim.cheddadi@inria.fr

Introduction

At the cellular level, plant growth results from the competition between the so-called turgor pressure and mechanical resistance from the cell walls. The turgor pressure itself results from a difference of osmotic pressure between the interior and the exterior of the cell: the plasmic membrane is semi-permeable, so that a higher solute (sugars, ions) concentration inside the cell leads to a water flux towards the cell and to a pressure rise; if the pressure exceeds a given threshold, the cell walls extend and the cell grows. Recently, different models of plant growth have attempted to incorporate mechanics of cell walls in tissue development [Boudon et al. 2015], but all of them make the assumption that turgor pressure is constant with time.
In this work, we go one step further and include a model of the regulation of turgor pressure in a multicellular framework.

Details of the model

The cell wall material is modeled as a continuous visco-elasto-plastic material characterized by an elastic modulus, a yield deformation, and a rate of synthesis of matter as in [Boudon et al. 2015]. Flows between cells occur through their membranes according to non-equilibrium thermodynamics, as a result from a difference in turgor and osmotic pressures. The value of pressure is not prescribed but emerges from the coupling of all these phenomena.

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Multiscale modelling of plant cell wall biomechanics: Interactions between mechanical properties and chemical processes

Mariya Ptashnyk[1], Brian Seguin[1]

[1]Division of Mathematics, University of Dundee, Scotland, UK
mptashnyk@maths.dundee.ac.uk

Introduction

The main feature of plant cells are their walls, which must be strong to resist high internal hydrostatic pressure (turgor pressure) and flexible to permit growth. The primary wall of a plant cell consists mainly of oriented cellulose microfibrils, pectin, hemicellulose, structural proteins, and water. The main force for cell elongation (turgor pressure) acts isotropically, and so it is the microscopic structure of the cell wall that determines the anisotropic growth of plant cells and tissue. The orientation of microfibrils and their tensile strength influence the wall's stiffness and direction of the cell expansion. It was also observed that changes in cell wall elasticity are strongly correlated with changes in the chemical configuration of pectin, see e.g. Hématy, Höfte, Wolf (2012). Pectin is deposited to cell walls in a methylesterified form. In cell walls pectin can be modified by the enzyme pectin methylesterase (PME), which removes methyl groups by breaking ester bonds. The de-esterified pectin is able to form calcium-pectin cross-links, and so stiffen the cell wall and reduce its expansion. On the other hand, the cross-links can be disturbed by the mechanical stresses in a cell wall.

In this talk microscopic models for plant cell wall and plant tissue biomechanics that take into account both the microstructure coming from the microfibrils and the chemical reactions between the cell wall's constituents will be presented. In the mathematical model we consider elastic deformation of a plant cell wall, the deposition of pectin, PME, and calcium to the cell wall matrix, interactions between PME and pectin, as well as formation and destruction of calcium-pectin cross-links. The interplay between the mechanics and the chemistry comes in by assuming that the elastic properties of cell walls depend on the density of calcium-pectin cross-links and the stress within cell walls can break the cross-links. To analyse the macroscopic mechanical properties, as well as for effective numerical simulations, the macroscopic models for biomechanics of plant cell walls and tissues are derived, Ptashnyk, Seguin (2014). The numerical solutions of the macroscopic models will demonstrate the dependence of mechanical properties of a plant cell wall on the density of calcium-pectin cross-links and on the orientation of the cell wall microfibrils.

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Mechanical basis for the helical pattern in twisting mutants of *Arabidopsis*

Hirofumi Wada

Department of Physics, Ritsumeikan University, Japan
hwada@fc.ritsumei.ac.jp

Helical growth is ubiquitous in plants, such as twinning tendrils and spiral order of leaves, petals or florets. A rapidly elongating root or hypocotyl of twisting mutants of Arabidopsis thaliana exhibits a helical growth with a handedness opposite to that of the underlying cortical microtubule arrays in epidermal cells: Ishida (2007). Using a continuum mechanics approach, we propose that the helical pattern observed is a direct consequence of the simultaneous presence of anisotropic growth and tilting of cortical microtubule arrays. By modeling a plant organ consisting of different tissues as a composite elastic body, we predict that the root helical pitch angle is a function of the microtubule helical angle and elastic moduli of the tissues: Wada (2012). The proposed model is versatile and is potentially important for other biological systems ranging from protein fibrous structures to tree trunks.

We also look at shapes of single cells with helical surface growth based on thin elastic plate theory with differential growth metric, which may be relevant to abnormal shapes of epidermal cells from twisting mutants observed in cell suspensions: Buschmann (2009).

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The role of memory-less tissue mechanics in the robustness of morphogenesis in plants

Dumond, Mathilde[1], Hamant, Olivier[1], Boudaoud, Arezki[1]

[1]Laboratoire de Reproduction et Développement des Plantes, Lyon
mathilde.dumond@ens-lyon.fr

How organs shape and size are determined is still poorly understood despite much progress in developmental genetics. Strikingly, organs have a reproducible shape while their cells have a highly variable behavior (Roeder (2010), Uyttewaal (2012)). How is such cellular variability buffered at the organ level? To answer this question, we chose to study Arabidopsis thaliana sepal, because each plant have a large number of almost identical sepals, which are accessible to live imaging. Moreover, sepals feature the stochastic formation of giant cells (Roeder (2010)) leading to a highly variable cell population.

In plants, morphogenesis is driven by internal hydrostatic pressure and controlled through the regulation of cell wall properties. Therefore, we first measured cell wall mechanics atomic force microscopy. Measured elastic moduli appeared highly variable at the supra-cellular scale, as well as at the intra-cellular scale. In addition, the underlying structure of the cell wall could be characterized using the correlation length of mechanical properties. We then built a model of sepal morphogenesis. This model consists of 2D approximation of the sepal, where only the outermost cell wall is represented. The mechanical properties of the cell wall and the internal pressure are the only parameters of this model, which uses the finite elements method to resolve the equilibrium between turgor pressure and mechanical properties at each time point.

We could reproduce the shape of a wild-type sepal assuming no variability of cell wall mechanics (Fig. 1). We then incorporated measurements in the model, assuming mechanical properties to be retained in time, but we found a high variability of organ contours. We therefore switched to the opposite hypothesis: cell wall mechanics is memory-less. The elastic modulus of the cell wall was spatially randomly chosen in the measured distribution and then reset at each time point. We found that sepal contours featured a low variability.

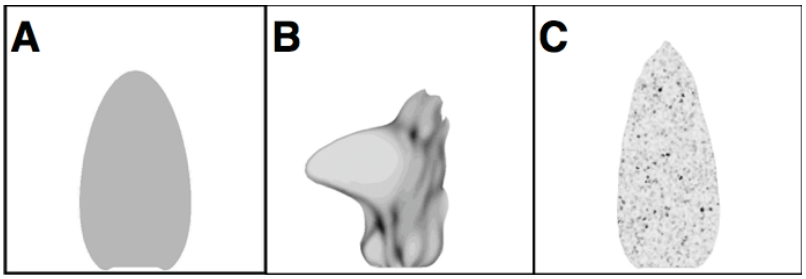

Fig 1: Shape of the simulated sepal when stiffness is homogeneous (A), when it is spatially variable (B) or when it is temporallly and spatiallly variable (C). (The darker the stiffer)

We concluded that processes that reset memory of cell wall mechanics buffer fluctuations of sepal shape. We are currently testing this idea, notably using lines showing diverse cell wall defects, from mutants for sensors of cell wall integrity to mutants for modifiers of cell wall.

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Actions of XTH family of enzymes in construction and remodeling of the plant cell wall

Kuki H[1], Shinohara N[1], Yokoyama R[1], Higaki T[2], Hasezawa S[2], Sunagawa N[3], Igarashi K[3], Tamura S[4], Ueda M[4], Nishitani K[1],

[1]Graduate School of Life Sciences, Tohoku University, Sendai, [2]Graduate School of Frontier Sciences,The University of Tokyo, [3]Graduate School of Agricultural and Life Sciences, The University of Tokyo, [4]Graduate School of Science, Tohoku University, Sendai, JAPAN
nishitan@m.tohoku.ac.jp

Molecular architecture of the cell wall in land plants is characterized by a framework of cellulose microfibrils, which are orderly embedded in an amorphous matrix consisting of various structural and functional components. The first molecular model of the cell wall of sycamore cell suspension cultures was advanced by Albersheim's group in the 1970s (Keegstra et al. 1973). This model envisaged pectin and xyloglucans as load-bearing components responsible for tethering cellulose microfibrils, the available surface area of which being coated by xyloglucans. Although the original model has been revised repeatedly and renamed as Type I and Type II cell walls by Carpita et al. 1993, the "tethered network model" has been widely accepted, and xyloglucan has been postulated to serve as load-bearing element at least in the Type I cell walls. The discovery of XTH families of enzymes capable of remodeling load-bearing xyloglucan tethers cemented the idea that "XTH catalyzed remodeling of xyloglucan crosslinks in the cellulose/xyloglucan network is responsible for the growth and development of the primary cell wall" (Nishitani 1997). More recently, this idea was further extended to the type II cell wall in rice, which contains less amount of xyloglucans (Hara et al. 2013). In the light of these ideas, the report on phenotypes of the *xxt1/xxt2* mutant by Keegstra's group was surprising in that Arabidopsis mutant plants lacking xyloglucans do not exhibit severe defects, and yet can grow to complete the life cycle (Cavaliera et al. 2008). Thus, the "tethered network model" of the primary cell wall is now seriously challenged (Cosgrove 2014). Very recently, Park et al. (2015) has proposed "biomechanical hotspots model" as an alternative for "tethered network model". The new model is based on structural findings obtained by solid-state nmr spectroscopy of intact cell wall, and envisages that the mechanically important xyloglucan is restricted to minor limited regions termed "hot-spots", that appear to be closely intertwined with cellulose.

These conflicting findings about the role of xyloglucans have let us to rethink the notion of cell wall construction and remodeling, which are essential not only for plant growth and development but for intelligent functions in land plants. My talk in this symposium will deal with re-examination of both "tethered network model" and "biomechanical hotspots model" by focusing (1) quantitative visualization of cell wall regeneration in Arabidopsis protoplast, and (2) novel roles for XTH family of protein in construction and remodeling of "covalent linkages" between cellulose microfibrils and xyloglucans.

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Keywords: Cellulose, Cell wall model, Xyloglucan, XTH (xyloglucan endotransglucosylase/hydrolase)

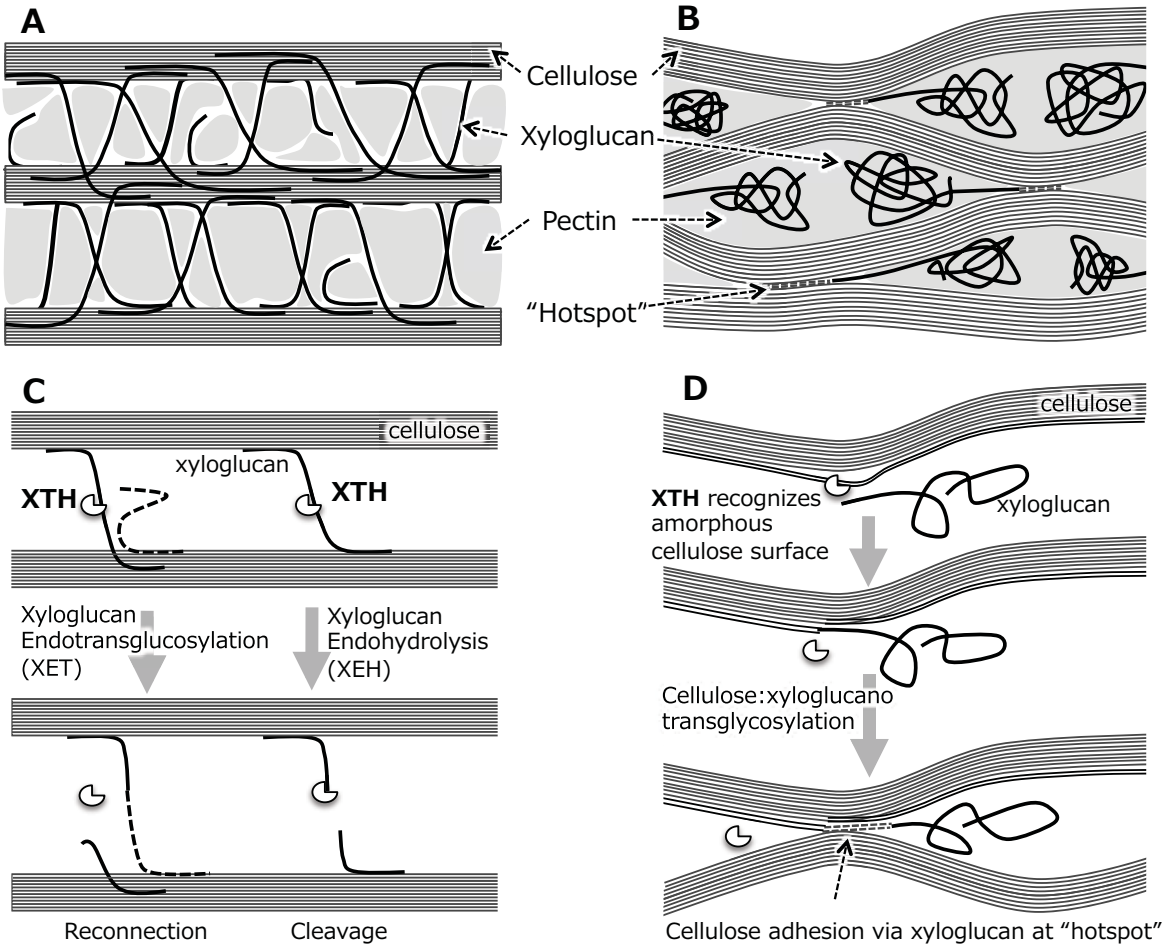

Fig. 1: Two hypothetical molecular models for the plant cell walls in land plants (A and B), and roles of XTH (xyloglucan endotransglucosylase/hydrolase) family of enzymes in their construction and remodeling (C and D). A; tethered network model, B; biomechanical hotspots model, C; a schematic representation of XTH-mediated reconnection and cleavage of xyloglucan tether in the "tethered network model", D; a hypothetical process by which the tight adhesion in the "biomechanical hotspots model" is formed via XTH-mediated heterotransglycosylation between cellulose and xyloglucan at a limited site.

Stop and Go: Cell wall pectin controls growth magnitude in the elongating hypocotyl

Firas Bou Daher[1], Marco Aita[1], Yuanjie Chen[1], and Siobhan A. Braybrook[1]

[1]The Sainsbury Laboratory, University of Cambridge, Cambridge, UK
firas.boudaher@slcu.cam.ac.uk

Introduction

Hypocotyl extension beneath the soil is an iconic example of rapid growth in plants. The dramatic extension of the hypocotyl (or analogous organ) required to push the shoot up an out of the soil is essential for seedling survival. This rapid organ extension is due to a wave of cell expansion within the organ. But we have very little idea of how the growth wave is controlled- how does a cell get the signal to stop or go?

The growth of every cell, and by extension a whole organ, is controlled by the cell wall. This unique and diverse cellular compartment must be strong enough to withstand turgor pressure, yet flexible enough to allow growth in a controlled manner. The cell wall may be considered as a material, with physical properties that dictate its behavior under stress (force) and strain (growth). In its simplest conception, the primary *Arabidopsis* cell wall is a fiber reinforced composite comprising strong cellulose fibers linked by hemicellulose and embedded in a pectin gel. Our previous work (Peaucelle et al. 2011, Braybrook et al. 2012) indicates that changes in pectin chemistry and its effects on material elasticity can drastically affect growth. Here we explore whether this is true in the hypocotyl system.

Kinematics of hypocotyl elongation: organ and cell

We have performed detailed kinematic analysis of hypocotyl extension in the dark yielding precise data on organ level elongation over time, segment elongation over time, and individual cell growth rates over time. These data have revealed intricacies in hypocotyl growth including: the presence of divisions in alternating files of epidermal cells and the presence of growth heterogeneity at the cellular level. This level of detail in hypocotyl elongation dynamics has allowed us to gain new insights into this growth process and has provided a framework within which to examine the mechanical aspects of growth.

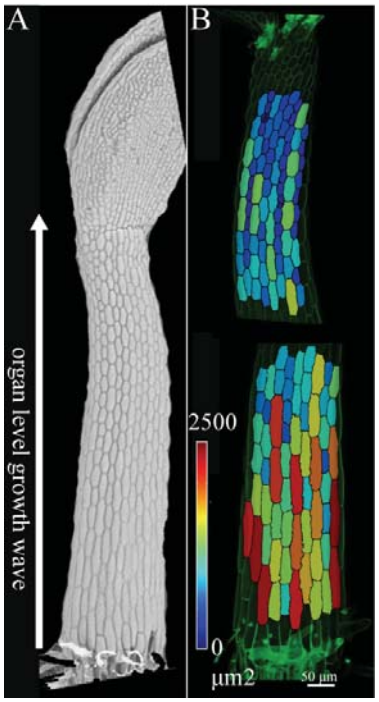

Fig.1: Hypocotyl extension at the cellular level. A) a scanning electron micrograph of a 24h post-germination Arabidopsis hypocotyl shows cell elongation in the base but not the top near the cotyledons. B) Cell surface area map of a 24h post-germination hypocotyl base and top region reinforcing the initial basal growth bias seen in (A) and illustrating difference between files of cells in the base. As each file starts with the same number of cells, it is clear that every other file must present cell division.

Turgor and wall elasticity- who controls growth?

In order to understand how these growth dynamics might be controlled we have examined the two inputs for growth- turgor pressure and wall elasticity. We have characterized turgor pressure (via plasmolytic analyses) in growing verses non-growing cells and seen no instructive variation in estimated pressure. However, using cell swelling-shrinking assays to calculate bulk elastic moduli for growing and non-growing cells we have determined that cell walls in expanding cells are more elastic. To underpin some of the underlying chemistry, we have observed changes in pectin chemistry in the epidermal cell walls using immunohistochemistry that indicate more elastic pectin is present in elongating cell walls and less elastic pectin in non-elongating cell walls. We examined microtubule (MT) orientation in growing and non-growing cells as read-outs of cell stress and strain (due to growth) and found that in non-growing cells there was no organization or orientation on MTs, whereas in growing cells there was a strong tendency for alignment. This adds weight to the idea that growing and non-growing cells exhibit very different cell wall mechanics as read out in stress and subsequent MT orientation.

Alterations in pectin chemistry change growth magnitude and dynamics

To further our inquiries regarding pectin and wall elasticity as it relates to growth control, we have examined the effect of changes in pectin chemistry on extension. A major form of cell wall pectin, homogalacturonan (HG), can be mechanically altered by changes in its esterification which allow or block cross-linking with Calcium ions to form strong bridges. Activity of PECTIN METHYLESTERASE (PME) enzymes removes methyl groups from the HG backbone, allowing formation of Ca-cross-bridges and rigidifying the pectin gel (Pelloux

et al. 2007). Conversely, activity of PECTIN METHYLESTERASE INHIBITOR (PMEI) proteins blocks PME activity therefore keeping pectin cells more elastic. Inducible PME and PMEI plants (Peaucelle et al. 2008) were used to explore the effect of forced pectin gel alterations on hypocotyl elongation kinematics. Induction of PME activity led to a decrease in final hypocotyl length and a reduction in elongation rate. Induction of PMEI had the opposite effect, enhancing growth. Both treatments had effects on the duration of cell growth and the size of the growing zone.

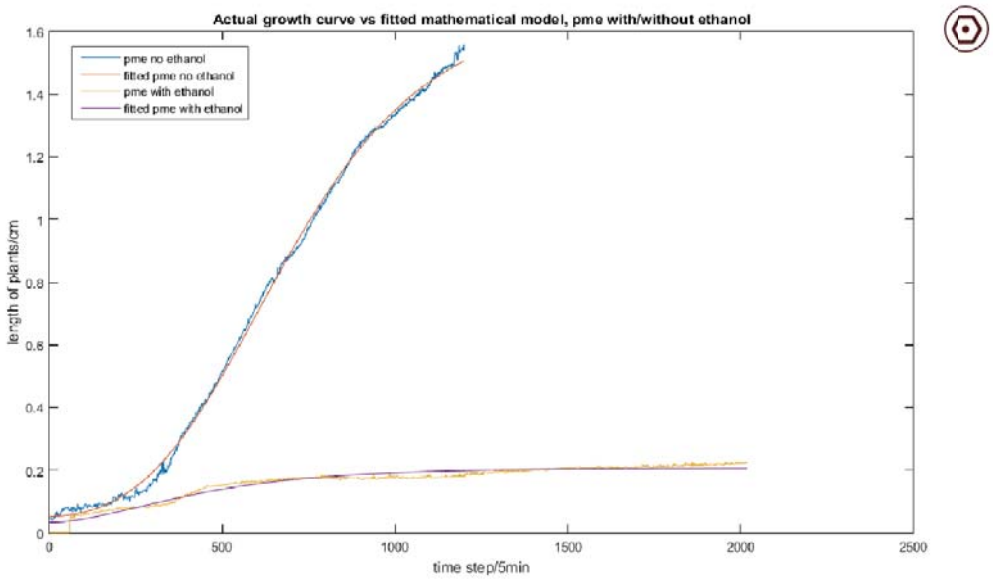

Fig. 2: Kinematic growth curves for inducible PME lines without (blue) and with (yellow) the presence of inducer. Hypocotyl length is displayed versus time steps of 5 minutes. Each example growth curve is smoothed with a best-fit.

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Quantification and comparison of phototropic and gravitropic sensitivities of three hardwood species by original isotropic light devices and model-assisted phenotyping

Coutand C[1,2], Adam B[1,2], Ploquin S[1,2], Lopez D[1,2],Moulia B[1,2]
[1]INRA, UMR 547 PIAF, F-63100 Clermont-Ferrand, France
[2]Clermont Université, Université Blaise Pascal, UMR 547 PIAF, F-63000 Clermont-Ferrand, France
catherine.coutand@clermont.inra.fr

Introduction

Phototropism and gravitropism are important features implicated in tree postural control. On earth, these mechanisms are intimately linked so that some special experimental design must be set to disentangle them. Moreover, to our knowledge, there is not study that has quantified the sensitivity of trees to light and gravity signals.

Rationale

In this study, a dedicated experimental design has been constructed in order to tilt young trees in an isotropic light environment (Fig. 1) (Coutand et al., unpublished results). This way, light is provided to the trees to ensure growth (the motor of tropic movements), but does not trigger any phototropic signal so that the gravitropic response can be isolated from the phototropic one. In parallel, some trees were tilted within an anisotropic light environment to get the photogravitropic response. These experiments were analyzed through the photogravitropic model A_RC (Bastien et al., 2015, Eq. 1) and its gravitropic-reduced AC version in the case of isotropic light (Bastien et al. 2013).

Tree gravitropic and photogravitropic movements were studied on three species: poplar, oak and beech, until gravitropic (Firn and Digby, 1995) and photogravitropic equilibriums (Galland, 2002) were reached, respectively. Tree gravitropic movements were recorded with varying the angle of tilting and graviphototropic responses were studied with varying the angle of tilting and the light direction and intensity.

Results

The experimental data revealed high differences of tree shape using isotropic or anisotropic light environments (Fig. 2), differences of threshold of gravitropic signal between species and suggested differences of sensitivity to light. The sensitivities to gravitropic and photogravitropic movements were then phenotyped thanks to The results validated the models, enabled to quantify tropic sensitivities to gravity and light and confirmed differences of sensitivities to light between species.

Conclusion

This study demonstrated the usefulness of isotropic light device to isolate the gravitropic response and was used for studying the short-time molecular responses to identify first actors within the gravitropic transduction cascade (Lopez et al., submitted; Richet et al, unpublished results). Moreover this study demonstrates the strength of model-assisted phenotyping to quantify the species sensitivities to light and gravity tropic signals.

Fig. 1 : Isotropic light device

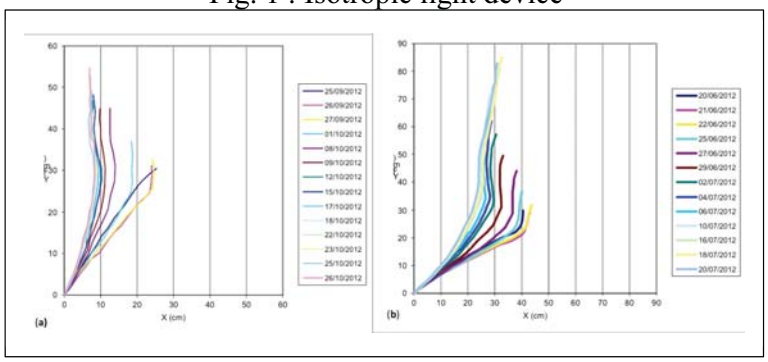

Fig. 2: Representative examples of poplar stem sucessive shapes after tilting the tree in an siotropic (a) or anisotropic (b) environment

$$\frac{\partial C(s,t)}{\partial t} = -v\big(A(L,t) - A_p\big) - \beta A(S,t) - \gamma C(S,t)$$

Eq. 1 : General equation of the $A_R C$ model: A= stem inclination angle (with reference to the vertical) , C= stem curvature, Ap angle of the light source (with reference to the zenith) , s= spatial coordinate – curvilinear abscissa-, L= Length of the reacting zone, t= time,

Aknowledgements
The study presented here belongs to the ANR project "TROPIC" (ANR-11-BSV7-0012) dedicated to study and model gravitropic and phototropic responses in trees.

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Cytosolic calcium increases in response to a change in the positioning of gravisensors: Results from space experiments

V. Pereda-Loth[1], J. Gérard[2], F. Bizet[3,4], and V. Legué[3,4]
[1]Université P. Sabatier, GSBMS, 133 Route de Narbonne, 31063 Toulouse, France,
[2]Université de Lorraine, UMR INRA-UL 1137 EEF, 54506 Vandoeuvre Les Nancy Cedex, France.
[3]Clermont Université, Université B. Pascal, UMR 547 PIAF, Campus Universitaire des Cézeaux, 8 Avenue Blaise Pascal, 63178 Aubière Cedex, France,
[4]INRA, UMR 547 PIAF, 63100 Clermont Ferrand Cedex 01, France
Valerie.legue@univ-bpclermont.fr

Introduction

Plants are the ability to sense and to re-orient their growth in response to various stimuli including light, gravity, wind and humidity. The response of a plant organ to the change of gravity direction called gravitropism can be regarded as a crucial factor in the posture control of stems (Moulia and Fournier, 2009) and in the progression for roots into the soil (Roy and Basshamm, 2014). In roots, specialized sensory cells, called statocytes, located in the root apex, perceive signal gravity. Even if it is well known that amyloplasts (located in the statocytes) are considered as gravisensors, the role of these organelles on transduction pathways, is not clearly elucidated (Perbal and Driss-Ecole, 2003). Some studies have demonstrated that cytoplasmic free Ca^{2+} concentration ([Ca2+]cyt) is affected by environmental stimuli including gravity (for review, Tatsumi et al., 2014). The regulation of this homeostasis involves a series of transduction events such as the synthesis and activation of calcium binding and targeted proteins including calmodulin proteins.

Methods

Two space experiments, called PolCa and Gravi-2 were performed on the International Space Station. The objective of these experiments is to study the impact of amyloplasts positioning on cytosolic calcium mobilisation and thus, better understanding the gravity perception in roots. The PolCa space conditions have provided a unique opportunity to observe a change of amyloplast positioning in statocytes after a swap from 1g-centrifuge to microgravity (*e.g.* 10^{-4} g) and *vice-versa*. More recently, the Gravi-2 experiment performed on-board the ISS in May 2014, offered us the opportunity to study the effect of amyloplast positioning in Lentil seedlings grown in microgravity and then, submitted to two g-levels (0.01g or 2g), as described in Gravi-1 experiment (Driss-Ecole et al., 2008).

Results

Since calcium is considered to be an important second messenger involved in root gravisensing, the cytosolic Ca^{2+} localisation has been analysed in statocytes and related to the positioning of amyloplasts. Our results confirmed a change of amyloplasts distribution in roots grown in microgravity. Interestingly, a swap of 10 min from microgravity to 1g (and *vice-versa*) is sufficient to induce some changes in amyloplast distribution. The observation of calcium precipitates using electron transmission microscopy combined with image analysis

showed that the calcium's distribution is not significantly different in roots statocytes grown in microgravity conditions, in comparison to roots grown on 1g-centrifuge. However, our observations revealed that calcium precipitates are affected following a swap to microgravity conditions or to 1g conditions.

All together, our results suggest that a new amyloplast positioning may play an important role in calcium signalling pathways.

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Involvement of a Wall Associated Kinase after gravistimulation in poplar stems

Kévin Tocquard[1], Nicole Brunel[1], Jérôme Franchel[1], Nathalie Fournier-Leblanc[1], Jean-Stéphane Venisse[1], Patricia Roeckel-Drevet[1]

[1]UMR PIAF, Clermont University, France
kevin.tocquard@gmail.com

Plants can re-orientate themselves according to the gravity vector. Response to gravistimulation is an essential mechanism whereby plants adapt to their environment. In angiosperm woody plants, reorientation of aerial parts involves the formation of tension wood which differs from normal wood by physiochemical and mechanical characteristics. Understanding the early processes leading to this wood formation is a challenging research question.

Our working hypothesis is that molecular actors involved in gravisensing and/or graviresponse could be localized at a continuum between cytoskeleton-plasma membrane-cell wall as described in the gravitational pressure model (Baluška and Volkmann, 2011). A change in the gravity vector would modify plasma membrane-cell wall mechanical relations with a tension between this two cell compartments at the upper side of the cell and a compression at the lower side. At the interface of this continuum, wall associated kinases (WAKs) could be good candidates as sensors and/or actors of the cell mechanical deformation. Indeed, WAKs have an extracellular part that can bind to the pectins of the cell wall, a single transmembrane region and an intracellular kinase domain which could signal deformations between membrane and wall to the cytoplasm. Using *in silico* approaches, we showed in poplar the most expanded WAK family never identified to date with 175 members (Tocquard *et al.*, 2014). We analyzed gene expression of the WAK family members in various organs and tissues of poplar tree and showed that WAKs were weakly expressed. Only 28 *PtWAKs* were expressed in the stem. One WAK is of particular interest showing differential expression after tilting of the poplar stem. The protein was localized in young xylem and bark cells devoid of amyloplasts. These data suggest that a WAK could be involved in the sensing/response to gravistimulation in a manner independent of the displacement of amyloplasts which is behind the starch-statolith hypothesis (Sack, 1997).

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Identification of proteins that potentially interact with Ca^{2+}-permeable mechanosensitive channels MCA1 and MCA2 in Arabidopsis

Aki Nakamura and Hidetoshi Iida

Department of Biology, Tokyo Gakugei University, Japan
m141819g@st.u-gakugei.ac.jp

Abstract

The Ca^{2+}-permeable mechanosensitive channels MCA1 and MCA2 are involved in an increase in $[Ca^{2+}]_{cyt}$ upon mechanical stress such as hypo-osmotic shock in *Arabidopsis thaliana* (1,2). The primary root of the *mca1* mutant is unable to enter hard agar medium form soft agar medium, suggesting that MCA1 is involved in sensing the hardness of agar (2). MCA1 and MCA2 are composed of 421 and 416 amino acid residues, respectively, and located in the plasma membrane. Both proteins have a transmembrane segment near the N-terminus, an EF hand-like motif and a coiled-coil domain in the internal region and a plac8 motif in the C-terminal region, and form a homo-tetramer (3). Although an N-terminal segment composed of 175 amino acid residues are shown to be sufficient for Ca^{2+} permeation when expressed in yeast cells, the molecular architecture and regulatory mechanism of MCA1 and MCA2 are unsolved. In the present study, to help elucidate the unsolved problems, we screened proteins five times independently that potentially interact with MCA1 and/or MCA2 using the split-ubiquitin membrane-based yeast two-hybrid system and identified two candidates that were selected repeatedly. We will discuss possible roles for these proteins in terms of protein-protein interaction.

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Integration of transcriptomic and proteomics approaches in characterizing short-term gravi-perception signaling networks in poplar wood

Richet N[1], Mauriat M[2], Lesage-Descauses M-C[1], Rogier O[1], Laurans F[1], Huguet S[3], Balzergue S[3], Lapalu N[4], Pilate G[1], Coutand C[5], Plomion C[2], Leple J-C[1]

[1]INRA, UR0588 AGPF, F-45075 Orléans cedex
[2]INRA, UMR 1202 BIOGECO, F-33610 Cestas, France
[3]INRA, UMR 9213/UMR1403 IPS2, F-91057 Evry Cedex, France
[4]INRA, UR1164 URGI, F-78026 Versailles, France
[5]INRA, UMR 547 PIAF, F-63100, Clermont-Ferrand, France
Jean-Charles.Leple@orleans.inra.fr

Summary

Plants organs are capable of sensing directional environment stimulus such as light, gravity, water availability and touch. Light and gravity are two of the most important environmental factors affecting plant stem growth and development. During the past 10-15 years, advances in our understanding of the molecular sensing and signaling actors in response to these stimuli have been mainly made in model annual plant. The goal of our study was to decipher the early molecular events operating in poplar wood stem submitted to changes in gravi-stimulation and so, prone to tension wood formation. As light and gravity early signaling may modulate each other we tilted plants in specific isotropic lightening devices enabling to ensure growth but triggering no directional phototropic signal (Fig. 1; Coutand et al., unpublished). Through RNAseq transcriptome profiling, proteomic and phosophoproteomic we identified candidates of the early signaling pathway of tension wood formation in poplar. Prominently, one receptor-like kinase is identified by the different approaches and appears as a good candidate for gravi and/or mechanical signaling. Moreover, several MAP kinases are also identified and could participate to this signaling network. Predicted gene network signaling models will be presented and discussed. This work was supported by the French ANR (project "TROPIC", ANR-11-BSV7-0012).

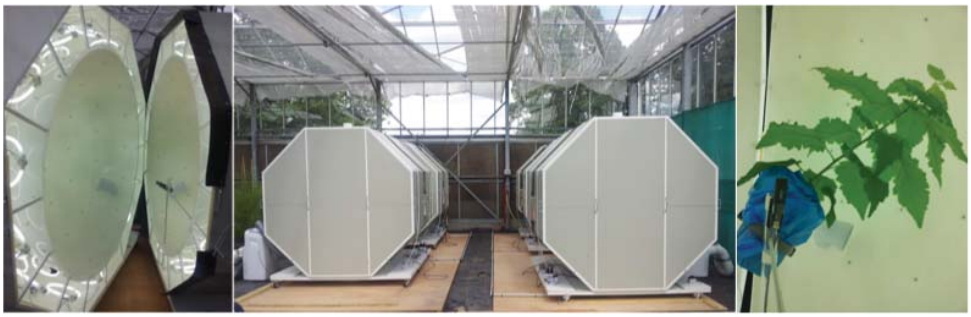

Fig. 1: Isotropic light illumination system. Going left to right: 1) the two hemispheres with their white circle neon lights and the sample attaching device, 2) the four modules used in this study placed in a greenhouse, 3) poplar plant fixed on the attaching device. Photograph courtesy of Richet N.

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DGE1, *DGE2* and *DTL* genes are involved in gravity signaling in gravity sensing cells of Arabidopsis

Masatoshi Taniguchi[1], Kenichirou Baba[2], Asako Yuasa[2], Masao Tasaka[2] and Miyo Terao Morita[1]

[1]Laboratory of plant environmental responses, Nagoya University, Japan
[2]Laboratory of morphological dynamics, Nara Institute of Science and Technology, Japan
taniyang@agr.nagoya-u.ac.jp

Higher plants have developed mechanisms for gravitropism, organ growth and movement in response to gravity. Forward genetic analyses using *Arabidopsis thaliana* have so far identified many genes involved in the process of gravity perception and asymmetric organ growth, however, the molecules and mechanisms for signaling processes remain unclear. Endodermal cells and columella cells of Arabidopsis are the gravity sensing sites, statocytes, in shoot and root, respectively. Inflorescence stems of Arabidopsis *sgr1*/*scr* (*shoot gravitropism 1*/*scarecrow*) and *sgr7*/*shr* (*shoot gravitropism 7*/*short-root*) mutants completely lack the layer of endodermis. On the other hand, *eal1* (*endodermal-amyloplast less 1*) retain the cell layer of which cells are do not fully develop as endodermal cells. All these mutant stems are agravitropic. To identify the novel genes involved in signaling process occurred in endodermal cells, we performed comparative transcriptome analyses using WT and the mutants. We focused some down-regulated genes in the mutants compared with WT, and named as *DLLs* (*DGE1*/*LAZY Like proteins*; *DGE1, DGE2, DTL*).

Phenotypic analyses of *dlls* mutants demonstrated that *DGE1*, *DGE2*, and *DTL* are involved in shoot gravitropism and that *DGE2* and *DTL*, but not *DGE1,* are required for root gravitropism. Expression analysis using transgenic plants expressing GUS under the control of each of their own promoters showed that *DLL*s are mainly expressed in the statocytes of shoot and root, although *DGE1* promoter activity was not observed in the statocytes of primary root. We then generated *dll*s triple mutants expressing each of *DLL*s under the control of the statocyte specific promoter: *SCR* (*SCARECROW*) promoter for expression in endodermal cells or *ADF9* (*Actin-Depolymerizing Factor 9*) promoter for expression both in columella cells and endodermal cells. These transgenic plants showed normal gravitropic response, indicating that *DLL*s function in the statocytes for shoot and root gravitropism.

To know in which process *DLL*s are involve, we first observed the amyloplast movement in the shoot and root statocytes of *dll*s triple mutant. Amyloplasts in *dll*s showed normal sedimentation after gravistimulation, indicating that the process of gravity perception is not affected in the triple mutant. We next investigated the formation of asymmetric auxin signaling after gravistimulation in *dge2dtl* double mutant and *dlls* triple mutant. Asymmetric signal in *dge2dtl* and *dll*s was reduced compared with that in WT 6 hr after gravistimulation. These results suggest that *DGE2* and *DTL* are involved in a signaling process between gravity perception and formation of asymmetric auxin signal in the primary root.

DLLs proteins share more than 26 % amino acid sequence similarity, although they contain no known functional or structural motif and domain. We focused on conserved 14 aa sequence at the C-termini (CDL, Conserved C-terminal in DGE1/LAZY1), and then tested whether DGE2 and DTL lacking the CDL rescue gravitropic phenotype of *dll*s *in planta*. The result indicated that CDL would be a novel functional motif in the DLLs protein family.

A laccase gene is involved in compression wood lignification in *Chamaecyparis obtusa*

Hideto Hiraide[1], Masato Yoshida[1], Saori Sato[1], Kunio Ihara[2], Miyuki Matsuo[1], Hiroyuki Yamamoto[1]
[1]Laboratory of biomaterial physics, Nagoya University, Japan
[2]Center for Gene Research, Nagoya University, Japan
hiraide.hideto@e.mbox.nagoya-u.ac.jp

Introduction

Trees show negative gravitropism in the stem during thickening growth as well as during elongation growth. When the stems of conifers are inclined, they form special secondary xylem called "compression wood" in the lower side. In compression wood, compressive growth stress is generated to lift the stem upward.

Generation of compressive growth stress is caused by chemical and anatomical features of compression wood different from normal wood. Especially, higher lignin content and different lignin distribution in the cell wall are believed to one of major factors [1].

This special lignification of compression wood will be controlled by molecular bio-mechanisms. To reveal the mechanism of compression wood lignification, we assess relationship between lignification of the cell wall and gene expression involved in lignin synthesis. The results suggested that laccase, which oxidizes lignin precursors (monolignol), was involved in compression wood lignification.

Materials and method

Fifteen Japanese cypress (*Chamaecyparis obtusa*) saplngs were used. Twelve were grown with the stem tilted at varying angles to induce compression wood to varying degrees. The angles were 10°, 20°, 30°, and 50° from the vertical; three saplings were used for each angle. The other saplings were grown with the stem vertical (0°) to induce normal wood as controls. One month later, the saplings were cut down to harvest differentiating and mature tracheids.

The mature xylem were then fixed in 3% glutaraldehyde in 0.1 M phosphate buffer (pH 6.98) and were embedded in epoxy resin for ultraviolet microscopic analysis. The image data of the ultraviolet photomicrographs with 256 gradations were analyzed using the ImageJ software (http://rsb.info.nih.gov/ij/).

Total RNA was purified from the differentiating xylem and analyzed by quantitative RT-PCR to reveal gene expression.

Results and Discussion

The transcript abundance of most of lignin synthesis genes (nine of twelve tested genes) was higher in compression wood than in normal wood (Fig.1a shows *CoPAL2*, which encodes phenylalanine ammonia-lyase (PAL), as representative).

Among the analyzed genes, the laccase gene (*CoLac1*) showed slightly different expression pattern than the others. *CoLac1* expressed very low in normal wood and very high in compression wood (Fig.1b). Such compression wood-specific gene is probably involved in compression wood lignification.

In compression wood cell wall, lignin content increases than that of normal wood, and the lignin increment is high in the outer part of secondary middle layer; this high lignified region is called "S2L". Ultraviolet microscopy observation [2] [3] indicated relationship of *CoLac1* expression with S2L thickness (Fig 2). S2L thickness in transverse section increased with increasing of *CoLac1* expression. To assess correlation between *CoLac1* expression and S2L thickness, we measured S2L thickness in 360 compression wood tracheids using ImageJ, and performed Pearson's correlation coefficient test. *CoLac1* transcript abundance showed a

positive correlation with S2L thickness ($P < 0.01$, $R = 0.585$). These results suggested S2L formation and thickening involved laccase encoded by *CoLac1*.

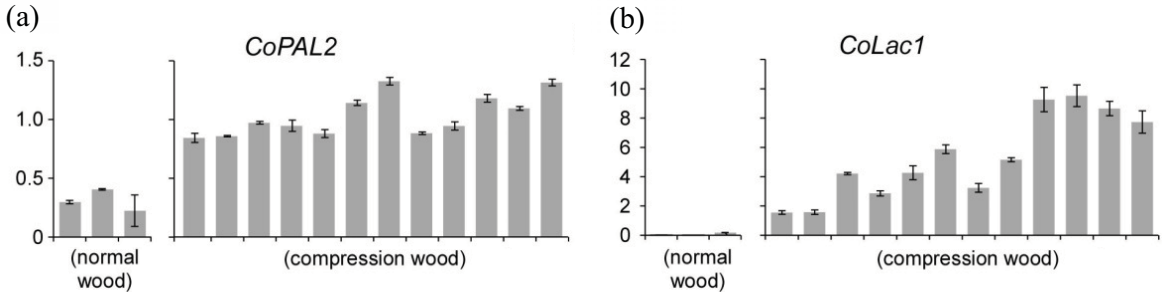

Fig.1 Expression of lignin synthesis gene in normal and compression wood
a) *CoPAL2*, b) *CoLac1*. Vertical axis indicates transcript abundance, representing the ratio of the transcript number of the target gene to normalization factor. [4]

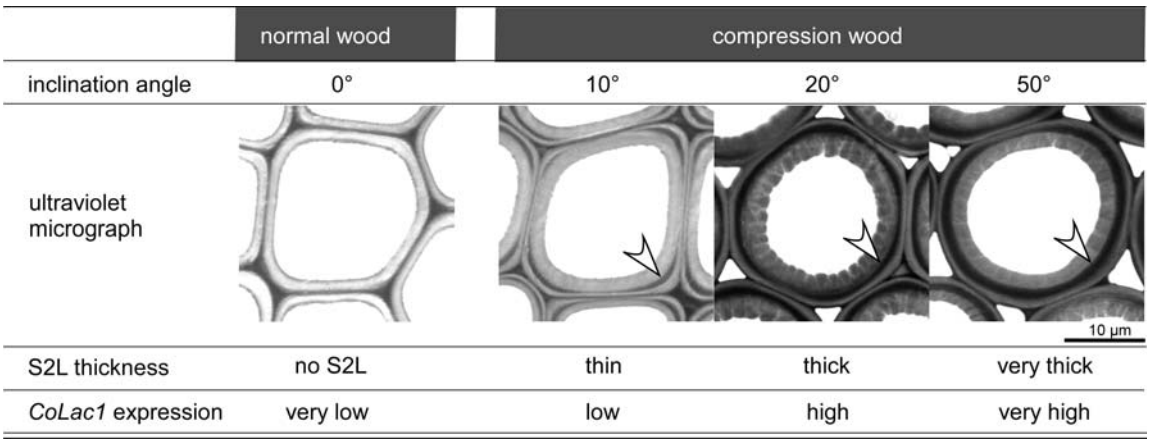

Fig.2 Relationship between *CoLac1* expression and S2L thickness
A tracheid under ultraviolet microscope was shown for each sapling grown with different inclination angles (0°, 10°, 20° and 50°). In ultraviolet micrograph, lignin shows dark, and darker region indicates higher lignin content. The arrowhead indicates S2L. In normal wood, S2L was not observed. On the other hand, in compression wood, S2L was observed and it was thicker when *CoLac1* expression is higher.

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Quantitative evaluation of feedback mechanisms between cell shape and cytoskeleton organization

Pauline Durand-Smet[1], Arun Sampathkumar[1], Elliot Meyerowitz[1, 2]

[1]Division of Biology, California Institute of Technology, 1200 East California Boulevard, Pasadena, California 91125, USA
[2]Howard Hughes Medical Institute, California Institute of Technology, 1200 East California Boulevard, Pasadena, California 91125, USA
pdurand@caltech.edu

Despite the significance of cellular morphology as a functional phenotype, it remains challenging to quantitatively relate morphological phenotype to the behavior of subcellular molecules. Molecular studies have identified many components controlling cell morphogenesis, but it is unclear how this information is translated into the physical world. In plant cells, growth requires synthesis of cytoplasmic components as well as expansion of the cell wall. The cell wall is a stiff yet flexible polymeric network that encapsulates cells and counterbalances stress created by turgor pressure inside the cell, thereby controlling cell shape. Thus, understanding the process of cellular morphogenesis requires solving a multiscale problem connecting the molecular components to the physical parameters controlling spatio-temporal regulation of cell shape. It is now well established that the cytoskeleton plays a key role in the biogenesis and morphogenesis of the cell wall. While the microtubules guide cellulose synthase complex movement (Paredez et al. 2006), the actin network is responsible for global distribution of cellulose synthase complexes (Gutierrez et al. 2009; Sampathkumar et al. 2013). It is also suggested that mechanical stresses orient the microtubules along their principal direction (Hamant et al. 2008; Sampathkumar, Krupinski, et al. 2014; Sampathkumar, Yan, et al. 2014). Nevertheless, to fully understand how plant cells are shaped and how external mechanical stresses influence this process, a quantitative approach to evaluate the mechano-response in single cells needs to be established.

Here we present a technique to confine single plant protoplasts into molds of defined shape to study single cell behavior using quantitative live cell imaging approaches coupled with physical measurements. The principle is to confine a protoplast expressing fluorescent cytoskeletal reporters into polydimethylsiloxane (PDMS) micro-wells of different shapes with sizes of 10 to 30 µm. The protoplasts are then monitored with a confocal microscope to evaluate changes in cytoskeletal organization and dynamics during the process of symmetry breaking (Fig. 1). These experiments will be the basis of assessing quantitatively how different shapes control cytoskeleton behavior by regulating the distribution of physical stresses.

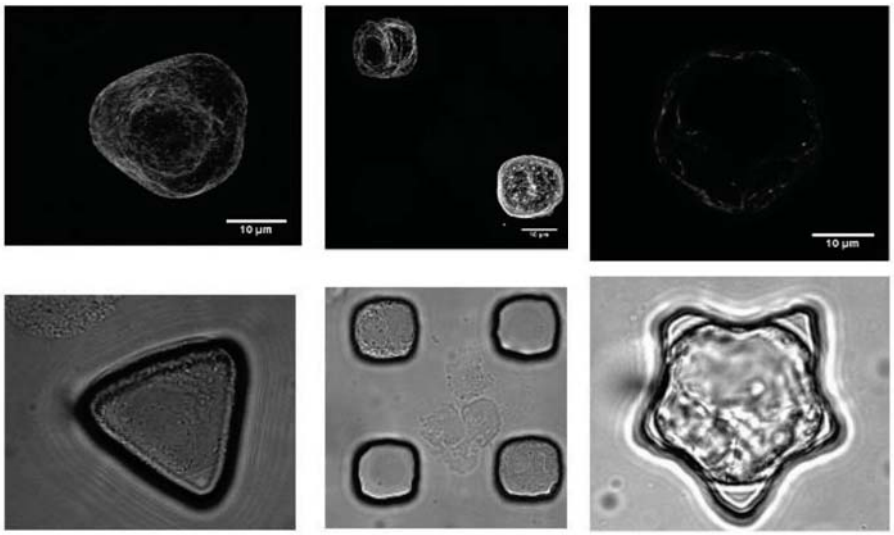

Fig. 1: Single protoplasts confined into micro-wells of different shapes. The pictures on the top show the actin network organization.

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An actin-myosin XI cytoskeleton determines plant posture by regulating organ straightening.

Haruko Ueda[1], Tomoo Shimada[1], Kentaro Tamura[1], Miyo Terao Morita[2], Ikuko Hara-Nishimura[1]

[1]Graduate School of Science, Kyoto University, Japan.
[2]Graduate School of Bioagricultural Sciences, Nagoya University, Japan.
hueda@gr.bot.kyoto-u.ac.jp

Introduction

Plants are able to bend nearly every organ in response to environmental stimuli such as gravity and light. The responses of organs to stimuli are restrained or even reversed so that the organ will stop bending and attain its desired posture. This phenomenon of organ straightening has been called autotropism and autostraightening and modeled as proprioception. However, where and by what machinery organ straightening is driven are unknown.

Results

Arabidopsis thaliana myosin XI gene family has 13 members. We found that the lack of two myosin XI members (XIf and XIk) resulted in a kinked gross morphology, in which inflorescence stems and pedicels failed to grow straight (Fig. 1). The *myosin xif xik* mutant exhibited hyper-bending of various organs in response to gravity and light (Fig. 2). We show that wild-type inflorescence stems possess a straightening mechanism to stop organ bending. In contrast, the *myosin xif xik* stems impair the straightening process during the courses of gravitropism.

Fig. 1 : Kinked gross plant morphology of a *myosin xi* sextuple mutant.

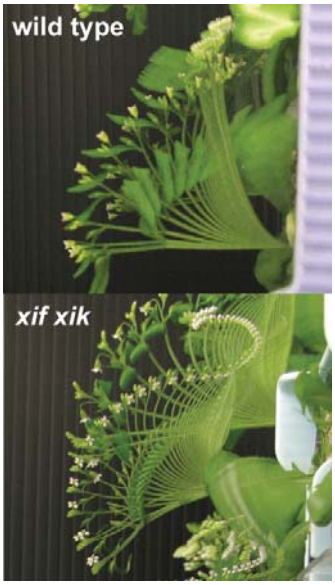

Fig. 2 : Maximum intensity projections of time-lapse images during gravitropism of inflorescence stems.

Expressing either *myosin XIf* or *myosin XIk* under the control of the *myosin XIf* promoter significantly rescued the defect in straightening of inflorescence stems of *myosin xif-1 xik-2*, suggesting that cells expressing *myosin XIf* have a key role in organ straightening. In a cross section of inflorescence stems, GUS signals driven by the *myosin XIf* promoter encircled the stem in a thin band, which consisted of xylem fiber cells and interfascicular fiber cells. The fiber cells developed extremely long bundles of actin filaments along the longitudinal axis of the cells. The stem of the heterozygous mutant *actin8* (*frizzy1/+*) showed a kinked gross morphology, enhanced bending in response to gravity, and an inability to straighten. All of these phenotypes are very similar to those of *myosin xif-1 xik-2,* indicating that actin filaments together with myosins XIf and XIk are required for organ straightening.

Conclusions

We conclude that the straightening of inflorescence stems is regulated by an actin-myosin XI cytoskeleton in immature fiber cells. The actin-myosin XI cytoskeleton enables organs to attain their new position more rapidly than would a series of diminishing overshoots in environmental stimuli. Hence, the long actin filaments in elongating fiber cells might act as a bending sensor to trigger the straightening system to adjust plant posture.

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A universal mechanism for hydraulic signals generation in natural and artificial branches

J-F. Louf[1], G. Guena[1], E. Badel[2,3], Y. Forterre[1]

[1]Aix-Marseille Université, CNRS UMR 7343, IUSTI Marseille cedex 13, France
[2]INRA, UMR 547 PIAF, 63100 Clermont-Ferrand, France
[3]Clermont Université, UMR 547 PIAF, 63000 Clermont-Ferrand, France
yoel.forterre@univ-amu.fr

Plants are constantly subjected to external mechanical loads such as wind, rain or even neighbours. These stimuli are known to affect the growth of the plants, a process called thigmomorphogenesis (Jaffe et al. 2002, Braam 2005, Telewski 2006). Typically, the bending of a tree stem leads to a local increase of the radial growth while the primary growth is rapidly stopped, suggesting a transport of the information from the stimulated zone to the apical zone (Coutand et al. 2000, 2009). Among the different hypothesis found in the literature to account for this long-distance transport (electrical signal, hormone transport), it has been proposed that local mechanical stimuli, like bending, could generate a hydraulic signal that could move through the xylem (Malone 1994, Julien 1993, Farmer et al 2014). Recently, such hydro/mechanical couplings have been directly observed by Lopez et al (2014), who showed that bending a whole living tree or a cut branch generates almost simultaneously an overpressure pulse in the vascular system. However, the physical mechanism responsible for this hydro/mechanical coupling and the basic properties of the hydraulic pulse (amplitude vs. bending deformation, dependence with wood properties) remained not understood.

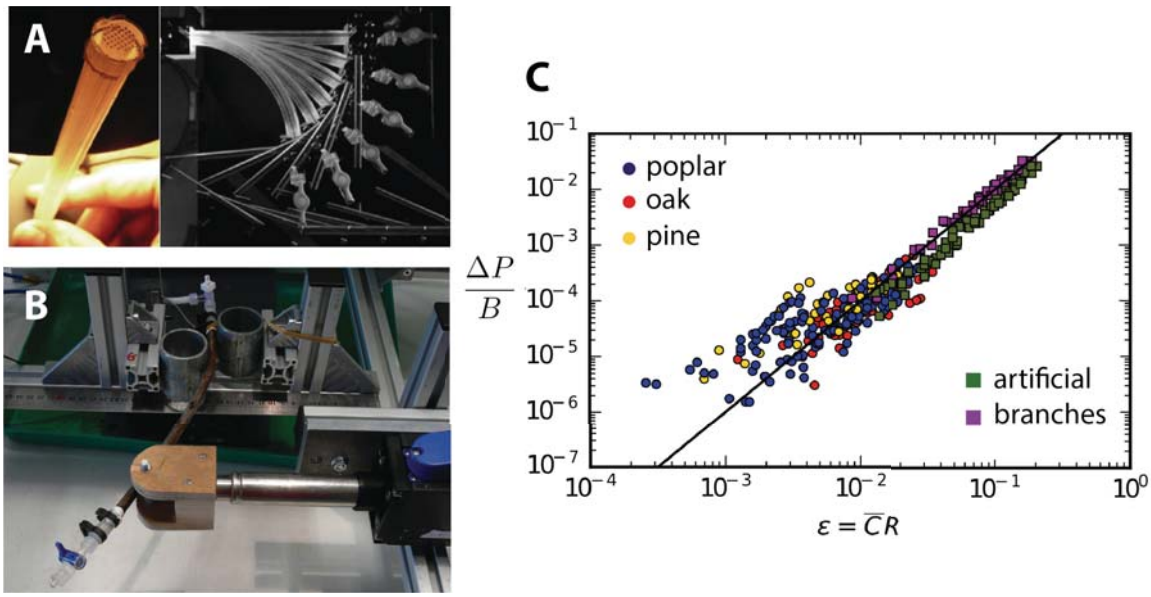

Fig. 1: A) Biomimetic branch. B) Bending set-up for natural branches (poplar). C) Hydraulic pulse amplitude ΔP normalized by the branch elastic bulk modulus B as function of the bending strain (circles: natural branches, squares: artificial branches).

In this work, we address this question by combining biomimetic experiments, experiments on natural tree branches and modelling. We first designed an artificial branch made of a transparent elastomer (PDMS) beam, perforated with longitudinal micro-channels and filled with a liquid (fig. 1A). We show that bending such a poroelastic beam generates a sudden increase of the mean liquid pressure, which scales with the beam elasticity and increases quadratically with the bending strain. A simple model is written to explain this non-linear response: when a soft porous beam is bent, its cross-section is squeezed to minimize the total elastic energy, thus reducing the volume of the conduits and expulsing the interstitial incompressible liquid. To validate this mechanism, we then performed experiments on natural branches of different wood species (poplar, pine, oak) (fig. 1B). We recover on these natural systems the same phenomenology: bending a branch in a closed geometry generates a mean increase of the xylem pressure, which varies quadratically with the bending strain. Once rescaled by the model prediction, both artificial and natural branches gather on the same master curve (fig. 1C), showing the universality of the mechanism of hydraulic signals generation in plants.

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A minimal model for plant tropisms as a noisy sensorimotor system

Y. Meroz[1], R. Bastien[2] , L. Mahadevan[1,3,4]

[1]School of Engineering and Applied Sciences, Harvard University, MA, USA [2]Department of Collective Behaviour, Max Planck Institute for Ornithology, Germany
[3]Organismic and Evolutionary Biology, Harvard University, MA, USA
[4]Physics, Harvard University, MA, USA

ymeroz@seas.harvard.edu

Introduction

Plant tropisms can be viewed as a sensorimotor system. Some stimulus (such as light or gravity) is applied from some direction, the plant then senses it, leading to a motor response according to the stimulus direction. These systems rely on stochastic processes, and yet are able to operate robustly in a noisy environment. In this work we take these processes into consideration within a mathematical framework, addressing known experimental observations including integration of intermittent stimuli [3,6,11], adaptation and desensitisation [4,5], enhancement [6], and the functional form of the dose-response curve [10].

Model

Recently, a minimal model was developed that accurately predicts the complete gravitropic, phototropic and proprioceptive control over the movement of different organs in different species in response to gravitational and light stimuli [1]. Here we extend this framework to include processes underlying gravitropism and phototropism, along different timescales, including [7,8]: (i) polarization of the PIN3 auxin transporter (phototropism) [2] or of statoliths (gravitropism), (ii) consequent auxin redistribution and lastly (iii) the motor response in the form of differential growth. We consider the underlying stochastic nature of the processes [9], describing the polarization process through a master equation with characteristic polarization and relaxation rates, and the auxin redistribution as a direct function of the polarization state. The stochastic nature of signal transduction is taken into account in the motor response by incorporating a memory kernel.

Results

We are able to retrieve the functional form of the memory kernel from previous experimental data. Moreover this mathematical description successfully captures experimental observations such as the integration of intermittent stimuli, enhancement, desensitisation due to pre-irradiation and a hyperbolic form of the dose-response curve. We show that the stochastic nature of the underlying processes, and the separation of timescales, are fundamental to plant tropisms and sensorimotor systems in general.

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On the role of gravity in shoot gravisensing

H. Chauvet[1], O. Pouliquen[1], Y. Forterre[1], V. Legué[2], B. Moulia[2]

[1]Laboratoire IUSTI, CNRS, Aix Marseille University, Marseille, France [2]Laboratoire PIAF, INRA Université Blaise Pascal, Clermont-Ferrand, France
hugo.chauvet@gmail.com

A plant accidentally put in a horizontal position rapidly bends and deforms to recover a vertical position. The ability of plants to feel gravity thus plays a key role in their development and adaptation to environmental changes (gravitropism and posture control). A crucial step in this gravisensing occurs in specific cells, the statocytes, which contain dense organites filled with starch granules (amyloplasts). The amyloplasts being denser than the surrounding intracellular fluid, they sediment at the bottom of the cell and are supposed to indicate the direction of gravity with respect to the cells (Morita 2010). However the mechanisms at work in statocytes and the link with the active bending of the plant at the macroscopic scale still need a better understanding (Moulia and Fournier 2009, Blancaflor 2015). In this study, we use an experimental approach to study gravitropic motions at the plant scale, and more specifically to investigate quantitatively the plant sensitivity to gravitropic stimuli and identify the sensed variable (e.g mechanical pressure by amyloplats, velocity or position of the amyloplasts in the cell ….).
An original experimental setup called ``gravitron'' has been developed to investigate the response of plant shoots to changes in both gravity intensity and direction. The system is based on an instrumented rotating table allowing full kinematical tracking of the tropic mouvement. These records were then interpreted in term of gravisensitivity using the relevant dimensionless quantity introduced by recent quantitative studies on gravitropic control (Bastien et al. 2013, 2014)

Using this setup we systematically measured the gravitropic response on a wide phylogenic range of plant species. Our observations show that the shoot response to a constant gravitropic stimulus is not sensitive to gravity intensity and only depends on gravity direction.
We also performed experiments imposing transient stimulus, i.e. inclining the plant from the direction of gravity during a finite time before being returned to the vertical. Experiments performed at different gravity intensities again show that the response of the plant to transient stimulus is independent of gravity intensity and solely depends on the time of exposition.

All-together, these results show that the sensors in statocytes are not sensitive to the pressure of the amyloplasts on some subcellular compartment nor to the displacements of these grains but rather to the position of the amyloplasts in the cell.

We will show that these results open new perspectives for the interpretation of the two classical laws of gravitropism (i.e. the “sine law” and the “dose response law”) and have major implications in our understanding of the mechanism at work in the gravitropic sensing.

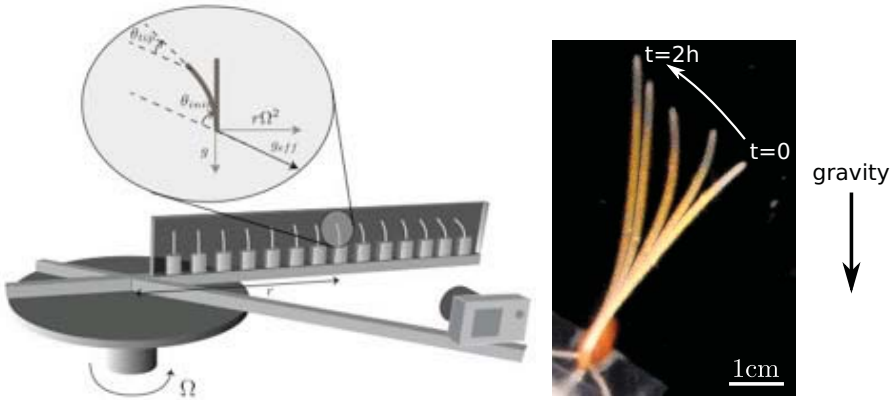

Fig. 1 : Experimental setup and example of the gravitropic response of a wheat coleoptile

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Tree acclimation to wind in a dense beech forest to investigate how mechanical cues are filtered and modify radial growth.

V. Bonnesoeur[1, 2], T. Constant[1, 2], M. Fournier[1, 2], B. Moulia[3]

[1] AgroParisTech, UMR 1092 LERFOB, 54000 Nancy, France,
[2] INRA, UMR 1092 LERFOB, 54000 Nancy, France
[3]INRA, Universite Blaise Pascal, UMR_A547 PIAF, Domaine de Crouël, 63039 Clermont Ferrand, France
vbonnesoeur@nancy.inra.fr

Introduction.

Climate change stresses the need of a better understanding of the environmental control of forest growth. Wind is an important climate factor well known to influence plant growth. More precisely, experiments where plants were bent in controlled conditions have proved trees can sense wind-induced strains and adapt their growth to, which is known as thigmomorphogenesis (Moulia et al., 2011). However these types of experimental conditions are often too simplistic compared to the complex pattern of mechanical stimulus experimented by trees in natural conditions. In greenhouse experiments, the mechanically stimulated plants are usually submitted to large and frequent amount of mechanical strain and compared to fully protected plants in order to emphasize thigmomorphogenetic responses. The very few field experiments consisted in guying trees and comparing their growth with control trees after several months. These studies have proved that mechanical stimulation of growth allocation is an important ecological process that can impact strongly forest ecosystem services as wood production, carbon storage or landscape protection against storms and strong winds. Actually , thigmomorphogenesis could account for 50% of the aerial growth (Meng et al., 2006). However the stimuli – i.e. the wind-induced strains in living cells - were not quantified and the time resolution (one to several years) was too low to give more insight about how thigmomorphogenesis takes place.

This work aims at accurately quantify thigmomorphogenetic responses of radial growth in a broaleaved forest (*Fagus sylvatica*, Northern Eastern France, public forest managed by ONF). We will particularly focus on wind perception by the trees and possible filtering of the different type of wind events by trees. We also want to study the between-tree variability of the mechano-sensing and growth response in relation with social status (dominant and suppressed trees) in very dense stands, undisturbed for a long time (no thinning). Assuming trees in this undisturbed stand are acclimated to usual winds, we monitored wind-induced strains in several trees of different status, then we manipulated this mechanical signal by adding or suppressing a giving amount of this natural stimulus (by guying or pulling some trees) and observed radial growth response.

Materials and methods.
We have monitored the longitudinal bending strain of 15 pairs of dominant-suppressed trees by setting up strain-transducers at breast height for the growing season. We have also monitored the secondary growth with automatic point dendrometer at breast height during the same period. In addition to the wind-induced strain regime, we manipulated mechanical stimulation to 24 trees. 6 trees were guyed to strongly reduce the strains in their trunk. 18 trees were submitted to additional artificial bendings of different strain magnitudes. These trees were bent once per day, on monday, tuesday and wednesday during 6 weeks. Three artificial strain magnitudes were imposed in reference to the wind-induced strain regime :

- a strain magnitude of 0.015% which corresponds to the mean daily wind-induced strain peak observed before the beginning of experiment.
- a strain magnitude of 0.04% which corresponds to the mean wind-induced strain peak observed during a depressional event
- a strain magnitude of 0.15% which corresponds to an intense and rare wind-induced strain peak a tree could sense during a storm, but remains far below wood strength and limit of elasticity.

The effects of the mechanical treatment on the secondary growth was investigated by using non-linear mixed effects model (Gompertz growth curve).

Results and discussion.
The trees submitted to an imposed strain of 0.15% showed significant growth responses, while all other treatments – guyed, natural wind strains, natural wind strains + 0.015%, natural wind strains + 0.04%, showed similar growth. Large strain magnitude had the following effects on secondary growth :

- a delayed growth start of 5.6 days
- an increased maximal radial growth rate of 0,024 mm/day (which represents an increase up to more than 100% compared with the other treatments)
- an increased radial growth increment 0,67 mm at the end of the growing season (which represents an increase up to more than 100% compared with the other treatments).

The mechanical effects on absolute growth on dominant and suppressed trees was similar. Thus, as suppressed trees are much smaller, they benefited more from thigmomorphogenesis than the dominant (mean relative growth rate increases from 0,8% to 2,4% on suppressed trees; but only from 2,1% to 3,1% on dominant). The growth responses were consistent with the thigmomorphogenetic syndrome. Moreover, in our experiment, trees only sense the strongest winds in an undisturbed stand, and not moderate changes of wind regimes or complete suppression of mechanical stimuli. As this result seems quite different from other field experiments (Meng et al. 2006 or guying experiments in general), the discussion argues about strain filter mechanisms in tree perception, concluding that the transduction scheme between perceived strains and growth response must change according to ecological demands and organs (root or shoot, radial or height growth): in dense stands, foraging for light (and so height growth) is a priority and then, only a great mechanical disturbance leads to cambial growth response, whereas in other conditions (no limiting light, or more limiting winds) and in other meristems (shoot or root elongation), thigmomorphogenetic growth could occur under different ranges of mechanical stimuli.

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Secondary growth regulation by strains induced by wind: From Stem Structure to Gene Expression

Eric Badel[1,2], Nathalie Leblanc-Fournier[2,1*], Jérôme Franchel[2,1], Mélanie Decourteix[2,1], Catherine Coutand[1,2], Bruno Moulia[1,2]

[1] INRA, UMR 547 PIAF, 63100 Clermont-Ferrand, France
[2] Clermont Université, UBP, UMR 547 PIAF, 63000 Clermont-Ferrand, France
*Nathalie.leblanc@univ-bpclermont.fr

Introduction

Mechanical signals are important factors controlling plants growth and development. External mechanical loadings, such as wind, lead to a decrease of primary growth, an increase of secondary growth, modifications of stem mechanical properties and biomass reallocation to roots (Telewski, 2006, Badel et al., 2015). Integrative approaches combining physical/modeling/kinematics at plant and organ levels demonstrated that strain-sensing (deformation) are involved in the process of mechanoperception (Moulia et al., 2011; Coutand et al., 2009). These results allowed the development of an integrative biomechanical model called S3m which postulates that mechanoperception is proportional to local strain and volume of the considered tissue (Moulia et al., 2011). In this study, we tested this biomechanical model at tissue level by studying the relation between local strain intensity, secondary growth and genes expression.

Strain-secondary growth relationship

Poplars were subjected to 3 successive daily bending per week during 4 months. Stem anatomical observations revealed that the bending stimuli modify the cambial activity both quantitatively and qualitatively. Using laser tomography, we followed the azimutal secondary growth. The results showed an ovalisation of the stem that resulted from a higher growth rate both in the stretched and the compressed part of the stem (figure 1). Furthermore, a biomechanical modeling revealed the direct link between the magnitude of the local longitudinal strain and the local radial growth of the stem according to what was expected with S3m model.

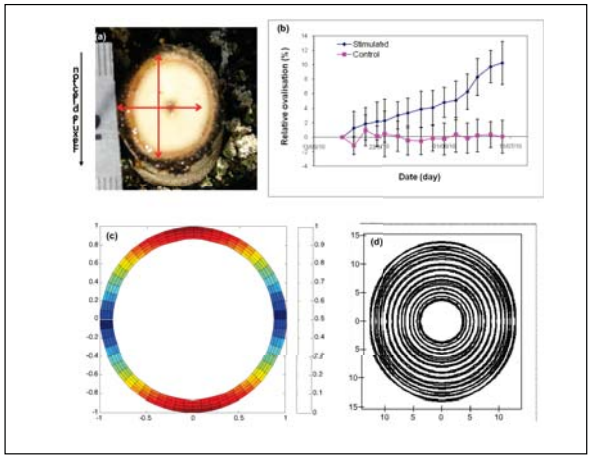

Fig. 1 : **Cambial activity induced by local strains applied to the stem: experimentations and modelling.** Plants were subjected to 3 successive daily bendings per week during 4 months. (a) Transversal section of a stimulated plant. (b) Relative ovalisation induced by

bending. (c) Strain field for living tissues. (d) Weekly growth rate measurements (blue lines) and growth rate modeling (red lines).

Local induction of *PtaZFP2* proportionally to the local strain levels

The local expression of *PtaZFP2*, a mechano-responsive gene known to be induced early after stem bending in poplars (Martin et al., 2014), was measured in different regions of the cross section of the stem (figure 2). Again, the magnitude of *PtaZFP2* gene induction was directly linked to the magnitude of the strain the tissues experienced, regardless the sign of the mechanical stimulation.

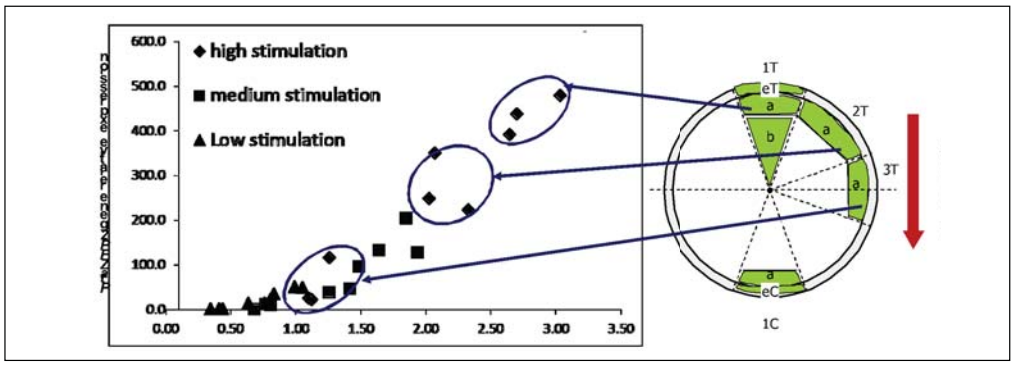

Fig. 2: ***PtaZFP2* gene expression induced by local strains applied to the poplar stem.** Plants were subjected to 3 successive daily bendings per week during 4 months. *PtaZFP2* expression was monitored by real-time PCR (left part of the figure). Plants were subjected to 3 different intensities of bending and different portions of the bent stem (a, on the right part of the figure) were collected 30 min after the last flexure. The relative transcripts abundance was determined by comparing to the gene expression level in untreated poplars using *EF-1α* transcript level as a reference.

In conclusion, both cambial activation and molecular responses suggest that few living tissues are able to respond to strains according to their magnitude and that the biomechanical model S3m is relevant at the tissue level.

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The Intermittent Nature of Leaf Growth Fields

Eran Sharon[1], Shahaf Armon[2]

[1]The Racah Institute of Physics, The Hebrew University, Jerusalem, Israel
[2]Shahaf Armon, Stanford University
erans@mail.huji.ac.il

Introduction

We present high resolution measurements of leaf surface growth. The measured growth fields are not smooth (Fig.1). Instead, they fluctuate over a range of scales in time and length. As we increase the measurement resolution, the measured growth fields appear rougher. We study the statistics of three scalar fields associated with the tensorial growth field: The growth rate, the isotropy and the directionality of growth. We find that the normal growth of a leaf involves switching between swelling and shrinkage. We identify the governing time and length scales of these fluctuations: The typical time scale is ~45 min. and the spatial correlation in the growth rate fields decays over distances of order millimeter. Measuring the growth in a low temporal/spatial resolution provides an apparent smooth field. The local growth was found to be anisotropic. Unlike the growth rate fields, the growth isotropy fields do not smoothened over time. Finally, we find significant differences between the growth fields measured during day time and those measured during night time. The growth at night is found to be more intermittent, with shorter correlation lengths and with no global directionality. These measurements present a new view on tissue growth, suggesting that the entire statistics of the fields and not just their means should be studied. In particular, the regulation of such fields and the link between their characteristics and the global geometry of a leaf should be studied.

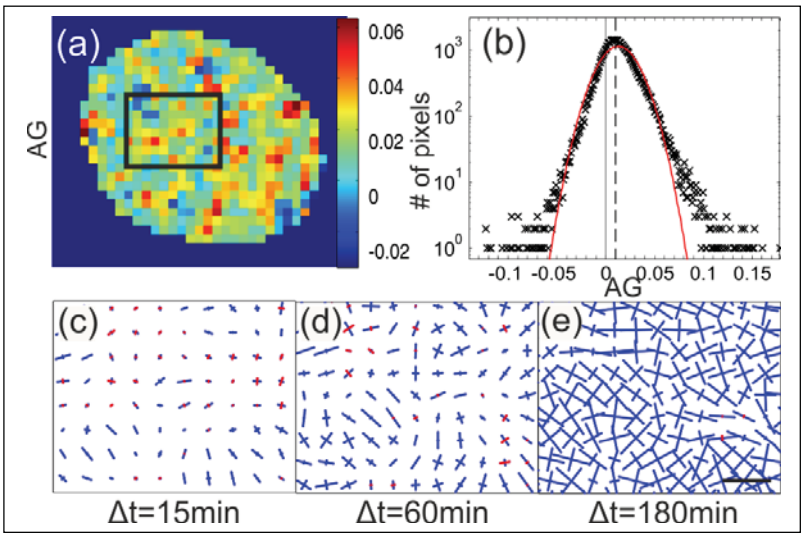

Fig. 1 : The dynamics of local growth in a Tobacco WT leaf in high spatial resolution ($250 * 250$ micron). (a) An example of a specific area growth field, AG, within $\Delta t = 15min$. (b) A histogram of all area growth events of $\Delta t = 15min$ during 2 days of measurement. Most probable value is ~1% (dashed line). A Gaussian distribution with the same mean and std is depicted in solid red line. (c-e) Principal growth measurements, taken from the same leaf area (marked with a rectangle in -a) with different time intervals. Left to right: $\Delta t = 15, 60, 180\ min$. At each point, the maximal and minimal growth vectors are depicted. The vectors are oriented along the principal directions and their lengths indicate growth values $\sqrt{\lambda_1} - 1$, $\sqrt{\lambda_2} - 1$ (scaled by the same arbitrary factor in all panels). Blue lines represent positive growth, red lines represent shrinkage. Scale bar: 0.5 mm.

On the stability of the growth pattern in the root

Tobias I. Baskin

Centre for Plant Integrative Biology, University of Nottingham, UK &
Biology Department, University of Massachusetts, Amherst, Mass. 01003 USA

baskin@umass.edu

Introduction

Roots explore their soil environment by means of growth. The growing region of the root contains a meristem where cells proliferate and expand slowly next to an elongation zone where cells divide rarely if at all and elongate rapidly. During growth, there is a continual flow of cells from meristem to elongation zone, and from elongation zone to mature (non-growing) zone, but despite the flux these zones maintain their original identities. Here I present the results of recent observations aimed at elucidating the basis for the stable zonation.

Results and discussion

Arabidopsis thaliana seedlings were grown on agar-solidified medium and imaged through a horizontal microscope. I obtained the velocity profile of the same root at repeated time intervals for 3 h. A representative example is shown in Fig. 1.

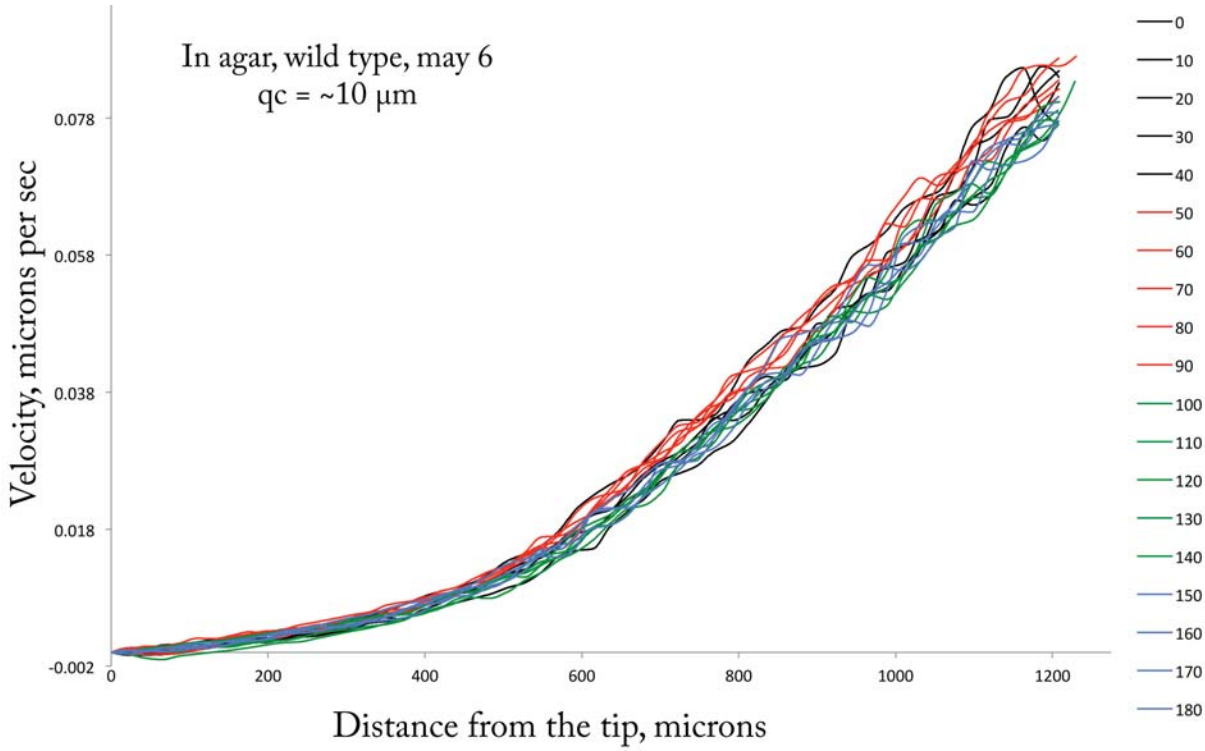

Fig. 1 : A set of velocity profiles for a single arabidopsis root. Time in minutes is given on the legend, with the same colour used for temporal bands. The quiecent center (qc) was at 10 µm on the *x*-axis.

The figure shows that the velocity profiles are congruent implying considerable stability to the zonation mechanisms. However, analysis indicates that the position where rapid elongation is first attained oscillates with a period of around 100 min. To examine the influence of

mechano-sensing, I am also subjecting roots of *botero* and *feronia* mutants to a similar analysis. Implications for the maintenance of the growth profile will be discussed.

Control of the kinematics of wood formation by a morphogenetic gradient

Félix P. Hartmann[1,2], Cyrille B.K. Rathgeber[1], Meriem Fournier[2], Bruno Moulia[3,4]
[1]INRA, Laboratoire d'Étude des Ressources Forêt Bois (LERFoB), Centre INRA de Nancy, F-54280 Champenoux, France
[2]AgroParisTech, UMR 1092, Laboratoire d'Étude des Ressources Forêt Bois (LERFoB), ENGREF, 14 rue Girardet, F-54000 Nancy, France
[3]INRA, UMR 547 PIAF, F-63100 Clermont Fd Cedex 01
[4]UMR 547 PIAF, BP 10448, Clermont Université, Université Blaise Pascal, Clermont-Ferrand, France
felix.hartmann@nancy.inra.fr

Introduction

In conifers, tracheary elements (tracheids) are produced by the vascular cambium, which is an undifferentiated tissue lying between the bark and the wood already formed. In temperate zones, a succession of annual growth rings can be easily seen on the cross-section of a fallen tree. The radial structure of a tree ring is characteristic and repeated almost unchanged from one ring to the other. The mechanisms which regulate wood formation and lead to the typical growth ring structure are not known. At the tissue level, differentiating tracheids are organized into radial files. Along these files, several developmental zones can be observed (Fig. 1). It has been suggested by several experimental works that graded-concentration profiles of some biochemical signals could guide cells during their differentiation by providing them with positional information (Bhalerao and Fischer, 2014). This is the so-called "morphogenetic-gradient hypothesis". The phytohormone auxin is the most prominent candidate as signaling molecule, but the role played by the peptide TDIF also got experimental support (Hirakawa *et al*., 2010). However, it is still not known whether a morphogenetic gradient can effectively control tissue growth and patterning. A developmental and biophysical approach relying on growth kinematics and on signal transport has thus been conducted through modelling to assess the hypothesis that this gradient could guide cell differentiation via concentration thresholds.

Model

In order to assess the morphogenetic-gradient hypothesis, we have developed a biophysical model for the development of a file of cells in the wood-forming tissue. The model includes cell division and enlargement processes through an explicit kinematics (in large deformations) of the growth of a cell file, and mechanisms of signal transport : diffusion in the apoplasm (for TDIF) and polar transport (for auxin). The signals are assumed to provide a positional information to the cells and determine the expansion rate of each cell (Fig. 2) so that the growth kinematics and the signal transport are fully coupled . Simulations were run using the forward Euler numerical scheme.

Results

We could demonstrate that both diffusive and polar transport can establish and maintain a concentration gradient during the expansion and development of the tissue, provided that some conditions are met. This gradient is able to control cell proliferation and the radial growth of the xylem. The morphogenetic-gradient hypothesis also accounts for the sizes of the developmental zones through the growing season. Nevertheless, it also proved out to be insufficient to reproduce the anatomical structure of the wood formed. Understanding the cell size pattern of wood remains thus a challenge for biophysics.

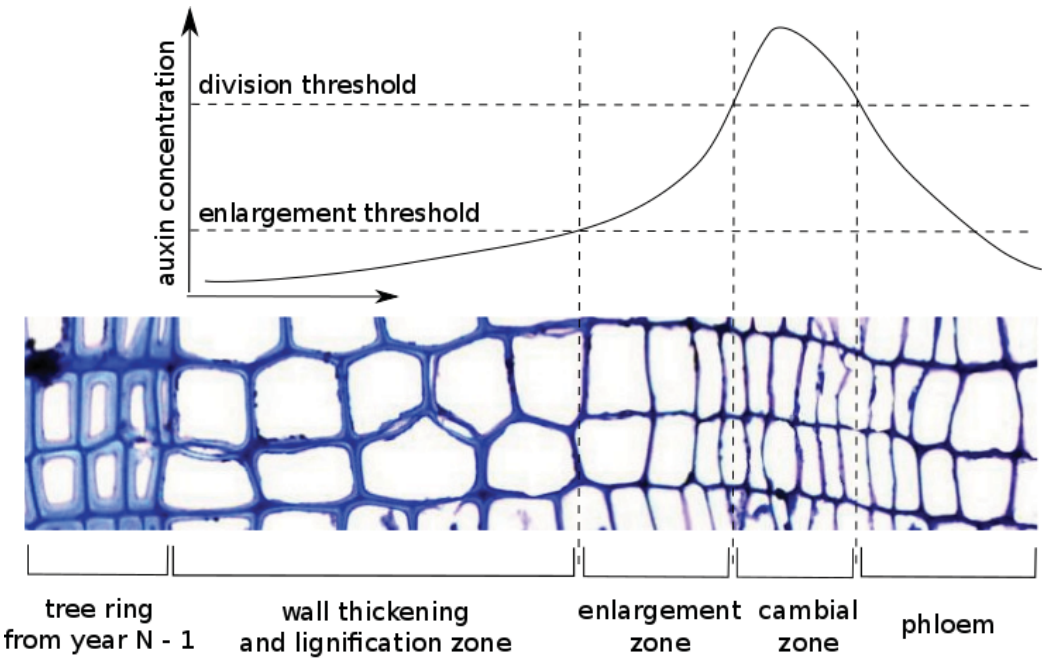

Fig. 1 : Developmental zones in the wood-forming tissue. Auxin concentration measurements across the tissue revealed a concentration gradient. It has been hypothesized that this gradient could guide cell differentiation via concentration thresholds.

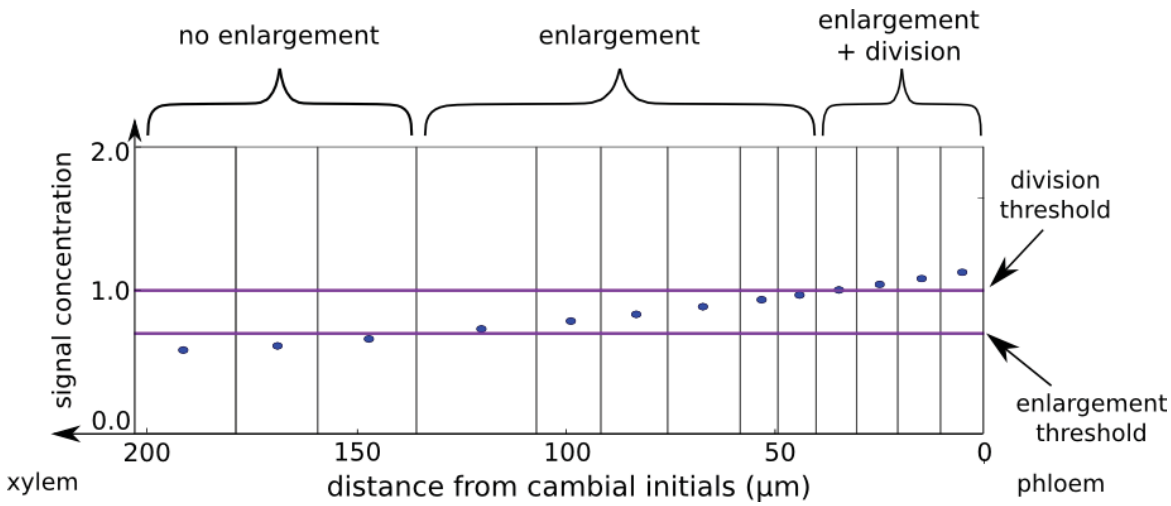

Fig. 2 : Schematic of the model. Tbe signal enters the file from the phloem side. A cell takes an identity (enlargement + division / enlargement only / no enlargement) depending on the local concentration of signal (blue dots) relatively to thresholds. An enlarging cell has an enlargement rate proportional to the signal concentration. When a cell with a division identity reaches twice its initial width, it divides.

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Cell wall basis of rhythmical growth changes in Arabidopsis hypocotyls

Ivakov A.[1], Flis A.[2], Apelt F.[2], Fünfgeld M.[2], Kragler F.[2], Scherer U.[2], Stitt M.[2], Vissenberg K.[3], Persson S.[1], Suslov D.[3,4]

[1]ARC Centre of excellence in plant cell walls, The University of Melbourne, Australia
[2]Max Planck Institute of Molecular Plant Physiology, Potsdam, Germany
[3]Plant growth and development group, The University of Antwerp, Belgium
[4]Department of plant physiology and biochemistry, St. Petersburg State University, Russia
suslov.dmitry75@gmail.com

Introduction

Plant growth involves two interrelated processes: the biosynthesis of biomass and the irreversible increase in volume. The first process is primarily defined by photosynthetic carbon assimilation, while the second one is driven by osmotic water uptake into cells and is controlled by cell wall extensibility. We have studied the regulation of these two processes in the course of rhythmical growth rate changes in *Arabidopsis thaliana* hypocotyls when grown in diurnal cycles. The diurnal variation in growth is known to depend on the interplay between light signaling, endogenous circadian clock, as well as metabolic inputs.

Materials and Methods

Arabidopsis plants were grown in 4 h light/20 h darkness cycles. The rate of hypocotyl elongation was continuously followed by infrared cameras. Cell wall biomass accumulation in hypocotyls was estimated from synthesis of cellulose, which is the most abundant component of plant dry matter. Cellulose synthesis was approximated by the speed of fluorescently-labelled CesA complexes movement in the plasma membrane and the number of complexes per unit cell surface area. Cell wall extensibility was estimated using frozen/thawed hypocotyls extended by the constant load (creep) method. Cell walls were stretched under 1.2, 1.6 and 2.0 g loads to mimic the action of different turgor on their extension. Creep was measured at pH 6, pH 5 and pH 5 with heat inactivation. pH 5 activates expansins, i.e. cell wall proteins mediating the “acid growth” response. Expansins lose their activity at pH 6, which is, however, optimal for the action of different enzymes (XTH and others) implicated in cell wall extensibility regulation. Heat inactivation was used to eliminate essentially all cell wall enzyme activities.

Results

Experimental plants grown in 4 h light/20 h darkness cycles demonstrated prominent rhythms in hypocotyl elongation rates. Growth peaked at the end of the night (EN) followed by a several-fold growth decrease with a minimum observed at the end of the day (ED). When Arabidopsis plants were grown without sucrose, plasma membrane CesA particles were absent in epidermal cells of hypocotyls at the end of the night, began appearing in large numbers in the middle of the light period and became abundant at the end of the day. They disappeared from the plasmalemma during the dark period and accumulated in small cellulose-synthase-containing compartments (SmaCC’s) just beneath the plasma membrane. In a set of experiments, plants were placed in extended darkness without sucrose, fed with exogenous sucrose while in darkness and treated with a photosynthesis inhibitor (DCMU), as well as were grown in 4 h light/20 h darkness photoperiod in the constant presence of sucrose. We concluded that the rate of cellulose synthesis in growing cells is regulated through the trafficking of CesA particles between the plasma membrane and intracellular compartments and not via their motility in the plasma membrane. The availability of metabolisable sugars from photosynthesis or the growth medium is necessary and sufficient to trigger CesA

mobilization to the plasma membrane while light signaling or endogenous rhythms do not appear to be involved.
Cell wall extensibility of hypocotyls from plants grown on sucrose frozen at the peak of their growth (EN) was compared with that of hypocotyls taken at the moment when their growth rate was minimal (ED). The extensibility of EN hypocotyls was 1.7-2.7-fold higher than that of ED hypocotyls at pH 5, the effect was only observed under higher loads (1.6 and 2.0 g). A comparable difference between EN and ED was seen at pH 6, but it was significant under lower loads (1.2 and 1.6 g). Heat inactivation completely eliminated differences in extensibility between EN and ED. Thus, cell wall extensibility changes do explain the diurnal variation of growth rate in Arabidopsis hypocotyls. These changes are mediated by at least two groups of cell wall proteins: the ones active at pH 5 and high loads, and others active at pH 6 and low loads. In a different experiment Arabidopsis plants were grown in 4 h light/20 h darkness cycles for four days followed by extended light for two more days. Even under continuous light, growth rhythms were observed every 24 h confirming their regulation by the circadian clock. The peak of growth was, however, shifted forward taking place at the end of the "day" ("ED") decreasing to the minimum at the start of the "day" ("SD"). Cell wall extensibility mirrored these growth rate changes being 2.2-2.7-fold higher in "ED" versus "SD" at pH 5 under higher loads (1.6 and 2.0 g). No differences between "ED" and "SD" were observed at pH 6 or pH 5 with heat inactivation. Thus only proteins active at pH 5 seem to be regulated by the circadian clock. Interestingly, mutant *elf3* plants with a defect in the circadian clock grew at a very high, nearly-constant rate in continuous light and demonstrated no differences in extensibility between "ED" and "SD" hypocotyls at pH 5, confirming that the circadian clock directly controls the fluctuations in wall mechanics in continuous light.

Conclusion

We have found that large differences in hypocotyl growth rates during diurnal cycles can occur under relatively constant rates of biomass synthesis. The rate of biomass accumulation is critically dependent on the availability of metabolisable carbon while not being directly affected by light signalling and the circadian clock. Rhythmic diurnal growth rate changes are controlled by proteins modulating cell wall extensibility. Some of these proteins were shown to be regulated by the circadian clock, while others appear to depend on light or metabolic signals.

Funding

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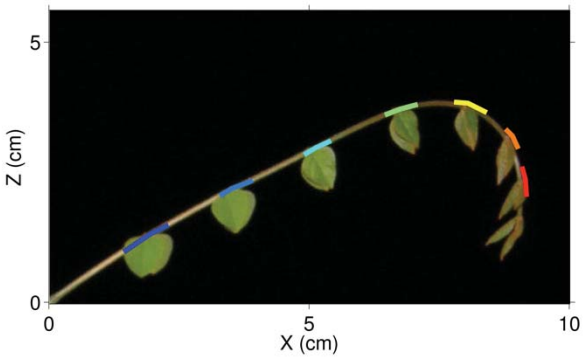
Interplay between Nutation and Leaf Development with or without gravity

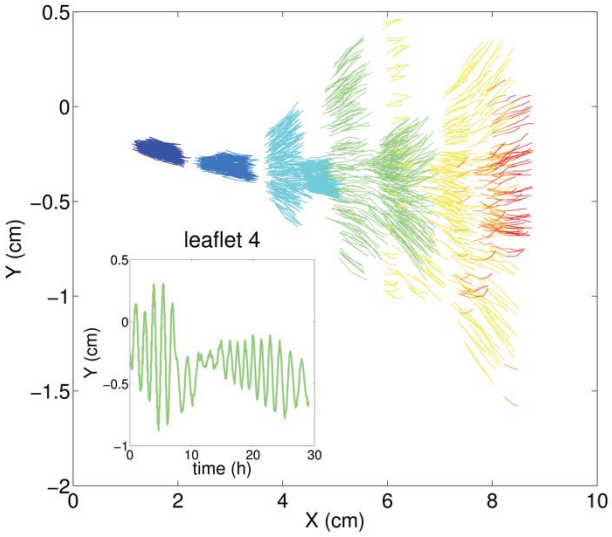
Julien Derr[1], Léa Laffond[1], Mathieu Rivière[1], Alexis Peaucelle[2], Stéphane Douady[1]

[1]Laboratoire Matière et Systèmes Complexes, Université Paris Diderot, France
[2]Institut National de la Recherche en Agronomie, INRA, France
julien.derr@univ-paris-diderot.fr

Introduction

Nutation is a very general phenomenum observed in plant which is characterized by an oscillatory bending movement of the plant during growth. It is known since Darwin that nutation must necessarily be linked to the growth mechanism of the plant[1]. More recently, a lot of debate took place to discuss the link between nutation and gravitropism[2,3]. Here we investigate the interplay between nutation and plant morphogenesis with or without gravity, in the case of the development of a typical compound leave.





Experiment

We focused on the case of Averrhoa Carambola. Its leaf is mostly coplanar ([X,Z] plane of the top photo) and is represented by a curve which unrolls until reaching a perfectly rectilinear shape. One can observe the leaf from the top view in the [X,Y] plane along time, by associating a different color to each leaflet (see the bottom figure). Nutation is characterized by this oscillatory bending in the Y direction happening on a given spatial zone, the "nutation zone". The inset shows typical time evolution of the Y deflection for one particular leaflet. We will discuss the oscillatory pattern as well as interesting beats observed, and how they are associated to discrete jumps in the "nutation zone" location. We'll also discuss the same kind of experiments performed in pseudo-zero gravity, and quantify their differences.

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Stress distribution in the cell wall – why does the innermost wall layer buckle after stress removal?

Dorota Kwiatkowska, Marcin Lipowczan, Dorota Borowska-Wykręt, Sandra Natonik

Department of Biophysics and Plants Morphogenesis, University of Silesia, Katowice, Poland
dorota.kwiatkowska@us.edu.pl

Introduction

In plants, mechanical stress at cellular and organ levels is transmitted mainly by cell walls. In living plant cells in the turgid state, walls are under tensile stress. The stress is either directly or indirectly turgor-driven. The first case works like stress in walls of a pressurized vessel. The second case are structural tissue stresses that appear because tissues building inner and outer portions of an organ differ in cell size and cell wall mechanical properties. Because the tissue stresses add to the directly turgor-driven stress, cell wall stresses *in situ* can be different from those expected in isolated cells. For example, in cylindrical organs, the maximal tensile stress *in situ* is in the longitudinal direction while in isolated cylindrical cells it is transverse.

Cell walls can be released from the turgor-driven stress by plasmolysis, while tissue stresses exist only *in situ* and disappear when the tissue is isolated. In consequence of stress removal the pattern of transverse bands appears on the inner surface of the outer periclinal cell wall of sunflower (*Helianthus annuus*) hypocotyl epidermis (Fig. 1). The striation represents the pattern of transverse folds on the inner surface of the wall, facing the protoplast. Hejnowicz and Borowska-Wykręt (2005) have postulated that the folds result from buckling of the inner wall layer under longitudinal compressive force exerted on this layer by the outer layer, when it shrinks elastically after abolition of the stress. We provide new evidence in support of this postulate.

Results and discussion

In theory, two scenarios can explain buckling of one of the wall layers: (1) initial tensile stress of the buckling layer is lower than that of remaining layers; (2) the stiffness of the buckling layer is higher than that of the others. In order to test which of these two scenarios of buckling is more probable in the case of growing cell wall, we created a computer model mimicking the deformation of cell wall layers after the tensile stress removal (Fig. 2). Using available realistic mechanical parameters as model assumptions we compare its outputs with measurement results and literature data for the real walls of growing epidermis of sunflower hypocotyl.

Following conclusions can be drawn from our model: A gradient of mechanical properties across the cell wall thickness is necessary to explain buckling of the inner cell wall layer after stress removal. Both the considered scenarios described above result in similar buckling pattern of the inner cell wall layer. However, we postulate that the first scenario is more probable. In growing cells in which new layers of the primary cell wall are added on the existing ones, the mechanical parameter in which the layers most likely differ is the initial stress in the cell wall layer plane. The tensile stress increases with the layer age up to a certain maximal value, i.e. the further the layer is from the protoplast, the higher its tensile stress. Support for this scenario comes from two observations: the recently formed cell wall layer cannot be under tension; if the second scenario were correct, the youngest layer would have to be the stiffest.

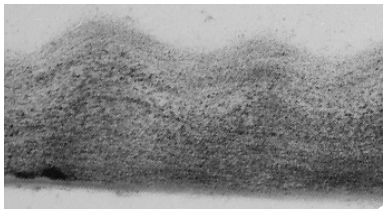

Fig. 1 : TEM image of transverse section of the sunflower epidermis wall after stress removal (upper surface faces protoplast)

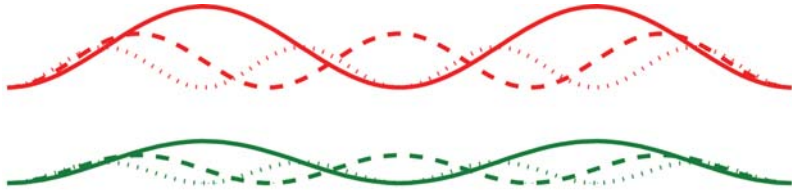

Fig. 2 : Estimated cell wall layer shapes: the shapes and deformations of wall layers are computed for assumed stiffnesses and initial stresses. We choose the layer shapes based on the minimal energy of the system, and compare them with empirical observations.

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Tooth wear of mammals and plant foods

Peter W. Lucas[1], Adam van Casteren[2], Amanda G. Henry[2], Khaled Al-Fadhalah[3], Abdulwahab S. Almusallam[3], Shaji Michael[3], Lidia A. Thai[3], Ali Shekeban[3], Jörg Watzke[2], Swapna Philip[1], Barth W. Wright[4], David S. Strait[5], Anthony G. Atkins[6]

[1]Faculty of Dentistry, Kuwait University, Kuwait
[2]Max Planck Institute for Evolutionary Anthropology, Leipzig, Germany
[3]College of Engineering and Petroleum, Kuwait University, Kuwait
[4]Department of Anatomy, Kansas City University of Medicine and Biosciences, USA
[5]Department of Anthropology, University at Albany, New York, USA
[6]School of Construction Management and Engineering, University of Reading, UK
peterwlucas@gmail.com

Introduction

Tooth wear is the fine-scale damage to dental enamel that threatens the life expectancy of herbivorous mammals. There must be strong selection pressure to avoid such tissue loss. Mechanisms that limit wear are probably both physiological (wear agent detection in the mouth) and anatomical (evolutionary increase in tooth crown height makes teeth last longer) in type. However, what exactly are the wear mechanisms themselves? This is an important issue for feeding contacts in any plant animal interaction, but the implications go further into paleontological contexts. A substantial literature promotes the idea that plant foods cause characteristic patterns of tooth wear from which the diets of fossils can be deduced. One of the most common statements is that tooth enamel is worn by seeds (Ungar and Sponheimer 2011), something very relevant to models of the evolution of human diet (Smith et al. 2015). Experimental studies that could establish the mechanisms involved in mammalian dental wear have not been very numerous. Thus we undertook a large-scale study with this specific aim, while offering it as a model study that could also be applied to invertebrate feeding. We focused on the most likely wear candidates: seed shells (densest plant tissue), silica phytoliths, silica (quartz) grit, all against enamel, and finally tooth enamel against itself. Rather than adopting traditional wear testing where the rate of tissue loss between surfaces is assessed after arbitrary intervals of time involving repeated contacts, we concentrated on the characteristics of individual wear events where single pieces of tissue were slid against an enamel surface at known forces. This allows damage to both the potential wear object and enamel to be assessed. The study is strengthened by recent theoretical advances in wear showing how the relative hardness of the surfaces, their toughness and the geometry of contacts play all a role in determining potential tissue loss (Atkins and Liu 2007).

Methods, Results and Discussion

The mechanical properties of 12 'seed' coverings (from monocots and dicots), phytoliths from three species of grass, grit from a Kuwait landscape and tooth enamel were measured by nanoindentation. Individual particles were then mounted on flat-ended nanoindenter tips and slid against enamel in a nanoindentation system (Fig. 1). Abrasive contacts (where tissue was lost via fracture) were seen between enamel and quartz and also between enamel and seed shell. Confirmation of tissue loss/exchange in these contacts involved employing a range of techniques depending whether there was transfer of a characteristic chemical element from one surface to the other (as with calcium in Fig. 1) or not, when detection by mechanical criteria was employed (Fig. 2).

Key results were that grit abraded enamel when grit particles were sufficiently sharp. In contrast, enamel appeared to abrade seed shell. The contacts that were made between phytoliths and enamel, and also when enamel was slid against itself, did not involve abrasion, but could be classified instead as rubbing. In rubbing contacts, both surfaces are deformed. This produces smooth contact surfaces that are permanently altered, but neither have incurred tissue loss (i.e, there is no wear).

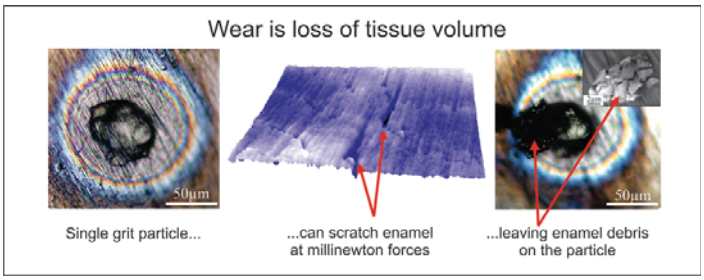

Fig. 1 : Method to gauge the effect of a potential wear agent on dental enamel. A particle (e.g. quartz grit - **left**) attached to the flat end of a nanoindenter probe is slid against an enamel surface at measured force. To prove damage, the enamel was scanned using AFM (**middle**), and the grit particle using SEM (**right**).

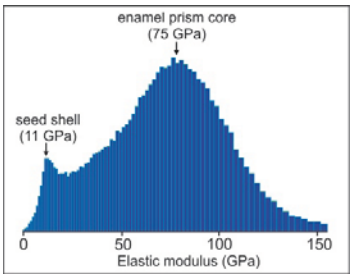

Fig. 2 : Quantitative AFM of enamel surface with adhering fragments from a seed shell contact. Fragments show the characteristic elastic modulus of the shell superimposed on enamel.

The implications of these studies seem broad. Rubbing contacts involve far slower rates of tissue loss than abrasive contacts because multiple contacts need to be involved before fragments eventually separate. Thus, unless grit is very rare, it must constitute the major threat to tooth tissue, supporting the contentions of both Sanson et al. (2007) and Damuth and Janis (2011). Seeds are unlikely to cause much wear to tooth enamel and thus should not be dismissed as part of hominin diets on the basis of wear surveys that are unsupported by experiment. Also supported is the hypothesis that, compared to quartz, silica phytoliths seem relatively harmless (Sanson et al. 2007). There are some grounds for supposing that phytoliths might function as mimics of 'grit', fooling a mammal into believing that it is ingesting gritty food that could wear its teeth badly, when actually it is not (Lucas et al. 2014). These contentions will be addressed in the talk.

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The mechanics of invasive cellular growth

Amir Sanati Nezhad[1], Mahmood Ghanbari[1], Carlos G. Agudelo[1], Muthukumaran Packirisamy[1], Anja Geitmann[2]

[1]Mechanical Engineering Department, Concordia University, Montreal, Québec, Canada
[2]Institut de recherche en biologie végétale, University of Montreal, Montreal, Québec, Canada
anja.geitmann@umontreal.ca

Introduction

Invasive behavior is the hallmark of a variety of cell types of animal, plant and fungal origin. To move against or invade a matrix requires the ability to mechanically invade this substrate. Cells with invasive or intrusive behavior thus have the common challenge to overcome the resistance of a substrate in order to accomplish their tasks. The strategies employed for this purpose differ significantly between walled cells and cells devoid of this stiff outer envelope. In cells devoid of a rigid cell wall a cytoskeleton mediated mechanism generates pushing and traction forces that are employed for cellular growth and invasion. Walled cells on the other hand are presumed to rely primarily on the force exerted by the cellular turgor pressure. This hydrostatic pressure can act onto an outside matrix if and when the cell wall yields. The spatio-temporally controlled yielding of the cell wall has therefore been postulated to regulate both the magnitude of the invasive force exerted and its direction, however evidence for this hypothesis is elusive.

Invasive growth in pollen tubes

One of the most rapid and highly targeted invasive activities is performed by the pollen tube, the delivery tool of the sperm cells in the flowering plants. This tubular extension is formed by a pollen grain upon contact with the stigma of a receptive flower. It can grow several hundreds of micrometers per minute and can become tens of centimeters long. It has to invade multiple tissues of the flower pistil to reach its target, the female gametophyte. To accomplish this task the pollen tube must not only exert invasive forces but also perceive the geometry and mechanical consistency of the growth substrate and follow directional cues emitted by the pistil and the female gametophyte. To investigate the directional growth behavior, the roles of chemical, mechanical and electrical guidance cues, and the ability to cope with mechanical obstacles we devised the TipChip, a microfluidic experimental platform that allows us to assess these parameters in quantitative manner (Fig. 1). Pollen grains are trapped individually within the microfluidic network, and the germinating pollen tubes are guided into microchannels in which they are exposed to test assays such as calibrated micro-cantilevers, highly localized electrical fields, or micron-sharp chemical gradients.

Quantification of invasive growth

Using two independent approaches we quantified the invasive and dilating forces exerted by growing pollen tubes. The former is the force exerted in growth direction, the latter maintains the tubular shape of the tube against compressive forces - a feature that is crucial to ascertain the delivery of the sperm cells through the tube. Both forces were found to be in the picoNewton range and the pressure that is necessary to generate them is in the hundreds of kiloPascals. This confirms that the tubes employ the internal turgor pressure to generate these forces. Interestingly, when exposed to mechanical obstacles, the tubes were able to modulate their invasive force and our observations suggest that they do so by regulating the cell wall mechanical properties.

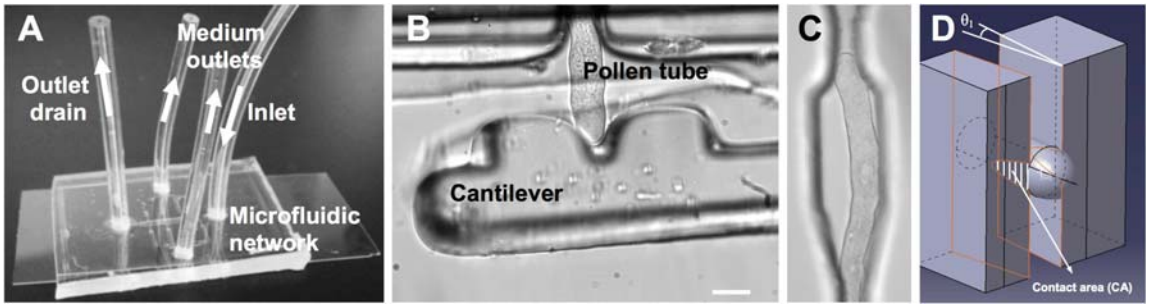

Fig. 1. TipChip - an experimental platform to quantify the invasive and tropic behavior of tip growing cells. **A.** Overview of the microfluidic platform. **B.** Quantification of the invasive force of a pollen tube through the deformation of a calibrated cantilever. **C.** Quantification of the dilating force of the pollen tube in a micro-gap setup. **D.** Finite element model used to calculate the dilating force from the interaction between the pollen tube and the elastic PDMS material of the micro-gaps setup.

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This abstract is based on data and text previously published in the following papers:

Agudelo C.G., Sanati Nezhad A., Ghanbari M., Packirisamy M., Geitmann A. (2015) Navigating a maze - Sensing and responding to mechanical obstacles during cellular invasive growth. Proceedings of the 59th Meeting of the Biophysical Society, February 7-11, Baltimore, Maryland

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Inference of Material Properties from Guard Cell Dynamics

Hugh C. Woolfenden[1], Michaela Kopischke[2], Silke Robatzek[2], Richard J. Morris[1]

[1]John Innes Centre, Norwich Research Park, Norwich, UK
[2]The Sainsbury Laboratory, Norwich Research Park, Norwich, UK
hugh.woolfenden@jic.ac.uk

Introduction

Stomata are microscopic pores on the surface of plant leaves through which gas exchange and water transpiration is performed. The pore is surrounded by a pair of highly specialised cells, called guard cells, that respond to abiotic and biotic stimuli in order to regulate the pore size and adapt to the environment. Guard cells translate these signals into a dynamic, reversible biomechanical output by changing the turgor pressure inside the guard cell pair. However, knowledge of (i) the material properties of the guard cell wall, (ii) the mechanical stress distribution, and (iii) how the guard cells stretch (during stomatal opening) beyond the plasma membrane elastically, remains limited. To address these questions, we have developed a three-dimensional elasto-mechanical model of a stoma using solid mechanics and the finite-element method.

Results

Our biomechanical model shows that strong and circumferentially oriented fibres are critical for correct stomatal function. Additionally, we can infer the material properties of both the cell wall matrix and the embedded fibre network by matching experimental results. The model fully describes the spatial distribution of mechanical stress for a given turgor pressure, thereby identifying stress 'hotspots' located towards the tips of the guard cells (coloured red in Fig. 1 and Fig. 2). By perturbing the model's parameters we can assess the physical effect on a stoma and predict whether the change contributes positively/negatively to pore size regulation. Ultimately we will generate a complete picture of the biomechanics that govern stomatal movements. This is an important step towards coupling biomechanics to the signalling networks, which is critical in understanding how plants respond to their environment.

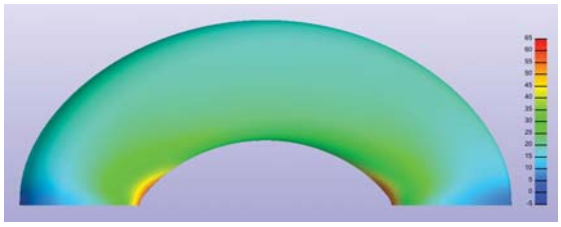

Fig. 1: Mechanical stress on outside surface of an open guard cell.

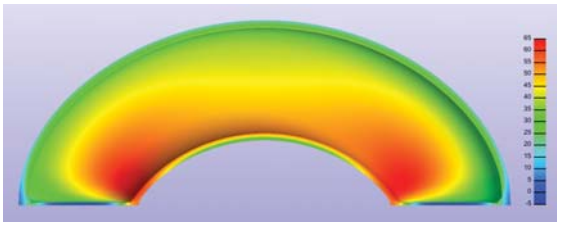

Fig. 2: Mechanical stress inside the open guard cell.

Micromechanics of oil palm fibres with silica bodies

Suhaiza Hanim Hanipah [1,2], Farah Nadia Omar [1], Loo Yu Xiang [1], Mohd Afandi P Mohammed [1*], Azhari Samsu Baharuddin [1], Jaafar Abdullah [3]

[1]Department of Process and Food Engineering, Faculty of Engineering, Universiti Putra Malaysia, 43400 UPM, Serdang, Selangor, Malaysia
[2]Faculty of Chemical Engineering, Universiti Teknologi MARA, 40450 Shah Alam, Selangor, Malaysia.
[3]Centre of Computed Tomography and Industrial Imaging, Malaysia Nuclear Agency, 43000 Bangi, Selangor, Malaysia
*afandi@upm.edu.my

Abstract
The surface of oil palm fibres contains embedded silica bodies or protrusions. Combined with the cellular structures within these lignocellulosic fibres make the mechanical characterisation of these fibres challenging. Here we reported a representative micromechanics model of an oil palm fibre which considers silica bodies on the surface and cellular structures within the cross section. This is made possible through X-ray micro-tomography analysis on a single fibre, which information was then imported to a finite element software, Abaqus. Initial investigation through a simplified representative finite element model showed that the effect of silica bodies towards the elasticity of an oil palm fibre was not significant (Omar et al. 2014). However, Currie and Perry (2007) discussed that in natural environment, silica bodies act as physical barrier (natural defence) to enzymatic degradation, fungal pathogens and outside heat. Therefore in the model proposed here, we simulated different scenarios of silica bodies on the fibre surface: 1) model with silica bodies (natural), 2) model after silica bodies were removed (after alkali pre-treatment), and 3) model without silica bodies. This study would be beneficial for bio-composites and bio-conversion (e.g. bio-compost) applications.

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Surface growth quantification of puzzle-shaped epidermal cells with subcellular resolution

Marcin Lipowczan, Joanna Elsner, Dorota Kwiatkowska

Department of Biophysics and Morphogenesis of Plants, University of Silesia in Katowice, Poland
marcin.lipowczan@us.edu.pl

Introduction

Striking feature of pavement epidermal cells in leaves of numerous plant species is their jigsaw puzzle shape. Although initially outlines of these cells are small isodiametric polygons, their shape and size change profoundly during the leaf expansion. The shape changes involve formation of invaginations, i.e. wavy anticlinal walls (Elsner et al., 2012; Szymanski, 2014), but also increase in complexity of an overall cell outline. In particular, the number of cell vertices (three-way anticlinal wall junctions) increases often several fold, and a cell outline defined by line segments joining the cell vertices often becomes convex in some places while concave in others. Since local growth distribution leading to such complex shape has not been recognized yet we aim at quantification of growth of an expanding *Arabidopsis thaliana* leaf epidermis at subcellular resolution using novel computation approach.

Material and methods

In order to follow formation of anticlinal wall invaginations and complex cell outlines, one needs more landmarks than only cell vertices. Therefore, we base our computations on sequential images of anticlinal walls of *A. thaliana* transgenic line *Lti6bGFP* with fluorescently labeled plasma membrane, supplemented by yellow-green fluorescent latex beads (Magsphere 0.3 µm) that adhere to cuticular waxes of outer periclinal cell walls (Fig.1). We obtain sequences of in vivo images with the aid of fluorescent microscopy, and use MATLAB codes for computations.

Results and Discussion

Using latex beads and cell vertices as landmarks we develop two approaches to characterize growth of the cell surface: (i) areal growth rate and anisotropy are computed for polygons defined by sets of landmarks; (ii) linear growth rates are computed for grooves formed above anticlinal cell walls.

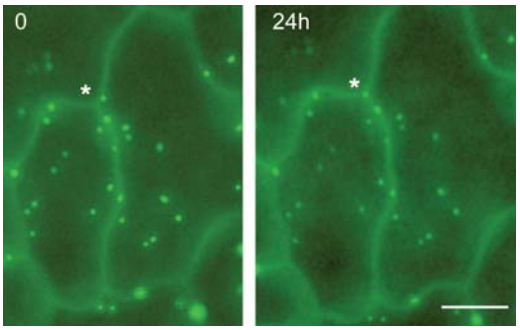

Fig. 1 : Two consecutive images of the same cells labelled with latex beads. Asterisk points to a bead that was not properly attached; others adhered strong enough to the

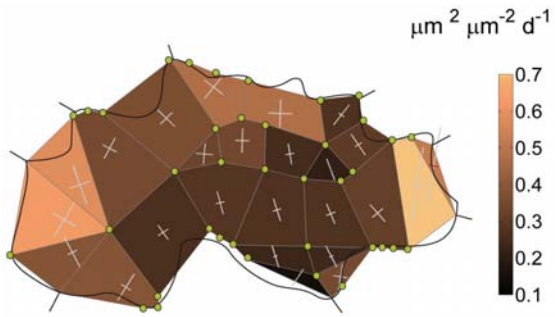

Fig. 2 : The areal growth rate colormap for regions (polygons) of an individual cell defined by latex beads and vertices. Crosses representing principal growth directions are

cell surface. Bar = 10 μm

overlaid on the colormap (red cross arm – shrinking).

Computations show that both growth of the outer periclinal wall of individual cells (Fig.2) and growth along the anticlinal walls are heterogeneous. Over this local heterogeneity the overall pattern of principal growth directions is superimposed, which is characteristic to the given leaf region (Remmler and Rolland-Lagan, 2012). We will discuss these observations with respect to existing hypotheses regarding puzzle-shaped cells morphogenesis (Szymanski, 2014).

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Morphogenetic motions: a tool to study growth in leaves

Mathieu Rivière[1], Julien Derr[1], Alexis Peaucelle[1,2], Stéphane Douady[1]

[1]Laboratoire Matière et Systèmes Complexes, Univ. Paris Diderot, France
[2]Institut Jean-Pierre Bourgin, INRA – AgroParisTech, France
mathieu.riviere@univ-paris-diderot.fr

Introduction

Growth is the pillar for plants to adapt to their environment and to generate perfectly regulated shapes. For these reasons, it remains an exciting botanical puzzle.
Plant growth comes from a competition between the cell-wall elasticity and the inner turgor pressure of the cell. As a consequence, spatial variations of turgor pressure and/or mechanical properties at the cellular level result in growth heterogeneities (see Fig. 1). From these emerge the final organ shape and also, during the process, a large variety of *morphogenetic motions*. These macroscopic motions can be read as an outward signal of the cellular mechanisms underlying shape regulation and growth itself. Here, we take benefit from the intimate link between growth and its related motions to gain a new insight on growth through a multiscale approach, from the organ to the cell-wall level.
Many biophysical and chemical changes (Peaucelle et al., 2011) linked with growth have been unearthed on *Arabidopsis thaliana*. We plan to assess to what extent the results on *Arabidopsis thaliana* are generalizable to other plants with more environment-sensitive growth, and presenting more enhanced movements, such as the compound leaves of *Averrhoa carambola* (see Fig. 2).

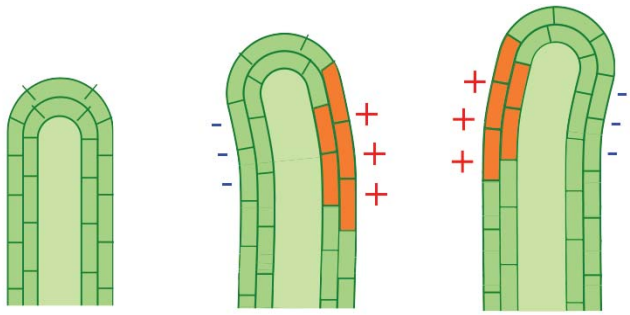

Fig. 1 : Differential growth leads to motions. (Mugnai et al., 2007))

Our approach in a nutshell

We assume that different states in the motions correspond to different growth status within the leaf's tissues. The first step is thus to characterize and link via quantitative measurements macroscopical growth and motions. To this end, we are currently developing a technique inspired from Bastien et al. (2013) combining time-lapse photography and Particle Image Velocimetry. Elongation is thus mapped along the leaf's rachis in time (see Fig. 3). Multiple cameras are used to decouple the actual growth from geometrical effects due to nutation (see Fig. 2b and Fig. 3). Camera calibration and 3D-geometry make it possible to go beyond these limitations. Morevover, by removing optical effects it allows us to perform precise growth measurements. The connections between growth and motions are then investigated through their relative amplitudes, spatial shifts, and time delays (Dornbusch et al., 2014).

Fig. 2 : Chronophotographies of *Averrhoa carambola*'s motions. (a) The typical unfurling motion of compound leaves (b) The widespread nutation motion.

In parallel we link the local growth status – discriminated by motions – to the biophysical parameters at the cell and tissue levels. Mechanics of the cell-wall is probed in a variety of situations thanks to Atomic Force Microscopy. We furthermore perform immunohistochemestry of the cell-wall. Ultimately, we plan to build a model in order to give full account on the emergence of morphogenetic motions in plants.

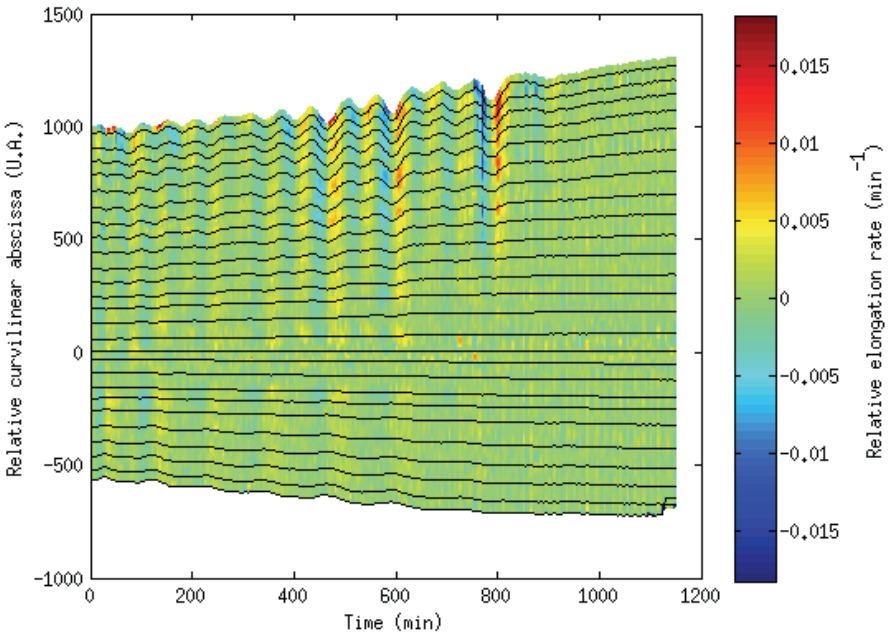

Fig. 3 : Elongation map of the rachis obtained with a single uncalibrated camera. Black lines represent the trajectories of a point of the rachis. Nutation is clearly visible ('contraction-elongation' oscillations). Even if we can observe a global elongation, nutation prevents precise growth measurements.

Acknowledgements
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Stress-Induced Changes in the Mechanical Properties of Leaves

Michal Sahaf[1], Eran Sharon[1]

[1]The Racah Institute of Physics, The Hebrew University of Jeruslaem, Israel

Introduction

Cell expansion, driven by turgor pressure, is the process through which the leaf grows in area by many orders of magnitude, after cell division had stopped. For a leaf to reach its normal shape there should either be no spatial fluctuations in the growth rate, or any such fluctuations should be counteracted before the leaf has been irreversibly deformed. It would make sense to assume, therefore, that stress build-up would serve to trigger a correcting response, which will manifest as a change in growth rate. That can happen if the mechanical properties of the leaf, which is a complex viscoelastic tissue, change as a result of mechanical stress or strain.

Methods

Most of the study of plant viscoelasticity was performed on dead tissue material [Kutschera 1996, Ryden 2003]. More recent studies such as [Hayot 2012] use nanoindentation to measure the properties of single cell walls in a living tissue. This study focuses on measurements of whole living leaves, using protocols of dynamic mechanical analysis (DMA) to measure the viscoelastic properties of the leaf.

We study the growth of young leaves of *Nicotiana Tabacum* under controlled stretching using a setup consisting of a stress-control system, and two cameras which collect 3D data for growth analysis. The images are then processed using PIV (particle imaging velocimetry) technique to measure the growth (strain tensor) with temporal resolution on the order of minutes, and spatial resolution on the order of tens of microns. Two types of measurements are performed: growth under constant, almost uniaxial stress, which allows for measurements of changes in growth patterns as a response to stress; and short-time periodical stress-strain measurements (DMA), which allows to obtain the viscoelastic properties of the leaf while causing minimal changes.

Results

We define an angle θ between the principal direction of the local growth tensor and the principal direction of the local applied stress tensor. On times of several minutes and up to ~1 hour we see a good alignment between the stress and strain, i.e., $\cos(\theta) \approx 1$ and the tissue yields to the stress. However, over several hours, we observe a decrease in this alignment, as can be seen in fig. 1, although the leaf is held under constant force.

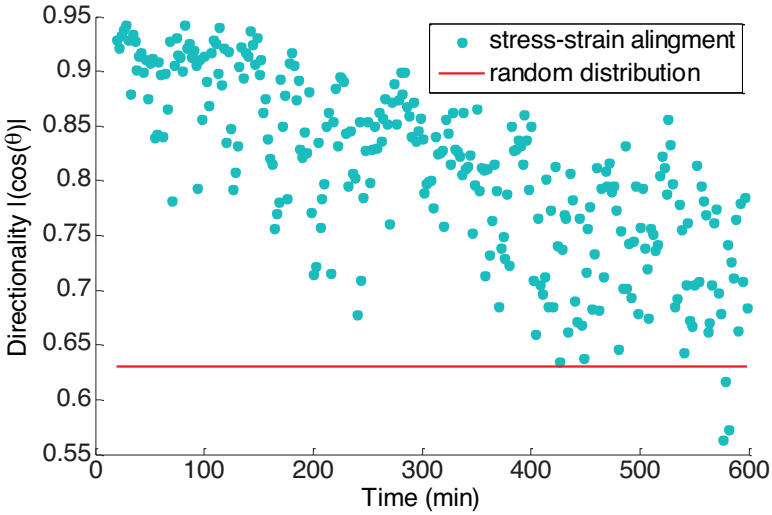

Fig 1: Stress-strain alignment in a leaf held under constant stress, over 10 hours.

When examining the visco-elastic properties of the leaf using oscillatory stress, we can observe the natural changes of its properties over time, and compare them to stress-induced changes. We find that while leaves naturally experience a decrease in Young's modulus over time, leaves subjected to mechanical stress exhibit an increase in Young's modulus in the direction of stress, and more a pronounced decrease in the direction perpendicular to the stress.

Discussion

We observe a process in which the effect of mechanical stress on growth decreases over time. This is accompanied by changes in the mechanical properties of the tissue. We notice different responses at different timescales: the initial response to mechanical stress is yielding, which usually would lead to the relaxation of stress; however, if stress is not relaxed and the leaf is subjected to it continuously, a change in its mechanical properties makes the leaf more resilient to strain. This can indicate a mechanism through which leaves can maintain their shape and resist deformation due to mechanical stress.

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Analysis of strain transfer from xylem to bark with use of DIC

Václav Sebera[1], Jiří Kunecký[2], Luděk Praus[1], Jan Tippner[1], David Ševčík[3], Petr Horáček[1], Štefan Surňák[1]

[1]Department of Wood Science, Mendel University in Brno, Czech Republic
[2]Institute of Theoretical and Applied Mechanics, Czech Academy of Science, Czech Republic
[3]Sobriety Ltd., Czech republic
vaclav.sebera@mendelu.cz

Introduction

Assessment of mechanical behavior of trees by means of advanced experimental techniques has been paid a big attention in recent years. One of the suitable non-destructive techniques to be employed is digital image correlation (NASA 2013, Sebera et al. 2014). This technique enables obtaining displacements and strains induced on trees when loaded. The goal of the study was to investigate strains and displacements induced on tree (*Corylus colurna* L.) surface when loaded by bending. Further, an influence of a bark on strain transfer from xylem to bark surface was studied. The study addresses issues of tree biomechanics that calls for a high quality deformation data measured on trees' surfaces in a non-invasive way. For these purposes, 3D form of DIC (3D-DIC) was used and tested against extensometers that are commonly used for such applications. Measured area of interest (AOI) on the tree was studied in both variants with bark and without.

Results

Full-field maps of vertical strains (ε_{yy}) obtained using 3D-DIC over the AOI are depicted in Fig. 1. Despite the fact that the strain maps of ε_{yy} are not as predicative as known for instance for isotropic or more homogeneous materials, they reveal a tension and compression parts at AOI. The latter is being located on left side (rather violet-to-blue shades) and the tension part is located on the right side (rather yellow-to-red shades) of the AOI. Furthermore, we see that strain fields are somewhat locally grouped and, especially on the barked variant, exhibit high variability. The coefficient of variation (CoV) for barked variant is for maximal compressive strains 62% and for maximal tensile strains 71%, meanwhile for debarked variant the CoV for maximal compressive strains is 37% and for maximal tensile strains 36%. Hence, the bark removal has a positive effect on measurement using DIC by decreasing variability of measured quantities and elimination of not correlated spots. However, to obtain data that are more interpretable, ie. overcome issues with the field discontinuities and their localness, and are more easily comparable to extensometers, the strain fields (ε_{yy}) were divided into 10 vertical bands that were averaged within a course of time.

The paired t-test carried out for strain fields (ε_{yy}) computed on barked and debarked AOI in course of force (rightmost red distribution) is showed in Fig. 2. It indicates the strain fields on barked and debarked AOI's differ less, especially at side bands where either high tension or compression strain occurs. This corresponds to a fact that the strains are highest in those bands (as results from Euler-Bernoulli bending theory) as well as from the fact that relative noise of the strain calculation is significantly lower here in respect to strain magnitudes. Each band of the AOI starts exhibiting p values below 0.05 at different loading level. Furthermore, the more the band at Fig. 2 is located towards the middle of AOI, the less the strains statistically differ. The latter is especially visible at compressive (left) side of the AOI where gradual increase of statistical difference occurs in first three bands from left.

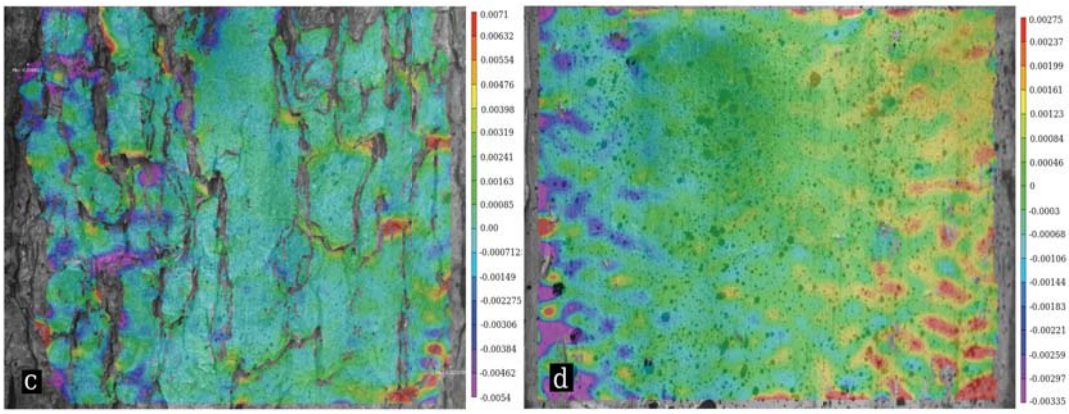
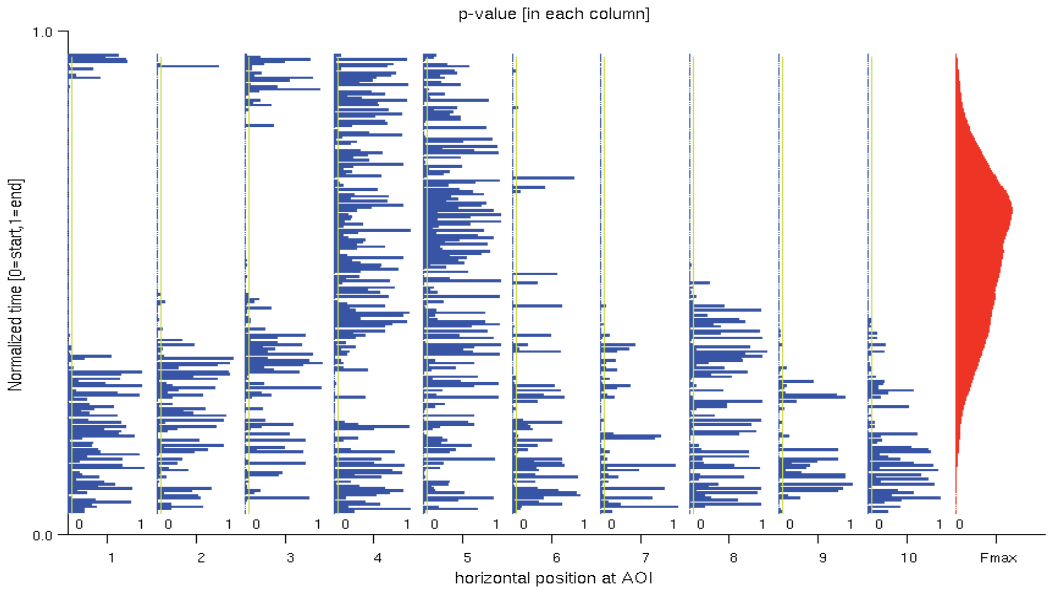


Fig. 1 : Vertical strain fields (ε_{yy}) at maximal load for barked (left) and debarked AOI (right).

Bands no. 4 and 5 reveal statistical difference during whole time of loading. The most left and most right bands achieve no statistical difference at certain load level, ie. 4.9 kN and 5.2 kN respectively. Based on this we can claim that it is possible to evaluate strains induced in the xylem based on values measured on a bark when such load level is reached.

Fig. 2 Paired t-test of ε_{yy} comparing debarked and barked AOI in 10 vertical bands (at α = 0.05). Length of the each bar denotes similarity, ie. the highest one has as zero bar length.



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Acknowledgement

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Division of labor in leaf functions among vein orders: an example of Fagaceae

Kiyosada Kawai [1], Naoki Okada[1]

[1]Graduate school agriculture, Kyoto University, Japan
email: k.kawaikx@gmail.com

Introduction

Leaf venation is a key trait determining leaf water relations, phloem transport and mechanical stability (Sack & Scoffoni 2013). However, since the contribution of leaf veins to mechanical properties has not been properly understood, our understanding of how leaf vein architecture adjusts these functions within leaves is limited. Here, we tested the hypotheses that leaf water use and mechanical properties would be more correlated with lower- and higher-order vein density (VLA), respectively.

Material and Methods

We studied 8 Fagaceae species growing in temperate forests in Japan. The species differed in secondary venation, leaf shape, leaf habits, size, and natural habitat. We quantified two mechanical properties [lamina tensile strength and tensile modulus of elasticity (E)] and two leaf water use traits [lamina hydraulic conductance (K_{lamina}) and carbon stable isotope ratio (δ^{13} C)], and nine traits relating to both vein architecture and leaf structure.

Results

Leaf mechanical properties and water relations varied significantly across 8 Fagaceae species. Primary and secondary vein density ($VLA_{1.2}$) correlated positively to lamina strength and leaf structural traits (leaf area, leaf thickness and leaf mass per area) but not to water use. In contrast, minor vein density (VLA_{min}) correlated to K_{lamina} positively but not to mechanical properties and leaf structural traits. The relationship between water use traits and mechanical properties as well as $VLA_{1.2}$ and VLA_{min}, were independent.

Discussions

Our results indicate that there is division of labor in leaf functions among higher- and lower-order veins, that $VLA_{1.2}$ influences leaf structural and mechanical properties, and that VLA_{min} affect leaf water use and, thus, potentially the gas exchange capacity. These findings suggest that vein hierarchy is associated with functional differentiation among vein orders to support leaf functions. Therefore, the evolutionary or accumulative shift in vein hierarchy might explain the variation in leaf structure and functions.

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Chemical treatment for the Degradation of the mechanical properties of miscanthus stems to facilitate fragmentation

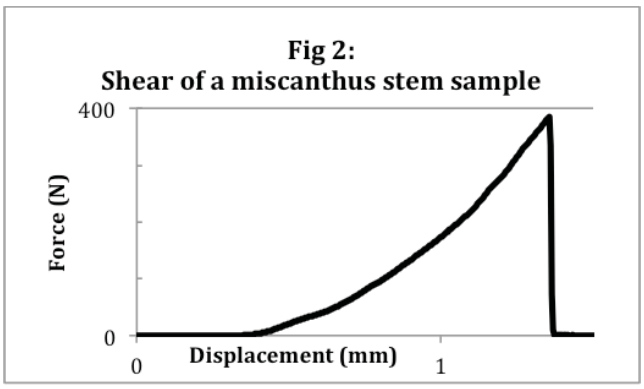
F. Mabille[1], A. Sadoudi[1], C. Barron[1]
mabille@supagro.inra.fr
[1] INRA, UMR IATE, Montpellier

Lignocellulosic plant biomass is a major source of renewable carbon polymers, but its use is hampered by its low biodegradability imposing energy-intensive pre-treatment. Degradability differences were reported between the different cell types of grasses and linked with heterogeneity of their composition, in particular their degree of lignification. In a biorefinery plant the pre-grinding step is an important part for any biotransformation. An histological deconstruction level, with dry fractionation processes, may be considered to optimize material flow to dedicated applications or transformations. The objective of this work is to propose a chemical pretreatment deconstruction resulting in a reduction of the mechanical proprieties of the grass stems and thus to a reduction of energy consumption for grinding.

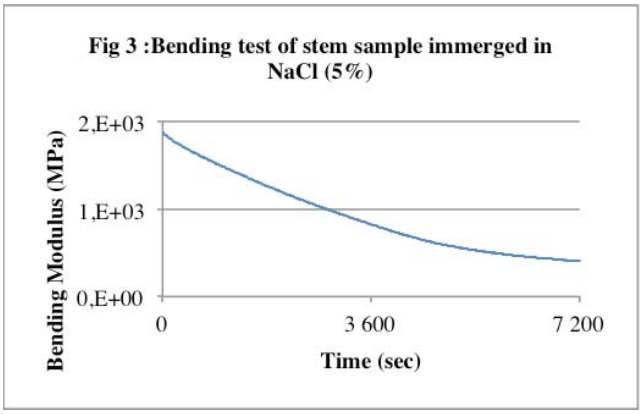
The mechanical properties before and after attack by chemical acid (H.Cl) or basic (Na.OH) pretreatment is performed in three steps. First, we carry on the end of a calibrated sample miscanthus stem, a shear test to measure resistance to breakage under a solicitation adapted to split this type of material. Secondly, the stem is subjected to dynamic bending under small deformation to measure the kinetics of deconstruction of the mechanical module. The loss of rigidity of the stem is thus measured for two hours in variously concentrated solutions. Finally the stem is dried at ambient temperature and humidity until it reaches its original water content and subjected to a shear test to quantify the loss of resistance to destructive solicitation akin to splitting solicitations.

Fig 1 : Shear device

The device (Fig 1) used for measuring the shear propriétées been developed in our laboratory. It measures the force required to shear a sample of stem or of straw under controlled speed (0.2 mm / s). The curve has three phases before rupture, an approach phase up to detection of contact, a compression phase more or less important depending on the nature and shape of the sample, and a shear phase until complete tearing.



The healthy part of the sample is then loaded in dynamic bending test at 1, 10 and 100 Hz for two hours immersed in a chemical solution more or less concentrated (0%, 1%, 2%, or 5%). Depending on the degree of concentration a significant deconstruction of the stem is observed with a decrease by a factor of ten of the module (Fig 3), which prolong if the test is continued. The sample is then rinsed and returned to the water content before immersion. Then, it undergoes again a shear test to quantify the decrease of the force required to the shear rupture. A significant change in the conformation of the bundles (Fig 4) could be observed with the chemical pretreatment.



This study shows the efficiency of a chemical pretreatment for reducing shear forces needed to break stems. These results lets presume a reduction in the energetic consummation during grinding, and will have to be confirme by technological tests on instrumented grinding tools.

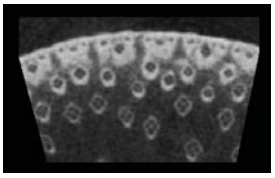


Fig 4 : Section of miscanthus sample

Influence of genetic pool on the stem microstructure and the lodging stability of flax

Christophe Baley, Alain Bourmaud

LIMATB, University of South Brittany, 56100 Lorient, France

Email: christophe.baley@univ-ubs.fr

The use of flax fibers as composite reinforcements is justified by the renewable character of these fibers as well as by their good specific mechanical properties [1]. Moreover, their mechanical performances are yearly reproducible, for a same variety [2] or for numerous batches [3]. In order to ensure a reliable culture of flax, the lodging stability of the plant could be considered as a key point. Thus, an important varietal selection work is performed in order to develop new varieties with high fibers yield and good disease or lodging resistance, to ensure a sufficient income for the farmer.

The lodging originates because of buckling of the stems due to abundant rain, strong winds or numerous visiting animals [4]; in case of severe lodging, the plants might be lying down on the ground. The lodging of flax stems is highly influenced by the stem stiffness, the environmental solicitations and especially the additional water weight. Consequently, the distribution of the water drops on the plant increases its mass and, with wind, the risk of instability becomes even more significant. Moreover, plants could react to environmental stimuli by different ways; this phenomenon, called thigmomorphogenesis, generally induces a decrease into the plant height conjugated to an increase of their diameter and ramifications [5]. Several parameters influence this stiffness: the diameter of the stem, the distribution and percentage of fibres in the section, the number of fibres, the thickening of the secondary cell walls (presence or not of one lumen), the stiffness and the diameter of the fibres.
In this paper, we studied different flax varieties in order to better understand the physiological or structural parameters having an influence on the plant stability.

In first time, we selected Marylin flax stem cultivated in the same geographical area over 4 successive years. Various parameters influencing the stem stiffness were analysed: the height and the diameter of the stems, their internal organization including the proportion of fiber bundles, the diameter and mechanical properties of elementary fibers. It was shown that the fiber yield and number of fibers increased with the height of the plants, the number of fibers being maximal at medium height. In addition, the fibers' diameter decreases with the height of the stem because of the growth mechanisms. Lastly, flexural tests on stems made it possible to correlate their stiffness with the Young modulus of the elementary fibers as well as their internal organization (Fig. 1).

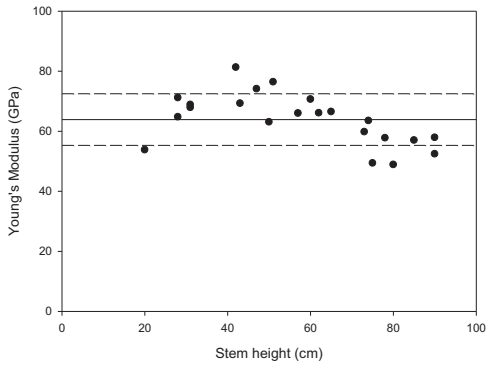


Fig. 1. Elementary fibers in Young's modulus estimated from the stem stiffness and its internal organization

In a second time, we studied two recent flax varieties (Eden and TDL25) with a distinct lodging resistance. The results of the mechanical characterization indicate that the Eden variety has a superiority concerning fiber stiffness. The analysis of the localized bending stiffness of a dried stem and of its fiber distribution in the corresponding cross section allowed us to estimate the fibers average modulus. Results obtained by this method were very close to the tensile tests values. The same procedure was used on green stems to approach the living state of the plant and to determine the Young's modulus of green fibers. The results highlighted a variation of fiber stiffness between the green state and the dried state (around +25%). The results enabled the use of a simplified buckling model, which confirmed the superiority of the Eden variety in lodging resistance. Thus, the analysis of the mechanical properties of flax stems and their structure could be a selection criterion.

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Strong or flexible: some ecological aspects of plant resistance to mechanical stress.

Niels P.R. Anten[1]; Sara Puijalon[2]; Frank Sterck[3]; Yusuke Onoda[4]
[1]Crop & Weed Ecology, Centre for Crop Systems Analysis, Wageningen University, P.O.Box 430 6700 AK Wageningen the Netherlands, niels.anten@wur.nl.
[2]Universite´ de Lyon, Lyon, F–69003; Universite´ Lyon 1, Villeurbanne, F–69622; ENTPE, Vaulx-en-Velin, F–69518; CNRS UMR 5023, F–69622 Villeurbanne, France.
[3]Forest Ecology and Management Group,Wageningen University, Droevendaalsesteeg 3, 6708 PB,Wageningen, The Netherlands.
[4]Department of Agriculture, Kyoto University, Kyoto 606-8502, Japan

Introduction

Classical ecological studies consider that leaf and stem characteristics have been designed to maximize the efficiency of light acquisition per unit of invested resource. Particularly in dense vegetation, plants should be designed to successfully compete for light with neighbours, through efficient conversion of resources to height increment and leaf production. But plants cannot violate the laws of physics and they must be designed to withstand the forces to which they are exposed and this imposes restrictions on the ability to effectively acquire light. This presentation deals with strategies that have evolved whereby plants meet these conflicting requirements. It first addresses the role of variation in wood and stem characteristics across tree species and how assessing their impact on tree biomechanics and on biomass per unit height may help understand ecological strategies in trees. Then, it moves to aquatic plants and addresses the strategies by which these plants deal with hydrodynamic forces, focusing on the tradeoff between being strong to resist or flexible to avoid them. Finally it compares the extent of the latter tradeoff between aquatic and terrestrial plants.

Main findings

Wood density variation in trees:

Wood density (WD) known to be both an important functional and a key determinant of forest carbon sequestration (Chave et al. 2009). The common thought has long been that low WD wood favors competition for light but that it comes at the expense of mechanical stability. However, by applying simple engineering theory to a broad dataset of tree mechanical traits, it was shown that trees with low WD wood can achieve the same height and mechanical stability (expressed in terms safety against global buckling and stem rupture by wind) as high WD trees but at a lower biomass investment. For branches however the opposite may hold: high WD might be more efficient in the sense of producing a branch of given length and resistance to rupture. Notably, high WD also entails a better ratio between flexibility and strength (Anten & Schieving 2010). High WD may also have benefits in terms of resistance to disease and drought tolerance.

Flexible or strong is there evidence for a avoidance tolerance tradeoff.

There are roughly two ways in which plants can deal with mechanical stress: either be strong and thus be able resist forces (tolerance) or be flexibility and thus avoid them (avoidance). A tradeoff may exist between the two strategies as strength is often associated with thick support structures made of rigid material. This hypothesis was first tested for aquatic plants, being an interesting model system as many of them are exposed to hydrodynamic forces that far exceed the forces typically experienced by terrestrial plants. For 28 aquatic species their ability to resist hydrodynamic forces (i.e., expressed as the maximum force that plants can resist without breaking) or avoid these forces (i.e. expressed as the inverse of drag force that plants experienced in a flow flume) was measured. A clear negative correlation was found between

the two, indicating that for this group plants a resistance-avoidance tradeoff with respect to mechanical stress indeed exists (Fig. 1a; Puijalon et al. 2011). Existence of this tradeoff may have important implications as it imposes constraints on the evolution of many morphological, anatomical and architectural traits.

Next, it was assessed whether this tradeoff also exists across woody terrestrial species. To this end mechanical traits of 39 woody species were measured. Drag force on selected shoots was measured by mounting them on a car and driving at constant speed and then measuring the force that the shoot exerted on a load cell, again low drag was interpreted as indicating avoidance. Crown reconfiguration was monitored by video imaging and streamlining was calculated. Safety was assessed as the ratio between the modulus of rupture and calculated bending stress from drag and was considered as a measure of resistance. Contrary to the results for aquatic plants no correlation was observed between resistance and avoidance (Fig. 1b). Species with thicker stems were more resistant to drag but did not experience larger drag forces. While crowns of such species were more rigid, as expected, and thus did not reconfigure easily they also exhibited more efficient streamlining. Thus, intriguingly the stress tolerance-avoidance tradeoff found for aquatic species did not appear to extend to terrestrial woody plants (Butler et al. 2012).

Why is there this apparent contrast between aquatic plants and woody terrestrial plants? This may have to do with the difference between water and air flow. In the experiment with the woody plants, it was observed that the species with more rigid shoots exhibited less flagging: an instability that may greatly increase drag. Flagging tends to increase with turbulence of the flow medium. The generally accepted way to determine the dynamic similarity between flow experiments under different conditions (such as water vs wind effects on plants) is to calculate the Reynolds numbers (Re), a dimensionless number that indicates the ratio of inertial to viscous forces. Flow tends to shift from laminar to turbulent with increasing Re values (Rott, 1990). It can be calculated that the Re values in the water flow experiment of Puijalon et al. (2011) were 5–10-fold smaller than during wind drag measurements of Butler et al. (2012). While drag forces exerted by water are typically much larger than those exerted by air, water is also denser, more vicious and generally moves slower than air. As such water tends to be less turbulent than air and the lower incidence of flagging may allow for flexible shoots to efficiently avoid drag. This strategy may be less feasible under air flow on land, where in addition, the greater effect of gravitational forces may already select for more rigid structures.

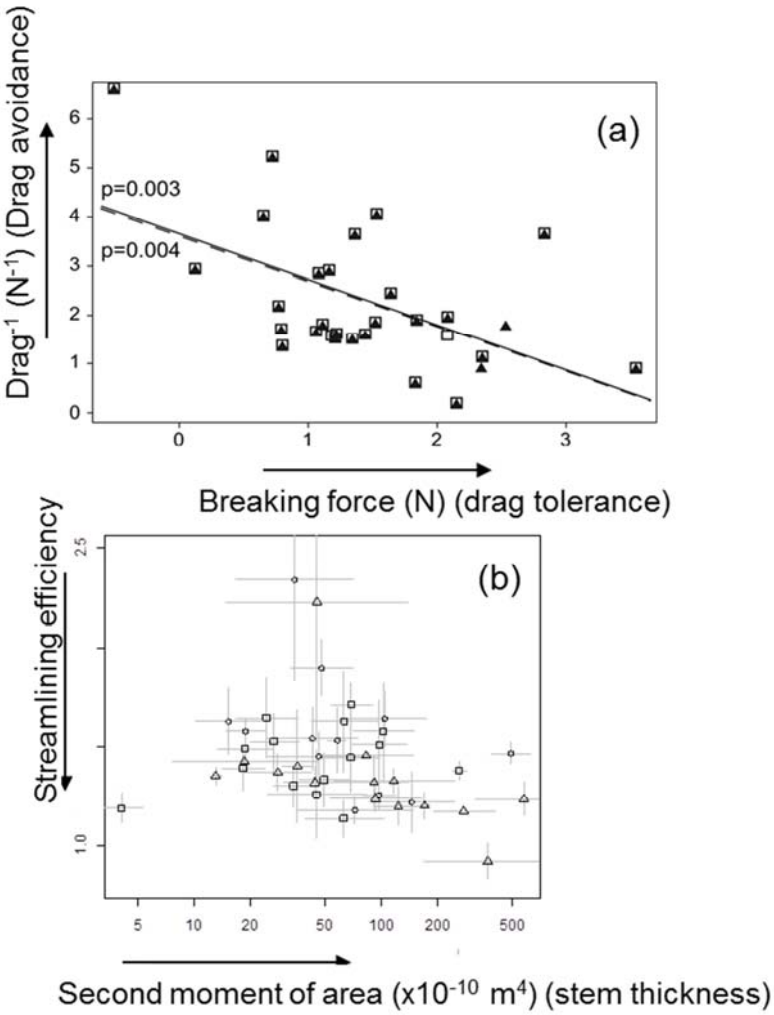

Fig. 1 (a) The relationship between drag tolerance (measured as the inverse of the drag force, $drag^{-1}$, experienced by plants under water flow at 0.9 m/s) and the maximum tensile force that plants can resist for 28 aquatic species (graph taken from Puijalon et al. 2011). (b) The relationship between streamlining efficacy (low values indicating a high degree streamlining) and the stem flexural stiffness (i.e., resistance to bending) (graph taken from Butler et al. 2012), with squares, circles and triangles indicating species from wet, dry and arid areas, respectively. Arrows indicate increasing values of tolerance, avoidance, stem thickness and streamlining efficiency. See also Anten & Sterck (2012).

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Modelling tree anchorage and estimating different contributing factors

Ming Yang[1,2], Pauline Défossez[1,2], Frédéric Danjon[3,4], Thierry Fourcaud[5]

[1] INRA, UMR 1391 ISPA, F-33140 Villenave d'Ornon, France
[2] Bordeaux Sciences Agro, UMR 1391 ISPA, F-33170 Gradignan, France
[3] INRA, UMR 1202 BIOGECO, F-33610 Cestas, France
[4] Université de Bordeaux, UMR 1202 BIOGECO, F-33610 Cestas, France
[5] CIRAD, UMR AMAP, Boulevard de la Lironde, F-34398 Montpellier Cedex 5, France
ming.yang@bordeaux.inra.fr

Introduction

Windstorms are the major hazard affecting European forests and causing timber losses. Tree uprooting is generally found to be the major failure mode for trees of large size during windstorms when soils are often saturated (Gardiner et al. (2010)). Understanding tree anchorage mechanism is essential for risk prediction of wind damage. This study investigates the tree anchorage failure as well as involved contributing factors with a mechanistic approach. Tree anchorage was modelled with the Finite Element Method. Effects of root architectural traits and material properties of soil and roots were evaluated through systematic sensitivity analysis. Different factors involved in tree anchorage have been classified with respect to their impacts on tree anchorage strength.

Materials and Methods

The anchorage model was developed in ABAQUS 6.13 (Yang et al. (2014)). The wind action on the tree is modelled by a horizontal displacement (1.2 m) applied on the top of a rigid stem (1.6 m in length). The stem is attached to the top of the root system in the soil (length X width X depth: 10 m X 10 m X 5 m) and drives the underground overturning. At the centre of the soil block corresponding to the root pattern location, a parallelepiped area (length X width X depth: 6 m X 6 m X 1.25 m) is partitioned. A scalar constitutive law derived from damage mechanics has been developed and implemented to model the individual root failure behaviour under tension, compression and bending. Roots are assumed to be linearly elastic and quasi-brittle, associated with the damage law. Soil material is defined to be homogeneous, linearly elastic and plastic with Mohr-Coulomb criterion. To introduce realistic material properties with least additional model complexity, the model was parameterised by a minimum of mass, stiffness and strength characteristics for roots and soil. Associated parameters were obtained from measurements either performed during the corresponding tree-pulling experiment (soil) or published previously for similar greenwood materials (roots). A species-specified description of root system architecture is attempted through root patterns described by main root classes associated with realistic dimensions extracted from extensive measurements for *Pinus pinaster*.

To evaluate impacts of main root classes on tree anchorage strength (maximum turning moment), 12 root system variations were designed by separately removing for each case one main root class (the taproot, windward shallow roots, etc.). To extract the most important contributing factors to anchorage strength, the Taguchi's method was applied to two main parameter sets, namely the set of root geometric parameters and the set of material properties.

Results

Anchorage strength predicted by the model agrees well with field results with respect to tree size and site conditions. Individual root stress and breakage patterns are identified and linked to the overall response to overturning with respect to root system variations. The simulated sequential root failure explains the two main measures characterising tree anchorage failure,

namely the sequential stiffness reduction and the overall physical failure of the root-soil system. The taproot, large shallow roots and windward sinker roots are found to be the most relevant architectural traits to tree stability to wind. Root system architecture, characterised by the rooting depth and the dimension of the root-soil cage, is found to be the predominant factor over root and soil material properties.

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Marker-tracking analysis of tree trunk deflection and root plate movement during pulling test

Jan Tippner[1], Václav Sebera[1], Luděk Praus[1], David Ševčík[2], Jan Čepela[2], Martin Brabec[1]

[1]Department of Wood Science, Mendel University in Brno, Czech Republic
[2]Sobriety Ltd., Czech republic
jan.tippner@mendelu.cz

Introduction

The motion of a root plate is usually not measured nor used in assessment of tree stability even despite its unquestionable contribution to its stability. Hence, there is a call for assessment technique that would provide complex information about tree mechanical behavior. The goal of this study is to test and examine marker-based tracking to obtain 2D and 3D displacement data of both root-plate top surface and deflection of the tree stem that are measured at the same time. For these purposes we used both mono- and stereo-vision systems that were synchronized. We examined solitaire Horse chesnut (*Aesculus hippocastanum* L.) with height of approx. 20 m. The tree was loaded by destructive pulling test, within which we acquired sets of images. Markers fixed to the tree were tracked in developed software.

Results

Marker tracking applied to pulling test was successful and it provided global information about tree displacement on both areas of interest, tree stem and root-plat top, see top row in Fig 1. The euclidean distance of tracked markers in course of time is displayed in bottom row in Fig.1. The algorithm in provided all information about the tree during single test and offer robust solution for solving both research and practical problems in tree mechanics.

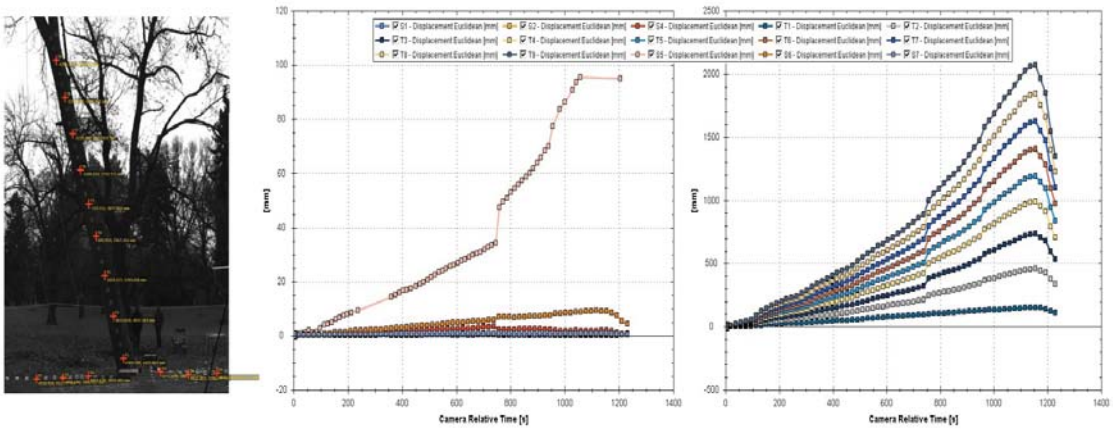

Fig. 1 : Marker tracking of tree during pulling test. Picture of loaded tree with defined markers (left); displacement of markers on ground (middle) and on the tree stem (right) in a course of relative time.

Acknowledgement

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It's Cool but What's it For? Multifunctionality and Interpretation of Function

Barbara Lachenbruch[1]

[1]Dept. of Forest Ecosystems & Society, Oregon State University, USA
barb.lachenbruch@oregonstate.edu

A critical question in biology is how, and whether, we can pin down the purpose of a structure, given that it may contribute to so many types of performance over the organism's lifetime. How do we go about deducing 'purpose?' This question may be even more important because of the novel climates, soils, and communities in which plants from now onward may be functioning. People who work with structure and function will increasingly be asked to provide guidance on the traits that are needed for a given management objective and environment. Moreover, because of advances in molecular biology but the rather low exposure of many molecular biologists to whole-plant biology, morphology, and function, science in general is at risk of generating catalogs of phenotypes and gene expression that lack functional interpretation. It is likely that we will increasingly be asked to help interpret the 'purpose' of the phenotypes expressed. In many cases these phenotypes will have structures that could have more than one function. This talk will concentrate on how we can get closer to understanding 'what something is for,' using examples from plant biomechanics and hydraulics.

One approach is to break performances (such as providing mechanical stability, or allowing a certain level of water transport) into the traits that contribute to those performances (such as the structure's geometry) in a process similar to 'factoring out' a number in mathematics. Once the contributing traits are listed, we can then ask how else they can be combined, to what other performances they may contribute, and the potential for compensations among traits and trait values. This approach is direct, but it has many complications. One complication is that a structure's function at time 1 may actually be to be a stepping stone toward the structure at time 2, and if we only look at one period we may well miss it. (Imagine asking the function of a floral bud at time 1, when its function will be much more visible once flowering and seed set occur at time 2.)

Another approaches is empirical: one can challenge the structure with different sets of conditions to learn effects on performance, inferring that the 'best' performance gives keys to what the trait's most important function is for the organism. Other approaches are historical, in which one seeks patterns in relation to evolution, and geographic, in which one seeks structural patterns in relation to environment and then infers the function.

The tools of biomechanics and hydraulics can get us only one step closer than the molecular biologists to understanding the performance of a set of morphological characteristics. Nonetheless, deep thinking and targeted experiments will advance our abilities greatly at deducing the important roles of multi-functional structures that we see.

Impact of the seeding rate on flax stem stability and fiber mechanical properties

Alain Bourmaud, Christophe Baley

Laboratoire d'Ingénierie des Matériaux de Bretagne (LIMATB),
University of South Brittany, France

alain.bourmaud@univ-ubs.fr

Abstract

These last years, we attended a significant development of the biocomposites; manufacturers are now considering semi-structural applications for these materials that require the use of vegetal fibres having efficient and reproducible mechanical properties (Lefeuvre et al., 2014). In a previous work (Baley and Bourmaud, 2014), we showed that flax fibre, in spite of slight differences from one variety to another, exhibit stable mechanical performances and can compete with glass fibres. Nevertheless, it is interesting to improve our knowledge in order to understand why some varieties are better than others on a mechanical point of view.

During the flax growing, the environmental solicitations are mainly due to sun, wind and rain, which can induce the plant lodging. The boundary conditions, i.e., the anchorage quality and the soil behaviour are also preponderant parameters for the plant stability and the lodging is highly influenced by the stem stiffness, the environmental solicitations and especially the additional water weight.

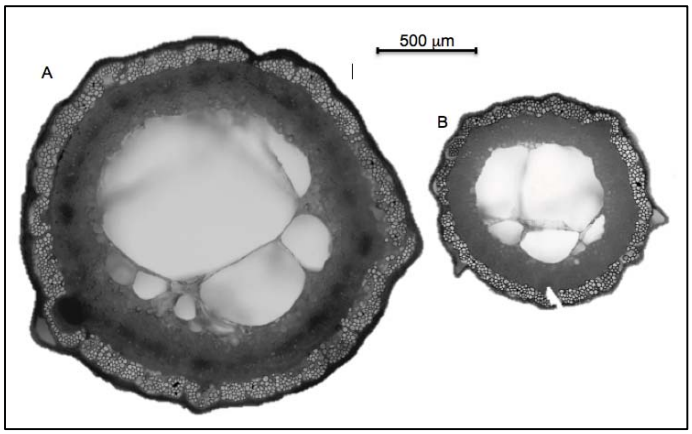

Fig. 1 : Aramis stems sections (middle height) for 1110 (A) (diameter=1.87±0.34 mm) and 2190 (B) (diameter=0.91±0.22 mm) plants/m^2

Moreover, the stem and fibre morphology, and consequently the plant stability are strongly linked to seeding rate. Indeed, the number of ramifications, of leafs, the quality of rooting, as well as the nutriment or water availability are key parameters for the stems stability and development. For flax, in order to reduce the lodging risk and maximize the fibre yield, the recommended number of plants is around 1500/m2 (Bert, 2013). In general, the increase of the seeding rate induces a decrease of the stem and fibre length (Easson and Long, 1992)

diameter as well as a yellowing and a decrease into the number of fibres, especially in the bottom of the stem. In this case, the risk of lodging is increased (Easson and Long, 1992; Gubbels and Kenaschuk, 1989) due to the lower cell wall thickening and to the poor rotting quality. Nevertheless, in case of poor or dried soil, a high seeding rate could be a solution to make easier the plantlet rising due to their collateral helps.

This work proposes a study of the impact of the seeding rate on these key parameters. We studied Aramis variety with 4 different seeding rates (1200, 1500, 1800 and 2500 seeds/m²). The results indicate the important impact of the seeding rate on stem morphological parameters; its increase induces a progressive decrease of the scutched fibre length and of the stem diameter (Fig. 1). In the same time the higher seeding rates allow to obtain improved scutched fibres yield (+11% between 1200 and 2500 seeds/m²) but, in an opposite way, induces a drop into the elementary fibres tensile properties and into the flax stem lodging stability, mainly due to the important stem diameter decrease. This work shows that a compromise must be found to optimize the fibre yield, the mechanical performances and the plant stability; it underlines the interest to use a conventional seeding rate, close to 1800 seeds/m^2.

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Structural Role of the Leaf Sheath

Margaret Julias[1], Daniel Robertson[1],and Douglas Cook[1]

[1]Plant Biomechanics Laboratory, New York University – Abu Dhabi, United Arab Emirates
douglascook@nyu.edu

Introduction

While all plants are important, the Poacea family of grasses and grains play a critical role for humans. The grains and grasses are under active research for increased crop production, as means for carbon sequestration and for bioenergy production (Cook et al, 2015). *Poacea* plants share a common structure which is very much like a telescoping radio antenna (Johnston, 2007). In the early stages of development, the leaf sheath is hypothesized to play a structural role in supporting the growth and elongation of the stalk. The purpose of this study was to investigate the sheath stalk interaction to gain new understanding regarding the structural role of the sheath.

Methods

Maize plants were grown in controlled laboratory environment to reduce environmental effects. A multi-modal imaging study was performed using scanning electron microscopy (SEM), high resolution computed tomography (μCT), and digital image correlation (DIC). DIC is a technique used to assess surface strains. In this study, DIC is used to assess growth patterns. SEM was used to understand the surface mechanics of the stalk and sheath, as an aid to understanding the apparent adhesive properties of these surfaces. μCT was used to obtain three-dimensional data regarding the stalk morphology, particularly to assess if contact between the stalk and sheath was continuous. Finally, DIC was used to visualize growth patterns of the sheath, as well as relative growth of the stalk relative to the sheath. This was accomplished by surgically creating a small "window" in the sheath tissue, and imaging the growth of sheath and stalk simultaneously.

Results/Discussion

This research project is currently in progress. Stalks are currently being grown in the laboratory. μCT scanning is being used to assess stalk/sheath contact area. Figure 1 shows a cross-section from μCT scan, showing a lack of contact between sheath and stalk. SEM images of stalk surface are shown in Figure 2, contrasting meristematic tissue, which is less organized and has fewer stomata, with intermodal tissue which is highly organized and has more stomata. Finally a DIC image of the stalk surface under a bending load is shown in Figure 3. This technique is able to identify areas of high strain before tissue failure occurs. Finally,

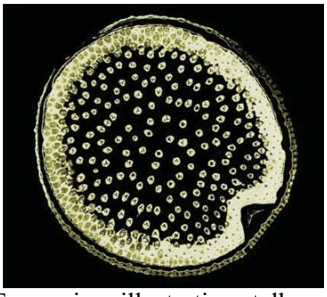

Figure 1: μCT scanning, illustrating stalk and sheath scan, showing lack of contact between the sheath and stalk

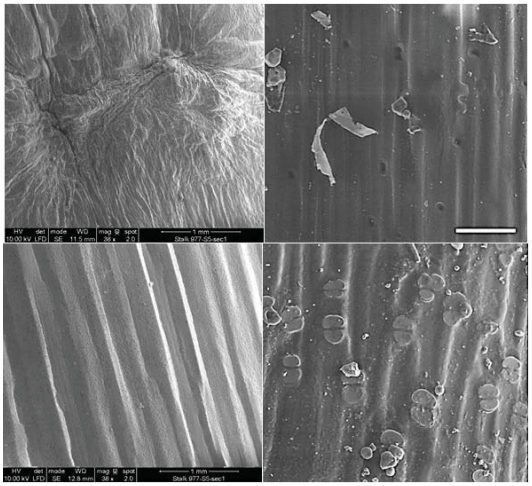

Figure 2: SEM images of stalk surface.
Top images: intercalary meristematic region.
Bottom images: intermodal tissue

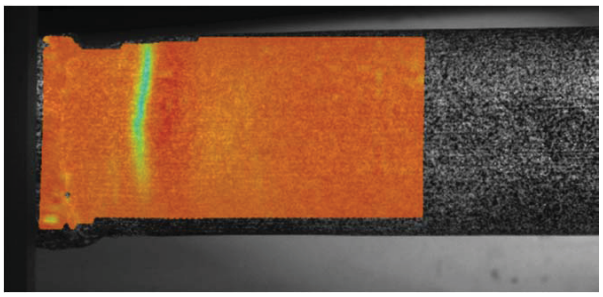

Figure 3: Digital image correlation (DIC) applied to stalk bending failure.
The yellow band indicates a region of high localized strain immediately prior to tissue failure.

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Forensic Analysis of Stalk Failure in Maize

Margaret Julias[1], Daniel Robertson[1], Brian W. Gardunia[2], and Douglas Cook[1]

[1]Plant Biomechanics Laboratory, New York University – Abu Dhabi, United Arab Emirates
[2]Monsanto Corporation, Huxley, Iowa
douglascook@nyu.edu

Introduction

Stalk failure causes damage and often death of the plant. This topic is particularly relevant in agriculture, but the basic biomechanics behind stalk failure are poorly understood. There have been many attempts to assess stalk failure resistance in maize. However, measurements such as stalk bending strength and rind penetration resistance have shown only moderate genetic relationships (Flint-Garcia et al., 2003; Peiffer et al., 2013; Hu et al., 2013). Furthermore, many previous studies of stalk strength have neglected to consider failure patterns, which can provide valuable information about the reasons and mechanisms of failure. This study describes a multi-modal failure analysis including forensic examination, image analysis, and structural engineering to gain new insights on patterns and potential causes of stalk failure in maize.

Methods

Maize stalk failure samples were carefully examined at several locations in North America and Africa during 2010, 2012, and 2014. To reduce the number of potential confounding factors, only stalks with no visible presence of disease or pest damage were included in this study. X-ray computed tomography was performed to obtain high-resolution geometric information of maize stalks at resolutions ranging from 78μm to 90μm per voxel. Customized computer code was developed to perform automatic analysis of the geometric features of stalk cross-section. Finally, tissue structures were investigated using stereo microscopy and scanning electron microscopy (SEM).

Results/Discussion

Maize stalks were found to fail in just three modes, including tensile snapping, longitudinal splitting, and buckling/creasing (see Figure 1). This is the first detailed description of maize stalk failure modes in the scientific literature.

Fig. 1: The three modes of maize stalk failure.

Overall, 91% of all failed samples exhibited creasing failure. Creasing was commonly aligned with the major diameter of the stalk cross-section, and the predominant direction of failure was in the direction of the minor diameter of the maize stalk (Figure 1). Furthermore, 89% of creasing failures occur within 3 cm of a junction between adjacent sections of the stalk (Fig. 2). These results indicate strong connections between maize stalk physiology and failure. An analysis of maize stalk geometry (Fig. 3) revealed that extreme changes in stalk geometry occur at nodal junctions, possibly acting as a stress concentrator.

Other potential stress concentrators were also found at the tissue level, including changes in tissue organization from smoother and organized internodal tissues to rougher and disorganized tissues near the node, and undeveloped brace root buds near basal nodes.

Presence of stomata was higher at this region (Fig. 4). These could also act as a stress concentrators, similar to the small holes in perforated paper.

SEM imaging revealed that tissue delaminations (a phenomenon often occurred in composite materials) were frequently observed under the rind surface at the failure region (Fig. 5). As the intralayer delamination grows within the stalk, severity of failure increases, which ultimately lead to complete destruction of the stalk.

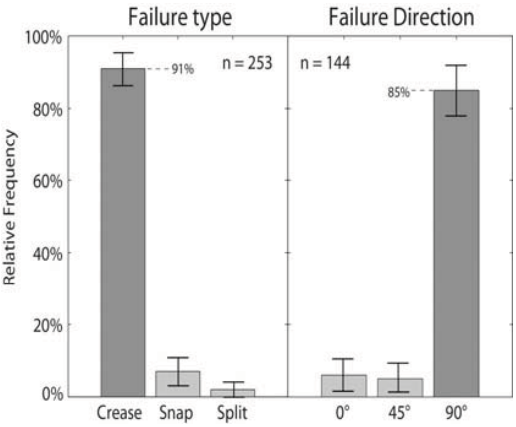

Fig. 2: Failure types and failure direction relative to stalk cross-section.

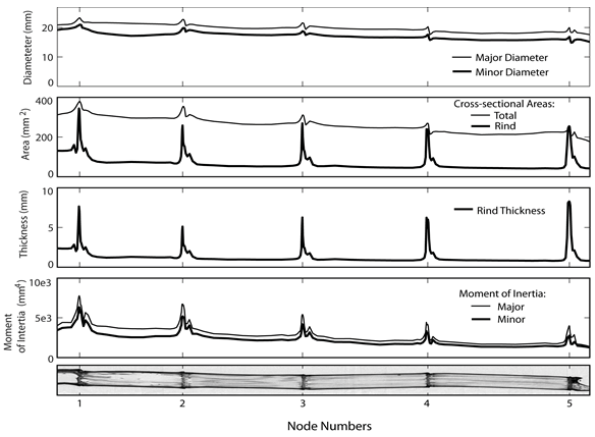

Fig. 3: Geometric changes of maize stalk

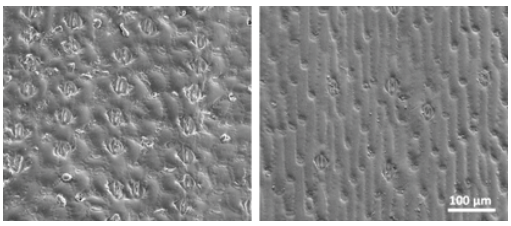

Fig. 4: Rind surface of node region (left) consists of more stomata than rind surface of internode region (right)

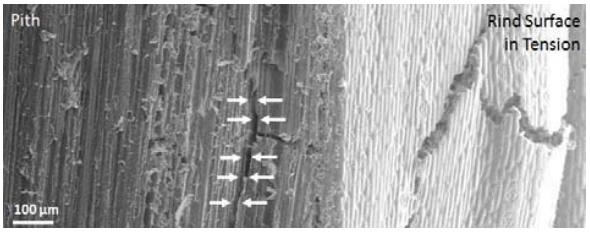

Fig. 5: Tissue delamination under rind surface

Discussion/Conclusion

The application of engineering failure analysis revealed distinct patterns in failure type, direction, and location. Maize stalk failure locations are spatially correlated with geometric variation, suggesting that geometric changes near the node may act as stress concentrators and may affect stalk strength and failure patterns. Observed differences in tissue structures may play a role in weakening the node region. However, this might have been compensated by the thicker rind tissues near the node. Further studies will be needed to elucidate the various effects of architectural and tissue variations on stalk failure.

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Numerical study of the stress distribution in bending trees: analysis of the effects of shear stress and maturation stress on tree resistance against wind loading.

JULLIEN Delphine[1], ALMERAS Tancrède[1]

[1]Laboratory of Mechanics and Civil Engineering, Montpellier University, France

delphine.jullien-pierre@univ-montp2.fr

As trees grow, they acclimate to resist the wind loading that they have experienced. During storms, wind loads can exceed the strength of the stem or the root system, resulting in either uprooting or stems breaking. Climate changes induce more frequent storms in places that were not subjected to these phenomena in the past, leading to disastrous damage in forest stands. Tree has also a special place in urban planning: shadow, greenery, air quality… But it represents a danger when branches or tree itself may break and fall. For reasons of safety and risk assessment, it is important to know more about tree failure.

Current simulations dedicated to this problem mostly represent stems as beams submitted to pure bending load. In this communication, we will present 3D simulations of stress distribution in bending stems, using a finite element models that considers the local orthotropic properties of material. It enables analysing the possible involvement of shear stress in the start of tree failure, as a function of the shape of the trunk and material properties of wood.

Our simulations show that the magnitude of shear stress remains low compared to that of axial stress, so that shear does not appear to play a major role in failure process. Our model enables to take also into account the pre-stresses in the trunk resulting from wood maturation. Results confirm the role played by maturation stress in the reinforcement of the structure, increasing its resistance to bending loads.

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Heterogeniety of Cell Wall in Relation to Wood Quality

Xu F[1], Ji Z[1], ZhangX[1], Kim YS[2]

[1]Beijing Key Laboratory of Lignocellulosic Chemistry, Beijing Forestry University, Beijing, China
[2]Dept. Wood Science & Engineering, Chonnam Nat'l University, Gwangju, South Korea
kimys@jnu.ac.kr

Woody plants have changed over hundred millions of years. By continually cross-talking to the surrounding environment, evolutionary optimization of its formation and structure is embedded in the cell walls of woody plants. Thin-walled precursor cambial cells undergo dramatic transformation such as cell expansion, secondary wall deposition, bordered pit formation, lignification and programmed cell death. During secondary xylem differentiation, 1) the mechanical strength to withstand the stem from diverse forces, 2) the conduit for radial and longitudinal transfer of developmental signals and nutrients, and 3) the defense system against biotic stresses are conferred in parallel with stem enlargement and diameter growth (Rao et al., 2012).

Thus, the cambium performs its taxa-specific meristematic task of cell production which varies in form, function and rate of production at different position in the trees with age and with season of the year (Schmitt et al., 2016). Complexity and heterogeneity in the cell walls in wood tissue, such as difference in size, shape, cell wall structure, texture and chemical compositions, are intrinsically stemmed from these activities of cambium. Much higher amount of secondary xylem (wood) on the inside than the secondary phloem on the outside is produced by the division of cambial cells, which exhibits also considerable variation in the structure and chemical composition. Primary cell wall is closely related to physiological aspects whereas secondary cell walls to material aspects of predominantly dead cells (Burgert & Keplinger, 2013). Secondary xylems are composite materials consisting of a highly ordered arrangement of cellulose embedded within a more or less amorphous matrix hemicelluloses and hydrophobic lignins.

Although wood is the most important sustainable and renewable material on this planet from an economic as well as an environmental perspective, serving as a raw material for the processing of a wide range of useful products, and in developing high-performance engineering applications, wood is not ideal solids nor ideal elastic materials nor ideal fluids. Despite overall similarities, there are substantial differences in structure and composition of cell walls. Differences in geometry and in cell wall architecture can be found in diverse cell types in wood, which are distributed axially and horizontally. The arrangement and geometry of diverse cell types are related to the different mechanical behaviors of the whole woods. Each tissue type differs from the others in its mechanical properties showing the internal structural heterogeneity (Tibaut et al., 2001). Due to the cellular heterogeneity, varying degree of anisotropy is intrinsically shown in the wood.

Chemically, wood is a hybrid biomaterial containing diverse polymers and low-molecular chemical extractable with organic solvents. Cellulose representing 40–50% of dry weight provides the specific anisotropic organization of the thick secondary wall because of its supra-molecular microfibril architecture. Although cellulose is considered as uniform in consisting of only cellobiose repeating units, however, celluloses in wood cell walls are heterogeneous in a variety of ways. Length, number, degree of crystallinity cellulose and

width of cellulose microfibrils also vary. Furthermore, microfibril angle differs in the different cell wall layers.

The hemicelluloses, comprising 20% in softwood and 30% in hardwoods, respectively, are highly heterogeneous complexes of polysaccharides. Hemicelluloses as heteropolymers occur in softwoods mainly as galactoglucomannan, whereas in hardwoods as arabinoxylan. Hemicelluoses can be divided into two categories: those in close affinity to cellulose and those coupled to lignin (Cosgrove & Jarvis 2012). Xyloglucan is restricted to inaccessible junctions between microfibrils. Due to the hydrophilic characteristics of polysaccharides, wood is highly sensible to humidity and shows strongly anisotropic properties resulting in geometric changes.

Wood's strength, rigidity and defense against various pathogens are principally enhanced by the lignins, which vary across different woody plant groups; gymnosperms 30% vs angiosperms 25%. Hardwood species are rich in syringyl (S) lignin, whereas gymnosperms are rich in guaiacyl (G). The topochemical distribution of lignin varies with cell type and within different cell wall layer (Fig. 1). Fibers are richer in S, while more concentrated G unit is present in the secondary walls of vessels, tracheids and rays (Zhang et al., 2012). Lignification is highly associated with the resistance to high water pressure rather than the contribution to mechanical support (Reyes-Rivera J. et al. 201).

In conclusion, woody plants constantly change their internal structures and the properties of their materials in response to changing environments until they reach the stable condition. The heterogeneity of wood cell wall provides us information to overcome the obstacles to bioconversion of woody biomass for renewable energy and to maximize the added-value of lignocellulosics by genetical design of woody plants.

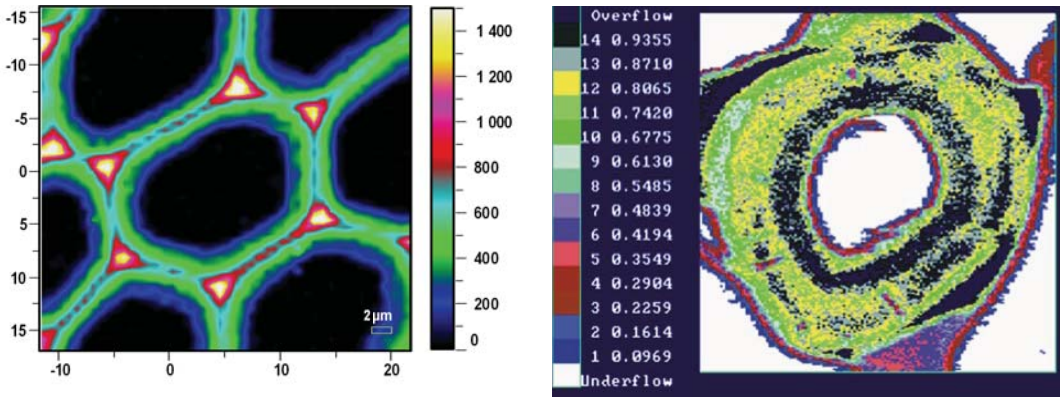

Figure 1. Raman image of lignin distribution in poplar cell wall, generated by integrating the intensity over the wavenumber range from 1570-1680 cm^{-1}(left; Ji et al., 2014); UV-scanning image (280 nm) of the sclereid differentiated in *Pinus radiata* callus cultures (right: Courtesy of Dr. R. Möller)

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Keywords: heterogeneity, wood cell walls, structural polysaccharides, lignin, topochemistry

Occurrence of xyloglucan in poplars for wind and earthquake

Rumi Kaida[1], Eiichi Obataya[2], Masato Yoshida[3], Futoshi Ishiguri[4], Jun Tanabe[4], Toru Taniguchi[5], Manabu Kurita[5], Kei'ichi Baba[6], Takahisa Hayashi[1]
r3kaida@nodai.ac.jp

[1]Department of Bioscience, Tokyo University of Agriculture, Japan, [2]Graduate School of Life and Environmental Sciences, University of Tsukuba, Japan, [3]Department of Biosphere Resources Science, Nagoya University, Japan, [4]Department of Forest Science, Utsunomiya University, Japan, [5]Forest Bio-Research Center, Forestry and Forest Products Research Institute, Japan, [6]Research Institute for Sustainable Humanosphere, Kyoto University, Japan

Xyloglucan occurs around cellulose microfibrils by hydrogen bondings not only in the primary wall but in the secondary wall. Its solubilization is very difficult with a weak alkali (i.e., less than 4% KOH) but requires with a strong alkali (e.g., 24% KOH) for the mercerization of the microfibrils. All the microfibrils could have been affected by xyloglucan, based on the analyses of elementary fibrils between wild type and transgenic poplar xylem overexpressing xyloglucanase (AaXEG2). This finding resulted in the fact that all the microfibrils, not only in the walls of growing cells but also in the xylem of tree stems, could be associated with xyloglucan by binding to the surfaces of elementary fibrils. Based on immunolabeling analysis with specific antibodies against xyloglucan, xyloglucan was observed in the G and S2 layers at a certain level as well as in the primary wall layer at a marked level.

We propose that the distinguishing character of xyloglucan should have structural roles in xylem. When poplar stems were subjected to static and dynamic analyses, a significant reduction occurred in the Young's modulus and bending strength of the transgenic lines compared with wild type poplars. Moreover, sway tests showed that the oscillation of transgenic stems continued longer, and its frequency was smaller than that of the wild type. The presence of xyloglucan reduces stem swaying and causes vibration control as well as vibration resistance in stems. When plants moved from the sea to the land, they might have developed the occurrence of xyloglucan through evolution to strengthen their cell walls. Xyloglucan could be required for tightening the walls through the cross-linking of cellulose microfibrils to resist the land stresses such as wind, rain, snow and earthquake.

Screening eucalypts for growth-strain

Nicholas T. Davies, Monika Sharma, Clemens M. Altaner and Luis A. Apiolaza

New Zealand School of Forestry, University of Canterbury, New Zealand
ntd14@uclive.ac.nz

Introduction

Eucalypt species are fast-growing and can produce high quality timber for appearance and structural products including Laminated Veneer Lumber (LVL). Eucalypts can contain large growth-strains which are associated with log splitting, warp, collapse and brittleheart. These impose substantial costs on processing (Yamamoto 2007). Costly, and only partially effective, mitigation strategies have been developed to reduce wood defects induced by growth-strain. As growth-strain is highly heritable, an alternative approach is to select and grow individuals which display low growth-strain. Until now measurement of growth-strain has been difficult, time consuming and expensive, preventing the assessment of the large number of trees needed by a breeding programme (Altaner 2015). As an example, the largest sample number in any reported growth-strain study was smaller than 230 trees (Solorzano Naranjo 2011). Traditionally selections are made when trees are older, not only increasing costs (e.g. trial management, sample handling) but also substantially extending the breeding cycle and delaying the deployment of improved germplasm (Altaner 2015). Developments at the University of Canterbury have resulted in a unique growth-strain measurement method supported by theoretical analysis (Entwistle 2014) - dubbed the "Splitting" test. It allows for rapid growth-strain assessment on young trees (Chauhan 2010).

Methods

The development of the splitting test, based on the pairing test (Chauhan 2010), has resulted in a simple and quick method of growth-strain measurement which can be used on small stems. The test relies on the release of stored strain energy in a single plane via cutting through the pith as can be seen in Fig. 1. Chauhan (2010) derived Equation 1 to estimate the deflection given growth-strain from the sample geometry.

$$Y_u = \frac{0.87\varepsilon L^2}{R_{avg}} \qquad (1)$$

Where: Y_u is the deflection, ε is the strain, L is the cut length and R_{avg} is the cross-section radius.

Fig. 1: Sample after the "Splitting" test has been performed. The opening is measured and used for the strain calculation along with diameter and cut length. This sample shows significant growth-strain as can be seen from the wide opening.

Data analysis was conducted in R (R Core Team 2015) and JAGS (Plummer 2015), utilizing a Bayesian approach to estimate the heritability of growth-strain at the family level. The effect of coppicing is included as a fixed effect. Specimen groups were grown during different time periods and for different rotation lengths, group is included as a random effect.

The "Splitting" test is physically constrained to positive values, as the opening cannot be reduced in the presence of compression at the stem surface and tension at the pith resulting in left censored data. Bayesian frameworks provide the ability to simulate partially observable data, and therefore reducing the systematic errors which occur due to left censoring. Here the left-censored initial values are sampled from a uniform distribution between -1.5 and 0.

Results and Discussion

This scoping study was substantially larger than any preceding investigations, assessing growth-strain and other wood properties on more than 600 *Eucalyptus bosistoana* and other eucalypt species at an early age (less than 2 years old). The results from these trials showed that growth-strain is heritable, and family rankings varied little whether grown from seed or coppiced from existing root systems. Tab. 1 shows the family mean Spearman rank coefficients of the tested wood properties whether grown from seed or coppice. Fig. 2 shows 20 families (8 half-sib replicates each) ordered by median growth-strain. The family rankings were similar (Spearman coefficient of ~0.77). In particular the top 3rd of the families, i.e. those with the lowest growth-strain were the best in both trials (Fig. 2). Growth-strain increased after coppicing. When plants are coppiced from existing root systems they emerge from the side of the old trunk resulting in a hockey-stick shaped lower stem. Given the nature of the testing procedure, it is suspected that the increase in growth-strain with coppicing was due to the formation of tension wood rather than an indicator that older trees will possess significantly higher growth-strain. Analysis revealed a narrow sense heritability (h^2) of 0.44 with a 95% credible interval of the posterior distribution from 0.20 to 0.67. Tab. 2 shows the h^2 values for the tested wood properties.

Tab 1: Spearman rank coefficients between the family means when grown from seed or coppiced from the existing root systems. Family mean growth-strain rank shows a strong relationship before and after coppicing (~200 plants from seed and ~200 from coppice).

Property	Spearman coefficient
Diameter	0.44
Dry density	0.30
Acoustic velocity	0.69
Stiffness	0.44
Growth-strain	0.77

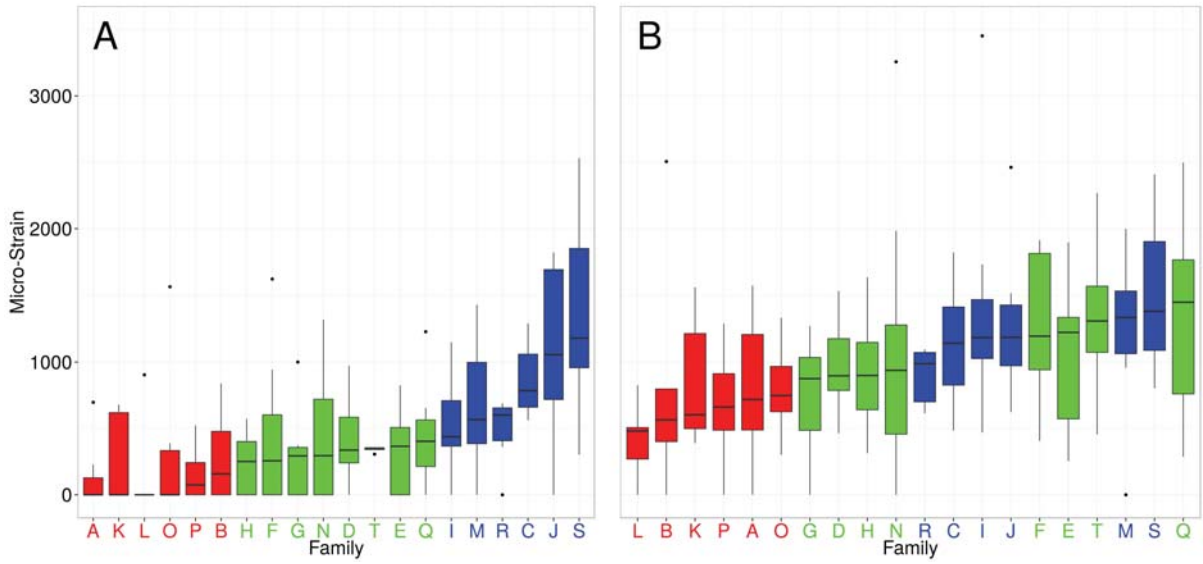

Fig. 2: High (blue), medium (green) and low (red) growth-strain families grown from seed **A** and the same individuals grown from coppice **B**. Note the large number of trees in the low growth-strain families showing closure (zero) during the splitting test and the higher magnitudes of growth-strain when grown form coppice (~200 plants from seed and ~200 from coppice).

Tab. 2: Narrow sense heritability of the measured wood properties. Large credible intervals are present on all heritability estimates, emphasising the need for large sample sizes. Growth-strain is under substantial genetic control (~400 plants from seed and ~200 from coppice).

Property	Heritability	95% Credible Interval
Diameter	0.27	0.087 - 0.46
Dry density	0.45	0.23 – 0.67
Acoustic velocity	0.84	0.54 - 1.14
Stiffness	0.38	0.14 - 0.62
Growth-strain	0.44	0.20 - 0.67

Tab. 3 shows the correlations between the family means of the measured wood properties while Tab. 4 shows the correlations at an individual tree level. The analysis included trees grown from seed and coppice. Acoustic velocity showed a good correlation with growth-strain at the family level but was only moderate at the individual tree level. Dry density showed moderate negative correlations with growth-strain. Diameter showed poor correlations with wood properties. All predictors were less strongly correlated with trees grown from coppice than trees grown from seed (data not shown). Testing for all of these properties can be conducted in less than five minutes per sample at trees as young as one year old. This for the first time allows a sizable wood quality improvement programme at reasonable cost.

Tab 3: Pearson correlation coefficients between family mean wood properties. The results indicated that growth-strain correlated most strongly with acoustic velocity (~200 plants from seed and ~200 from coppice).

	Dry density	Acoustic velocity	Stiffness	Growth-strain
Diameter	-0.09	-0.40	0.26	0.34
Dry density		-0.19	0.45	-0.39
Acoustic velocity			0.75	0.86
Stiffness				0.46

Tab. 4: Pearson correlation coefficients between measured wood properties at the individual tree level. The results indicated growth-strain correlated most strongly with acoustic velocity (~200 plants from seed and ~200 from coppice).

	Dry density	Acoustic velocity	Stiffness	Growth-strain
Diameter	0.33	-0.15	0.33	-0.03
Dry density		-0.55	0.96	-0.45
Acoustic velocity			-0.36	0.59
Stiffness				-0.34

Over the last decade, the New Zealand Drylands Forest Initiative (NZDFI) has obtained the largest collection of seed in the world for a number of naturally durable eucalypt species, including *E. bosistoana*, with the aim of establishing a fast-growing, naturally durable, super-stiff, sustainable plantation timber resource in New Zealand. The basis is a breeding programme which gives wood properties the same priority as growth, form and tree health. This novel approach to tree improvement also includes very early screening (age 1-2) to ensure a timely deployment of improved germplasm (Altaner 2015). With the “Splitting” test, screening of the entire genetic stock is now a practical solution to remove growth-strain induced wood defects. A 10,000 tree trial consisting of ~200 families each with 50 half-sibling replicates of *E. bosistoana* has been established. The trial will be harvested at an age between 18 and 24 months (late 2016 - 2017) and evaluated for growth-strain, as well as improved for early form, growth, stiffness, volumetric shrinkage and basic density. As the tests are destructive the superior individuals need to be rescued by coppicing. Propagation of coppice cuttings is also providing a fast route to deploy improved material to the forestry sector. Additionally, a number of long-term field trials have been established (as early as 2009) throughout New Zealand to provide longer term studies of wood properties in particular heartwood formation.

Due to the nature of the "Splitting" test, strains which result in the closure of the specimen cannot be measured, and as a result are recorded as zero. Fig. 2 shows a number of individuals exhibited closing, particularly when grown from seed, indicating an atypical stress pattern in the stem (Dawson 2011) with greater contraction at the pith than the periphery. The inverted stress-profile may be a result of tension wood being formed by the trees at young age (i.e. at the centre of the stem) to straighten the stem followed by normal wood with lower axial tensile growth-stresses at the periphery. Trees can form reaction wood in response to wind loading (Coutts 1995). A closing sample may indicate increased sensitivity to wind loading and the development of reaction wood at a young age in that genotype. In order to better understand the mechanisms for this unusual behaviour, a new trial has been established in which initial bending will be induced in the stems at a young age followed by straightening.

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Gradient of residual stress within logs affecting deformation of sawn timber

Kana Yamashita[1], Takeshi Fujiwara[1], Yuji Ikami[2], Yukari Matsumura[2],
Hideo Kato[3], Miyuki Matsuo[4], and Hiroyuki Yamamoto[4]

[1]Department of wood properties, Forestry and Forest Products Research Institute, Japan
[2]Department of wood processing, Forestry and Forest Products Research Institute, Japan
[3]Department of wood engineering, Forestry and Forest Products Research Institute, Japan
[4]Laboratory of biomaterial physics, Nagoya University, Japan
zaikana@ffpri.affrc.go.jp

Introduction

During the growth of a normal tree the stem becomes progressively compressed in the central part of the tree and tensioned close to the periphery (Ormarsson et al. 2009). This stress state (gradient of the longitudinal stress component in the radial direction) can cause deformations in timber sawn from the stem and reduces the yield. In order to establish appropriate sawing methods for improving the deformation of sawn timber, it is necessary to understand the stress state and its influence on the deformation of the sawn timber during sawing.

The diameters of logs supplied in the market are increasing for the Japanese cedar which is the major plantation species in Japan. Okuyama and Sasaki (1979) analyzed the crooking of the quarter-sawn board in connection with the longitudinal residual stresses using logs with small and middle diameters. In this study, the gradient of the residual-stress state and its released strain during sawing were investigated to reveal their influences on the deformation and the unevenness of the sawn timber from large diameter logs.

Materials and methods

Six green logs of Japanese cedar (*Cryptomeria japonica* D. Don) were used. The logs were 4 m in length and more than 35 cm in diameter of small-ends. Three logs were used for measuring the radial gradient of the released-strain in the longitudinal direction. They were sawn into 30-40 mm thick flitch. Strain gauges were pasted in the longitudinal direction on the radial plane across the diameter at intervals of 25 mm. Released-strains were measured as the strain when the flitch was cross-cut at distances of 20 mm each from the gauge center.

The other three logs were used for sawing experiment to measure the released-strain in the longitudinal direction during sawing. The logs were sawn into 120 mm thick two-sided cant and then cross cut equally into two cants 2 m in length. Strain gauges were pasted in the longitudinal direction on the radial plane across the diameter at intervals of 40 mm. The cant was anchored on the carriage with clamps and sawn into flat-sawn timbers with 40 mm thick by bandmill from the bark to the pith and to the bark direction. The released-strains of the anchored cant were measured during sawing. Width and thickness of the sawn timber were measured at the both ends and the middle of the timber following to the measurement of bow and crook.

Results and discussion

The radial gradients of the released-strain in the longitudinal direction were shrinkage in the central part and elongation close to the bark (Fig. 1), which were the same trend with the previous studies for Japanese cedar (Watanabe et al. 1963, Kitahara et al. 2000, Nogi et al 2003). The radial gradient tended to be steep near the pith and gentle near the bark.

Radial gradient of the released-strain is reported to be varied by growth rate (Okuyama and Sasaki 1979, Kitahara et al. 2000) and MFA (Yamamoto 1998) which is larger in the juvenile wood. In order to generalize the radial gradient of the released strain, further studies are required.

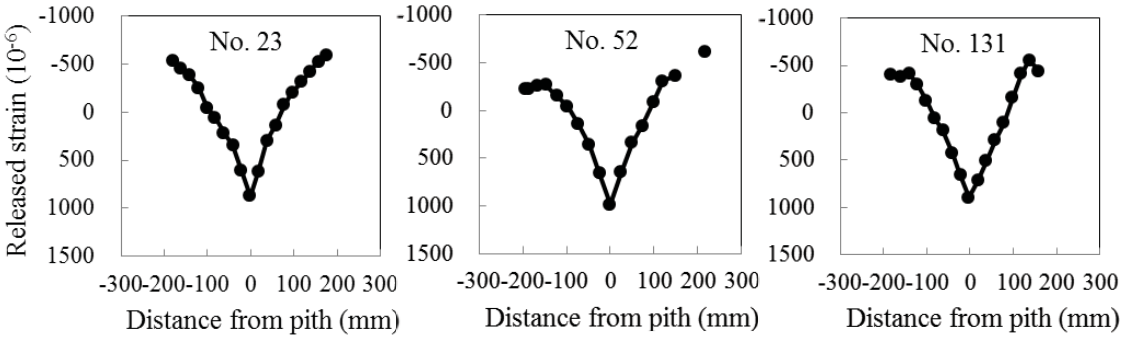

Fig. 1 : Radial gradient of the released strain in the longitudinal direction within logs

In the sawing experiment, the distribution of the strain of the anchored cant changed whenever a timber was sawn successively. The released-strains had radial gradient from the sawn side to the anchored side. Until sawing the timber including pith, the released-strains were elongation at the sawn side and shrinkage at the anchored side. This result suggested that the cant was warped due to the release of the tension residual-stress at sawing. In the sawn timber, bow and unevenness in thickness were observed. The convex sides of bow fronted to the anchored side. The timber was thicker at the middle than at both ends, which might be caused by warping of the cant.

Those trends were different after the timber including pith was sawn. The released-strains in the cant showed shrinkage at the sawn side and elongation at the anchored side, and became much smaller. In the sawn timber exhibited smaller bow and its convex sides fronted to the sawn side. The timber was thinner at the middle. This result suggested that the residual stress in the cant had reduced after sawing the timber including the pith.

Bow and thickness unevenness of the sawn timber were smaller in the timber sawn from the periphery of the cant at the beginning of the sawing, which was similar with the result by. Okuyama and Sasaki (1979). One of the reasons of such trend was supposed to be the gentile gradient of the released-stain in the longitudinal direction near the bark.

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Modeling hygrothermal recovery of wood in relation with locked-in strains during tree life

Sandrine Bardet, Joseph Gril

[1]Laboratoire de Mécanique et Génie Civil, Université Montpellier, CNRS, France
sandrine.bardet@umontpellier.fr

Introduction

Heating of green wood involves a complex set of deformation processes, including reversible thermal strain, reversible shrinkage due to decrease of moisture content, and irreversible viscoelatic recovery of growth stress activated by temperature called hygrothermal recovery (HTR) by Yokota (1962). This leads to an irreversible tangential swelling and a radial shrinkage above 70°C (glassy transition of lignins) due to the release of locked-in strains (Kubler 1959, Gril 1993, Salmen 1990). Locked-in strains are first generated in the maturation process and then by the loading history applied to the wood before tree felling. A attempt to model HTR behavior is proposed, based on the hypothesis that rheological conditions during maturation are equivalent to those of hygrothermal treatment.

Experimental data

RHT experiments were realized by Bardet (2012), in order to measure HTR on a Japanese softwood in directions (R, T, L) and to study the influence of distance from pith (specimen from outer zone, middle zone and inner zone) and of the thermal process.
Specimens were cut from a Japanese Cypress (*Chamaecyparis obutusa*) recently felled, with dimensions 20mm in the observed direction and perpendicular section 4x4 mm2. They were kept in water at 3°C before testing. The testing device was a Thermo Mechanical Analyser (TMA) equipped with compression attachment, so that the specimen were submitted to a slight compression stress (equivalent to 5 g) and immersed in water, the system controlling the temperature both during heating and cooling. Variations of dimensions were recorded with an accuracy of 0.01 µm. The specimens were submitted to a thermal process as follows: heating at 3°C/min from room temperature to 85°C, constant temperature during 240 mn cooling at 3°C to 25°C. This process was repeated one or 2 times. A typical curve for a tangential test is given in figure 1.

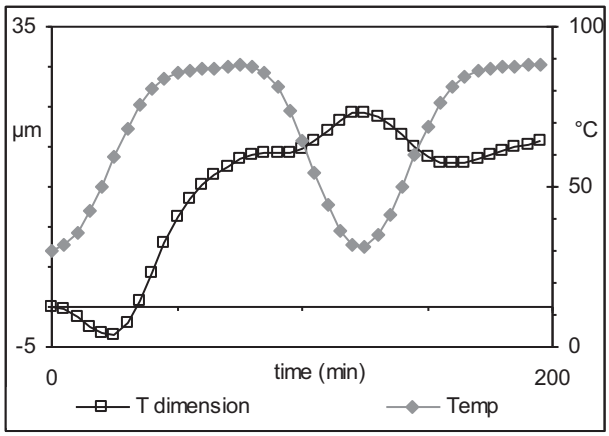

Fig. 1 : Typical hygrothermal recovery curve for tangential test, outer zone (variations of dimensions and temperature against time)

The first heating leads to a large increase of dimension (around 20 µm) related mainly to hygrothermal recovery of growth strains. The first cooling causes a slight additional increase of dimension explained by an increase of fibre saturation point leading to a swelling (reversible phenomenon). This is made evident by the second heating causing a slight decrease of dimension corresponding to the reverse reversible phenomenon.

A method to separate hygrothermal recovery and reversible deformation is applied by subtracting a curve from one to another. This method can be applied to tangential, radial and longitudinal tests from outer, middle and inner zone.

Modeling

Locked-in strains are first generated in the maturation process and then by the loading history applied to the wood before tree felling. Wood maturation corresponds to biochemical processes happening after the deposition of secondary layers, such as lignin polymerisation, completion of cellulose crystallisation or cross-linking in the amorphous regions of the cell-wall material. This phenomenon induces maturation strains, which can be simulated by presuming that rheological conditions during maturation are equivalent to those of hygrothermal treatment (Gril and Thibaut 1994). Loading history applied to the wood depends on the action of subsequently formed wood layers, new cells are submitted to longitudinal tension and previous cells to compression. Finally, wood in the tree is pre-strained: near the bark wood is under tension in the longitudinal direction, under compression in the tangential direction and almost stress-free in the radial direction, near the pith wood is under compression in the longitudinal direction and under tension in both tangential and radial direction.

Loading history is simulated using a parabolic model (Bardet 2002) submitted to a succession of steps with different temperature, humidity and mechanical conditions. The first step, maturation, is modeled by an imposed strain (1%), at 80°C for one week. Then tree life is modeled by an imposed stress at 25°C for several years. This leads to spectrum of locked-in strains. These locked-in strains due to maturation and stress history are partially released by cutting specimens from the tree (elastic recovery). Remaining locked-in strains are released by heating the wood in water or steam, this viscoelastic recovery is thermally activated and is related to the experimental HTR.

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Retrospective analysis over ten years of the static loading of beech tree crowns based on allometric relationships between branch dimensions and biomass distribution

Constant T.[1,2], Morisset J.-B.[1,2]

[1]INRA, UMR 1092 LERFOB, 54280 Champenoux, France
[2]AgroParisTech, UMR 1092 LERFOB, 54000 Nancy, France
constant@nancy.inra.fr

Introduction

In tree biomechanics functioning, taking the effect of the crown weight is always a challenging task requiring strong assumptions or detailed description. This task is even more challenging when you are looking to a difference of loading between two dates. The emergence of new technologies such as T-LiDAR in forestry research offer new ways for measuring detailed information about the spatial distribution of the woody volume, and to infer the mass distribution. But, these kind of data got at different dates for assessing the change of loading are still rare, especially for period corresponding to forest management operation such as thinning. At the opposite of this optimal situation, the changes of crown weight were often deduced from allometric relationships describing the whole crown weight from scaling laws based on dbh. Well adapted for ecological or theoretical studies, this method is poorly suitable for looking to individual trees (Almeras 2009). For such cases, the variation of the weight of the crown can be derived from final weight measurement decreased with respect to an observed temporal variation of some characteristics of the crown, like the horizontal crown projection area (Barbacci 2008), but these assumptions remain somewhat coarse. In this work, the objective is to develop a most accurate method for carrying out a retrospective analysis of the mechanical loading due to the crown.

Material and Methods

Starting from a final destructive measurement of the crown, this approach was based on the assumptions that allometric relationships could be used to derive the temporal variation of branch characteristics. The relationships between branch characteristics of beech trees were investigated by means of different databases where the insertion diameter, the total length, the chord length, the position of the balance point of the branch, and the weight were measured. In these analyses, some data (insertion diameter, branch length) are more numerous than others (weight, balance point).

Results and Discussion

Highly significant relationships were found between, (i) the insertion diameter and the branch length, (ii) a variable homogeneous to a volume, built by combining the insertion diameter and the length, and the branch mass and (iii) the position in length of the centre of gravity and the branch length (Fig. 1).

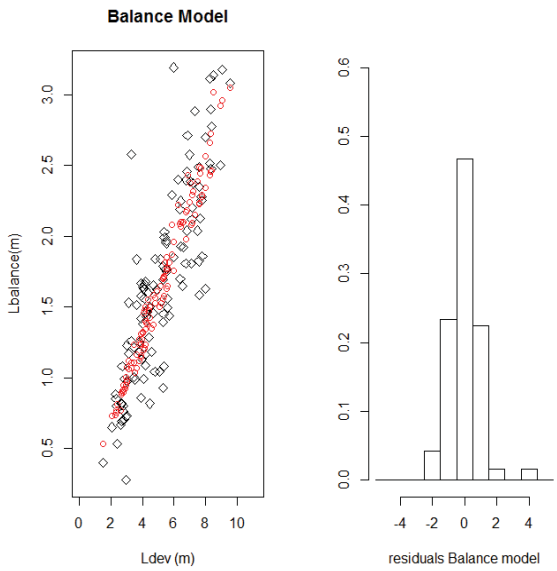

Fig. 1 : Relationship between the length of beech tree branches, and the location along the length of the balance point.

These last results suggest that the mass distribution of a beech branch can be successfully compared to a paraboloid with the same length and diameter. Furthermore, starting from the measured final configuration of each branch, making the assumption that the insertion angle of a branch does not vary a lot over ten years, and that the ten last annual ring formed at the branch insertion were measured, the previous relationships can be combined to compute the variations along ten years of the branch weight and bending moment.
The established relationships suggest that once again that mechanics is an important factor in the design of tree architecture.

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The motor function of wood: mechanisms underlying the generation of maturation stress during cell-wall formation

Tancrède ALMERAS

[1]Laboratory of mechanics and Civil Engineering LMGC (CNRS - U. Montpellier), France
tancrede.almeras@umontpellier.fr

Plant movements have fascinated scientists since at least the end of the 19th century. The occurrence of these movements rises many questions about the way they are regulated by the plant (mechano-perception, signal transduction and trigger of the movement) as well as about the nature of the "motor", namely the mechanisms that provide the mechanical energy for these movements. Rapid movements (such as e.g. seed ejections or carnivorous plants) are often based on the accumulation of elastic energy, either by osmotic pressure or by desiccation. The motor of slower movements of plant young stems and leaves is also well-identified, based on changes in cell turgor pressure inducing differential growth and thus bending movements.

However, for stiffer organs such as lignified stems, the magnitude of the mechanical stress provided by these turgor-related mechanisms is not enough to generate significant bending movements. The motor forces are provided by the so-called "maturation stress", a mechanical stress that is set-up in lignified cells during the formations of secondary cell-walls. Occurrence and magnitude of these maturation stresses (up to tens of MPa) in wood have long been evidenced experimentally. Their consequences on the internal state of stress of tree trunks and the way they induce stems movements are well understood and consistently explained by biomechanical models. Nevertheless, at sub-cellular scales, the mechanism by which motor forces are generated during cell-wall formation is still a matter of debate and an active research topic.

Woods generating mechanical stresses of large magnitude are called reactions woods. They are classically divided into "compression woods" that generate compressive stress on the lower side of tilted stems of gymnosperms, characterized by large microfibril angle (MFA) and high lignin content, and "tension woods", generating tensile stress in the upper side of tilted stems of angiosperms, and typically characterized by a specific "gelatinous" layer, devoid of lignin and having a very low MFA. Many works have evidenced that this classification is a simplification, some examples not conforming to it, such as compression wood found in some angiosperm species, tension wood found in gymnosperms, and angiosperm tension woods in which the G-layer is not apparent. Nevertheless, we will here concentrate of the mechanisms involved in typical compression wood and G-layer tension wood.

Comprehension of how compression wood works resulted from the analysis of structure /properties relationships, namely the link between MFA and the magnitude of maturation strains. By analogy with previous modeling of the drying shrinkage of compression wood, researchers have assumed that the source of mechanical energy was a dimensional variation (here swelling) of the lignified matrix, and that this movement was directed across the stiff microfibrils, so that, if the MFA is large, the stress is directed in the direction of the cell axis. In order to model jointly the maturation stress of both normal and compression wood as a continuum, it was necessary to assume that a second process was also at work, namely a shrinkage of the cellulose network along the microfibril direction. This model does account correctly for both the sign and order of magnitude of the maturation stress, in both the

longitudinal and tangential directions of woods, for both normal and compression woods. However, it is still limited in at least two aspects: (1) it provides an explanation for the role of lignification and MFA in the process, but does not identify at the molecular level what causes the swelling of the matrix and the shrinkage of the cellulose network; (2) it is not sufficient to explain the large tensile stress found in tension wood.

Regarding tension wood, a number of new experimental observations have been reported in the recent years, and various models have been proposed to explain the link between its structure and its biomechanical function. Most of these models are based on a description of the interaction between the unlignified matrix and cellulosic microfibrils. In this presentation, I will ty to summarize the models that have been proposed and confront them to the body of knowledge that has been accumulated in the literature. I will argue for a possible model based on the swelling of the matrix in a connected microfibril network. Although a lot of progress has been achieved in the biochemistry and molecular biology of the formation of the G-layer, the mechanism that induces the swelling of its gel-like matrix material is still not fully understood.

Recent progress on the study G-layers located in other tissues than wood (e.g. cortical fibers of annual plants) and on tension woods without visible G-layer may contribute to build soon a consistent picture of the mechanisms generating in high magnitude stress in plants, encompassing normal woods and all kinds of reaction woods.

Modifications in the cell wall during the development of tension wood in a G-layer and a non-G-layer species

Bruno Clair[1], Shan-Shan Chang[2], Jean-Romain Roussel[1], Françoise Quignard[3], Jacques Beauchêne[1'], Tancrède Alméras[2]

[1]CNRS, Ecologie des Forêts de Guyane (EcoFoG), Kourou, France
[1']CIRAD, Ecologie des Forêts de Guyane (EcoFoG), Kourou, France
[2]Laboratoire de Mécanique et Génie Civil (LMGC), CNRS, Université Montpellier, France
[3]Institut Charles Gerhardt, UMR 5253 CNRS-UM-ENSCM, Montpellier, France
bruno.clair@cnrs.fr

To recover verticality after disturbance, angiosperm trees produce "tension wood" allowing them to bend actively. In poplar, the tension stress has been shown to take place in a specific unlignified layer of the cell wall called G-layer (Yamamoto et al. 2005, Fang et al. 2008) , and to be supported by the cellulose microfibrils (Clair et al. 2011). However, the driving force of the stress generation in cellulose remained unclear.
Even more unknown is the mechanism of tress generation in species that do not produce G-layer.

Thanks to the measurement, by nitrogen adsorption-desorption, of the mesoporosity of the cell wall and its changes during maturation of poplar (*Populus deltoides* × *P. nigra*) tension wood and opposite wood, we have evidenced the progressive increase in the pore size during the development of the G-layer until its stabilisation in mature wood (Chang et al. 2015). This observation strongly supports the hypothesis of the swelling of the G-layer matrix during maturation as the origin of maturation stress in poplar tension wood.

Following this observation, similar mesoporosity measurements were performed on *Simarouba amara*, a species described to be devoid of G-layer in tension wood. Results show that, during the maturation of the cell wall, *S. amara* tension wood cell wall presents a mesoporosity similar to what is observed in poplar tension wood. A large scale study of the development of tension wood cell wall was therefore carried out on tilted seedlings, saplings and adults *S. amara* trees.

Microscopic observations were done on tranverse sections of normal and tension wood after staining or observed under UV light to assess the presence/absence of lignin. They have shown that *S. amara* produces a cell-wall layer with all of the typical characteristics of a G-layer, but that this can be observed only as a temporary stage of the cell-wall development because it is masked by a late lignification. Being thin and lignified, tension wood fibres cannot be distinguished from normal wood fibres in the mature wood of adult trees.

These observations indicate that the mechanism generating the high tensile stress in tension wood is likely to be the same in species with a typical G-layer and in some of the species where the G-layer cannot be observed in mature cells. A larger observation of the diversity of

tension wood development on numerous non-G-layer species would now be needed to allow for the generalisation of these observations.

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Evaluation of non-cellulosic polysaccharide distribution in differentiating and mature poplar tension wood fibres: abundance of rhamnogalacturonan I, presence of acetylated glucomannan and absence of xyloglucan in the G-layer

Guedes F T P[1], Laurans F[1], Quemener B[2], Secerovic A[1], Assor C[2], Boizot N[1], Vigouroux J[2], Lesage-Descauses M-C[1], Leplé J-C[1], Déjardin A[1], Pilate G[1]

[1] INRA, UR0588 Amélioration, Génétique et Physiologie Forestières, F–45075 Orléans cedex [2], France ; 2UR 1268, PVPP, INRA, rue de la Géraudière, BP 71627, 44316 Nantes, France
gilles.pilate@orleans.inra.fr

Introduction

Tension wood (TW) is produced by temperate hardwood trees in order to support their increasing load, orient their axes and cope with environmental cues such as wind. TW fibres often harbour a supplemental layer, the G-layer, rich in crystalline cellulose, containing matrix polysaccharides but no lignin. The tensile force responsible for the specific TW properties originates from the G-layer and is transmitted to cellulose microfibrils soon after their deposition during G-fibre maturation. This force is potentially generated from physical changes in the high porosity hydrogel recently identified in the G-layer.

In search for molecules with gelling properties…

In order to identify molecules potentially involved in hydrogel formation, the distribution of the different classes of non-cellulosic polysaccharides was evaluated during xylem fibre differentiation by immunolocalization, both in TW and in opposite wood (OW), a wood devoid of G-fibres. In parallel, differentiating G-layers were isolated and their polysaccharide composition determined.

We found a few promising candidates…

These complementary approaches provided us with data on the evolution of non-cellulosic polysaccharides during G-fibre differentiation: contrary to previous reports, we did not find any evidence for the presence of xyloglucan in poplar G-layers, but evidence was found for an abundance of rhamnogalacturonan type I pectins (RG-I) in the G-fibre, with an apparent progressive loss of RG-I side chains during G-layer maturation. Likewise, glucomannan deacetylation seems to take place during G-layer maturation. Both RG-I and deacetylated glucomannans may be involved in the formation of a gel. These novel results will be discussed in the light of previous studies on the subject.

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Biomechanical aspect of stem movement and the growth strains in monocotyledonous giant bamboo

Yan-San Huang[1,6], Chin-Mei Lee[2], Li-Fen Hung[5], Fu-Lan Hsu[3], Hui-Ting Chang[4] and Ling-Long Kuo-Huang[5]

[1] Department of Forestry, National Chung Hsing University
[2] Division of Forest Utilization, Taiwan Forestry Research Institute
[3] Division of Forest Chemistry, Taiwan Forestry Research Institute
[4]School of Forestry and Resource Conservation, National Taiwan University
[5] Department of Life Science, National Taiwan University
[6] Corresponding Author (yansanhuang@ntu.edu.tw)

Abstract

Plant biomechanics is an interdisciplinary science describing behavior of plants subjected to plant form and function. For coniferous and broad-leaf trees, growth stresses accumulate in the cell walls during the secondary growth year by year and a specific regular distribution pattern was formed to keep the trunks and branches at an equilibrium situation. These growth stresses represent an important physiological mechanism enabling the trees to adjust to environmental conditions. In woody trees, righting movement of branch or trunk depends on the mutual interaction of gravitational disturbance, phototropic response, and gravitropic correction. Four factors are involved in gravitropic correction, *i.e.* asymmetric growth strain, eccentric growth increment, heterogeneous longitudinal elasticity (MOE), and initial radius, which are associated with reaction wood production. In this paper, we review our former researches including (1) Growth strains in the trunk and branches of Taiwan red cypress, (2) Biomechanical modeling of gravitropic response of branches, and (3) Effects of diurnal self-weight change on the surface strain of the branch of *Araucaria cunninghamia.* For Taiwan red cypress (*Chamaecyparis formosensis*), growth strains and microfibril angles of branches are larger than those of trunks. Because the computed rates of gravitropic correction and gravitational disturbance had the same order of magnitude, the studied horizontal branch samples were close to biomechanical equilibrium.

Effects of the self-weight change of branches on the growth strain measurement are discussed in detail using the experimental results in which the self-weight change was directly measured by using strain gauges. Our current work refers to investigate the growth strains of the giant bamboo (*Dendrocalamus giganteus*), the biggest bamboo in the world, and the results showed that the number of secondary wall layers in the fiber cells increased during

ageing of the bamboo and growth strains also increased. Besides, we also investigate the righting process of the artificially tilted saplings of *Swietenia mahagoni*. We find that in order to avoid the influence of bending stress on the measured surface growth strain, the measurement should be made before the tilted trunk bending up apparently. In the analysis of gravitropic response, the four factors mentioned above and density variation over the cross section throughout the stem are considered in the further modified model to enhance the efficiency of evaluation.

Analysis of the mechanical interaction between a growing root and a granular substrate using the Discrete Element Method

M. Fakih[1,2] *, J.-Y. Delenne[3], F. Radjai[2,4] and T. Fourcaud[1]
[1] CIRAD UMR 51 AMAP, CIRAD-CNRS-INRA-IRD-UM, Montpellier, France
[2] CNRS UMR 5508 LMGC, CNRS-UM, France
[3] INRA UMR 1208 IATE, INRA-CIRAD-SupAgro-UM, France
[4] <MSE>[2] CNRS UMI 3466 CNRS-MIT, CEE, Massachusetts Institute of Technology, USA
*e-mail: mahmoud.fakih@cirad.fr

Introduction

Among numerous factors that explain the variability of root architecture, including genetics, environment and developmental instability (Forde, 2009), the mechanical interaction between growing roots and the soil substrate has often been under-estimated. We hypothesize that the heterogeneous structure of soil at the particle scale, evidenced by the broad distribution of forces, can significantly influence root growth trajectories and its ramification system. We use discrete-element numerical simulations to investigate, as a first step, the growth of a single root inside a granular material.

Objectives

The objectives are 1) to understand and quantify root-soil mechanical interactions at both the grain and plant scales, and 2) to develop a general biophysical law of root-soil interactions that can be used in Functional Structural Plant Models (FSPM) (Fourcaud et al., 2008).

Numerical Modeling

For this purpose we developed a numerical model of root-soil system based on the Discrete Element Method (DEM) (Radjai & Dubois, 2011). This approach is suited for the investigation of the effect of soil disorder on root variability at the particle scale. The simulations allow us to characterize the reorganization of grains under the action of a growing root. By return, the forces experienced by the root during its growth can be estimated numerically. These interactions are analysed using methods and tools provided by the physics of granular media.

The root is modelled using chains of connected spheroline elements. The growth is initiated from a circular element placed at the free surface of a granular bed prepared by random pluviation. This circle plays the role of meristem, which is constantly replicated at a given rate and pushed forward under the action of elastic forces. As a result, the root grows as a line of fixed thickness equal to the diameter of the circle and with prescribed stiffness and bending moment. The orientation of the meristem at every growth step is determined by the dynamics of the whole root under the action of its internal elastic forces and reaction forces exerted by the grains. Since the model is two-dimensional and the pore space is not open, we use two different diameters for the particles: a large diameter for the interactions between particles and a smaller diameter for the interactions between particles and root. The difference between the two diameters corresponds to the width of a gap at contact points through which the roots can pass. The ratio r of this gap to the root diameter is an essential model parameter.

Parametric Study

Several parameters being involved in the mechanical behaviour of the root-grains system, we first carried out a parametric study of their influence on forces at the root tip and the overall root trajectories. The major parameters affecting root trajectories and reaction forces were identified to be the root stiffness and gap ratio r, as discussed above. Our first results exhibit 3 different growth regimes: $r \ll 1$, $r \sim 1$ and $r \gg 1$. By analysing the evolution of reaction forces

exerted by grains on the root tip, we find that for all regimes, the mean force during the growth increases with root stiffness as a power law. For all values of root stiffness tested in each regime, we find a broad distribution of the forces experienced by the root apex. The distribution has the same functional form when the forces are normalized by the mean force for each stiffness. It is characterized by a decreasing power law for forces below the mean and an exponential fall-off for forces above the mean. This distribution seems thus to reflect the broad distribution of forces inside the granular material. It is also interesting to note that, since the forces scale with the mean force, the standard deviation of forces also scales with the mean force.

Perspective

The modelling approach developed in this work will be applied to characterize the shape of the roots and the influence of soil properties, such as size polydispersity and cohesion between grains, on their development. The numerical results will be compared to experimental data from the Rhizoscope genotypic platform developed at CIRAD, which allow observing real plant roots growing in ballotini (Ahmadi et al., 2014).

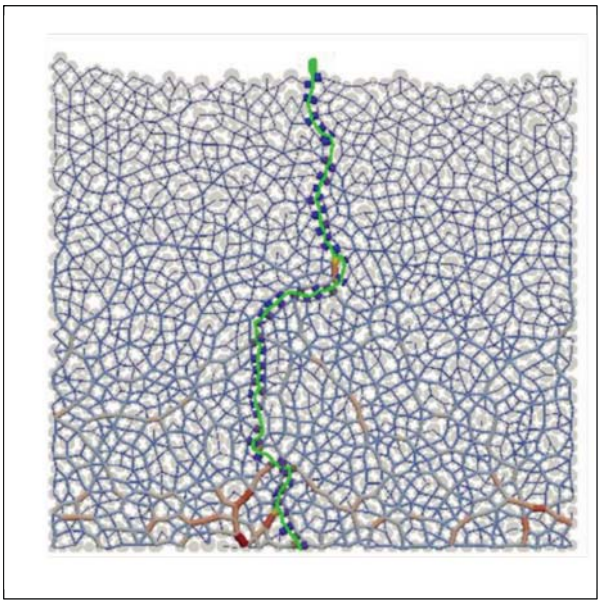

Fig. 1 : DEM simulation of root growth and a map of force chains in the granular bed.

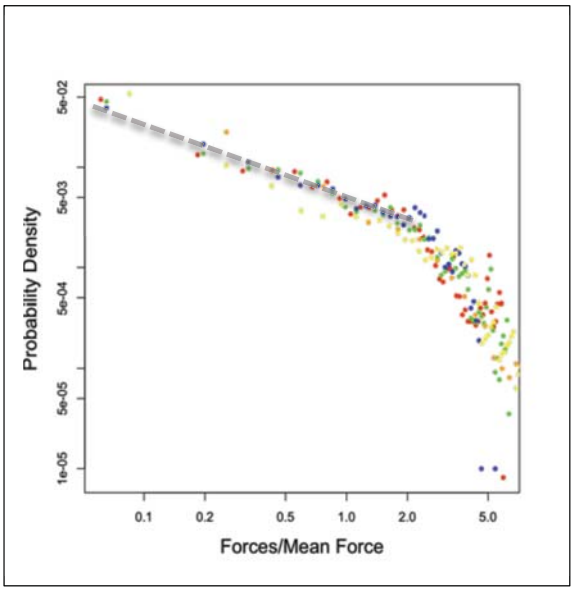

Fig. 2 :Probability density function of forces for several root trajectories

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Mapping of the structure of a corn kernel by three complementary experimental techniques.

F. Mabille[1], A. Sadoudi[1], T. Ruiz[1], R. Sidiboulenouar[2], C. Coillot[2]
mabille@supagro.inra.fr
[1] INRA, UMR IATE, Montpellier
[2] Laboratoire Charles Coulomb – BioNanoNMRI, Université Montpellier 2

Corn is an annual herbaceous tropical plant, native to Central America. With more than 1 billion tons a year, corn is the most cultivated cereal in the world before the wheat and rice. Corn is used in many industrial field : animal feed (which consumes about two thirds of the production), food (biscuit, oil, drink, starch ...), pharmaceutical industry (cholagogues property, diuretics and anti lithiasic), chemical industry (glue, starch, biogas, biofuels, bioplastics ...). On the plant, the cob of the corn is surrounded by a leaf. Depending on the variety, the ear of corn has very variable dimensions ranging from 5 to 45 cm long with a diameter of 3 to 8 cm. At maturity, it usually contains between 400 and 500 grains.
Corn grain is a caryopsis formed of three distinct histological sections: the germ, the endosperm, and envelopes of the fruit. The endosperm, which can be schematized as a mass of starch granules embedded in a protein matrix, is surrounded by a peripheral casing, called aleurone layer. The corn endosperm has two types of texture. The endosperm part next to the germ is white and floury and has crumbly texture. peripherical endosperm is colorful, its texture is compact and glassy. Depending on the variety, the proportions of mealy / glass parts are highly variable and impact the thermo-hydro-mechanical properties of the grains.
The harvest of corn is a significant water content ranging from 0.35 to 0.45 g_{water}/g_{db}. To allow their conservation, corns must be dried to a moisture content of about 0.14 g_{water}/g_{db}. During this operation, the relative deformations of the various histological parts generally lead to the occurrence of microcracks in the glassy endosperm, which can spread to sizes of the order of the scale of the grain.

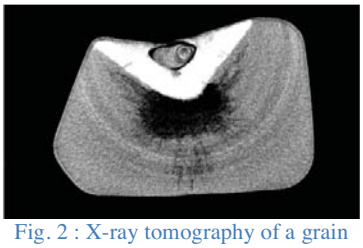
Fig. 2 : X-ray tomography of a grain

This fragmentation of the vitreous endosperm is detrimental to some industrial applications that require the largest possible semolina. The study of the mechanical impact of water transfers in the histological structures of the grain during a drying process, is a scientific question related to the control of corn fragmentation properties. The work presented is a combination of three experimental techniques used for volume mapping.
Tomography X (Montpellier RIO Imaging) allows initially to map the different areas according to their density. The low density areas, such as central mealy area or invasive micro-cracks in the glass endosperm, can thus be located and quantified (Fig. 1). The germ clearly appears in white, the peripheral part of the glassy endosperm in gray and the mealy and porous part of the center endosperm in the black. Some radial cracks not opening at the periphery, can be observed in the glassy area, begenning at the interface with the farinaceous area. Mapping kinetics of sorption and desorption is performed by NMR (Fig. 2).

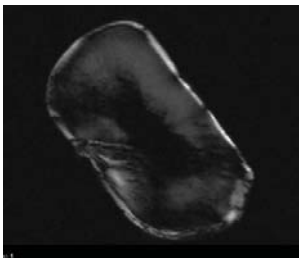
Fig. 1 : NMR image of a grain

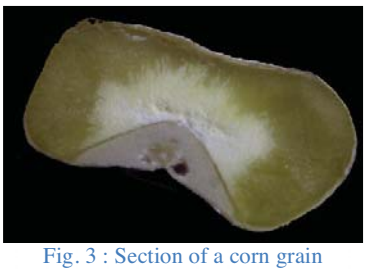
Fig. 3 : Section of a corn grain

These images allow to identify areas and estimate the water transfer ratio. The grain is then again analyzed X-ray tomography to quantify the impact of an thermo-hydro-mechanical stress on hydro-textural state and the developpment of cracks in the grain. Finally, the grain is subjected to destructive mapping in real images (Fig. 3) to determine the histological areas, and thus validate previous observations.
These three techniques are used together to determine quantitatively the volumes of the different histological parts of a grain, to quantify and locate water in these compartments, and to estimate the evolution of the states of porosity and cracking in the grain.

Use of vibrational properties to categorize the different types of wood within trees - as compared to interspecific diversity

Iris Brémaud[1], Pierre Cabrolier[1]

[1]WOOD team, Laboratory of Mechanics and Civil Engineering, CNRS, Université de Montpellier, France
iris.bremaud@univ-montp2.fr

Introduction

Different types of wood are formed during the life of trees, either systematically, or in response to environmental constraints. These different types of wood can be characterized by differences in both microstructure and chemistry (e.g. in reaction Vs. normal wood) or mostly in chemistry (e.g. in heartwood vs. sapwood). Viscoelastic vibrational properties (specific dynamic modulus of elasticity E'/ρ and damping coefficient tanδ) can constitute interesting markers of such differences, as E'/ρ is primarily affected by orientation of wood elements (microfibril angle or sometimes grain angle), while tanδ is strongly dependent on the chemical composition of the cell wall matrix substance (amorphous polymers and extractives). Secondary metabolites (extractives), in particular, can modify damping to a great extent, and are strongly connected to botanical classification. In our previous contribution to the International Plant Biomechanics Conference, we presented how vibrational properties, and especially damping coefficient, could be considered as a marker of taxonomical classification (Brémaud et al. 2009). The present paper aims at observing the way in which such diversity in vibrational properties is built, through the different (micro-) structural and chemical features associated to the different types of wood formed during the life of trees, either systematically or in response to occasional stresses. Experimental results on heartwood Vs. sapwood and on compression Vs. normal wood are presented for contrasted species. These results will be compared to other information from the literature in order to build a typology of wood types within trees on the basis of vibrational properties.

Materials and methods

Samples of heartwood and sapwood from the same tree were collected from 16 species (4 softwoods, 2 temperate hardwoods and 10 tropical hardwoods). Total number of tested specimens amounted to circa 250 for heartwood and circa 130 for sapwood. Samples of compression wood and of normal or opposite wood were collected from 3 softwood species (Brémaud et al. 2013) and from 1 atypical hardwood (Cabrolier and Brémaud 2015, presented to PBM8). For a sub-set of studied species, the sampling covered full radial variations from pith to bark.

Experiments were conducted on small specimens in longitudinal direction ($150\times12\times2mm^3$, L×R×T or L×T×R). Vibrational properties were measured using non-contact forced bending vibrations (Brémaud et al. 2012). Systematic tests were done on "air-dry" wood conditioned at 20°C and 65%RH. For a sub-set of specimens, vibrational measurements were also conducted at different relative humidities and on water-saturated wood. FTIR-ATR spectra were obtained in order to get some comparative information on chemical composition.

Results and discussion

The analysis of the relation between damping coefficient (tanδ) and specific modulus of elasticity (E'/ρ) provides a way to separate effects of orientation (microfibril angle or grain angle) from those of changes in chemistry. When orientation effects only are involved, a

general and "standard" trend curve between tanδ and E'/ρ is observed (Brémaud et al. 2012). When chemical composition is changed, tanδ is increased or decreased at a given value of E'/ρ. When observing compression Vs. normal wood of softwoods, E'/ρ was decreased by high microfibril angle (as is well known), however damping was lower than expected, due to more condensed type of lignins in compression wood (Brémaud et al. 2013). However this figure was less clear in the case of compression wood formed in an atypical hardwood (Cabrolier and Brémaud 2015). This raises the question of a potential of the type of lignins to discriminate vibrational properties between softwoods and hardwoods. This might be explored by comparing sapwoods of different species (that are supposedly not yet impregnated by extractives). However, if sapwoods do exhibit, in average, a "standard" damping, this is less true when observing different species separately: in some species sapwood has "atypical" damping also. In some case this was found to be due to precursors compounds present in sapwood, having an opposite effect to the final extractives in the heartwood (Yano, 1994). When comparing wood types formed in a same tree, the heartwoods had a much higher inter-specific diversity than the sapwoods did. The heartwood formation resulted in differences in damping between sapwood and heartwood from 0% to -50%. When observing the moisture-dependence of vibrational properties, different trends were observed depending on wood type, and also on species. The observation at higher moisture content can be better related to the situation in the living tree, on one hand. But mostly, analysis of these trends in moisture-dependence of damping can help to study some hypothesis about the link between chemical nature of extractive, and their "mechanical activity" (Minato et al. 1997, 2010).

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Nanocharacterization of chemical and mechanical properties of plant cell walls and lignocellulosic bioinspired assemblies

Chabbert B[1,2], Muraille L[1,2,3], Bercu B[3], Aguié-Béghin V[1,2], Molinari M[3]

[1]Inra, UMR 614 Fractionnement des Agroressources et Environnement, Reims, France
[2]Université de Reims Champagne Ardenne, Inra, UMR 614 Fractionnement des Agroressources et Environnement, Reims, France
[3]Laboratoire de Recherches en Nanosciences, Université de Reims Champagne Ardenne, Reims, France
chabbert@reims.inra.fr

Lignocellulosic resources have been recognized to be the most promising sustainable and renewable source of materials for the elaboration of composites (Thakur et al, 2014). Notably, plant cell walls have are multilayered materials resulting from the assembly of different biopolymers: cellulose, hemicelluloses and lignins. These components are interconnected through a variety of covalent and non covalent bonds that make a highly organized network. This structural organization plays a key role in the regulation of plant cell growth, mechanical properties and biodegradation.

Chemical and structural variations within cell wall layers will not only affect the mechanical properties of the fibers but also the different preparation steps such as the defibration, and the interface properties between the fibers and the matrix in the composites (Salmen and Burgert, 2009, Burgert and Keplinger, 2013). Therefore, anticipating and controlling the mechanical properties of the composites implies to evaluate a multi-scale analysis of the morphological and physicochemical properties of the fibers (Le Digou et al, 2014). Nanoscale investigation of the chemical and physical properties of the cell wall could provide a more comprehensive view on the molecular and supramolecular mechanisms that promote their physicochemical properties and reactivity is still challenging

In order to get information of the fiber properties at nanoscale level, the potentialities of atomic force microscopy (AFM) were assessed in different modes while comparing plant cell walls and of bioinspired polymer films. To this end, non-lignified and lignified systems were obtained using cellulose nanocrystals, hemicelluloses and lignin as single, binary and ternary nanocomposites (Muraille et al, 2015). Thanks to the use of adhesion measurements via tip functionalization with lignocellulosic polymers (Estephan et al, 2011), of nanoInfrared absorption, nanomechanical measurements, and by comparison between native cell walls (poplar, hemp) and lignocellulosic bioinspired films, we will try to understand the relationship between the composition and the supramolecular organization of lignocellulosic polymers and their nanoscale properties.

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ROLE OF LIGNIN IN THE STRUCTURE OF WOOD FIBRES

Lennart Salmén
Biorefining, Innventia, Sweden
lennart.salmen@innventia.com

Abstract
Wood materials are nowadays being viewed with increasing interest, as a green resource. Its utilization in new types of applications is also highly desired. Thus a better understanding of the wood ultrastructure and how the wood components are interacting in building up its properties is highly demanded. In the wood, the cellulose fibril aggregates dominates properties especially along the grain why the contribution from the other wood polymers and its role in the arrangement of the cell wall structure is often neglected. However their importance both in affecting transverse fiber properties and in their role as a spacer affecting cellulose aggregation during processing cannot be neglected. As the cellulose microfibrils make up a highly irregular lenticular three-dimensional structure the role of the matrix polymers in-between may be viewed in different ways. The understanding of the load bearing capacities of the lignin in different structural elements may be a key factor.

It is generally considered that lignin is contributing to the compressive resistance of the wood cell wall. Thus compression wood has higher lignin content as well as a more cross-linked lignin, presumably stiffer than the cell wall of normal wood. Clearly, in order to pose strong compressive strength, the rather thin cellulose fibril aggregates have to be prevented from collapse. With regard to the stress contribution of the different components the views are still unclear.

With the use of dynamic FTIR spectroscopy information regarding the response of the different wood polymers to mechanical forces at the molecular level may be gained. The interaction between and with the different hemicelluloses as well as of the lignin may to a higher extent be examined. Utilizing polarization FTIR the orientation and the degree of orientation of these components in different structures have also been analyzed indicating orientation effects both for the hemicelluloses and lignin. The stress related spectroscopic signals show clearly that the cellulose is directly contributing to the load bearing capacity of wood (Gierlinger et al. 2006; Salmén and Bergström 2009). When using the same tools focusing on the lignin component in wood no contribution what so ever is detected (Gierlinger, Schwanninger et al. 2006; Salmén and Bergström 2009). Examining of higher lignin-rich material, i.e. primary cell wall material, shows that lignin indeed may be detected to be deformed and contributing to the stress transfer in a material. Thus it could be questioned whether or not the lignin in the fiber cell wall wood structure is more acting as filler taking upon compressive loads than contributing to tensile strength properties, as indicated in Figure 1.

When chemically attacking the wood structure, however only mildly as in the initial phase of the chemical cooking process, the structure seems to be altered in the way that lignin is taking part in the stress transfer, see Figure 2. Thus by partly removing some of the hemicellulose and lignin the possibilities of a direct connection in stress transfer between cellulose and lignin seems to occur. It could be questioned if what is seen is the development of LCC:s in the initial phase of cooking. The yield of the wood for these conditions is around 80 to 85%. The fact that the strength properties of cellulose products are at its maximum at this yield could well be explained as due to such bonds formed. The implication for our understanding of the wood cell wall structure will be further dealt upon.

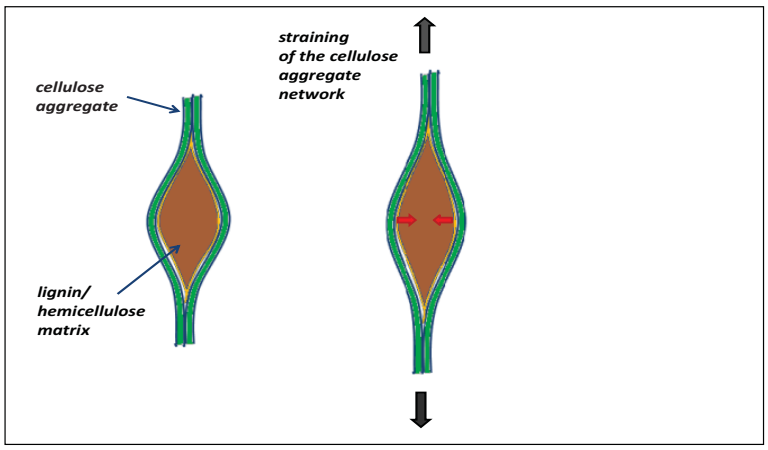

Fig. 1 : Cellulose aggregate structure showing the straining of the cellulose aggregate network, indicating the compressive resistance of the lignin/hemicellulose matrix. The elliptical spaces among the cellulose aggregates have a length-to-width ratio of about 2 and a minor diameter across the ellipse of 5 to 10 nm.

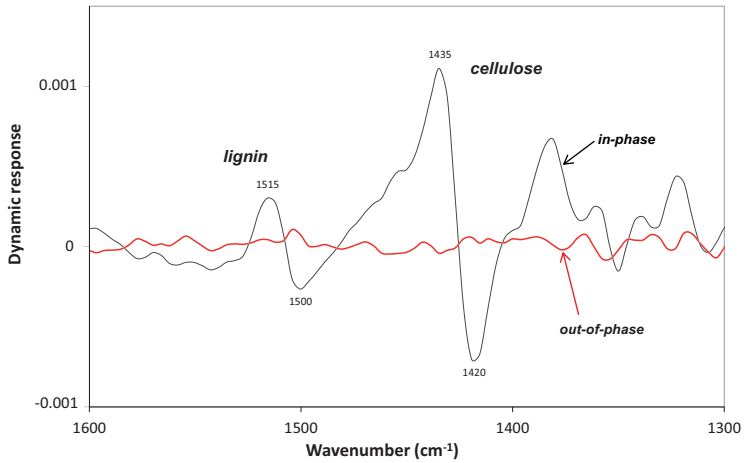

Fig. 2 : Dynamic FTIR spectra of a paper made of pulp subjected to only an initial impregantion stage of cooking redering a yield of 80%. Both the cellulose, at a wavenumber of 1430 cm^{-1}, and the lignin at a wavenumber of 1507 cm^{-1}, show split peaks indicating that they take part in the stress transfer in the material.

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Water sorption in S2 layer: role of the crystalline-amorphous interface

Karol Kulasinski[1,2], Robert Guyer[3,4], Dominique Derome[2], Jan Carmeliet[1,2]

1 Chair of Building Physics, ETHZ Swiss Federal University of Technology Zurich, Switzerland

2 Laboratory for Multiscale Studies in Building Physics, Empa, Swiss Federal Laboratories for Materials Science and Technology, Dübendorf, Switzerland

3 Solid Earth Geophysics Group, Los Alamos National Laboratory, New Mexico, United States

4 Department of Physics, University of Nevada, Reno, Nevada United States

dominique.derome@empa.ch

Introduction

Biopolymers, such as cellulose or hemicelluloses, are of increasing interest due to their potential use in biofuels industry and as building blocks in new materials. The interaction of many biopolymers with water is known to rearrange their internal structure, make them moisture sensitive and influence their physical properties. We study the different components, a cellulose microfibril and a microfibril aggregate in terms of the effect of water sorption.

Water sorption in wood microfibril simulated with Molecular dynamics

Using Molecular Dynamics (MD) simulations we constructed and investigated a three-phase model of a cellulose microfibril aggregate that consists of crystalline cellulose, amorphous hemicellulose and lignin. The model is studied with MD simulations, where the explicitly present atoms are moved by integration of the Newton equation of motion. The MD models have size of a few nanometers or several thousands of atoms and are put in full periodic boundary conditions. A typical time scale of a simulation is 10 ns with resolution of 1 fs. Construction of valid models of crystalline and amorphous celluloses, hemicellulose (galactoglucomannan) and lignin has been performed as the indispensable step to construct more complex models of S2 layer.

Water molecules are adsorbed in the amorphous hemicellulose and an excess of sorption on the interface, while no sorption occurs in the crystalline cellulose. The water molecules, adsorbed on the interface push away the polymer chains, forcing the two phases to separate. This causes the breaking of hydrogen bonds, particularly pronounced on the interface. As a result, the stiffness, particularly in shear, decreases non-linearly, which is explained by breaking of numerous hydrogen bonds due of sorption.

Role of the crystalline-amorphous interface

The existence of two different regions in moisture response are demonstrated. At low moisture content value (below 0.05) the moisture is uniformly adsorbed within hemicellulose, breaking a small amount of the hydrogen bonds. The microfibril does not swell and the porosity does not change whereas the stiffness can even increase due to additional H_2O-polymer hydrogen bonds bridges. As the moisture content increases, water molecules are adsorbed in the bulk amorphous phase, but also preferentially at the interface, leading to a swelling, proportional to the porosity increase and a mechanical weakening.

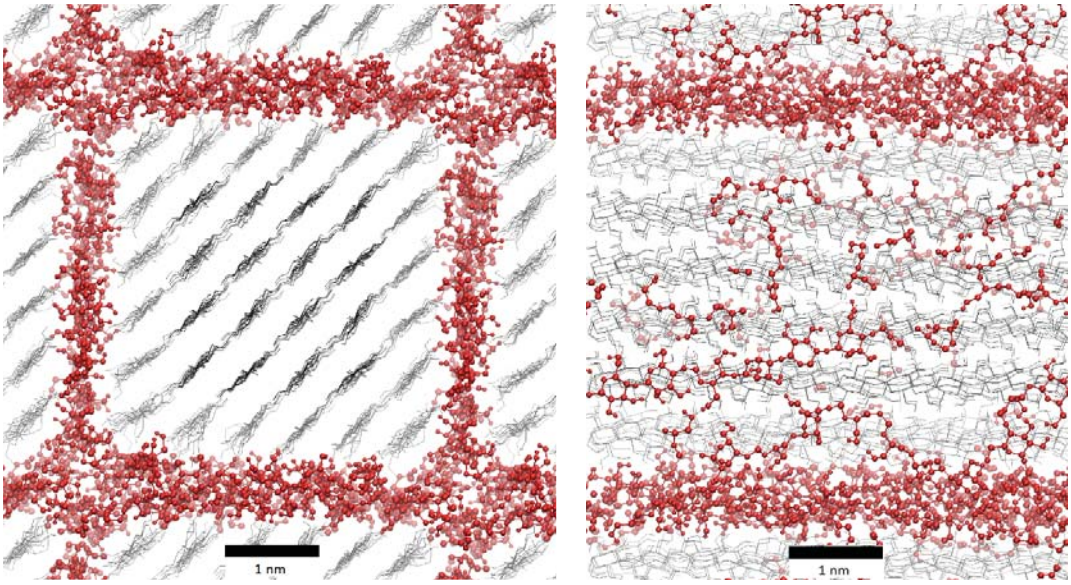

Fig. 1 : Structure of a cellulose microfibril in periodic boundary conditions. View (left) across the chains, and (right) along the chains.

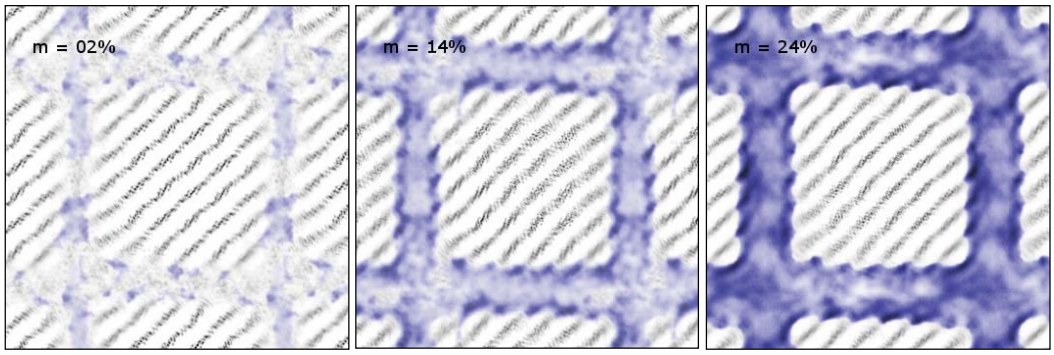

Fig. 2 : Density profiles over 2 ns trajectory of the microfibril (grey) and adsorbed water (blue). Adsorption shown at 3 stages: (left) m=2%, (center) m=14%, (right) m=24%.

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Micromechanical and Structural Characterization of Genetically Modified Poplar

M.Özparpucu[1,2], M. Rüggeberg[1,2], I. Burgert[1,2],

[1] Institute for Building Materials (IfB), ETH Zürich, 8093 Zürich, Switzerland
[2]Laboratory of Applied Wood Materials, 8600 Dübendorf, Switzerland
merveo@ethz.ch

Abstract

Wood represents a hierarchical, anisotropic fibre composite material with excellent mechanical properties. Despite its wide use as construction material, its mechanical behavior has not been entirely understood. While the mechanical role of cellulose in the secondary cell wall has been subject of various studies, the specific influence of the single matrix components, hemicellulose and lignin, and their interaction in the wall under mechanical load remain partly unraveled.

In the present study, we elucidate structure-property relationships of transgenic poplar tissue (3-4 months old). In these mutants, amount and composition of either lignin or hemicelluloses had been altered. We carried out micromechanical analysis together with X-ray diffraction, Fourier transform infrared spectroscopy (FTIR) and Raman analysis, to link the mechanical behavior to the ultrastructure and the biochemical composition of the cell walls. The mechanical parameters extracted from the experiments were normalized to density to reveal changes in mechanical properties at the cell wall level. By using genetically modified poplars and characterizing the altered cell wall structure in comparison with that of wildtype plants, we aim at elucidating the specific influence of the single components on the overall mechanical behavior of the plant cell wall and thus gain a better understanding of wood cell wall mechanics.

Experimental characterization of hydrogel swelling under plant cell wall environment

C. Assor[1, 3], F. Quignard[2], T. Alméras[3] and O. Arnould[3]

[1] UMR Ingénérie des Agropolymères et Technologies Emergentes, Sup'Agro, INRA, Montpellier, France
[2] Institut Charles Gerhardt - UMR 5253, Equipe Matériaux Avancés pour la Catalyse et la Santé , Montpellier, France
[3]Laboratoire de Mécanique et Génie Civil (LMGC), CNRS, Université de Montpellier, France
carole.assor@supagro.inra.fr

Pectins are one of the major components of primary cell walls and the middle lamella of dicotyledons (Carpita and Gibeaut 1993). Pectins play a central role in the control of cellular adhesion and thereby of the rheological properties of the wall. During the plant development, pectins are involved in several "active" phenomena as germination, growth cell acting on the cell turgor, cell wall matrix swelling and cell adhesion. A set of well-known enzymes modulate their structures to make their properties adapted to the plant requirements. Although there is an established literature on pectin structure and the way by which they are modulated by enzymes in the plant cell walls (Senechal at al., 2014), their interactions with others cell wall polymers and their mechanical behavior upon physiological conditions remain unclear. Some relevant experiments were carried out to characterize pectin swelling at high concentrations (>30% w/w) from samples under film and gel forms and different counterions under osmotic stress (Zsivanovits et al., 2004), but any were established under physiological conditions.

In this study, an original device was designed to characterize the mechanical behavior of hydrogels submitted to osmotic stress on conditions that mimic plant cell wall environment (Figure 1). Cylindric samples (10mm of diameter X10 mm of height) are placed in a stainless steel closed chamber in which water uptake, due to osmotic stress, occurs through a porous stone located at the bottom of the gel. At the opposite side of the sample, a force cell allows to measure the stress developed by the flow of water through the gel. As sample deformation is prevented by locking the longitudinal direction, the stress determined from the force cell correspond to the water pressure. The measurement of change in force during the time test duration allows determining the kinetic of water uptake as well as the maximum stress (Figure 2). The small sample size tested will permit both to characterize extracted polymers from the plant cell walls and to apply enzymatic treatments on polymers during measurements.

A set of tests was established on a model hydrogel made of alginates (with high (G) and low (M) galacturonate units ratio) at different content (1, 2 and 3% of their dry mass) formed in a 0,1M of various counterions solutions (cobalt, calcium and manganese). In parallel, the water uptake of these samples under free deformation conditions and characterization of their elastic properties were done. The integration of experimental results exhibits a good relationship between G', P and ε that validate the device.

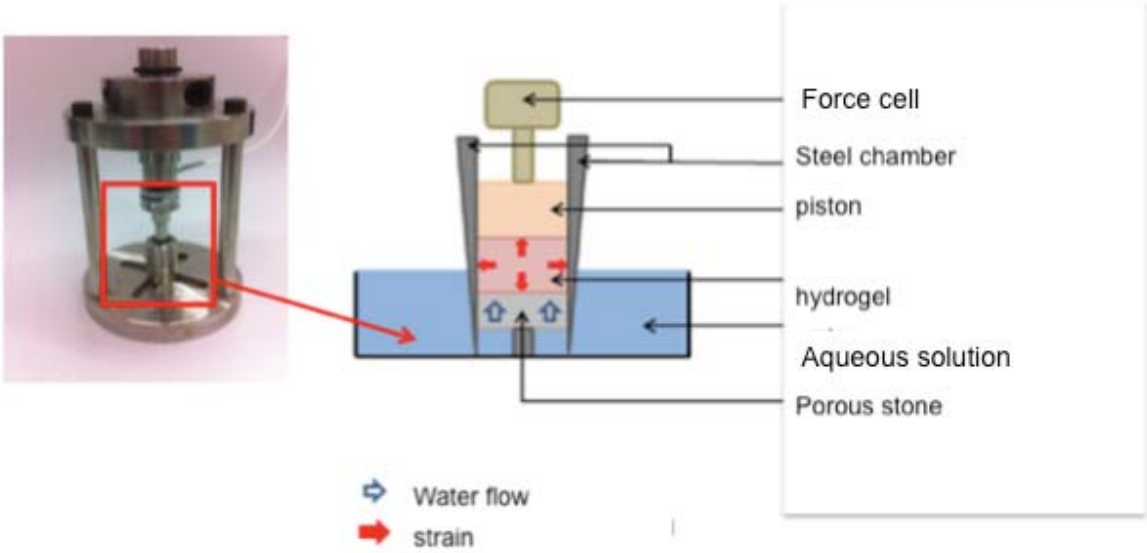

Figure 1: Experimental device develop to characterize swelling of hydrogel in aqueous solutions (Halle mécatronique, LMGC, Montpellier, France).

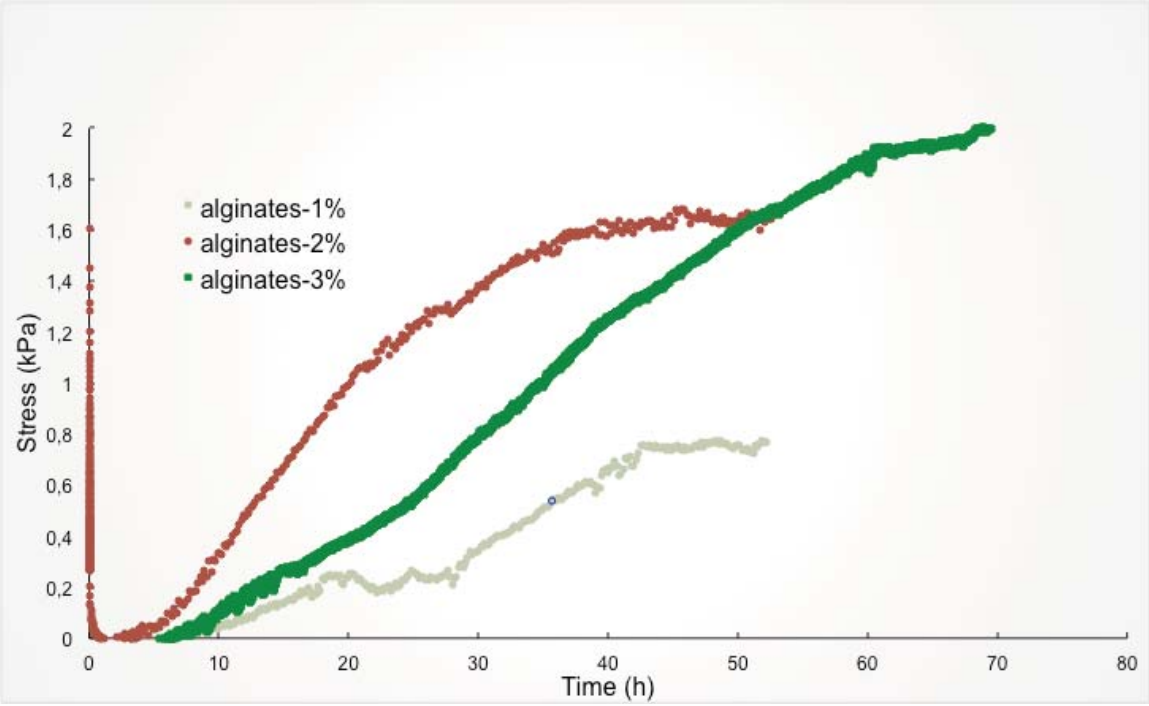

Figure 2: Evolution of the stress during water flow through the gel in the experimental device at different alginate ratios (Figure 1).

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Nondestructive Predictors of Stalk and Stem Strength

Daniel Robertson, Margaret Julias, Shien Yang Lee, Douglas Cook

Division of Engineering, New York University – Abu Dhabi, United Arab Emirates
djr7@nyu.edu

Introduction

Accurate measurements of stem strength are vital to ecological and evolutionary questions in plant biomechanics. However, few studies describing bending test methodologies are found in the scientific plant literature. We hypothesized the most commonly employed method of measuring bending strength induces premature stem failure and produces inaccurate strength measurements. We also hypothesized that stem *flexural rigidity* and *area moment of inertia* (two engineering quantities used to characterize bending stiffness) are strong predictors of stem strength that outperform current nondestructive testing methodologies.

Methods

Four mechanical testing regimes (internode loaded 3-pt bending, node loaded 3-pt bending, 4-pt bending and transverse compression) were applied to bamboo culms, giant reeds, and maize stalks. Flexural rigidity measurements were obtained by deflecting stems by 5mm and moment of inertia calculations were obtained from computed tomography (CT) scans. Rind puncture resistance (the most commonly employed nondestructive predictor of stalk strength) was measured to provide a benchmark against which to compare flexural rigidity and moment of inertia predictions.

Results

The most commonly employed testing methodology in the literature (internode loaded 3-pt bending) was found to induce premature stem failure and under predict stem strength by 40% to 60% (Fig. 1, $p<0.001$). A modified 3-pt bending method was therefore created which accurately measured stem strength. Flexural rigidity and area moment of inertia were better predictors of stalk strength than rind puncture resistance. For maize rind puncture resistance predicted 18% of the variation in stalk strength, while moment of inertia predicted 73% and flexural rigidity predicted 81% of the variation in stalk bending strength (Fig. 2).

Discussion

Sound testing methodologies are fundamental to scientific research. Presenting the current study at Plant Biomechanics 2015 will enable attendees to improve the accuracy and efficiency of future ecological, evolutionary, and agronomic plant biomechanics studies. Handheld tools for acquiring flexural rigidity measurements are currently being developed in our laboratory that will enable nondestructive (i.e. do not damage the plant) acquisition of strength data, thereby enabling analysis of stalk strength throughout a single plants lifecycle.

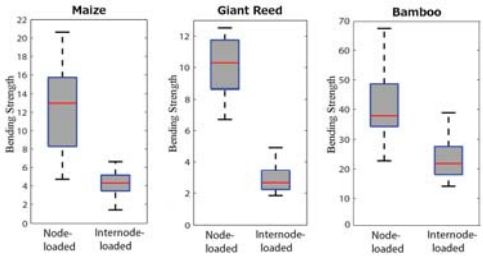

Fig. 1: Bending strength box plots

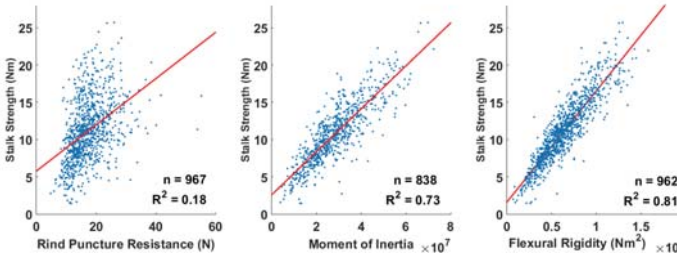

Fig. 2: Scatter plots and regression lines for maize

Impacts of Wind Loading on Tree Form and Wood Properties

John R Moore[1]

[1]Forest Systems, Scion, New Zealand
john.moore@scionresearch.com

Summary

The term thigmomorphogenesis was coined by Jaffe (1973) to describe the response of plants to mechanical stimuli, including wind-induced movement. However, plant responses to wind loading were studied long before this time, with the earliest observation of the effects of wind on plant form dating back to 300 B.C. Trees exhibit a range of responses to wind, ranging from modifications to stem shape, reductions in height growth, increases in diameter growth, changes in branch structure, and changes in wood properties (Telewski 1995). The effects of wind on stem shape have been widely studied and the requirement for a tree stem to resist wind loading has been put forward as a theory to explain stem shape (Metzger 1893; Larson 1963). This theory assumed that the cambium produces new wood in such a way to equalize the distribution of stresses along the outer surface of the stem. This is often referred to as the "uniform stress theory" and has been the subject of much debate the scientific community (Niklas and Spatz 2000; Mattheck 2000; Morgan and Cannell 1994; Dean et al. 2002). Experimental evidence for the impacts of wind on trees has come from a number of studies in which trees were either mechanically flexed or guyed to prevent them from swaying (e.g. Burton and Smith 1972; Mitchell 2003; Moore et al. 2014; Jacobs 1954, 1939; Mickovski and Ennos 2003; Watt et al. 2009; Jones et al. 2013). In this keynote paper, I present an overview of research examining the effects of wind loading on tree form and wood properties, with a particular focus on studies that have been carried out in radiata pine (*Pinus radiata* D. Don).

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Plant Wind Damage

Barry Gardiner[1, 2], Bruno Moulia[3, 4], Peter Berry[5]

[1]INRA, UMR 1391 ISPA, F-33140, Villenave d'Ornon, France
[2]Bordeaux Sciences Agro, UMR 1391 ISPA, F-33170, Gradignan, France
[3]INRA, UMR 547 PIAF, F-63100 Clermont-Ferrand, France
[4]Clermont Univ., Univ. Blaise Pascal, UMR 547 PIAF, F-63100 Clermont-Ferrand, France
[5]ADAS High Mowthorpe, Duggleby, Malton, North Yorkshire YO178BP, UK
barry.gardiner@bordeaux.inra.fr

Introduction

In addition to the requirement to grow almost all land plants need to balance the need to photosynthesise, to transport water, to reproduce, and to provide mechanical support under static and dynamic loading. One of the major sources of dynamic loading on plants is the wind. The subject has been given a detailed treatment in de Langre (2008) and there are reviews of the damaging impact of wind on forests by Gardiner *et al*. (2013) and on crops by Baker *et al*. (2014). In this paper we focus on the physics of wind motion and wind loading in plant canopies, we show how plants are damaged by the wind, and we make a comparison of the similarities and differences in the types of wind damage to different plant groups.

Wind in the Boundary Layer

The air in the boundary layer of the earth's atmosphere is in almost constant motion primarily due to pressure variations created by differential heating of the earth's surface by the sun. Depending on the latitude and the meteorological conditions the winds generated can have very different intensities, direction and persistence. In addition the wind flow becomes increasingly turbulent close to the earth's surface where plants grow. Wind speeds are found to vary at different time scales from yearly, to approximately every 4 days (the synoptic scale), with a peak at around 12 hours (the semi-diurnal scale representing day to night differences), down to turbulence at time scales between minutes and tenths of seconds. This description is a gross simplification of the complexity of motions and dynamics in the atmospheric boundary layer and within plant canopies but it does illustrate that plants live in an environment with wind speeds varying over a range of time scales from seconds to years and they need to have mechanical structures that can withstand both short-term gusts and more persistent mean winds. Furthermore, the wind environment will change over the lifetime of a plant with generally increasing wind speeds and wind loads as a plant grows and increases in height. Therefore plants have adapted to these changing mechanical requirements through a range of morphological, chemical and physical adjustments at every level from the cell wall, tissue and the whole plant in order to provide better biomechanical performance (Gibson, 2012).

Mechanics of bending in stem and roots

The force on plants from the wind is due to two types of drag. The first is viscous drag on the elements of the plant where the flow has to come to zero at the plant surface and the second is pressure or "form" drag due to the pressure differential that develops from the front to the back of each element of the plant. When the wind speed is low, viscous drag dominates (Reynolds number < 103), but as the wind speed increases pressure drag becomes dominant and is usually of the order of 3 times the viscous drag (Patton and Finnigan, 2013). Even when there is no wind there is loading on a plant due to its self-weight. Plants have adapted to deal to this loading and the internal stem structure and morphology of plants is adapted to avoid buckling, cellular collapse or other types of stem failure (Niklas and Spatz, 2012). In

addition the plants need to maintain an orientation that maximises their opportunities to capture light for photosynthesis. They maintain the position of the stem and leaves and branches either through turgor pressure (Dumais and Forterre, 2012) or in woody plants by use of internal differential growth stresses formed during cell maturation. (Fournier *et al*., 2014).

When a plant is subject to wind loading there is the direct loading of the wind on the plant canopy and also additional gravitational loads of the displaced parts of the plant (Peltola, 2006). The plant does not topple or uproot if the elastic restoring forces in the stem and the root system together with the counterbalancing load of the displaced root system are able to resist the combined wind and gravitational forces (Coutts, 1986) and it does not break if the modulus of rupture of the plant material is greater than maximum stress produced at the outside of the stem. Spatz and Bruechert (2000) demonstrated that plants are structured in a way that they approach their biomechanical limits within controlled safety margins but that snow or strong winds can cause these margins to be exceeded and lead to damage.

Wind damage to crop plants

Wind damage to plants can vary on scales and intensity from damage to the cuticle of individual needles and leaves to regional scale damage in which whole plant communities are destroyed such as following a hurricane or typhoon (Figure 1). Wind damage to crop plants often consists of damage to leaves or young plants through abrasion induced by the wind, leading to faster water loss and desiccation. Damage from wind-blown soil or sand can be a particular problem where crops are grown on sandy soils. Wind can also cause leaf tearing in many species, which can lead to temporary increase in water loss. In addition in grasses leaf folding can damage the cuticle and may lead to severe water loss and even wilting and death.

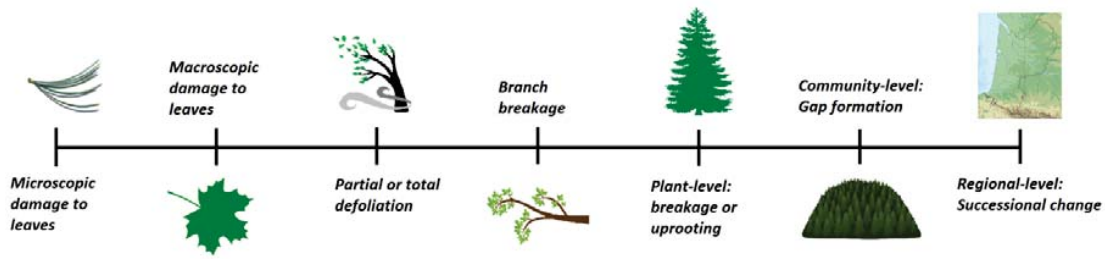

Figure 1: Wind damage to plants occurs at scales from surface damage to leaves and needles to regional scale damage from major storms (Courtesy Tommaso Locatelli)

Many crops are susceptible to wind damage by lodging (Figure 2). Lodging can take the form of stem lodging due to the bending or breaking of the lower culm internodes or root lodging due to failure of root anchorage. Stem lodging is due to the strength of the culm not be sufficient to prevent bending and Brazier buckling (Niklas and Spatz, 2012) from the combined moment due to wind loading of the leaves and seed heads of the crop and the overhanging weight of the displaced top of the plant (Baker *et al.*, 2014).

Figure 2: Root lodging in wheat (left) and stem lodging (brackling) in barley (right). Photographs Pete Berry.

Wind damage to forest trees

Wind damage to woody plants has been studied for more than 50 years because of the economic importance for forestry (Gardiner *et al.*, 2013). Damage due to wind can take many forms (Figure 3), and be exacerbated by wet snowfall or salt deposition. Young trees can become loosened around the root collar by swaying and topple due to inadequate rooting. In older trees leaders, branches and crowns may break; and whole trees may be wind-thrown when stem and root plate overturn or may experience wind-snap (Figure 4) when the stem breaks. The mechanism for damage to trees is very similar to that for cereal crops although the exact details may be slightly different. So, for example, tree stems almost never buckle like cereal culms except under exceptionally rare circumstances when stems are exceedingly thin (Figure 13) such as under-storey saplings or when trees are planted or regenerate at very high densities.

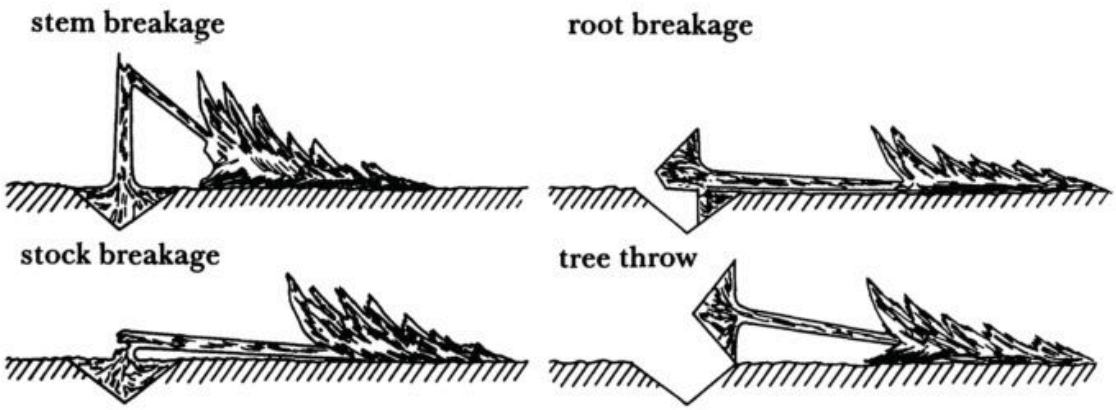

Figure 3: Phenotypes of wind damage to trees. Courtesy of Helmut Mayer.

Figure 4: Overturning of whole trees with shallow roots (left) and stem breakage of well rooted trees (right). Photographs Barry Gardiner and Chris Quine.

Wind damage can also occur to the cuticle of woody plant leaves and needles from windblown sand or ice just as in crop plants but sand and ice can also cause substantial damage to tree bark. Such leaf or needle damage may lead to large increases in "cuticular" conductance leading to increased water loss and an increased possibility of water stress. Recovery from cuticle damage is better if the leaves are still expanding and also seems to be better when the plants are growing under mild water stress. In extreme cases this cuticular damage leads to tree "flagging" where all the needles or leaves on the side of the prevailing wind are killed and leaves only survive in the lee of the tree (Figure 5).

Figure 5: Flagged trees in New Zealand. Courtesy John Moore.

Comparison of wind damage to crop plants and forest trees

Table 1: Comparison of wind damage to crop plants and forest trees, management options and available risk models

		Crop Plants	Forest Trees
Breakage	*Mode*	Brazier buckling of stem	Snap with fibre separation on tension side and local fibre buckling on compression side. Stem buckling almost unknown
	Location	Between nodes and also sometimes at the nodes	Typically at ⅓ to ½ tree height or at a heavy branch whorl but not systematically studied
	Key factors	Stem height, stem diameter, stem wall thickness, stem yield stress	Stem diameter, Modulus of Rupture of the wood, number of branch knots, presence of rot
Uprooting	*Mode*	Soil cracks and soil depression on leeward side. Very little soil movement upwind	Soil depression downwind and soil lifting upwind. Usually there is almost whole root removal from the soil
	Location	Generally roots sink into ground and rotate about a point within the root system either underneath the stem or slightly upwind. However, plants with a large number of widely spread roots fail more like trees.	Generally root plate hinges about point downwind of stem with most of the root plate and soil lifting out of ground. Trees with large tap roots in more sandy soils may fail more by root breakage very close to the stem in an analogous way to stem breakage
	Key factors	Soil wetness, soil shearing strength, root spread, tap root length and stiffness, soil bulk density, root rot	Root architecture, taproot size, rooting depth, size of upwind shallow roots. Role of soil wetness still uncertain but anecdotal evidence of increased risk after heavy rains
Spatial Location		Field margins are particularly vulnerable, partly due to overlapping field operations, e.g. increased seed rate and N fertiliser	Just inside forest edge or at a newly created edge. Upwind felling almost always causes damage to downwind stands. Increased damage in extremely wet areas due to ponding or poor drain maintenance
Management	*Species*	Big differences in damage levels and type of damage between different cereals (e.g. barley often breaks near the head, wheat near the base)	Difficult to clearly establish species differences. Spruces and poplars appear to be most vulnerable among conifers and broadleaves respectively, silver fir and oak the least vulnerable
	Progeny	Breeding has led to large reductions in wind risk	Little or no evaluation of vulnerability of different progeny
	Plant spacing	Increased spacing can reduce damage	Important influence on tree stem form and strength. Change in spacing following thinning increases risk for a few years until trees acclimate.
	Fertiliser	Reduces stem and root strength, increases the load that must be supported	Evidence not totally clear on impact of fertilisers although suggestions is that it increases risk
	Ground preparation	Rolling after sowing to consolidate soil is helpful	Ground preparation that increases rooting depth (e.g. breaking up hardpan) improves stability but ground preparation that limits root spread (e.g. ploughing) reduces stability
	Height control	Systematic and proven use of plant growth regulators in reducing lodging	No direct equivalent in forestry. Closest equivalent is "oceanic" forestry or tree topping
Risk Modelling	*Available models*	Generalised model for cereals (Baker model)	Mechanistic models (ForestGALES, HWIND, FOREOLE) and statistical models (e.g. "*LOTHAR*")
	Application	Selection of improved genotypes, crop management practices, risk prediction, advice	Forest planning, forest management, urban forest risk management, risk mitigation, advice
	Sensitivity analysis	Sensitivity analysis of key factors	Limited sensitivity analysis but no complete parameter sensitivity analyses yet conducted.

The similarities and differences between wind induced failure in crop plants and trees have been summarized in Table 1 along with the impact of different management options, and the risk modelling tools available for foresters and agronomists.

Although many of the damage processes are very similar there are some important differences. For example, soil wetness is known to be a critical component of crop failure whereas the evidence for trees being at higher risk after heavy rainfall is mainly anecdotal. Stem breakage is found to be controlled by $\pi MORdbh^3$ in tree trunks and $\pi\sigma d_0^3$ in cereal stems; where *MOR* is the Modulus of Rupture of the green wood, *dbh* is the diameter at 1.3m above the stem base, σ is the stem yield stress and d_0 is the diameter of the plant stem at its base. Breakage is therefore controlled by the third power of the plant stem diameter and some measure of the material strength. The type of breakage is different with trees breaking by catastrophic rupturing of the whole stem (Figure 4) whereas cereal plants break by localized collapse and buckling (Figure 2). Overturning or root lodging is dependent on stem volume in trees, whereas it is dependent on the effective root ball diameter cubed in crop plants. However, both these factors have units of length cubed and the lack of a good correlation of tree susceptibility to overturning against some measure of root characteristics is probably due to the difficulties of measuring whole root systems in trees rather than an inherent lack of correlation. The exact form of overturning is slightly different with tree roots being lifted out of the ground (Figure 4) whereas the roots of cereal crops tend to be pushed down into the soil (Figure 2).

Resume

Farnsworth and Niklas (1995) "see plants as representing a multitude of solutions to multi-goal optimization which has no definitive answer and so leads to a set of more or less efficient designs". This is just as true for the mechanical requirements of plants and Fournier *et al.* (2013) show that trees have to balance 4 biomechanical traits: 2 safety traits against wind and self-buckling and 2 motricity traits to sustain an upright position. In this paper we have shown how tree have to withstand wind at a range of time scales from very short-period gusts to long term persistent winds. When wind damage does occur to plants it can vary from surface damage to needles and leaves due to abrasion from sand or ice or plant parts clashing, to breakage of individual branches and trees, damage to whole plant communities, up to regional scale damage that brings successional change in the ecosystem. Plants have adopted a range of morphological, chemical and physical adjustments to provide better biomechanical performance under this wind damage risk. These adaptations vary between plants, and with plant ontogenetic stage, but understanding the nature of these adaptations is critical for understanding plant structure and development, and the role of wind disturbance in plant ecosystems.

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Integrating trees and vegetation in urban microclimate simulations: a multiscale approach

Dominique Derome[1], Thijs Defraeye[1,2], Jonas Allegrini[1], Jan Carmeliet[1,2]

1 Laboratory for Multiscale Studies in Building Physics, Empa, Swiss Federal Laboratories for Materials Science and Technology, Dübendorf, Switzerland
2 Chair of Building Physics, ETHZ Swiss Federal University of Technology Zurich, Switzerland
dominique.derome@empa.ch

Introduction

In dense urban environments, much higher air temperatures are found due to higher adsorption of solar radiation, reduced longwave radiation to the sky, anthropogenic heat generation and reduced ventilation due to lower wind speeds. This urban heat island effect leads to increased energy consumption for cooling of buildings and strongly reduces thermal comfort of the inhabitants, which can even lead to health problems. One of the measures to mitigate urban heat islands is to increase the amount of vegetation.

Not only does vegetation provide shading, but it also reduces the air temperatures due to transpiration, during which latent heat is extracted from the surroundings of the plant. Evaporation from water bodies, soil surrounding the plants or porous building facades, wetted by rain or irrigation, contributes in the same way. As the temperatures of these surfaces are reduced, they also provide cooling to the surroundings by radiation and increase of thermal comfort. However, increased relative humidity of the air due to evaporation may lead to decrease of thermal comfort.

There is a complex interplay between the heat and water transport processes in the soil, the vegetation and the surroundings: vegetation determines the local environment by turbulent airflow around and through the plants and absorption, reflection and transmission of solar and longwave radiation in the canopy, while on the other side, the local environmental determines highly the evapo-transpiration process of vegetation. Several aspects of these two-way couplings are not well understood. One of the reasons is that limited attempts have been made to look at all parts of the vegetation water cycle, covering air-soil-stem-leaves-air. The present study will shed more light on this cycle by presenting the combined efforts in numerical modelling of all aspects of this cycle, considering adequately the different scales of the problem and their interrelations.

Urban microclimate modeling

The airflow as well as transport of heat and water vapour in urban areas are solved by computational fluid dynamics. In addition, an Eulerian multiphase model for transport of wind-driven rain was developed, which captures droplet deposition on urban surfaces, including buildings, soil and vegetation (Kubilay et al. 2014). The EM model includes the impact of turbulent dispersion of raindrops. For an accurate assessment of droplet impact on smooth and rough surfaces, such as droplet spreading, splashing, bouncing but also absorption evaporation, water film forming and run-off, additional models have been developed (Martin et al. 2015). These models are CFD-based and rely on the Volume-of-Fluid method. The combination of these models provides an accurate moisture load on building facades and vegetation, at very high spatial resolution, and is also an important boundary condition for the soil water budget. Next to environmental water transport, the water uptake of rain in the soil as well as convective evaporation back to the environment and root water uptake are looked at using finite element modeling. For root water uptake, a specific focus is on the rhizosphere, as

its resistance to moisture transport is highly dependent on the soil moisture content. The evaporation to the air domain is tackled using conjugate modelling (Defraeye et al. 2012). The remaining part of the water cycle still to be tackled is the vegetation, as discussed below.

Integrating vegetation in urban microclimate modeling

The water transport from the roots through the plants up to the leaves is modelled using a simple resistance network. For the exchange of heat and moisture of vegetation with the airflow through the plant canopy, a porous medium model is made. This model incorporates the impact of vegetation on momentum transport and turbulence generation and destruction within the plant canopy, but also on solar radiation absorption and transmission. The impact of stomatal opening is parameterised by variable convective heat and mass transfer coefficients of the leaves. The three components of the vegetation system, e.g. rhizosphere, wood trunk and leaf canopy, are documented at a lower scale using heat and mass transport modeling, namely at the scale of root/soil interface, xylem/phloem, and leaves (Defraeye et al. 2013, 2014). Using this approach, as shown in Figure 1, first results on the impact of vegetation on the local microclimate in streets (temperature, relative humidity and velocity) and on thermal comfort of pedestrians are presented.

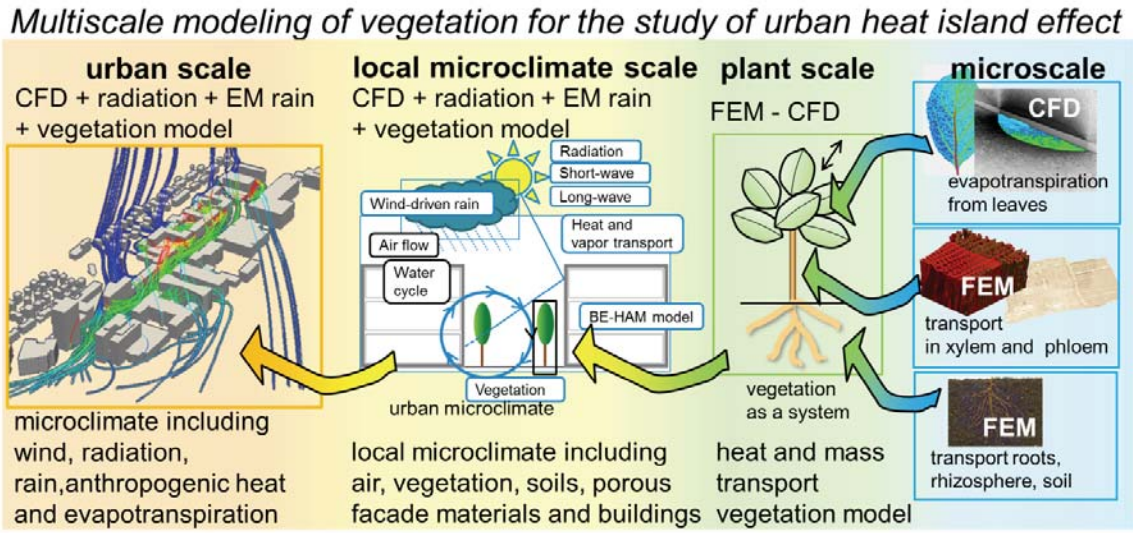

Fig. 1 : Schematic representation of the multiscale approach designed to take into account vegetation in urban microclimate simulations.

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Modelling trees response to wind forcing using Terrestrial LiDAR data.

Toby Jackson[1], Pasi Raumonen[2], Alexander Shenkin[1], Yadvinder Malhi[1]

[1]Ecosystems Laboratory, University of Oxford, UK
[2]Department of Mathematics, Tampere University of Technology, Finland
tobias.jackson@linacre.ox.ac.uk

Introduction

Wind damage is an important form of disturbance in both tropical and temperate forests and has a significant influence on the global carbon cycle (Espírito-Santo et al., 2014). Large-scale models can be used for predicting damage to single species forests (Hale et al., 2015). More advanced models can capture the dynamic response of a single tree whose structure has been mapped (Sellier et al., 2008). Here we introduce our model of trees response to wind forcing based on terrestrial LiDAR data.

The model

The motion of a tree in the wind is related to its wood density, elasticity and branching architecture as well as its surroundings. We use Terrestrial LiDAR point cloud data and quantitative structure modeling to accurately describe the geometry of a tree in a forest (Raumonen et al., 2013, Calders et al., 2015). Utilizing engineering structural mechanics approaches, we model trees as systems of beams. Increased structural accuracy can be achieved through different meshing techniques and wood property measurements derived from fieldwork.

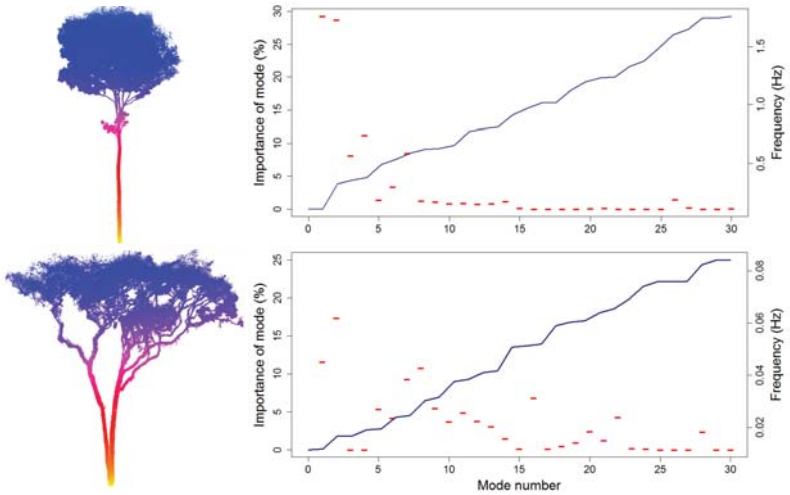

Fig. 1 – Top – point cloud of a tree with relatively simple branching architecture, and plot of the frequency and importance of its first thirty sway modes. Bottom – as in top, but for a tree with more complex branching architecture. Tree point clouds courtesy of Alvaro Lau Sarmiento, Wageningen University.

The preliminary results in figure 1 show that architecture affects the complexity of a tree's response to wind forcing and questions the general relevance of the fundamental sway mode (Moore and Maguire, 2004). The motion of the first tree can be well described by <5 modes whereas that of the second tree requires >10.

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Evolutionary optimization of fluid transportation systems in plant leaves

Natalya Kizilova

Department of Theoretical and Applied Mechanics, Kharkov National University, Ukraine
n.kizilova@gmail.com

Introduction

The long-range fluid transport in plant leaves is provided by the xylem and phloem vessels composing complex networks of different geometry. The rotate, palmate, pinnate, arcuate and parallel venation types are considered as consequent types appeared during the evolution, though the nowadays evolutionary new and ancient forms coexist. As it was shown by detailed measurements on the digital images of the fresh cut leaves of different size, shape and venation type, the principles of construction of the venation include the Murray's law between the diameters of the veins in the bifurcations $d_0^3 = d_1^3 + d_2^3$, where d_0 and $d_{1,2}$ are the diameters of the parent and daughter veins (fig.1) [1]; the Rosen's law for the branching angles

$$\alpha_1 = \arccos\left(\frac{(1+\xi^3)^{4/3}+1-\xi^4}{2(1+\xi^3)^{2/3}}\right), \qquad \alpha_2 = \arccos\left(\frac{(1+\xi^3)^{4/3}+\xi^4-1}{2\xi^2(1+\xi^3)^{2/3}}\right),$$

where $\xi = \min\{d_1, d_2\}/\max\{d_1, d_2\}$ is the branching asymmetry [1,2]; the linear relationship between the mean diameter and length of the vein $L/<d>=\text{const}$ [2,3]; the Hack's law governing the relationship between the longest length L_Σ along the branch of veins and the leaf blade area S supplied through this branch $L_\Sigma/\sqrt{S} = \text{const}$ [3].

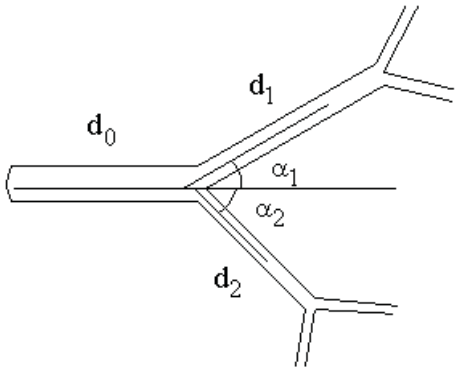


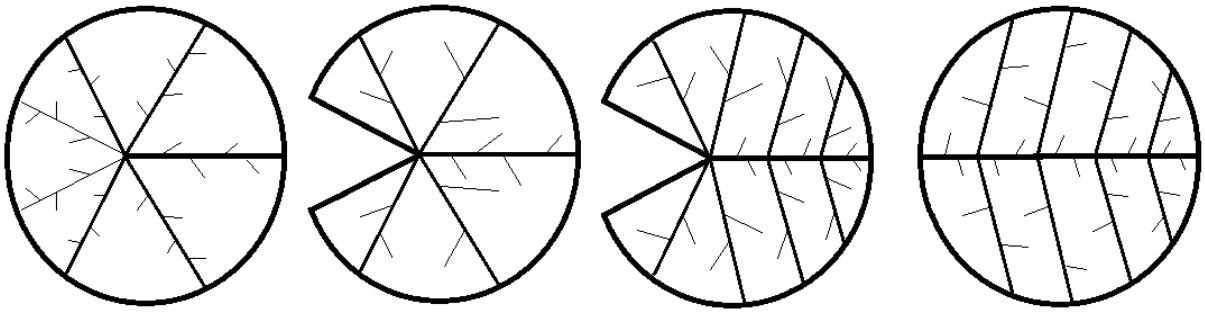
Fig.1. The schematic view of the bifurcating veins

It was shown the structure of the leaf venation systems corresponds to the optimal pipeline providing the fluid delivery and distribution at minimal total energy expenses [1-3]. A model of the branched pipeline of porous rigid tubes with permeable walls was developed, and it was shown the balance of the inflow of the plant sap to the branch and its percolation and absorption in the corresponding area of the leaf blade leads to the fulfilment of the Hack's law [3]. Validity of the Murray's law was also confirmed by the measurements on the plant xylem conduits [4]. In this paper the comparative analysis of the hydraulic properties of the leaf venations of different evolutionary types is studied.

Materials and methods

Digital images of 1200 fresh cut leaves have been analyzed using the in-house developed software [3]. The 150-250 single bifurcations per leaf blade, depending on its size, have been

recognized and the diameters $d_{0,1,2}$, lengths $L_{0,1,2}$, and branching angles $\alpha_{1,2}$ have been measured. The following plants have been studied: *Tropaeolum* L. (peltate shape, radial venation), *Acer campestre* (palmate venation); *Vitis aestivalis* (cordate shape, pinnate venation); *Cotinus obovatus, Fraxinus ornus* (ovate shape, pinnate venation).



a b c d

Fig. 2 : Models of different shapes and venation types: radial (a), palmate (b), pinnate (c), pinnate and oval shape (d)

Validity of the Murray's and Rosen's laws have been confirmed and no significant differences between the venation types of different evolutionary ages have been found.

Numerical computations of the average hydraulic conductivity Z_h/V per unit volume of leaf blade have been carried out basing on the model [3] of the fluid flow through the branched pipeline of thin rigid tubes with permeable walls due to the pressure drop between the inlet (P_{in}) and the constant pressure inside the porous leaf blade which is supposed to be equal to the atmospheric pressure (P_{atm}). Different numbers of the main veins (n=3-9), values of the angles at the leaf base ($20-160^o$), diameter-dependent branching angles of the main and secondary veins according to the Rosen's law ($\alpha = 30-90^o$) have been used.

It was shown the value Z_h/V slowly decrease with transition between a→b→c→d (fig.2) when the number of the main veins is taken the same for each shape. The increase from n=3 to n=5 main veins lead for noticeable decrease in Z_h/V for each shape, while the next increase to n=7 and n=9 was not favorable for the leaf hydraulics. The lowest values of Z_h/V have been detected for the thin secondary veins with $\alpha_1 \sim 90^o, \alpha_2 \sim 0^o$.

Conclusions

The leaf blade shapes and venation types of different evolutionary ages possess geometrical construction obeying the Murray's and Rosen's laws that is optimal for the fluid delivery and distribution at the minimal energy costs. The hydraulic conductivity Z_h/V per unit volume of the leaf blade depends on the venation type, number of the main veins and the branching angles of the secondary veins. One may conclude that some optimization of the conducting system has been carried out by evolutional optimization.

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Diffusion and bulk flow of sugar and water in leaves I: Experiments

Hanna Rademaker[1], Katrine Engholm Villumsen[1], Kaare Hartvig Jensen[1], Helle Juel Martens[2], Alexander Schulz[2], Tomas Bohr[1]

[1]Department of Physics and Center for Fluid Dynamics, Technical University of Denmark, Denmark
[2]Department of Plant and Environmental Sciences, University of Copenhagen, Denmark
hanra@fysik.dtu.dk

Introduction

Plants need to distribute both water and sugars throughout the whole organism. This distribution is achieved through a combination of bulk pressure driven flow and diffusion. In the leaf, where the water is evaporated and the sugars are loaded into the vascular transport system, both processes are of importance, but the details of this interplay are currently not known. Here we present new insights from theoretical analysis and experimental studies, both on real plants and on biomimetic devices.

Experiments

We study the leaf architecture at vein branchings with different light and electron microscopy techniques, examining how the conducting sieve elements are merged at a Y-junction. The results are of importance in the context of fluid transport efficiency.

We further investigate osmotic transport processes in 3D-printed biomimetic devices, modeling the passive sugar loading from the mesophyll into the phloem. We focus on the relevance of bulk flow and diffusion for this loading type.

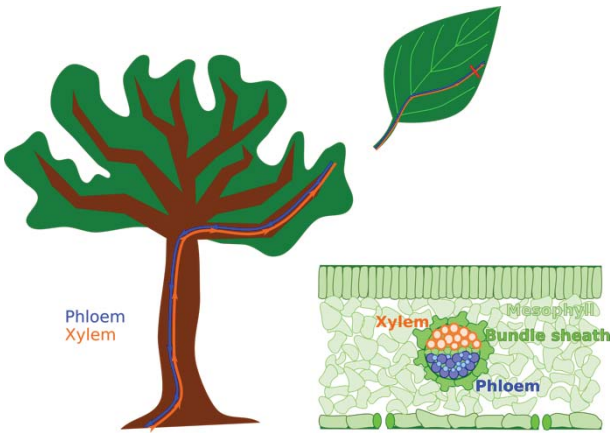

Fig. 1 : The vascular system of a plant. Water is taken up at the roots and elevated to the leaves in the xylem (orange). A small fraction of this water is used to produce sugars, which are transported in the phloem (blue) to roots, fruits or young leaves. The leaf cross section shows the arrangement of xylem and phloem inside veins.

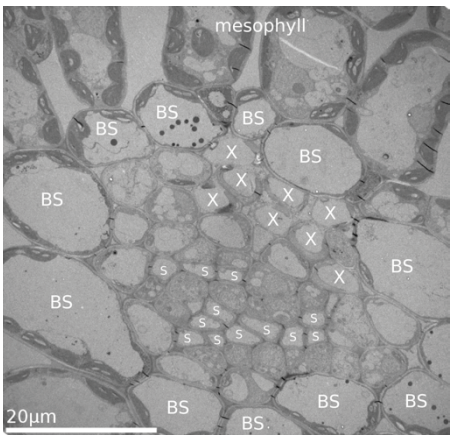

Fig. 2 : TEM image of a vein cross section in an apple leaf. The image shows the sieve elements (S) of the phloem and the xylem vessels (X), surrounded by bundle sheath cells (BS). In the top of the image mesophyll cells are visible.

Diffusion and bulk flow of sugar and water in leaves II: Theory

Tomas Bohr[1], Hanna Rademaker[1], Katrine Engholm Villumsen[1], Kaare Hartvig Jensen[1], Helle Juel Martens[2], Julia Dölger[1], Johannes Liesche[2] and Alexander Schulz[2]

[1]Department of Physics and Center for Fluid Dynamics, Technical University of Denmark, Denmark
[2]Department of Plant and Environmental Sciences, University of Copenhagen, Denmark
hanra@fysik.dtu.dk

Introduction

Plants need to distribute both water and sugars throughout the whole organism. This distribution is achieved through a combination of bulk pressure driven flow and diffusion. In the leaf, where the water is evaporated and the sugars are loaded into the vascular transport system, both processes are of importance, but the details of this interplay are currently not known. Here we present new insights from theoretical analysis and experimental studies, both on real plants and on biomimetic devices.

Theory

Taking as the starting point, the Kedem-Kachalsky equations for the coupled water-sugar transport through a porous membrane, we can describe the loading of sugars into the sieve tubes in cases, where this does not involve active sugar transporting proteins. For the polymer trap loaders (active symplasmic loaders), we find that the bulk water transport plays an essential role. We show corresponding results for a family of 3D-printed biomimetic devices, modeling the passive sugar loading from the mesophyll into the phloem.

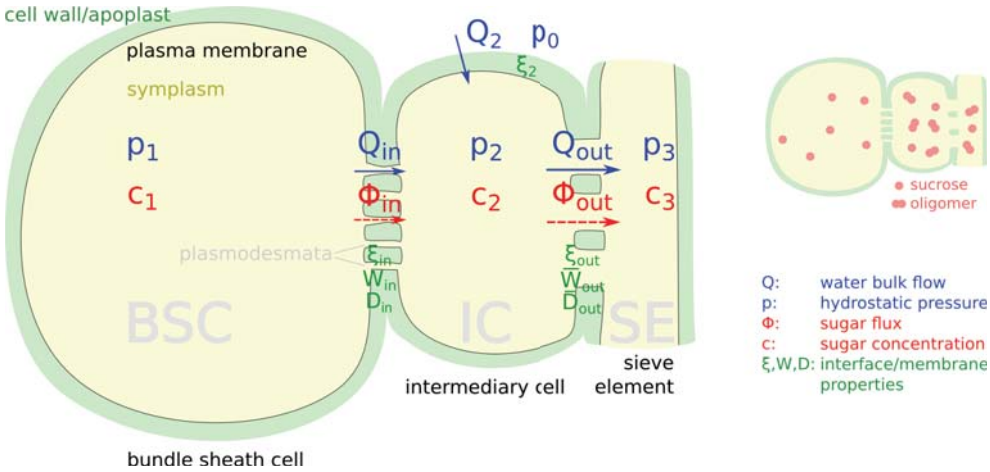

Fig. 1 : The uptake of sugar and water into the cells of the phloem transport system can be achieved in several ways. One type is called 'polymer trap' loading (Rennie and Turgeon (2009)), where sucrose coming from the bundle sheath cell enters the companion cell through tiny pores, called plasmodesmata. The sucrose is then enzymatically converted to larger sugar molecules, which can not diffuse back. Our theoretical study (Dölger et al.(2014)) suggests, that a water bulk flow significantly enhances the sugar transport through the plasmodesmata.

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An overview of tensile water in living and dead cells: The role of negative pressure in water transport and in the water balance of leaves.

Mel Tyree[1,2], Dongmei Yang[1] and Yiting Ding[4]

[1]College of Forestry, Northwest A&F University, Yangling, Shaanxi, China
[2]Center for Nano- and Micro-Mechanics, Engineering Mechanics, Tsinghua University, Beijing, China
[3]Technology and Engineering Center for Space Utilization, Chinese Academy of Sciences, Beijing, China

mel.tyree@cantab.net

Tensile water (water under negative pressure) is generally believed to occur in xylem conduits and the generation of tensile water is central to the Cohesion Tension Theory of sap ascent in plants. The first part of this keynote address will concern the experimental evidence for tensile water in dead xylem cells (Tyree & Zimmermann 2002). I will also briefly highlight some of the recent controversies raised by those who opposed the C-T theory.

The last half of the talk will address new concepts of negative pressure in living cells (= negative turgor pressure). Except for works like Oertli (1986) and Oertli et al. (1990) the concept of negative turgor has received little experimental or theoretical consideration until the micromechanical models of Ding et al. (2014). Using ABAQUS Ding et al. (2014) simulated the stresses and strains in micro-capsules the size and shape of palisade cells and determined theoretically that the pressure difference between the inside minus outside of cells should generate conformational changes in the shape of the cell walls, cell volume and the concentration of solute in the cell wall assuming an active semipermeable membrane, Fig. 1.

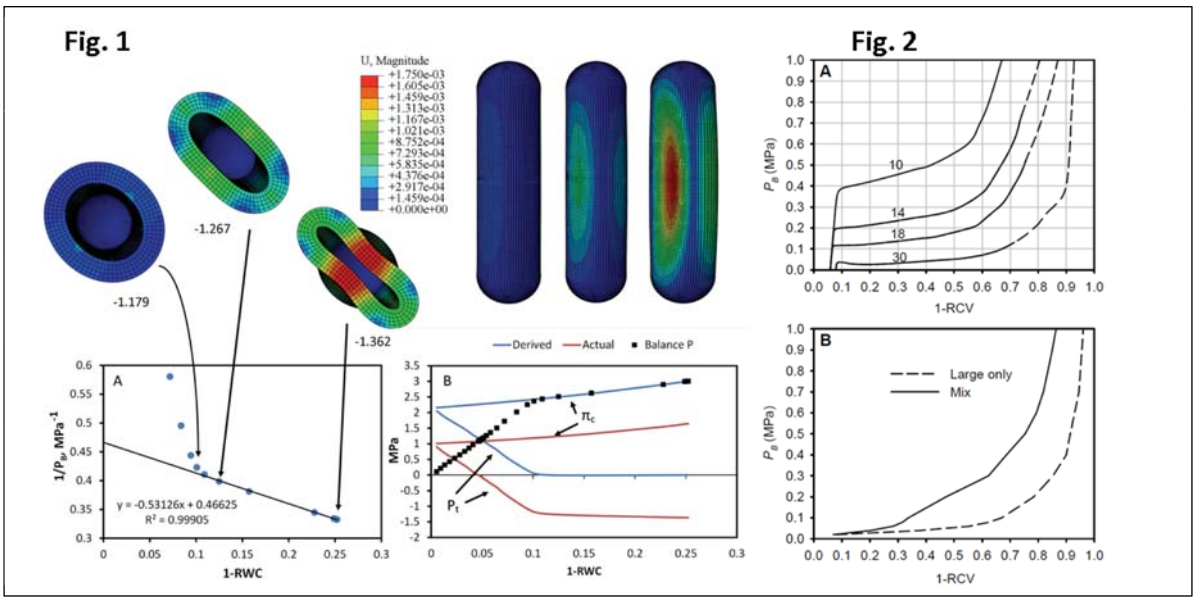

Fig. 1: Color figures (A) left and (B) center from Ding et al. 2014) showing development of negative turgor in pressure-volume curve format followed by collapse (when slope of P_t versus relative volume loss changes form steep negative to shallow negative). P_B = balance pressure, P_t = turgor pressure, π_c = cell osmotic pressure.

Fig. 2: right hand graphs (A) and (B) theoretical predictions of negative turgor (measured with a pressure bomb) on tissue with the cell membrane destroyed by heating. (A) four cells of different diameter in μm (numbers next to graph). (B) the theoretical curve for large cells only or a mix in a tissue where the cell volumes are of each cell size are in the ratio of 55:15:15:15 for cell sizes of 30: 18:14:10 μm diameter.

Ding et al. (2014) addressed the theoretical pressure volume curves of single cells with different diameters and shapes: capsule cylinders to spheres. In Yang et al. (2015) a few of these solutions were used to predicted the pressure-volume curves of theoretical mixed tissues consisting of living cells (intact membranes) and dead cells (disrupted membranes). Shown in the two right-hand graphs (A) and (B) are the predictions for dead cells (membranes disrupted by heating to 60 ^{o}C for 5 minutes). Other model predictions were made for pressure volume curves of living cells (graphs not shown in abstract).

Experimental evidence was gathered to test predictions from the micro-mechanical analysis in Ding et al. 2014 that negative turgor exists in small leaf cells. The pressure chamber and psychrometer techniques were used on living and dead-tissue of species with small and large palisade cells of *Robinia* and *Metasequoia*, respectively.

Results proved that *Robinia* lamina with small cells had about 0.3 MPa more negative turgor than *Metasequoia* lamina with large cells when measured in dead tissue. However psychrometer measurements of sap expressed from dead tissue revealed that classical pressure-volume analysis on living tissue gave the correct value of original osmotic pressure regardless of the theoretical magnitude of negative turgor, which was contrary to predictions of micro-mechanical models for unicellular systems.

The reason for the disparity between results of multi-cell tissues and the theory of unicellular systems was explained by the non-collapse of palisade cells in *Robinia* in the balance pressure, P_B, domain of PV curves. Failure to collapse means that negative turgor pressure, P_t, smoothly increases without an abrupt change of slope of P_t versus cell lumen volume. Models confirm that pressure-volume curves of such a leaf would have the observed experimental behavior including an *x*-intercept that was smaller than expected based on anatomical estimates of apoplastic water volume.

The collapse or non-collapse of cells under negative turgor is critical to the behavior of theoretical pressure-volume curves of binary mixes of cells. The factors that change with collapse are the x- and y-intercepts, where the y-axis is $1/P_B$ and the x-axis is relative water loss (1-RWC), RWC = relative water content. Collapse is defined as the point along the x-axis when there is an abrupt change in slope of P_t versus (1-RWC). When collapse occurs in both cell populations at $P_t \cong 0$ then the y-intercept = $1/\pi_o$ =1/(original osmotic pressure) and the x-intercept = the volume of the symplast relative to the total water volume in leaves. When one cell group collapses at negative P_t in the P_B domain then the y-intercept is underestimated but the x-intercept is about right. When one cell group does NOT collapse in the P_B domain then the y-intercept is about right but the x-intercept is too small. Hence by examination of pressure-volume curves the P_t at which collapse occurs can be roughly ascertained. Knowing what values of π_o and symplast water are 'about right' requires independent estimates of π_o using thermocouple psychrometers and of apoplastic water volume using quantitative anatomical analysis.

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Keywords: Cohesion-Tension Theory, Tensile water, negative pressure, negative turgor, pressure-volume curves.

Seeding Strategy of the Autochore Plant and Bursting Mechanism of the Fruits

Sakamoto J[1], Endo Y[2], Ito K[3]

[1]School of Mechanical Engineering, Kanazawa University, Kanazawa, Japan
[2]AW Engineering Co. LTD., Anjo, Japan
[3]Graduate School of Natural Science and Technology, Kanazawa University, Japan
sakamoto@se.kanazawa-u.ac.jp

Plant seeds are moved in a variety of ways. Wind, insects or animals carries them sometimes. Dispersion by bursting pericarp is a typical movement of plant seeds that is self-active and independent of other creatures [1]. Hereafter, pericarp means wall of fruit, which contains and disperses the seeds. If a pericarp can burst powerfully and spread seeds widely, it is advantageous in expanding the breeding grounds of a plant. So, the bursting mechanism of a pericarp of existent plants seems to be adapted mechanically and structurally. There are various kinds of autochore plant that have different bursting mechanism of fruit. For example, the impatience fruit accumulates stress to burst by swelling, and the pericarps perform roll up motion while bursting. Fruit of the Vicia angustifolia accumulates stress to burst by drying up, and the pericarps scatter seeds by their spiral motion. Fruits of Japonica orixa eject their seed in the air with the inner pericarp from outer pericarp using stress accumulated by drying up. In both autochore plant fruit, pericarp stress occurred by swelling or drying makes its bursting. Stress accumulated in pericarp has important role for the bursting mechanism and seed spreading. We focused on bursting fruit of the Impatiens, the Vicia angustifolia and Japonica orixa.

At first, we clarified that fruit of the Impatiens have outstanding function to disperse seeds far away [2]. In the study, swelling and burst motion simulation of the fruit was performed by using finite-element method. We considered an optimization problem to maximize peak velocity of the pericarp tip with varying swelling stress distribution as design variables under stress constrain. Optimal burst motion of the fruit was obtained by solving the problem as shown in Fig.1, and compared with the real fruit motion. It became clear that pre-burst stress distribution of the pericarp is advantageous to make it large and fast motion for spreading seeds [3].

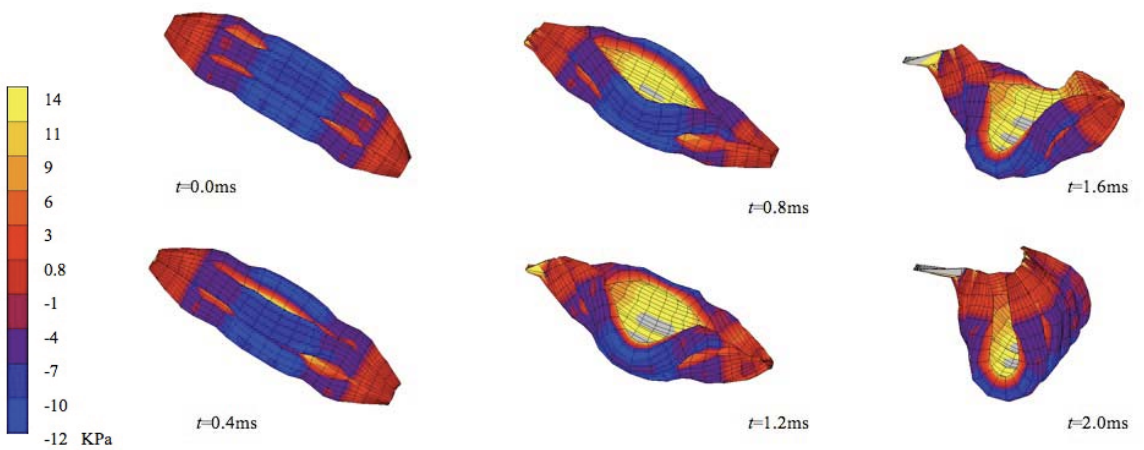
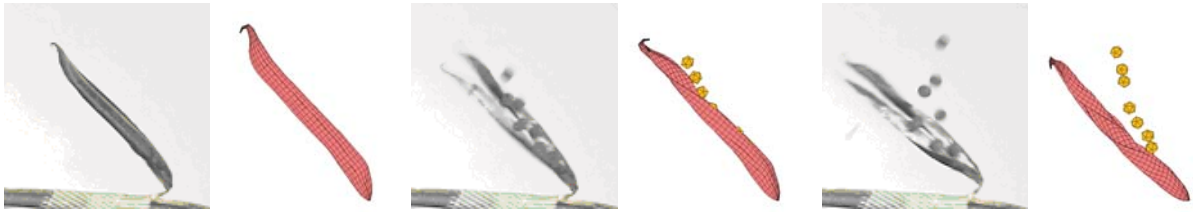


Fig. 1 : Simulation of bursting motion of the Impatiens fruit obtained by solving the optimization problem to maximize peak velocity of the pericarp tip.

In fruit of the Vicia angustifolia, laminated structure and fiber tissue arrangement in the pericarp seems very important to consider the bursting mechanism. Anisotropic mechanical properties of the pericarp were determined, and the finite-element model considering the anisotropy was created based on 3D scanning of actual fruit of the Vicia angustifolia. Computer simulation of the pericarp bursting motion by finite-element method was compared to actual motion to validate the finite-element model, and mechanics of the fruit bursting was considered. Configuration and time at maximum deformation occur in the simulation was consistent with actual motion. It was validated that structure and mechanical properties of the FE model was effective. Parametric study changing the fiber direction in the pericarp was carried out to evaluate optimality of the fiber direction for spreading seeds. It was clarified that optimal fiber direction was found to achieve faster and larger motion of the pericarp, and the optimal fiber direction was almost same as actual one. Furthermore, as shown in Fig.2, FE model of total fruit composed of a pair of pericarps and included seeds was developed and effectiveness of the model was ensured [4].



t=0msec *t=1msec* *t=2msec*

Fig. 2 : Sequential images during burst of a fruit of Vicia angustifolia taken by high-speed video camcorder and the corresponding computer simulation.

In the inner pericarp of Japonica orixa fruit, fiber direction of the inside surface is different to outside one. It seems that difference of the fiber direction is one of important factor of deformation of the inner pericarp. A FE model of Japonica orixa fruit to simulate seed ejection was developed. The FE model of fruit can reproduce the seed ejection phenomenon by the simulation in which applies dry shrinkage to the pericarps.

Simulation method of bursting motion of autochore plant fruits by using finite-element method was established through these studies. It is effective to study influence of fiber directions, material properties or stress distribution on bursting motion of fruits. We can discuss optimality of these parameters to bursting motion by using the simulation method.

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Keywords: Bursting Motion, Autochore Plant, Fruits, Seed, Simulation, Finite-Element Analysis

Geometry in Closing Motion of Leaflet of Sensitive Plant (*Mimosa Pudica*)

Hidetoshi Kobayashi[1], Keitaro Horikawa[1], Nakamichi Tominaga[1], Takeshi Yamauchi[2]

[1]Department of Mechanical Science and Bioengineering, Osaka University, Japan
[2]Department of Materials Science and Technology, Niigata University, Japan
hkoba@me.es.osaka-u.ac.jp

Introduction

In early spring, the leaves and flowers of most plants are folded or rolled to fit the small inside space of buds. Then, they come from tiny buds and grow to become big leaves and flowers. Therefore, the unfolding of a corrugated simple leaf such as Hornbeam and Beech leaves, was investigated by using vector analysis by Kobayashi (1998). While, sensitive plant (Mimosa pudica) quickly closes leaflets as a response to a mechanical or thermal stimulus, as shown in Fig.1. The closing motion of the leaflets faced each other across the midrib looks like very complicated motion which includes twisting of leaflet and overlapping each other as making one line along the midrib for the compact storage. For a design of man-made deployable structures, therefore, it is quite useful to investigate the closing mechanism of the leaflet of sensitive plant.

Three installation angles

In this study, the quick folding motion of leaflets was carefully observed by using stereo-video-cameras, then the folding manner of leaflet, the location of pulvinus connecting leaflets to the midrib and the cross-sectional shape of midrib etc. were also observed by using various microscopes. From the observations, it was found that the leaflets accomplish the complex folding motion by their one-way rotation with the ingenious combination of three installation angles relating to the leaflet, as shown in Fig.2, midrib and pulvinus of sensitive plant leaves. They are α, β and γ where α is the apex angle of the midrib cross-section, β is the angle connecting the pulvinus with the midrib, and γ is the angle between the axis of pulvinus and the rachis of leaflet. We confirmed that this understandings concerning folding motion of leaflets is true by the theoretical analysis based on the understandings including α, β and γ . The motion of the leaflet tip obtained from the analysis coincided well with the trajectory captured by the stereo-video-cameras.

Fig. 1 : Closing leaflets of sensitive plant.

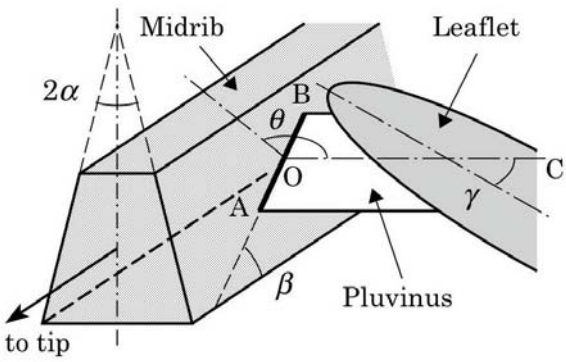

Fig. 2 : Three angles of closing leaflets of sensitive plant.

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Carpel Closure by Protodermal Tissue Adhesion in *Arabidopsis thaliana*

Rieko TSUJINO, Aika YOKOI, Hitomi ICHIKAWA, Megumi IWANO, Seiji TAKAYAMA and Mitsuhiro AIDA

Graduate School of Biological Sciences, Nara Institute of Science and Technology, Japan
m-aida@bs.naist.jp

Introduction

A characteristic feature of flowering plants is the fusion of carpels, which results in the formation of a closed, vessel-like structure of the gynoecium. This closed structure contributes to protection of ovules inside, separation of the sites of pollination and fertilization, and promotion of seed dispersal. Carpel fusion can occur in two ways: when primordia are initiated as a united structure early in their inception, the fusion is referred to as congenital, whereas a fusion that occurs after discrete primordia are formed is termed post-genital (Fig. 1).

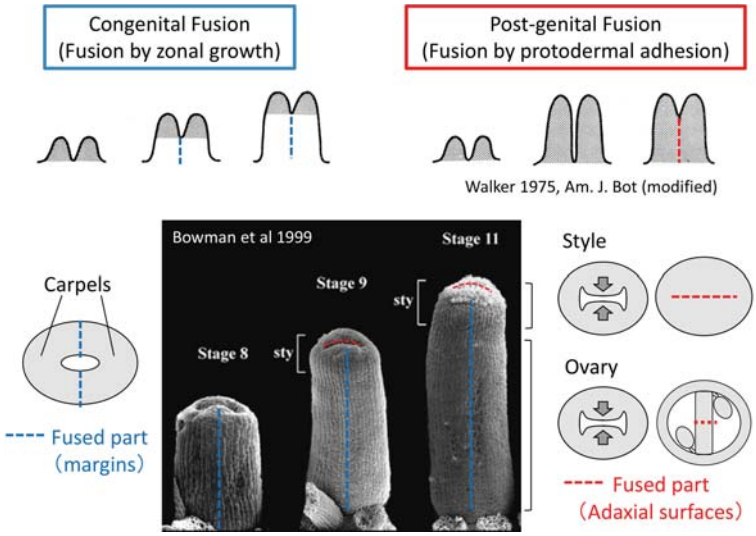

Fig. 1 : Two types of carpel fusion in *Arabidopsis*

Results

We analyzed morphological and molecular characteristics of contacting protodermal cells during post-genital carpel fusion in *Arabidopsis thaliana*. The fusion was associated with deformation of surface cell walls, disruption of the layered organization of the protoderm and reduction in expression of a protodermal marker, *PDF1*. Electron tomography suggested that the contacting surfaces of the cell walls were covered with procuticle, which is involved in preventing cell-cell adhesion. Subtending the procuticular layer was a layer extending from the middle lamella of the inner cell walls, which is important for maintaining cell-cell adhesion.

Conclusions

-Post-genital fusion of carpels in *Arabidopsis* is associated with changes in the shape of the surface cell walls.

-Disruption of the layered organization and reduction of protodermal marker expression suggest a loss of epidermal fate.

-Surface cell walls are covered with procuticle, which is involved in preventing cell-cell adhesion.
-Cortical layer of the surface wall is similar to the middle lamella, which contains the cell adhesion molecule pectin.

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Seed Ejection Simulation of Autochore Plant Fruit in case of *Japonica Orixa*

Koji Ito[1], Jiro Sakamoto[2]

[1]Graduate School of Natural Science and Technology, Kanazawa University, Japan
[2]School of Mechanical Engineering, Kanazawa University, Japan
mritkj@stu.kanazawa-u.ac.jp

Introduction

The bursting of a plant fruit is a very interesting phenomenon in a biomechanical viewpoint because it is a mechanical phenomenon directly related to reproduction of the plant. If a plant has a fruit that can burst powerfully and thus spread its seeds widely, it has a reproductive advantage. In this study, we focused in *Japonica orixa*, which is a kind of autochore plant. Fruits of *J. orixa* eject their seed in the air with the inner pericarp. The purpose of this study is to create a mechanical model of *J. orixa* fruit for finite-element analysis and to simulate seed ejection behavior in order to consider the ejection mechanism.

Japonica orixa

Japonica orixa is an autochore plant, which is a deciduous shrub of the Rutaceae growing in valley along swamp. The height is about 1-5 m. Its ovary is divided into four, the number of mericarp is 1-4, and the size of a fruit is about 10 mm. It bursts after drying, and blackish brown seed ejects with inner pericarp from outer pericarp. Fig.1 shows an exterior view of *J. orixa* fruit, and Fig.2 shows a seed and inner pericarps taken out from fruit.

Fig.1 *Japonica Orixa Fruit*

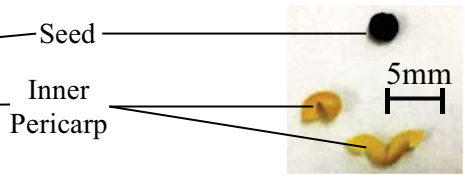


Fig.2 Inner Pericarps and a Seed

Seed ejection process

First, outer pericarps shrink by drying, and they sprits in joint parts (Fig.3 (a)). Then, inner pericarps dry and deform, and it makes outer pericarps extend (Fig.3 (b)). As deformation of outer pericarps finish, it restricts deformation of inner pericarp. Strain energy is stored in inner pericarp due to its deformation with restriction. At last, base of inner pericarp is broken by inner stress, and the seed and inner pericarp eject (Fig.3 (c)). A part of the strain energy of the inner pericarp converts into the kinetic energy of the seed ejection.

(a) (b) (c)

Fig.3 Seed Ejection Process

Observations

We made observation of inner pericarp tissue by optical microscope to clarify the deformation mechanism. Fig.4 and 5 shows the inside (seed side) and outside (outer pericarp

side) surface of the inner pericarp, respectively. Fiber direction of the inside surface is different to outside one. Although the fibers of inside surface align with the longitudinal direction, the fibers of outside surface cross the longitudinal direction. It seems that difference of the fiber direction is one of important factor of deformation of the inner pericarp.

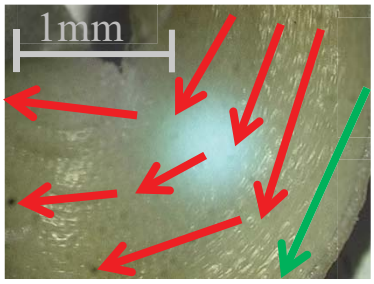


Fig.4 Inside (Seed Side) Surface of Inner Pericarp

Longitudinal Direction

Fiber Direction

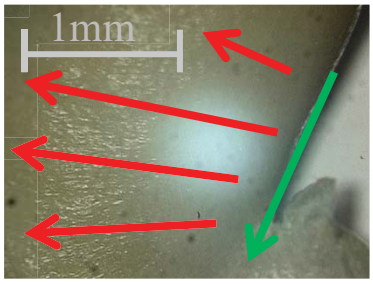


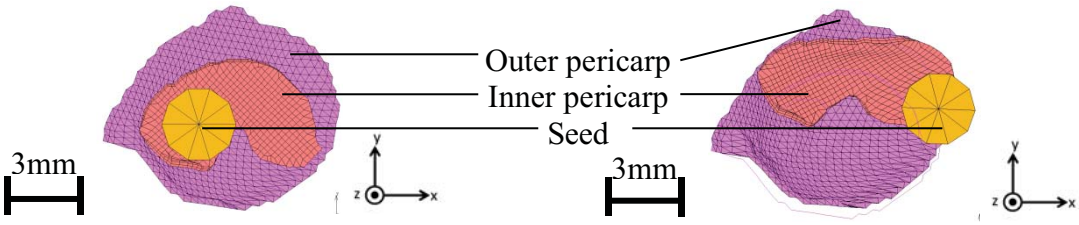
Fig.5 Outside (Outer Pericarp Side) Surface of Inner Pericarp

Finite-Element Analysis

Shape of a *J. orixa* fruit was scanned by using 3D laser scanner, and material properties were given as considering its anisotropy. The finite-element model has different fiber direction of outside and inside as same as the ovation. MSC.Marc was used to non-linear finite element analysis with large deformation and contact problem. We gave pseudo thermal shrinkage to the inner pericarp equivalent to drying shrinkage. 3 steps with changing constraint conditions were performed to simulate seed ejection process as follows. (1) Base of the inner and outer pericarp was fixed and thermal shrinkage was applied to the inner pericarp. (2) Whole of the outer pericarp was fixed completely as keeping thermal shrinkage to the inner pericarp with its base fixation. (3) Fixation of the inner pericarp was released.

Results

Fig.6 shows the symmetric half model of *Japonica orixa* fruit while initial and ejected condition. Table.1 shows seed ejection velocity and its kinetic energy obtained by the analysis and observation. Although seed ejection phenomenon was simulated in the analysis, quantitative agreement with observation was not achieved.



(a) Initial Condition (b) Ejected Condition

Fig.6 The Finite-Element Model of *Japonica orixa* fruit

Table.1 Comparison of FEA and Observation on Seed Velocity and its Kinetic Energy

	FEA	Observation of *J. orixa* seed
Ejection velocity [km/h]	0.957	30.0
Kinetic energy [10^{-6}J]	0.0939	868.0

Conclusion

A FE model of *Japonica orixa* fruit to simulate seed ejection was developed. In spite of quantitative disagreement between the analysis and observation, the FE model of fruit can reproduce the seed ejection phenomenon by the simulation in which applies dry shrinkage to

the pericarps. It would be more accurate model has high fidelity to real fruit of *Japonica orixa* by optimizing parameters such as material properties, boundary conditions of the model.

Curving the fold, an actuation mechanism for plant reconfiguration.

Jeremy Segers[1], Denis Terwagne[1]

[1]Faculté des Sciences, Université Libre de Bruxelles (ULB), Belgium
Denis.Terwagne@ulb.ac.be

Plants are artful combiners of mechanics and geometry to perform motion in constrained environment. For the purpose of reproduction, ice plants (family Aizoaceae) that are most often found in arid or semiarid parts of southern Africa have developed a protective mechanism to resist seed desiccation and allow opening of the seed capsules when the rain comes.

When the seed capsule is wet, its valves open and free the seeds (see Fig. 1-B1-B2). This unfolding opening motion of the capsule valve around a hinge has been recently attributed to the swelling of an inner cellular structure that induces bi-directional geometric constraints in each valve; and which can be modeled as a simple origami folding [Harrington (2011)]. This folding mechanism is of great inspiration for a burgeoning community of mathematician, physicist and engineers, which endeavor to crack the complex relation between the mechanics and the geometry of origami folding.

In this spirit and inspired by the sophisticated mechanism of the opening and closing of the ice seed plant valves, we present a model experiment of the mechanism (see Fig. 1-A1) based on an origami folding. By imposing a curvature to the fold, we actuate its opening and closing (see Fig. 1-A2). This imposed curvature induces inner mechanical constraints that allow a control of the deflection angle, which ultimately leads the fold to close completely. Along our study, we thoroughly analyzed and characterized this mechanism as a function of the geometrical and mechanical parameters.

We find two closing regimes as a function of the fold geometry. The first regime shows a monotonic and continuous increase of the deflection angle as the imposed curvature to the fold increases. The second regime corresponds to a saturation of the deflection angle at a maximum value and an increased attachment capacity of the fold.

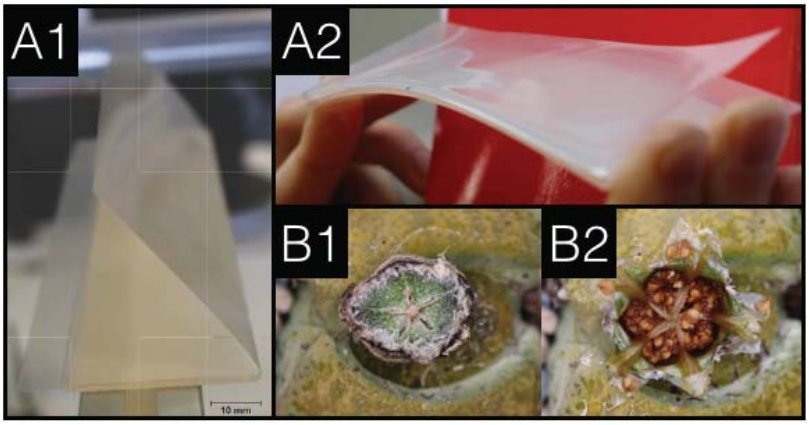


Fig. 1 : A) Origami folding A1) without any imposed fold curvature and A2) with an imposed fold curvature, the deflection angle is maximum; B) seed capsule of a *Lithops lesliei* plant that is B1) dry and closed and that is B2) wet and opened revealing the seeds.

The physical insight gained by the analysis and the characterization of this origami folding should allow us to better understand the underlying closing mechanisms behind the ice seed plant opening and closing mechanism. Possible out comings might also arise in fields ranging from deployable engineered structure to soft robotics and medical devices,…

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Smart wooden actuators

M.Rüggeberg[1,2], C.Vailati[1,2], M. Hassani[1], F.Wittel[1], I.Burgert[1,2]

[1] Institute for Building Materials (IfB), ETH Zürich, 8093 Zürich, Switzerland
[2]Laboratory of Applied Wood Materials, Empa, 8600 Dübendorf, Switzerland
mrueggeberg@ethz.ch

Plant tissues take up and loose water when exposed to fluctuating relative humidity. This hygroscopic behavior comes along with dimensional changes. Nature makes use from these properties and has developed plant organs which are actuated in response to changes in relative humidity. Pine cones, wheat awns and orchid tree seedpods generate complex and reversible movements as a response to changes in relative humidity for effective seed dispersal (Dawson, Vincent et al. 1997, Elbaum, Zaltzman et al. 2007, Erb, Sander et al. 2013). The actuation is achieved by transforming the dimensional changes to bending and twisting movements by creating bi-layered structures with specific fiber orientations in the individual layers.

When using wood as a construction material its hygroscopic nature and dimensional instability represents an inherent drawback. Inspired by the plant model systems, we take advantage of the moisture sensitivity of wood and its ability to swell and shrink for creating smart, autonomously controlled wooden actuators. (Rüggeberg and Burgert 2015). We have glued and tested wooden bilayers using beech and spruce wood (Figure 1) with perpendicular fibre orientation which reversibly bend under changing relative humidity. We compare the experiments with numerical predictions using a rheological model of beech and spruce (Hassani, Wittel et al. 2015). A history-dependent moisture-stress analysis using the developed material model under changing climatic condition is performed, to predict the true stress and deformation state, hereby including all possible deformation mechanisms as a function of moisture content.

In outdoor applications, such wooden bilayers respond to the daily alteration of relative humidity. As sensor and actuator are incorporated in the wood itself, the movement of these elements is autonomous and solar driven. Possible applications are smart, actuated sun shelters, facades, roof elements or tracking systems for solar panels for autonomously directing to the sun. The mechanical properties of wood and its good machinability allow for upscaling the size of the actuators and by coupled deformation of bilayers we aim at amplifying the amplitude of the movement to overcome the diffusion induced limits in the velocity of actuation. A prototype of a carrier for solar panels has been built and successfully tested in the lab and the field.

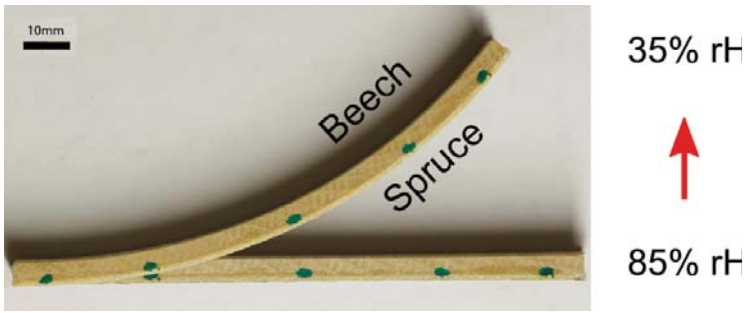

Fig.1: Actuation of a wooden bilayer strip using beech and spruce. The bilayer was glued at 85% relative humidity. Curvature is shown 24 hours after transfer to 35% relative humidity.

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Pine cone as a source of inspiration for moisture induced self-shaping flax/PP biocomposite

Antoine Le Duigou[1] and Mickael Castro [2]

[1]Laboratory of Material Engineering of Brittany (LIMATB), Polymer and Composites, South Brittany University, France

Antoine.le-duigou@univ-ubs.fr; mickael.castro@univ-ubs.fr

Introduction

Plant fibres are already typically used for textile applications and for several years as reinforcing agent in structural polymer composites. However, as highlighted by several recent review [4], plant fibres typically suffer from high water absorption resulting in anisotropic swelling. Water uptake in plant fibres, such as flax for example, attains around 20 wt% [7] while their axial and transverse free swelling values reach 0.1% and 20-25% respectively [8]. When embedded in a polymer matrix, the swelling of plant fibres can lead to cracking and debonding [1], and consequently to a loss of the composite properties [1].

Basically, to protect themselves, or to disperse seeds, plants have developed original movement strategies without any external source of energy but rather only influenced by humidity gradients [2, 5]. The motion of natural actuator, pine cone [9], wheat awn [3] and seed dispersal [2] is reached thanks to a bilayered structure where each tissue has a specific architecture, *i.e.* a swellable matrix whose movement is hindered by stiff cellulose fibrils [2].

The motivation of the present work is to convert the observed "natural" weaknesses of vegetal fibres encountered in the field of structural polymer composites into the driving force of a water-induced actuation phenomenon, thus following one of the first indications of the biomimetic paradigm. The purpose of the present article, inspired by humidity-driven bending elements such as pine cones, is to develop a new recyclable bio-based autonomously self-shaping material induced by humidity variation. The bending ability of the different manufactured bilayered biocomposites is quantified when immersed in water and finally compared to theoretical prediction.

RESULTS AND CONCLUSIONS

Water-responsive biocomposites are manufactured as a bilayer with opposite swelling behaviour. One layer is actually composed of flax fibre (bundles and singles fibres) and the other one is polypropylene as it is an apolar polymer matrix (Figure 1A). Flax fibres, in the proposed composite, play both role of reinforcing and swelling agent, and thus, their orientation is of high importance [6]. A typical evolution of the water responsive biocomposite actuator before and after sorption is shown in Figure 1B, i.e. exhibiting bending during immersion and straightening while drying (Figure 1B).

The curvature is then evaluated as a function of the thickness ratio between the structured active (flax/PP) to passive (PP) layer (*m* ratio), as it is known to be a crucial parameter for bending ability. Bending curvature reaches a maximum average curvature K around 0.052 ± 0.008 mm^{-1} for a given *m* ratio of about 0.5. An increase of *m* above 0.5 does not induce any curvature increase. This is due an increasing moment of inertia and thus stiffness.

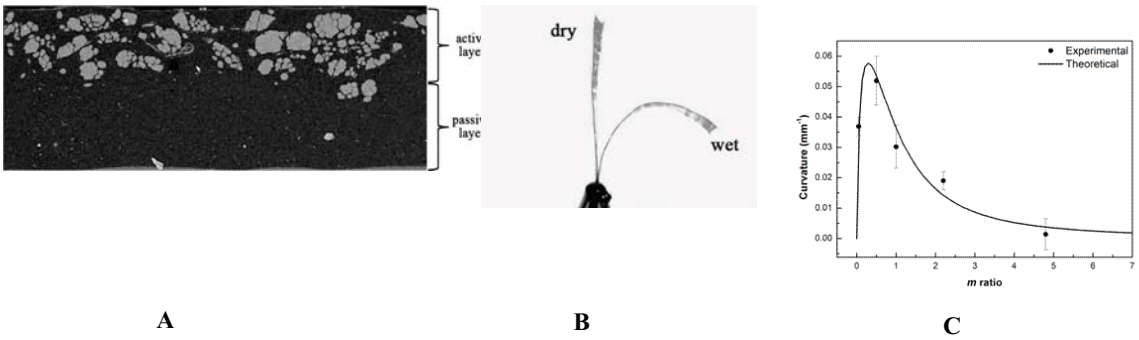

Fig. 1 (A) Cross sectional SEM image of biocomposite actuator, (B) Biocomposite actuator before and after sorption step, (C) Evolution of curvature as a function of layer thickness ratio. (solid line is theoretical prediction).

Bending curvature is then compared to theoretical prediction given by a modified Timoshenko's model for hygromorphic bilayer [9]. Theoretical model fits rather well the experimental curvature (Figure 5). Thus predictive bending actuation can be achieved by monitoring biocomposite actuator properties and *m* layer thickness ratio.
Complementary works will be presented on the relationship between water uptake and bending ability. Durability of these water-responsive biocomposites will be discussed as well as their potential applications.

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Wound reactions of herbaceous plants

O. Speck[1,2,3], S. Anandan[1,2], C. Paul-Victor[1,2], A. Cegna[1,2], K. Schmauder[1], A. Rudolph[1], T. Speck[1,2,3]

[1] Plant Biomechanics Group and Botanic Garden, University of Freiburg, Germany
[2]Freiburg Materials Research Center (FMF), Germany
[3]Competence Network Biomimetics, Germany
olga.speck@biologie.uni-freiburg.de

Introduction

In the course of 3.8 billion years plants and animals have evolved the amazing capacity to deal with various types of wounds and injuries. Since a high evolutionary pressure on the development of self-repairing functions can be assumed, independent evolution of self-repair mechanisms can be expected in different lineages of plants and animals. This explains the huge number of different "solutions" in living organisms to deal with damage. In all examined plants self-repair processes typically can be subdivided into a fast self-sealing phase followed by a markedly slower self-healing phase. Using "self-repair" as an umbrella term the two phases are characterized by anatomical and biochemical modifications plus changes of biomechanical properties. Over the last years a variety of comparative studies were carried out for a deeper understanding of self-repair processes in plant fibers and in organs of plants growing under extreme ecological conditions [1-4].

Material and Methods

Comparative anatomical and biomechanical studies were carried out on species of the Mint family (*Mentha spicata, Mentha longifolia, Mentha aquatica*, and *Leonurus cardiaca*) having distinctly square stems with marked bundles of collenchyma fibers in the corners of the stems. Additionally species from different plant families with succulent cylindrical plant organs (*Sansevieria cylindrica, Rhipsalis baccifera ssp. mauritiana and Euphorbia tirucalli)* were studied. Furthermore, two varieties of *Linum usitatissimum* with different mechanical properties were analyzed to study self-repair in fiber-reinforced composites. The selected test plants were injured along the stem in longitudinal and in transversal direction (Fig. 1) and wound reaction was analyzed comparatively [5-8].

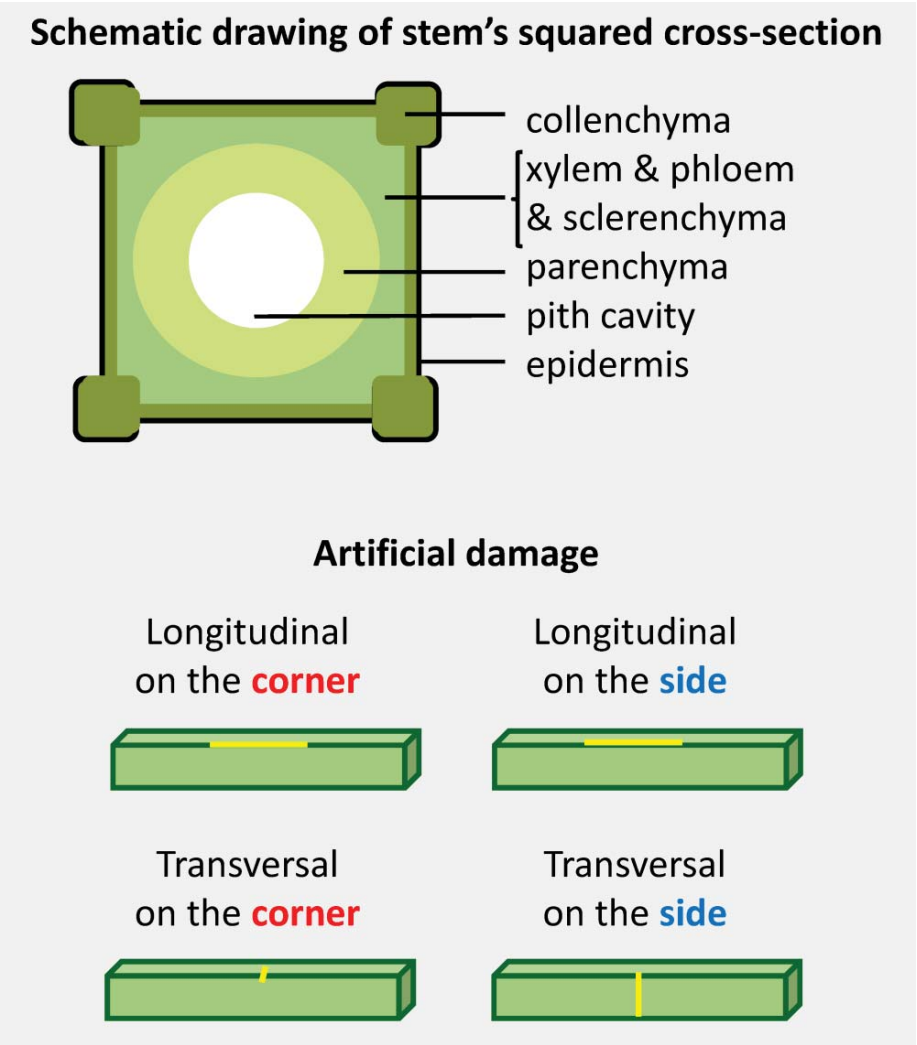

Fig. 1: Types of damage as applied in species of the Mint family.

Results

According to the wound direction (i.e. longitudinal or transversal), the wound position (i.e. on the collenchyma fibres bundles or on the fibre-reinforced composite tissue), and the wound size (i.e. razor blade or lancet), different reactions were detected during a healing period ranging between three to four weeks: (1) discharge and coagulation of latex (fig. 2) or discharge of mucilage, (2) rolling in or overlapping of the wound edges comprised of cuticula, epidermis and underlying tissues, (3) formation of a boundary layer by the deposition of lipophilic substances (lignin, suberin, cutin) in or on the cell walls in the wound region, (4) opening or closing of the wound by (internal) movement of entire plant organs, and (5) formation of a wound periderm. After the healing time injured plant stems showed significant changes in elastic and viscoelastic properties compared with undamaged control plants [8-9].

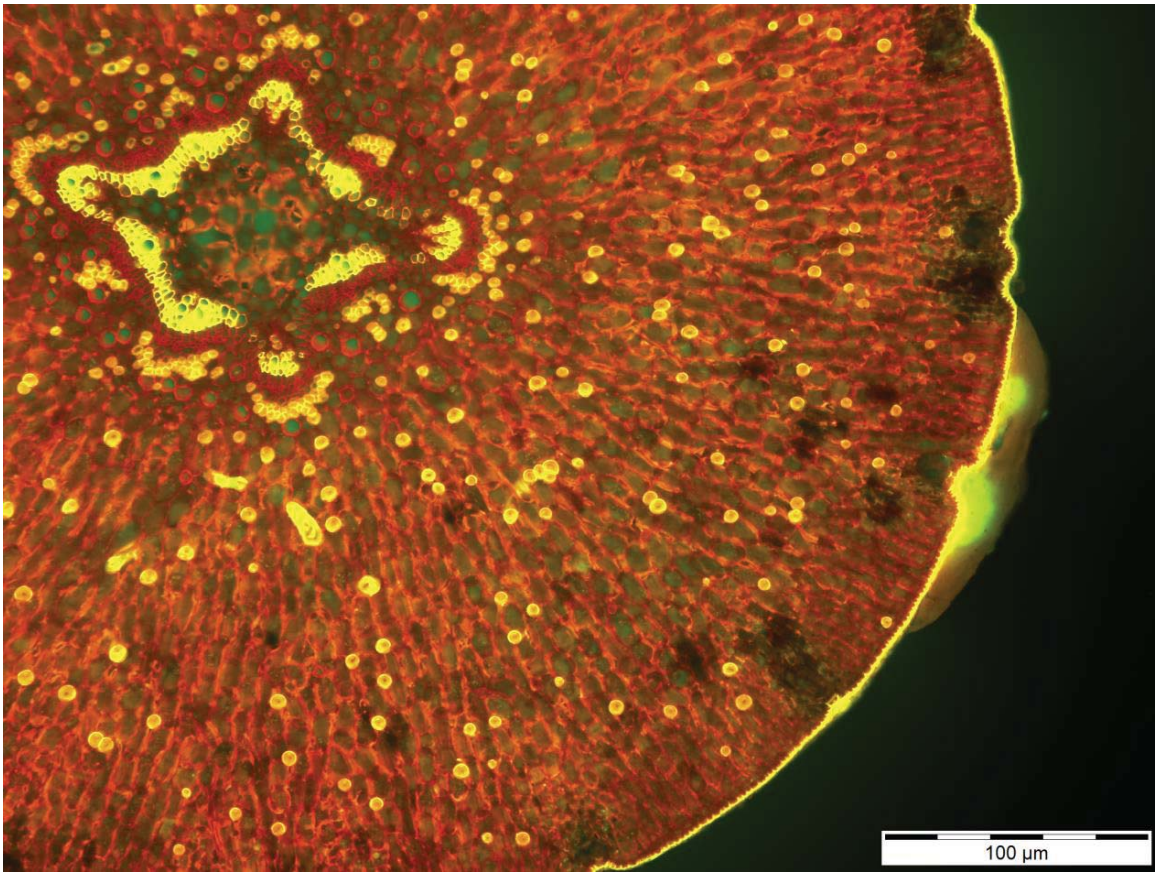

Fig. 2: Cross-section of an *Euphorbia tirucalli* stem stained with a special staining for lignin (Acridine Orange). The damage in longitudinal direction of the stem is rapidly sealed by the discharge and coagulation of latex, from [7].

Biomimetics

Besides detailed new quantitative insight in fundamental processes of structural and functional wound repair in plants, these studies provide a valuable source of bio-inspiration for the transfer into innovative biomimetic self-repairing materials and structures.

Acknowledgement

The two projects “Self-repair mechanisms in plants growing under extreme ecological conditions” and “Self-repair of plant fibres as a role model for self-healing fibre-reinforced composites” are part of the European Marie Curie Initial Training Network "Self-Healing Materials: from Concepts to Market" (SHeMat) funded in the 7th Framework Programme of the European Commission.

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Bio-inspiration by plants for construction technology and architecture: a short overview of recent work in the CRC 141

Thomas Speck

Plant Biomechanics Group and Botanic Garden, University of Freiburg, Germany
Freiburg Centre for Interactive Materials and Bioinspired Technologies (FIT), Germany
Competence Network Biomimetics, Germany
thomas.speck@biologie.uni-freiburg.de

Introduction

Biological materials and structures are based on the combination of a few basic molecular components but due to their hierarchical organization and often multi-layered structuring they are finely tuned and highly differentiated. Biological materials and structures are characterized by multiple networked functions and (often) possess excellent mechanical features, many-fold self-x-properties and a pronounced adaptability to changing environmental conditions. Based on recent developments in computational design, simulation and fabrication new options exist for the transfer of these principles to the macro-scale of building construction and other fields of technology. The aim of the Transregional Collaborative Research Centre 141 (CRC 141) 'Biological Design and Integrative Structures – Analysis, Simulation and Implementation in Architecture' is not only to increase the performance in construction technology and architecture, but also to transfer the inherent ecological properties of natural constructions to architectural solutions. Examples are the efficient usage of limited resources and closed material cycles whose implementation will allow for increasing sustainability in architecture and technology (Antony et al. 2014, Knippers, Speck 2012).

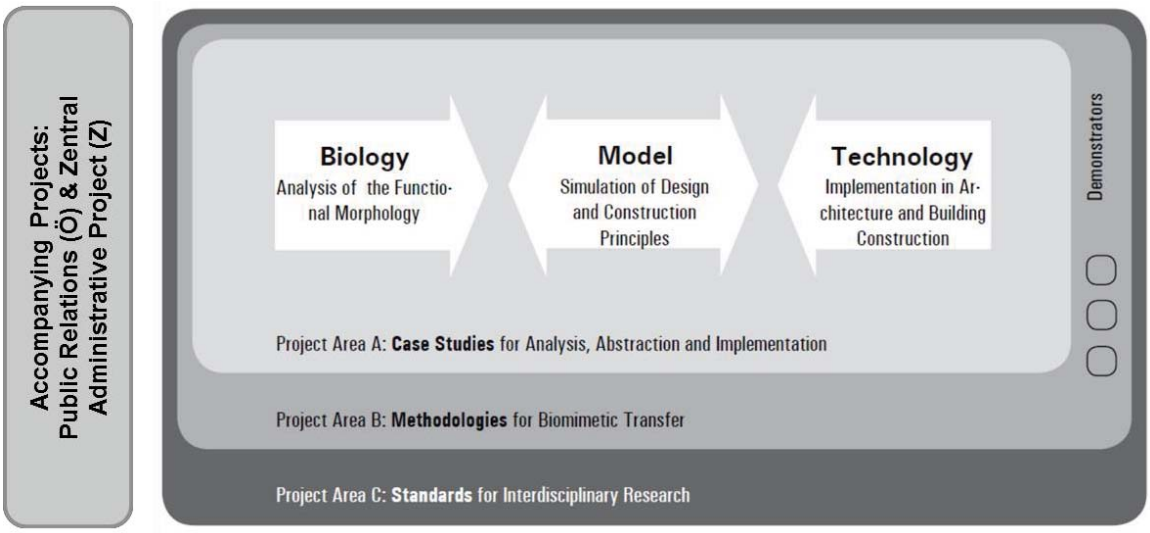

Fig. 1: Structure of the Cooperative Research Center 141 „Biological Design and Integrative Structures - Analysis, Simulation and Implementation in Architecture". © ITKE University of Stuttgart and Plant Biomechanics Group University of Freiburg.

The Transregional Collaborative Research Centre 141 represents an interdisciplinary research initiative based on the co-operation between scientists from the universities of Stuttgart (coordinating university), Freiburg and Tübingen as well as the satellite institutions Stuttgart State Museum of Natural History (SMNS) and Fraunhofer Institute for Building Physics (IPB). All scientific projects of the CRC 141 are handled by natural scientists (biologists, physicists, chemists, geologists), mathematicians, material scientists, engineers and architects on an

interdisciplinary basis (Fig. 1). Each project is headed up by at least an architect and/or an engineer and also a natural scientist. From the fifteen approved interdisciplinary CRC 141 projects, seven run with contribution of principles investigators from the Plant Biomechanics Group Freiburg. Those will be introduced in short project descriptions subsequent to the above general introduction to the central ideas of the CRC 141. A more detailed description of the organizational structure of the CRC 141, of the individual projects, and of the scientists involved can be found online (www.trr141.de).

The main focus of the research program of the CRC 141 lays on analysing design and construction principles in biology and on the subsequent transfer of these principles to architecture and building constructions. Evolution – a process during which all species constantly adapt to a permanently changing environment through mutation, recombination and selection – can be considered as guiding principle, as in this process the efficient usage of natural resources is of significant advantage. This results in structures made of only a limited number of chemical elements and basic molecular components that are directly available as part of a natural material cycle. Additionally they are processed under ambient conditions, which means in a typical temperature range between -50 and +40° C and at ambient pressure that is – with the exception of deep sea regions – close to 1 bar. Based on genetically controlled self-assembly living organisms form hierarchically organized, finely tuned and highly differentiated materials and structures with a multitude of interconnected functions which occasionally fulfil contradictory functional requirements (Fig. 2). In this respect, biological structures differ fundamentally from most building constructions being made of a large variety of materials which are used in the form of individual components. These components are independently optimized for a few target functions under stationary boundary conditions. Technical materials are typically produced with highly energy consuming methods of manufacturing including high process temperatures of several hundred degrees Celsius and high pressures of up to several hundred bar. Design principles such as adaptability, multi-functionality or hierarchical structuring have so far been used in technology and especially in building construction only to a very limited extent, even though their omnipresence in nature is a well-known fact (Speck et al. 2015a, 2015b, Knippers, Speck 2012).

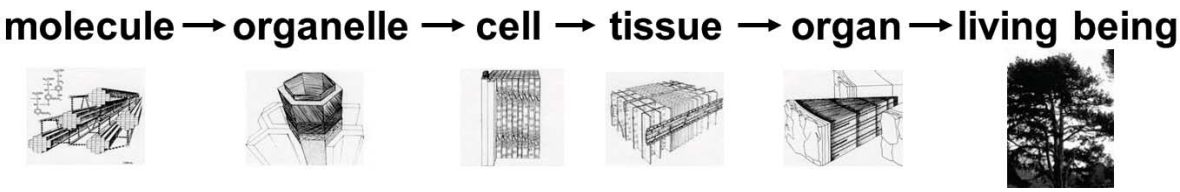

Fig. 2: Biological "production method": genetically controlled self-assembly. Biological materials are built by constructing from small to big with an increase in complexity by hierarchical growing. These materials are based on hierarchies and functionalized surfaces and interfaces at different length scales. © Plant Biomechanics Group University of Freiburg.

Results and Discussion

In the past a central paradigm of building construction has been the use of as many identical parts as possible and to arrange them as simply as possible, an approach that is in total contrast to all natural structures. However, over the last few years, this idea has changed fundamentally, especially driven through the introduction of computational manufacturing processes. These novel manufacturing processes not only increase the possible level of geometric differentiation and individualization, but also allow for the production at reasonable costs of porous, multi-layered or fibre-based materials with locally adjusted mechanical, physical and chemical properties. This development has been further pushed on by recent developments in simulation technologies that have focused on multi-scale models and the interplay of mechanical

phenomena at various hierarchical levels. Together with a multitude of new quantitative methods in biology for structural investigations covering diverse hierarchical levels, this progress in computational simulation and fabrication offers new options for the analysis of biological structures and for the transfer of their design and construction principles to the macro-scale of building construction and other fields of technology (Ahlquist et al. 2015, Schleicher et al. 2015, Speck et al. 2015a, Knippers, Speck 2012).

Using this advantageous situation, the CRC 141 aims for implementing ideas from living nature into bio-inspired materials and structures for a novel, smart and ecologically friendly architecture. To reach this goal, the entire program of the CRC 141 is conceptualized as a dialogue: the functionally important features of the biological systems are abstracted in a model which represents the properties of interest and the underlying design principles. In a next step this model serves as a basis for simulating functional morphology and biomechanics and by this provides the basis for technical implementation. In the process of 'reverse biomimetics', the results represent also the foundations for further investigations and a more detailed understanding of the biological systems. This reiterative approach will finally lead to a closed heuristic spiral which eventually results in an increased knowledge in all scientific fields involved namely biology, material sciences, building construction and architecture (Fig. 3) (Speck, Speck 2008).

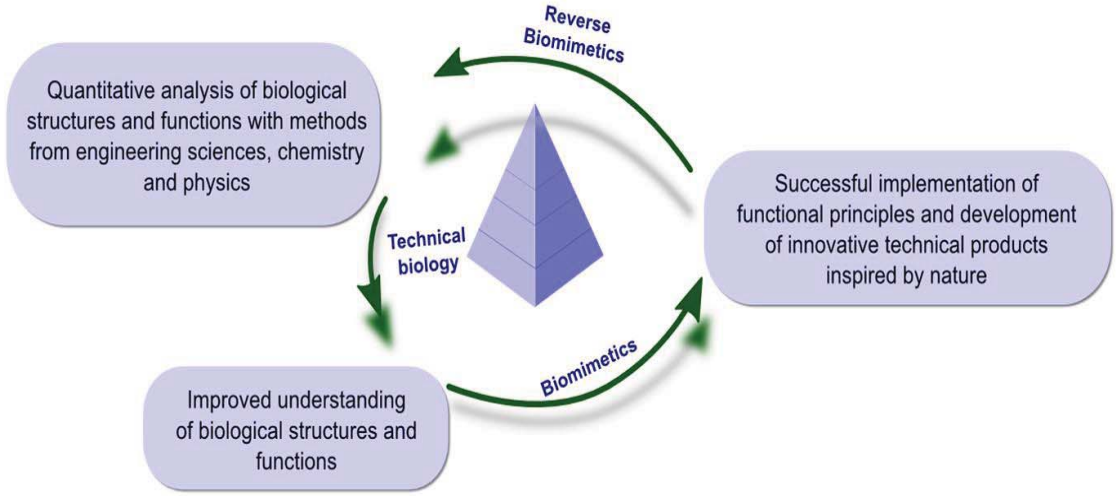

Fig. 3: Relation and mode of operation in technical biology, biomimetics and reverse biomimetics. The closed heuristic spiral connecting technical biology, biomimetics and reverse biomimetics causes a continuous increase in knowledge in biomimetics and biology. © Plant Biomechanics Group University of Freiburg.

In order to achieve the central goals outlined above in the Transregional Collaborative Research Centre 141 'Biological Design and Integrative Structures – Analysis, Simulation and Implementation in Architecture' several key questions have to be answered (Speck et al. 2015a):

(1) How can the structure of hierarchically organized, multifunctional and adaptive biological systems be modelled and simulated? The computational simulation will not only enable a deeper understanding of biological structures and systems but also of their evolutionary development. In parallel, it will serve as a basis for transfer to technical applications and for novel developments in the field of building construction (Schleicher et al. 2015, Lienhard et al. 2011).

(2) How can computational fabrication methods be used to transfer the design and construction principles of nature to the macro-scale of building construction? The aim in the CRC 141 is not only to increase the efficiency of building structures but also to develop new bio-inspired products with features such as multi-functionality, hierarchical structuring or adaptability that until now have not been used in building construction (Speck et al. 2015b, Ahlquist et al. 2015, Schleicher et al. 2015, Knippers, Speck 2012).

(3) Under which conditions do the abstraction and transfer of structural and functional principles from biology lead to a more sustainable technology that 'inherits' not only the functional but also the ecologically beneficial properties of biological role models? The development in building construction is increasingly directed towards resource efficiency, sustainable use of building materials and closed material loops, which are self-evident for most natural systems (Speck O. et al. 2015, Antony et al. 2014).

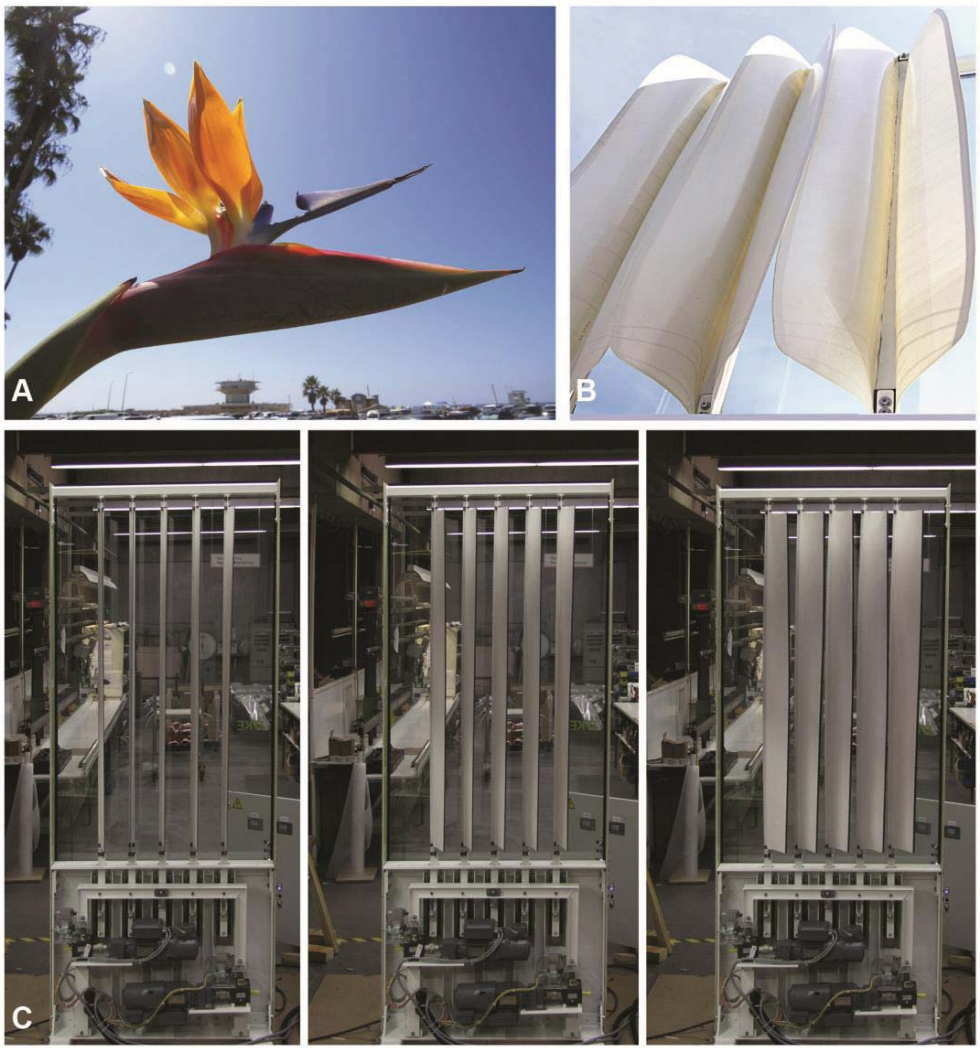

Fig. 4: Biological concept generator Bird-Of-Paradise flower with violet perch (A) and demonstrators of the biomimetic façade shading system, double flectofin® (B) and simple flectofin® in different positions of closure for shading (C) © PBG University of Freiburg and Julian Lienhard, ITKE University of Stuttgart.

The “research philosophy” of the CRC 141 and recent results are presented for four projects dealing with (1) bio-inspired highly energy dissipating structures for load bearing systems and facades, (2) deployable systems in architecture inspired by the kinematics of planar, curved and corrugated plant surfaces (Fig. 4), (3) highly load-bearing branched beams for construction

technology inspired by plant branchings, and (4) the conditions that have to be fulfilled in order to allow for a transfer of structural and functional principles from biology to a more sustainable architecture (Speck o. et al. 2016, Antony et al. 2014). Examples for structural materials with a high energy dissipation capacity include fibre-reinforced graded foamy structures and ultra-thin-layer materials which are inspired by fruit peels (e.g. pomelo) and seed coats (e.g. macadamia, coconut), as well as by damping and insulation of different bark structures (e.g. redwood- and pipevine-bark) (Thielen et al. 2015, Knippers, Speck 2012). Different active and passive movements of flowers, as well as trap movement of various carnivorous plants and passive movements of cone scales form different conifer species serve as concept generators for deployable systems in architecture (Schleicher et al. 2015, Speck et al. 2015b, Lienhard et al. 2011). Examples for light-weight materials with excellent mechanical properties are biomimetically optimized branched and un-branched fiber-reinforced composite materials with gradient structure inspired by bamboo, horsetails and branched stems of dragon trees and columnar cacti (Ahlquist et al. 2015, Haushahn et al. 2014, Müller et al. 2014).

Acknowledgements

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Energy dissipation in plants – from puncture resistant seed coats to impact resistant tree barks

Georg Bauer[1,2], Stefanie Schmier[1,2], Marc Thielen[1,3], Thomas Speck[1,2,3]

[1]Plant Biomechanics Group Freiburg, Botanic Garden of the University of Freiburg, Germany,
[2]Freiburg Centre for Interactive Materials and Bioinspired Technologies (FIT), Germany,
[3]Freiburg Materials Research Center (FMF)
georg.bauer@biologie.uni-freiburg.de

Introduction

In biology, there exists a variety of cells, tissues and organs that need to be protected in order to ensure the survival and/or the fertility of the whole organism. Among the plant kingdom, the cambium of trees and plant embryos are examples of these crucial structures. Thereby, the bark (the term 'bark' is used here to describe all outer tissue layers of a tree stem, surrounding its cambium) can be seen as the protective layer of a tree's stem, and the seed coat and/or fruit wall take over the protection of a plant's embryo.

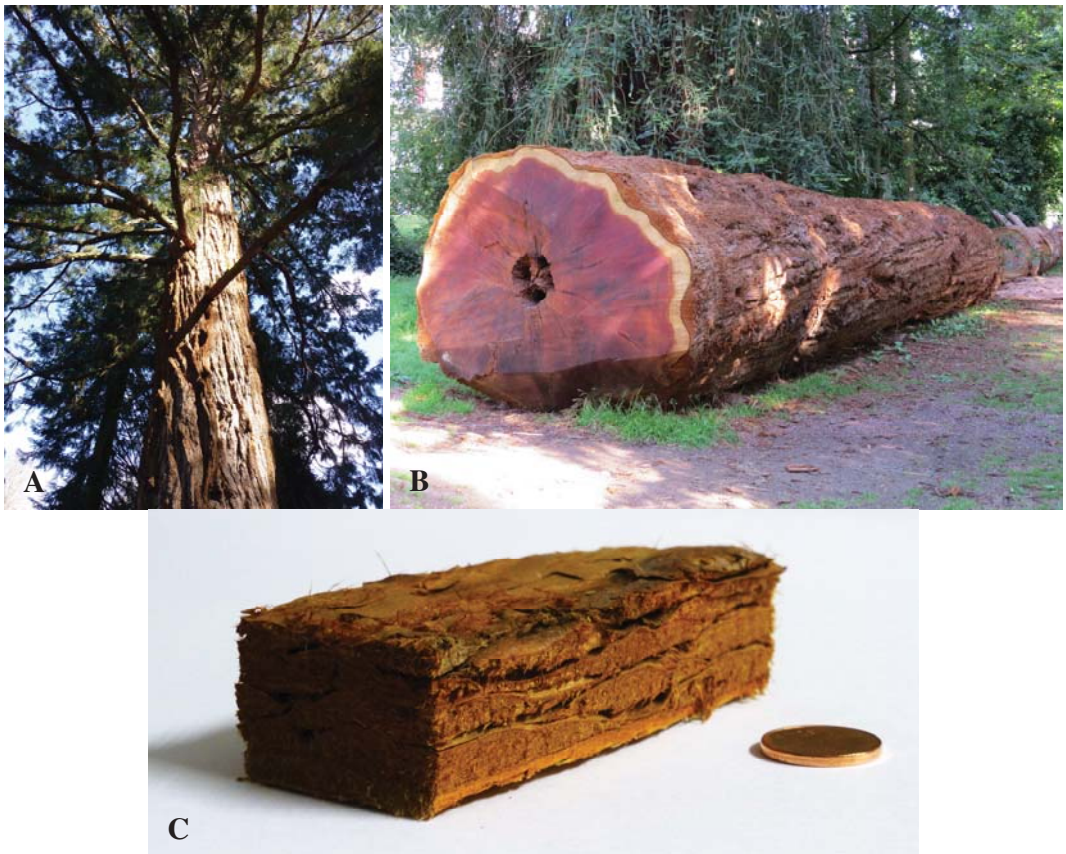

Fig. 1: Impact resistant and heat insulating tree bark: (A & B) Giant Sequoia (*S. giganteum*) in the Botanic Garden, Freiburg showing the thick and protective bark. (C) Prepared sample: layered and fibrous structure of the bark of Giant Sequoia.

Impact resistant tree barks

The vascular cambium of a tree – a thin meristematic tissue layer surrounded only by the tree bark – generates the secondary xylem and the secondary phloem of a tree by cell proliferation in the outwards (forming the phloem) and the inwards (forming the xylem) direction of a stem's cross section. The vascular cambium is thus essential for the growth and survival of the whole tree and cannot be reestablished by any other tissue in a tree stem. Consequently, protecting the cambium from damage, heat and chemical/biological decomposition can be seen as of major importance for the survival of a tree. This function (i.e. protection of the cambium) is provided by the tree bark. For example, a tree's bark is a physical barrier against microorganisms and an insulation layer protecting the stem's more central parts from overcritical heat during forest fires (Bauer et al., 2010). Furthermore, among other hostile interventions high impact rockfall events represent another scenario for a tree when it becomes important to protect its cambium from external hazards, as they occur regularly in mountainous regions (Volkwein et al., 2011). One example of a tree species which is native in summer-dry mountainous regions, and is thus frequently exposed to forest fires and rockfall events is the Giant Sequoia (*Sequoiadendron giganteum*; see Fig. 1).

In order to examine a tree's potential to protect its cambium from rockfall events, free fall tests were conducted with the bark of a Giant Sequoia logged in spring 2015 (Fig. 2). While a bark sample was placed on top of an instrumented anvil and a drop weight was dropped through a guided tube onto the bark sample, a highspeed camera recorded the impact (for a detailed description of the testing device we refer to Thielen et al. 2015). Typical force-time curves (Fig. 2B) display a wide force peak during impact. Thus, the fibrous and multilayered tree bark is capable of dissipating high amounts of energy (up to 90 %) during such an impact event. Further investigations of the anatomy of the bark of different tree species (including also less impact resistant tree barks) will illuminate the correlation between inner bark structure and energy dissipation capability.

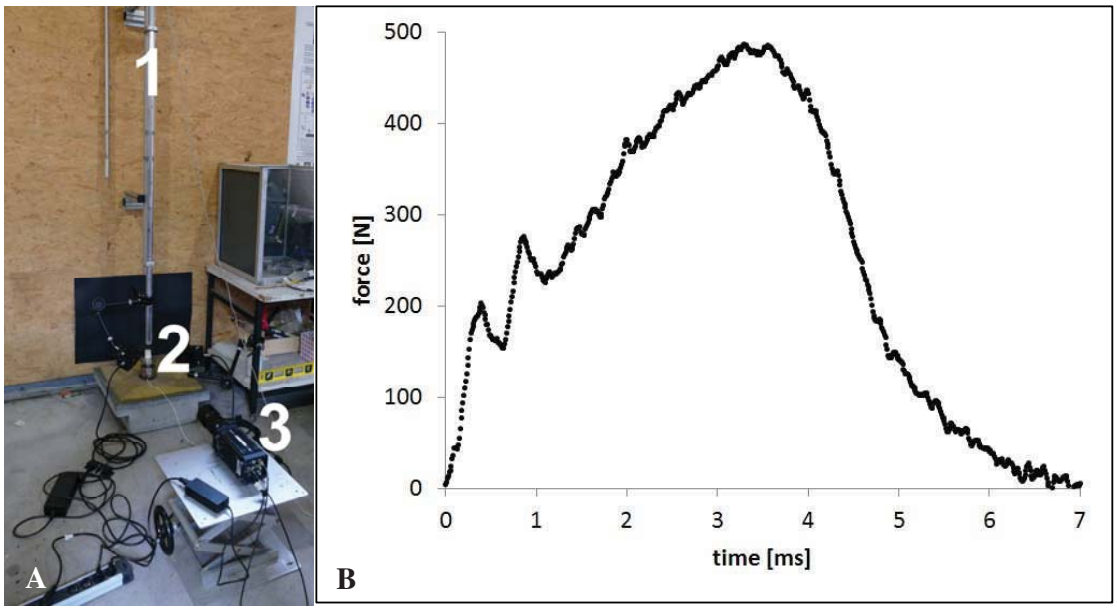

Fig. 2: Free fall test rig used for impact tests of the Giant Sequoia tree bark (A), including a guided tube (1), an instrumented anvil (2) and a highspeed camera (3). (B) Typical force-time curve of an impact test with a sample of the Giant Sequoia tree bark at 3.8 J impact energy.

Impact resistant fruit walls and puncture resistant seed coats

In order to resist dropping from great heights or having to withstand predator's mouthparts, plant embryos need to be surrounded by e.g. impact resistant fruit walls and/or puncture resistant seed coats (Seidel et al., 2010).
A very sophisticated example of an impact resistant fruit wall represents the coconut which botanically represents a drupe (Fig. 3). The thin exocarp, the thick fibrous mesocarp as well as the hard and tough endocarp surround the endosperm and the embryo. Embryo and endosperm are thus protected from high forces occurring when the fruit falls from the coconut tree which can reach up to 30 m in height, or when predators try to penetrate the fruit wall in order to feed from the nutritive endosperm. The combination of the fibrous mesocarp and the tough and hard endocarp represents an effective impact protection as it allows for damping impact energy very efficiently and for puncture resistance.

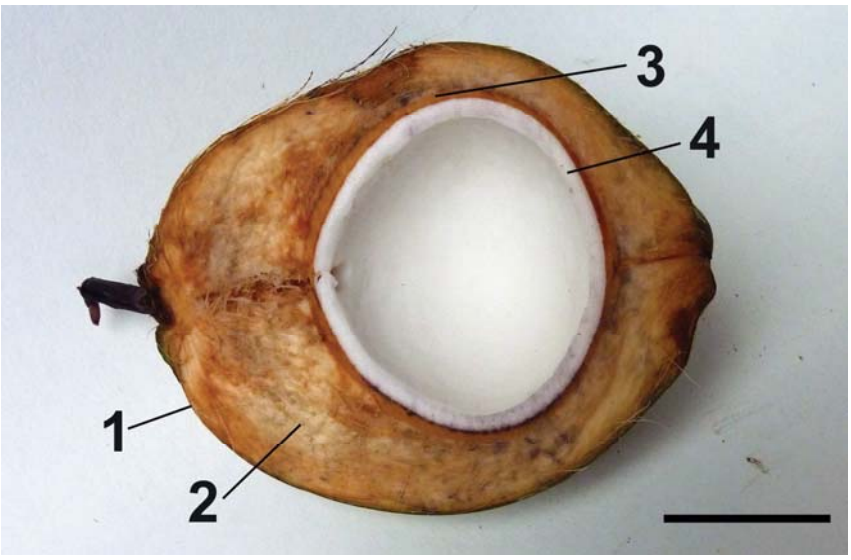

Fig. 3: Impact resistant multi-layered fruit wall of the coconut (*Cocos nucifera*). The exocarp (1), the fibrous mesocarp (2) and the tough and hard endocarp (3) serve as protective layers surrounding the endosperm (4) and the embyro. Scale bar: 5 cm.

The multilayered and fiber-containing tough endocarp is much denser compared to the surrounding meso- and exocarp. Its resistance to high velocity impacts was examined using an impact pendulum (Fig. 4A). Samples cut from the endocarp were placed on the sample holder and the instrumented impact pendulum was released to transfer its potential energy to the test sample.

Typical force-time curves of such impacts reveal multiple peaks during fracture of the endocarp (Fig. 4B). Comparing samples cut along different directions (meridional vs. equatorial plane) reveal differences in their mechanical behavior. These variations in the nominal stress and in the impact resistance imply a different structuring of the outer and inner layer of the endocarp (e.g. fiber content, fiber orientation etc.).

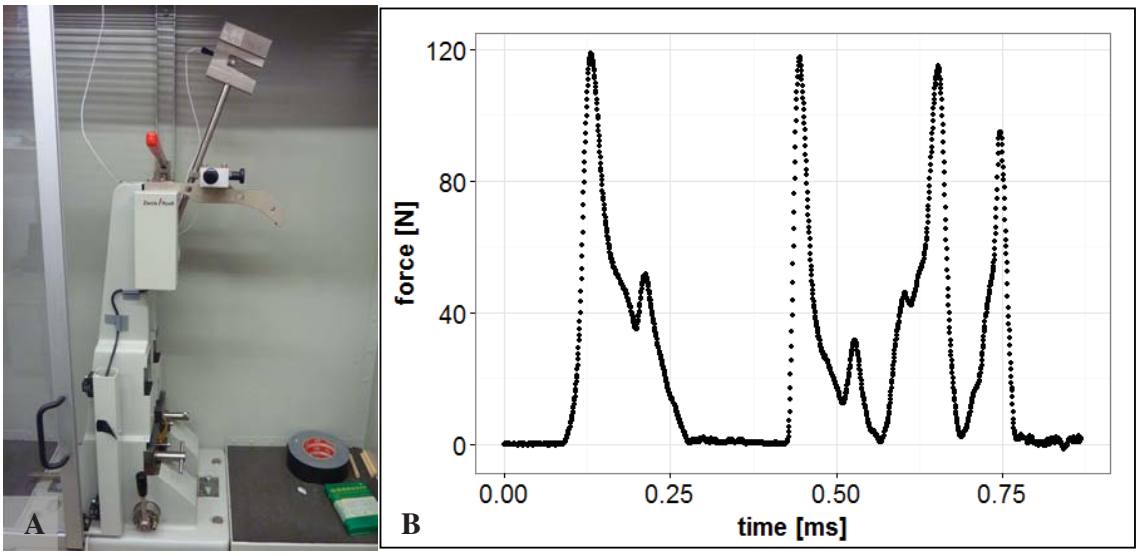

Fig. 4: Impact pendulum used for the impact tests of the coconut endocarp (A). Typical force-time curve of an impact test with a cocnut endocarp sample at 7.5 J impact energy (B).

Fig. 5: Assortment of hard shelled fruits and seeds in the diaspore collection of the University of Mainz.

Future anatomical studies of the coconut will focus on the structuring of the different endocarp layers. The non-uniform composition of the endocarp is most likely adapted to differences in the stress distribution caused by the ellipsoidal shape of the fruit. Further

investigations will additionally include other puncture resistant fruit walls and seed coats (Fig. 5), as e.g. done for the macadamia nut which botanically is a seed (Schüler et al. 2014). The aim is to investigate the puncture resistance of additional biological, fibrous and/or multilayered systems beside the abovementioned impact resistance of fruit walls of the coconut to have a source of inspirations for puncture resistant materials.

Technical implications

Concluding from these studies, a high impact resistance is achieved by layered (micro-laminated) and/or fiber reinforced structures. On the basis of these and upcoming results, technical bio-inspired structured porous and/or fiber-reinforced and/or layered concrete and ceramic materials exhibiting a high impact and/or puncture resistance will be developed and built with special emphasis on materials for application in building construction.

Typically, the magnitude of a biological structure differs from that of a man-made system. To manage the scaling up of technical structures derived from the biological role models without any degradation of mechanical properties the theory of Weibull (1951) shall be taken into account. Those models are well established in the material sciences for brittle materials. Therefore the investigation of size-volume-property-relations of biological role models will be of high interest. By this token the project shall answer the question whether the Weibull models also can be applied in order to explain the failure behaviour of non-brittle biological materials and structures (such as for example bark failure).

Acknowledgements:

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A model of plant and microbe symbiosis: Ricardian approach

Toshiko FURUKAWA[1], Takuya IIMURA[2]

[1]Department of biological sciences, Tokyo Metropolitan University, Japan
[2]Department of business administration, Tokyo Metropolitan University, Japan
furukawa-toshiko@tmu.ac.jp

Introduction

Modeling the interactions of species is an important topic in biology, ecology, and in any environment related disciplines. In the past decades we have witnessed a series of successful mathematical modeling of such interactions, for example, evolutionary game theory (Maynard Smith and Price 1973) and complexity theory (Waldrop 1992). In this work, we focus on *symbiotic* interaction among many interaction types between plants and microbes, and propose a new---but its origin is old---simple model of symbiosis, which exploits a famous international trade model in economics that dates back to David Ricardo (1817). Symbiosis is the relationship that both parties have benefits. Each, however, should pay considerable amounts of costs, and symbiotic status occurs only when benefits far exceed costs (Hopkins 1997, Paracer and Ahmadjian 2000). We regard the exchange of carbon and nitrogen performed by legume and rhizobium---a typical plant and microbe symbiosis---as a trade that exploits the *comparative advantage* in the costs of fixing the carbon and nitrogen owned by legume and rhizobium, respectively. We construct a model of such exchange on the basis of observable data such as C/N ratio, which means that the model is testable.

The model

Plant: We denote by x_p^c and x_p^n the amounts of carbon and nitrogen, respectively, taken by a plant per day. We assume the following four for the plant.

(P1) The growth rate g_p of the plant per day is increasing in x_p^c and x_p^n.

(P2) The growth obeys Liebig's law of the minimum.

(P3) The ratio of the minimum x_p^c and x_p^n implied by this law is a constant and independent of g_p.

(P4) The efficiency of carbon fixation is constant, whereas that of nitrogen self-intake depends on the environment.

Microbes: We denote by x_m^c and x_m^n the amounts of carbon and nitrogen, respectively, taken by a population of microbes. We assume the following four for the microbes.

(M1) The growth rate g_m of the microbes per day is increasing in x_m^c and x_m^n.

(M2) The growth obeys Liebig's law of the minimum.

(M3) The ratio of the minimum x_m^c and x_m^n implied by this law is a constant and independent of g_m.

(M4) The efficiency of nitrogen fixation and carbon self-intake are both constant.

The results

Under the above assumptions, we establish (i) a sufficient condition for a symbiotic status to occur in terms of the comparative advantage, and (ii) the equilibrium exchange rate, which predicts the trade ratio of carbon and nitrogen. We also discuss the estimation method of parameters and computation of equilibria.

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How the pomelo peel (*Citrus maxima*) absorbs impact energy by distributing stresses

Marc Thielen, Thomas Speck

Plant Biomechanics Group, Botanic Garden, Faculty of Biology, University of Freiburg, and Freiburg Materials Research Center (FMF), Germany

marc.thielen@biologie.uni-freiburg.de

Introduction

Pomelos are the largest citrus fruits and have an extraordinary thick peel, which dissipates energy during impact and assures the fruit´s integrity even after being shed from 10-15 meters height (Figs 1a & b). This protection against splitting open is crucial for the species insofar as it prevents the fruit from being afflicted by mould spores and microbes and thus ensures that the seeds stay germinable and that the fruit pulp, which is devoured together with the contained seeds, remains attractive for fruit eating animals dispersing the seeds (Thielen et al. 2012).

At first glance the biological tissues constituting the peel do not seem to be well suited to resist impacts from such heights, especially as considerable amounts of kinetic energy – owed to the weight of pomelo fruits of 1-2 kg and the height of fall – are involved. Due to the (internal) hierarchical structuring, the pomelo peel, however, is able to dissipate considerable amounts of kinetic energy without visible external damage of the fruit. Quasi-static and dynamic compression tests were performed to elucidate the mechanism of how this is achieved.

Results and Discussion

The main part of the peel, the whitish albedo, consists of parenchymatic cells which constitute the (living) struts of this foam-like tissue (Fig. 1c), and is interspersed by a three-dimensional network of vascular bundles (Thielen et al. 2013). On the tissue level, this network is responsible for structural damping by distributing local impact stresses and strains to large peel volumes. Thus the peel volumes involved in damping are (markedly) larger than could be expected from the impacting contact area alone (Seidel et al. 2013). Figure 2 clearly shows that the vascular bundles, attached to the foam-like tissue, act as stiffening elements. Due to their comparably high density, they help to distribute stresses to neighbouring less stiff tissue volumes and prevent the occurrence of uniform linear compaction bands. Another, however not yet experimentally confirmed hypothesis is that vascular bundles may act as ‘guy wires’ which help for translating compressive stresses into tensile stresses (Thielen et al. 2013, Niklas 1992).

On a cellular level the struts of the foam-like peel tissue – which in fact represent living parenchyma cells containing cell sap – act as hydraulic dampers. The vacuoles of these cells are filled with incompressible and viscous cell sap, which exerts hydrostatic pressure (turgor) against the cell walls. When these cells are bent or compressed, the cell walls first are tautened, then stretched and eventually cell fluids may be forced to flow through the semi-permeable vacuolar membrane (tonoplast) and finally through the cell wall, which then may lead to additional energy dissipation due to the need to overcome the viscous resistance (Thielen et al. 2013, Thielen et al. 2015).

Impact tests with cylindrical peel samples lead to the observation that the parenchymatic tissue itself behaves as auxetic structure (Fig. 3). Auxetics are metamaterials (or structures) having a negative Poisson ratio. They expand laterally when being stretched and contract laterally when being compressed (Lakes 1987). In contrast to the mechanistical models of conventional open pored foams, which typically are set up under the assumption to consist of so-called Ashby or

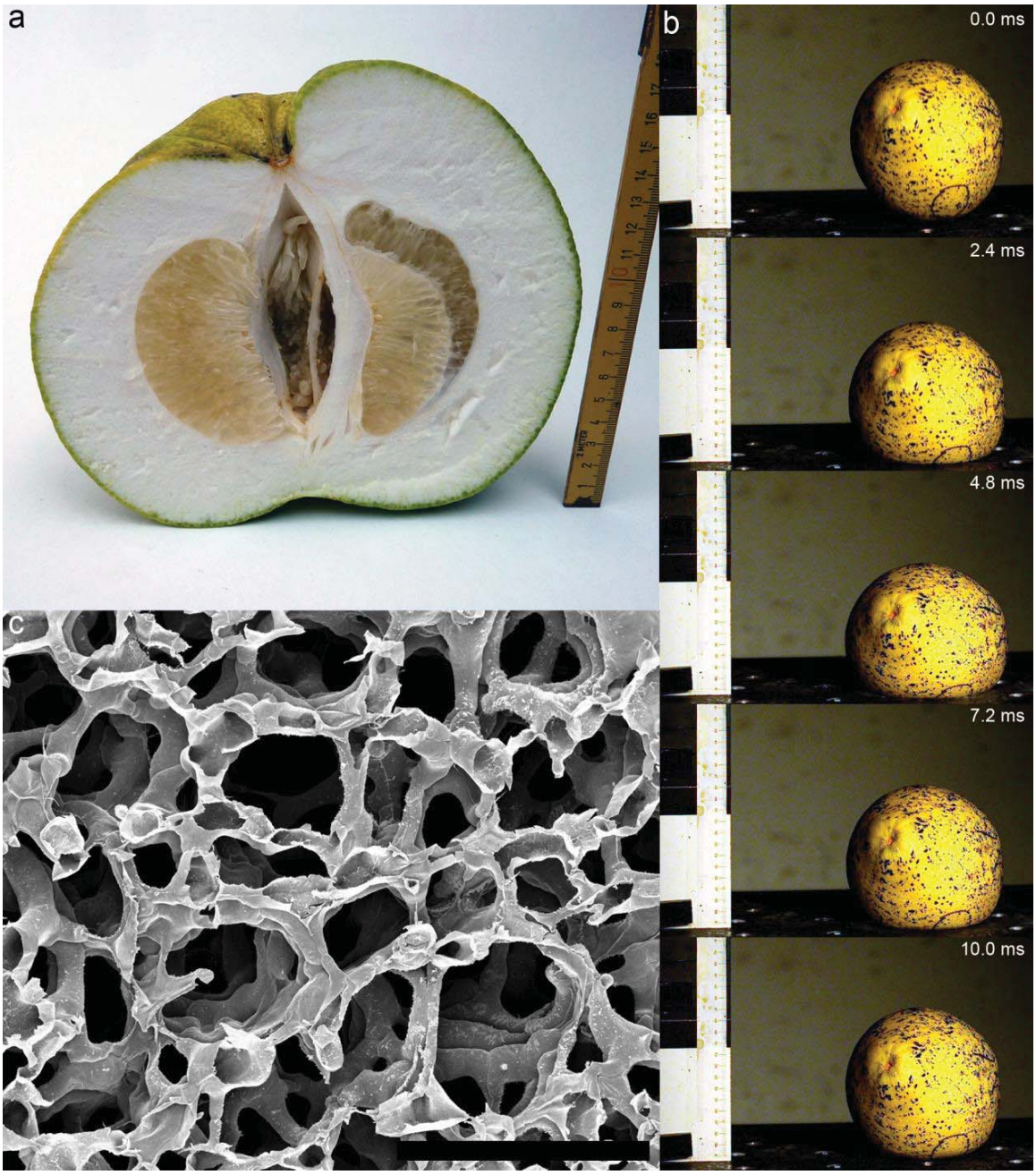

Fig. 1: (a) Longitudinal section through a pomelo exposing the thick whitish part of the peel – the so called albedo. (b) Images from a high-speed video of a pomelo impacting on a metal plate after being dropped from 6.5 m height, showing the pronounced deformation during the impact event. (c) SEM image of the albedo showing it´s foam-like structure. Scale bar: 400 µm. © Plant Biomechanics Group, Freiburg & Institute of Lightweight Engineering and Polymer Technology Dresden

Kelvin cells, auxetic foams can best be represented by re-entrant cells whose struts bend inwards when being compacted. In case of intact pomelo peel this behaviour results in a global stiffening effect, as neighbouring tissue volumes are pulled into the vicinity of the impact thus increasing the energy dissipating peel volume.

Fig. 2: Pomelo peel sample with black patterning applied for showing the deformation, submitted to increasing quasi-static compression (from left to right). The vascular bundles (stained red with phloroglucinol/hydrochloric acid) behave as stiffening elements, distributing the strains and preventing uniform linear compaction bands.

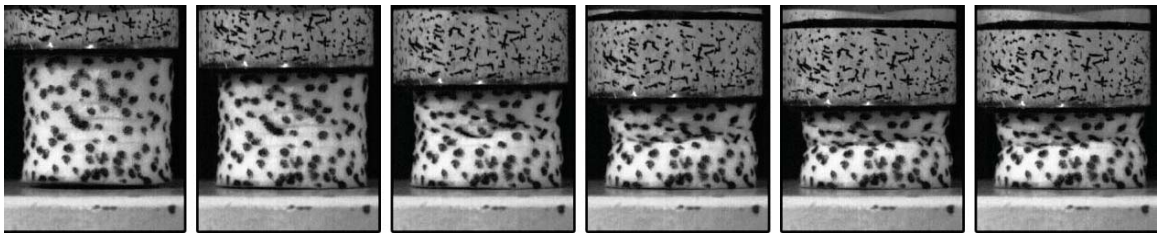

Fig. 3 : Impact loading of freshly cut, cylindrical pomelo peel sample. The lateral contraction of the peel expresses it´s auxetic behaviour (negative Poisson ratio) during impact compression.

Conclusion and Outlook

Due to described ‘tricks’, the relatively flexible peel tissue is able to withstand severe impacts without bottoming out by distributing mechanical stresses occurring during impact. Further investigations envisage to quantify the auxetic effect and to develop biomimetic structures for impact protection.

Acknowledgements

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New insights into the passive nastic motions of pine cone scales and false indusia in ferns

Simon Poppinga, Thomas Speck

Plant Biomechanics Group, Botanic Garden, Faculty of Biology, University of Freiburg, and Freiburg Materials Research Center (FMF), Germany
simon.poppinga@biologie.uni-freiburg.de

Introduction

A central research focus in the Plant Biomechanics Group Freiburg deals with plant movements which are increasingly recognized as worthwhile concept generators for a biomimetic transfer into technical kinetic systems (Burgert & Fratzl 209; Lienhard et al. 2011; Knippers & Speck 2011, Poppinga et al. 2013a). Recently, we showed how the traps of various carnivorous plants move quickly to catch their prey (Vincent et al. 2011, Poppinga & Joyeux 2011, Poppinga et al. 2012), and discussed different trap types in an ecological and evolutionary context (Poppinga et al. 2013b). Moreover, several active and passive types of motion in plants were quantitatively analyzed, abstracted and transferred in finite element models as basis for an implementation in architecture, e.g. as deployable façade structures (Schleicher et al. 2015). Passive nastic plant motions are of particular interest for biomimetic transfers into technical systems because the respective structures do not consume metabolic energy and are sensor, actuator, and regulator in one. Over the last years the structural basis of different hygroscopic plant movements and how they are mechanically evoked was analysed and it could be shown how the involved actuation principles can be used for a transfer into bioinspired technical materials and structures (e.g. medical devices for drug release, sun-shading systems) (Fratzl et al. 2008; Reichert & Menges 2010; Turcaud et al. 2011; Elbaum & Abraham 2014; Reichert et al. 2014; Taccola et al. 2015). In the following we present new insights into the functional morphology and biomechanics of two examples of passive hydraulically moving plant organs.

Functional morphology and biomimetics of cone seed scale movement in *Pinus*

A prime example for passive nastic motion is the pine cone opening and closing mechanism. It is state-of-the-art that each involved cone seed scale is built up as a tissue bilayer (as seen in longitudinal section), with a highly swellable lower sclereid layer and a much lesser swellable upper fibrous sclerenchymatous layer (i.e., the resistance layer). The bending movement of the scales which altogether results in the opening/closing of the cone is based on the different swelling/shrinking properties of these tissues (Harlow et al. 1964; Dawson et al. 1997; Reyssat & Mahadevan 2009).

The pine genus (*Pinus* spp., Pinaceae) comprises 113 species with very different cone and scale size and architecture (Richardson 2000) (Fig. 1A). We conducted comparative studies which take into account the link between seed scale morphology and anatomy and bending kinematics. A better understanding of the scaling laws that apply to both pine cone seed scale architecture and kinematics will help for the development of advanced hygroscopic biomimetic flaps in architecture and for other technical applications. We investigated the cones and cone scales of 8 pine species so far, regarding their bending kinematics, biomechanics and functional morphology. For each species, three different scale morpho-types have been taken into account, so that the total number of investigated scale types sums up to 24. The cone size of the studied pine species ranges from 3 cm to 25 cm and scale

dimensions range between 1 cm and 5 cm. Moreover, the general shape of the scales was found to be very diverse, from more or less flat structures to complex double-curved shells. The kinematics were analyzed as measurements of the angular changes (in relation to the cone axis) of the scales over time owing to swelling (in humid environment and under water to ensure total "wetting") and desiccation (in dry environment, i.e. in a phytochamber at 25°C and a relative air humidity of 30%). By observation of longitudinal sections we were able to measure the thickness of the tissue layers inside each scale type and to analyze the cell types in the different layers. We observed intra- and interspecific differences in angular velocity and maximum angular change of scale movement. Such differences even occurred between different cones of the same pine species. From the respective analyses of functional morphological follows that the angular velocity depends: (1) on the thickness of the hygroscopically active tissue layer, and (2) on the ratio of this active tissue layer to the rather immobile resistance layer. Interestingly, the maximum angular change is independent from the length of the bending zone, i.e. from the area of a scale which undergoes bending deformation during scale movement.

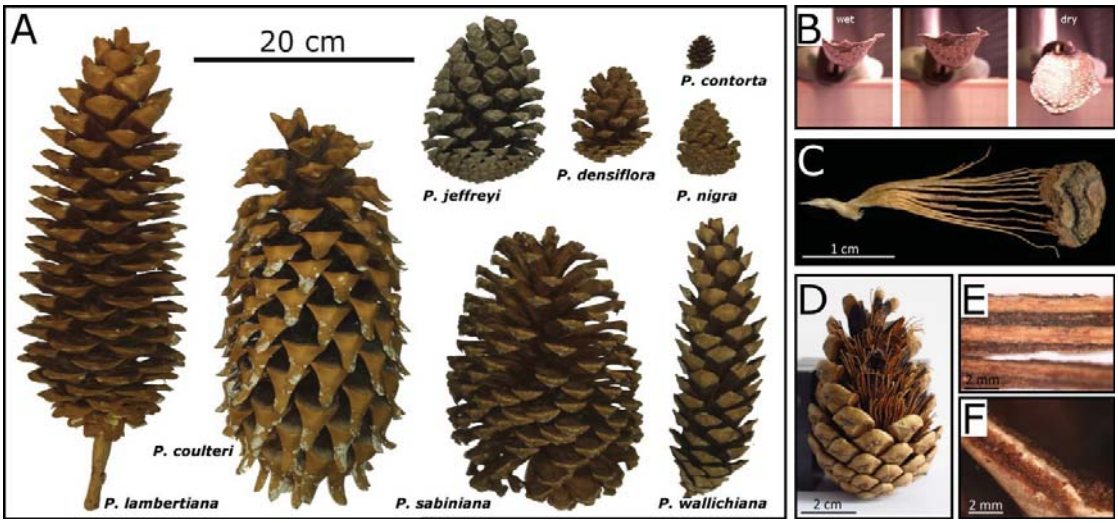

Fig. 1 : Pine cone diversity and seed scale movement. (A) Cones of different pine species showing the huge variety in size and shape. Image adapted from Poppinga & Speck (2014). (B) The scales of *P. wallichiana* show a combined flattening and bending motion during desiccation. (C) The sclerenchymatous tissue consists of several "cables" that extend into the scale periphery. (D) The sclerenchymatous tissue and the sclereid matrix can easily become separated manually. (E) & (F) Details on the sclerenchymatous "cables" (bright) and the sclereid matrix (brown).

Apart from the bending movements of most scales we discovered that the scales of *P. wallichiana* perform additionally "in-scale" movement causing a distinct change of the scale shape additionally to the overall bending movement. In the wet state these scales possess a V-shaped profile, as seen in cross-section. During the desiccation process the scales first begin to flatten and then perform "normal" bending motions (Fig. 1B). This complex two-stage shape change has not yet been described and analyzed before and we are just beginning to understand the structural prerequisites for this complex motion pattern. During our morphological investigations of the scales we observed several structural peculiarities which have not yet been understood in terms of how they cause (or, influence) the deformation. The resistance "layer", i.e. the sclerenchymatous tissue, is actually not a continuous layer but extends in form of cable-like structures into the scale periphery (Fig. 1C). Moreover, these

sclerenchymatous "cables" also bend on their own by swelling/desiccation which indicates a so far unknown micro-structural anisotropy in the "cables". What's more, we found that the hygroscopically active sclereid matrix is not tightly connected to the rather immobile resistance layer which is thought to be a prerequisite for a technical bilayer but can be easily separated by manual pulling (Fig. 1D-F).
Together with colleagues from the Institute of Computational Design of the University of Stuttgart (ICD, Prof. Achim Menges and co-workers) we conducted 3D-scans at various degrees of humidity which allowed for a digitization of the actuation of the pine cone. These data are currently being used to develop simulation techniques for predicting shape changes due to hygroscopic swelling. The application-oriented teamwork with the ICD will lead to the construction of the pursued biomimetic hygroscopic flaps. By applying special printing techniques using copolyester with embedded cellulose fibers, 3D printing of artificial, hygroscopically moving scale flaps was possible and allows for mimicking the biological role models. Moreover, together with colleagues from our industrial partner, we performed µ-CT-scans of moving pine cone seed scales at ~5 µm resolution. This enables us to non-invasively quantify the deformation of the involved tissues in space and time. Additionally to these analyses of extant *Pinus* species, fossilized and semi-fossilized cones (~15.000 and ~20.000.000 years of age) have also been investigated. The scales of these entirely carbonized cones are still capable of hygroscopic movement which highlights the extremely high structural reliability of this actuation mechanism.

Sporangium exposure and spore ejection in the fern *Adiantum peruvianum*
The ornamental Peruvian maidenhair fern (or: Silver-Dollar, *Adiantum peruvianum*) is commonly cultivated in tropical greenhouses of Botanic Gardens owing to its decorative pinnules and glossy black stems (Fig. 2A). We investigated the two different processes involved in spore release in this species (Poppinga et al. 2015). The sporangia are situated on the undersides of recurved marginal lobes, the so-called false indusia (Fig. 2B). Alike true indusia, which are epidermal outgrowths and do not appear on *A. peruvianum*, they mechanically protect the sporangia. The false indusia are strongly curved and have a reniform shape. They stay closed until the water supply through several veinlets breaks down. By evaporative forcing they then flap open and expose the sporangia, a movement that can be considered as the first step in spore liberation. Only mature false indusia (with visible dark-brown coloration) flap open completely and only in this developmental stage the opening is accompanied with spore release.
The opening process is very variable in terms of duration. The fastest false indusium opened in 50 min, whereas others needed 8 hrs for opening. As the speed of hydraulic movement strongly depends on the thickness of the moving organ (Skotheim & Mahadevan 2005), this may partly be explained by differences in false indusium dimensions, as well as by differences in pinnule remnants (the false indusia have been cut off the pinnules for testing), in the respective developmental stage and also by variations of the environmental conditions. The movement itself is very complex and comprises a rotation of the false indusium around its connection line to the pinnule in direction to the pinnule margin, plus a complete curvature inversion and a flapping of the lateral parts towards the center of the false indusium. This "inside-out flapping" leads to a barrel-shaped form of the dried-out false indusium (Fig. 2C-E). Sporangia become orientated more or less towards the pinnule margin and point in almost 360 degrees around the rotational axis of the "barrel", which we hypothesize to promote effective spore scattering for wind dispersal. The curvature inversion is accompanied with an asymptotic decline of mass of the false indusium (total mass loss ~42% compared to the initial hydrated state). The motion is reversible as false indusia fully re-close under water. In contrast to the carnivorous Venus flytrap, where curvature inversions of trap lobes incorporate

fast snap-through transitions because elastic energy is stored in the lobes and released (Forterre et al. 2005), the inversion of the false indusium is a very continuous movement without any sudden acceleration. This leads us to hypothesize that the desiccation-driven deformation of the false indusium is not accompanied by an accumulation of elastic stretching energy and that both states of curvature are not separated by a distinct energy barrier.
The false indusium is built up as a functional bilayer with an adaxial layer constituted of a single row of tubular cells and an abaxial layer consisting of several rows of much thinner, tangentially elongated cells. Water evaporates through the outwards facing walls of the tubular cells which strongly deform owing to the cohesion forces that build up inside the cells. In contrast, the abaxial cells deform to a much lower degree. We hypothesize that the stresses hereby generated are sufficient to actuate the observed overall false indusium deformation. The exposed sporangia then begin to eject their spores. Their well-investigated catapult motion is based on a cohesion-force driven splitting of the sporangium into two cups and a tensioning and cavitation-triggered relaxation of the annulus, which acts as the throwing arm (Haider 1954; Noblin et al. 2012). We found that spores from the apical cup become "actively" thrown out and that the recoil of the very fast relaxation motion (duration 40 µs) entails a bouncing of the sporangium and "passive" scattering of spores from the basal cup (Fig. 2F). Spores travel with a maximum velocity of ~5 m s^{-1} and we measured a launch acceleration of ~6300 g. Moreover, spores often adhere to each other and become expelled as clumps. Also, sporangia fragments and whole sporangia become sometimes ejected which presumably influences throwing distance and dispersal.

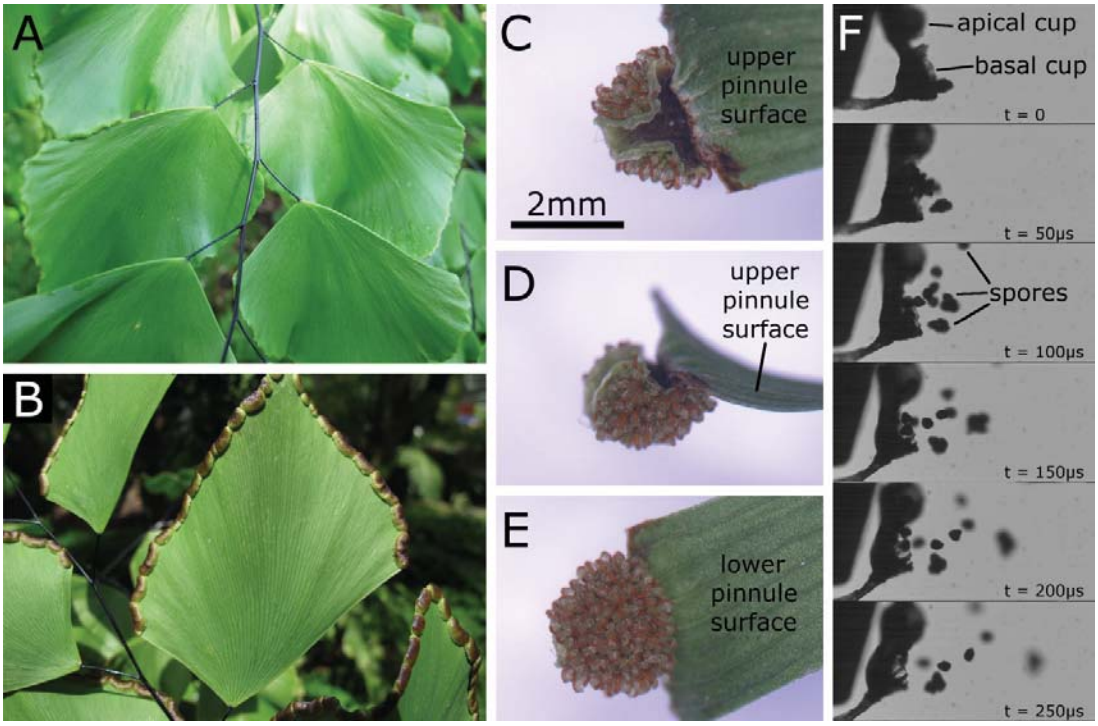

Fig. 2 : False indusium movement and spore ejection in *A. peruvianum*. (A) Detail of a pinnate leaf; note the ornamental black rhachis. (B) Lower pinnule surface with several recurved marginal lobes, the false indusia. (C)-(E) Views from different angles on a fully open false indusium. The sporangia have shed their spores. (F) High-speed time series of spore ejection from a leptosporangium of *A. peruvianum*. The recoil of the fast relaxation entails spore shedding from the apical and basal cup.

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A passionate climber: Functional morphology and biomechanics of the adhesive tendrils in *Passiflora discophora*

Holger F. Bohn[1,3,4], Felix Günter[1], Stefanie Schmier[1,4], Siegfried Fink[2], Thomas Speck[1,3,4]

[1]Plant Biomechanics Group Freiburg, Botanic Garden, University of Freiburg, Germany, [2]Chair of Forest Botany, University of Freiburg, Germany, [3]Freiburg Materials Research Center (FMF), Germany, [4]Freiburg Center for Interactive Materials and Bio-Inspired Technologies (FIT), Germany
holger.bohn@biologie.uni-freiburg.de

Introduction

Climbing plants save a substantial amount of resources by clinging to external supporting structures instead of developing a self-supporting trunk [Isnard, Silk 2009, Rowe, Speck 2004, 2015]. Passion flower species typically climb with unbranched axillary tendrils which coil around rod-shaped supporting structures with suitable diameter. The tendrils of a few rather primitive species, most prominent in *Passiflora discophora*, show a different attachment mode [Jørgensen et al. 1987]. They are branched and terminate in persistent adhesive pads, allowing the plant additionally to ascend flat supportive structures. On smooth surfaces semi-spherical attachment pads are formed, whereas on substrates with cavities the pad tissue opportunistically fills out the cavities resulting in a perfect form closure with the substrate (Fig. 1). Simultaneously with the formation of attachment pads the tendril axes coil, herewith pulling the shoot closer to the substrate and forming an elastic damping element.

We analyzed the structural development, functional morphology and mechanics of the adhesive tendrils and pads in *P. discophora*, aiming to understand the structure-function-relationship of the attachment process. This knowledge may be used to develop novel high load-bearing bio-inspired technical anchoring systems.

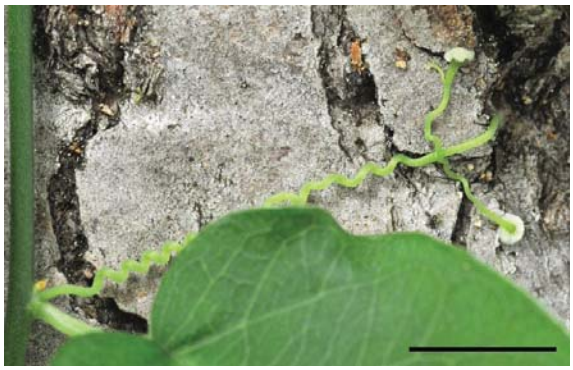

Fig. 1: Adhesive tendril on a young shoot of *Passiflora discophora*. The tendril is branched and terminates in three adhesive pads (white tissue) which are attached to the bark of a supporting tree. The main axis and often also the secondary axes are coiled adding viscoelastic damping properties to the tendril. Scale bar: 5 mm

Results and Discussion

Young tendrils of *P. discophora* establish contact with a substrate by firstly interlocking with their hook-shaped tips. This touch stimulus induces callus-like growth of epidermal cells at the tendril tips resulting finally in the formation of adhesive pads [Bohn et al. 2015]. The opportunistic growth of the pad tissue establishes an optimal form closure with the substrate

even filling minute gaps and fissures (Fig. 2). Additionally we found an extracellular substance at the interface between the pad tissue and the substrate forming a several micrometer thick continuous layer (Fig. 2b).

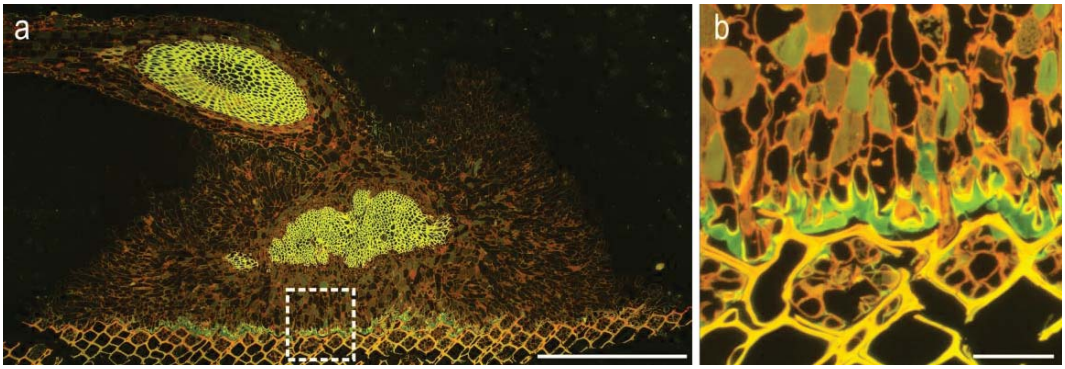

Fig. 2: Anatomy of fully grown adhesive pad attached to conifer wood. Fluorescence micrographs of acridine orange-stained median longitudinal section. a) The pad consists of a central lignified tissue which extends into the tendril axis (stained in yellow) and a surrounding unlignified tissue (stained in orange-brown). The elongated cells of the unlignified tissue are orientated in good approximation radially around the central part. b) Detail from rectangular box in a. At the interface very tiny cells of the pad tissue grow into the cells of the climbing substrate enhancing the mechanical interlocking. Furthermore peripheral cells of the pad tissue are embedded in an extracelluar substance (stained in green). Scale bars: a) 500 µm, b) 50 µm

Histochemical analyses showed that the substance consists of lipids. Therefore we conclude that the substance might be some kind of cuticle derivative which enhances the form closure of the pad with the substrate surface and may additionally act as an extracellular glue. After two to four weeks the tendril is fully grown and enters the stage of final differentiation. During this phase the tendril desiccates and the living cells die but it remains functionally intact.

Comparing these data with results from studies on similar attachment systems of other plant species [Steinbrecher et al. 2010, Seidelmann et al. 2012] reveals general principles of this attachment mode, specifically: (1) branched tendrils with multiple attachment pads, (2) formation of papillose cells allowing an optimal form closure with the substrate and – in several species – additional secretion of a gluing substance, (3) coiling of the tendril axes, and (4) permanent attachment even after senescence of the tendrils.

To analyze the mechanical properties of the adhesive tendrils we conducted micro-tensile testing both, on the level of the complete tendril and on the level of individual adhesive pads (Fig. 3a,b). The force-displacement curves of the adhesive tendrils show a characteristic shape (Fig. 3c). At the beginning the coiled tendril is stretched and elastically deformed. During the following phase the tendril becomes further elongated until the coiling is removed, which already represents a viscoelastic and/or plastic deformation. When fully stretched further loading of the adhesive tendril leads to a straining of the tendril tissue. Finally, when reaching an overcritical pulling force the system fails by rupture of the main tendril axis in the majority of the cases (Fig. 3c). This result is by and large independent of the developmental stage of the tendrils (alive or senescent) and of the type of substrate they were attached to. Thus on the level of the complete system, the adhesive pads possess a larger safety factor then do the

tendril axes. Furthermore, prior to rupture tendrils show a high extensibility making them a displacement optimized highly energy dissipating attachment system.

Currently we perform further mechanical analysis on the level of the individual adhesive pads. At first, due to the minute size of the adhesive pads we had to developed an advanced experimental technique allowing us to pull adhesive pads parallel (shear) and normal (adhesion) to the substrate. With these tests we aim to gather information on the force distribution and failure modes of the single adhesive pads and to compare the mechanical properties on the level of individual pads with that of the complete adhesive tendril.

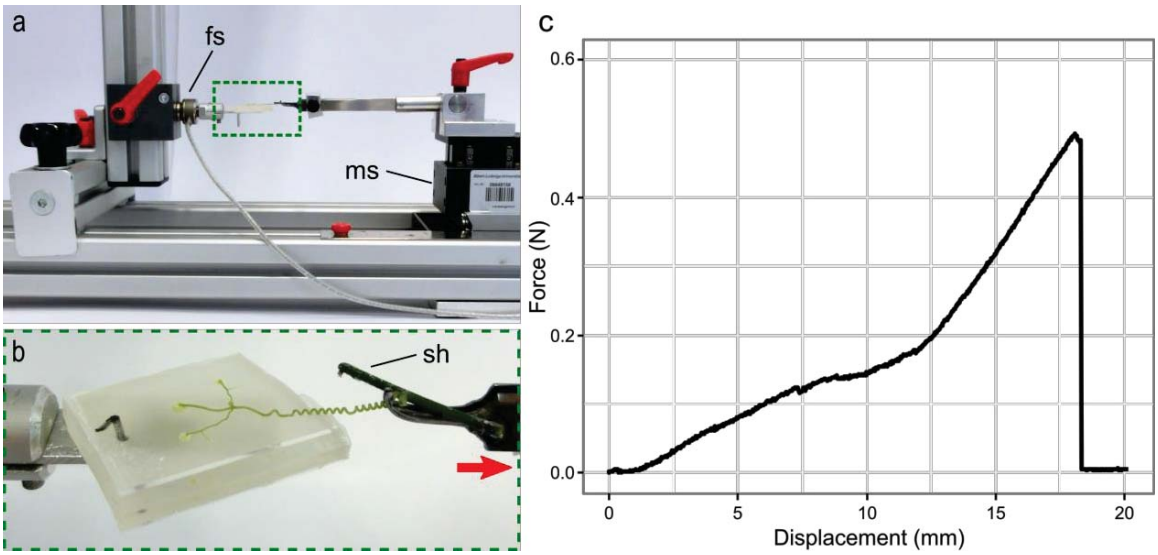

Fig. 3: Micro-tensile testing of a complete adhesive tendril under (near) natural loading conditions. a) Custom build micro-tensile testing device equipped with a linear motor-stage (ms) and a highly sensitive force transducer (fs). b) Detail from a. Fully grown adhesive tendril mounted for the pulling test. To minimize impact on the tendril during the test, load is applied to the shoot (sh) from which the tendril originates (red arrow: pulling direction). c) Typical force-displacement-curve for a fully grown adhesive tendril.

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Biophysical dynamic module for the polarization of auxin efflux carriers PIN-FORMED (PIN)

Valeria Hernández-Hernández[1,2*], Naomi Nakayama[3], Adriana Garay[1], Rafael Barrio[4], Carlos Villarreal[4], Elena R Alvarez-Buylla[1,2] , and Mariana Benítez[1,2*]
[1] Instituto de Ecología, Universidad Nacional Autónoma de México,
[2] Centro de Ciencias de la Complejidad, Universidad Nacional Autónoma de México,
[3] School of Biological Sciences, University of Edinburgh,
[4] Instituto de Física, Universidad Nacional Autónoma de México,
[*]**e-mail: valeriahhdez@gmail.com, mbenitez@iecologia.unam.mx**

Introduction

Physical cues have long been thought to play instructing role in developmental processes, however, developmental biology has mainly focused in the role of molecular components. Only in recent years scientists have started to elucidate the role of mechanical forces in plant development (Nakayama et al., 2012; Uyttewaal et al., 2012).
The molecular and mechanical processes affect each other in non-lineal ways making the study of morphogenesis a challenging task. Given the complexity of biological development, mathematical and computational models have become an important tool to integrate experimental evidence, study the collective dynamics of different components, and to make novel predictions that can be tested in real systems. The combination of experimental work with computational models is a powerful methology in the study of plant development.
The root of *Arabidopsis* is an ideal system to try to address questions such as how physical and biochemical processes feedback each other during plant growth and morphogenesis. In the apical-basal axis, three different developmental stages or zones can be clearly distinguished. The first one is the root apical meristem (RAM) that contains the quiescent cells (QC), the meristematic cells with high division rates and the transition domain, where this division rates starts to decrease until cells stop dividing (Ivanov and Dubrovsky, 2012). The second stage is the elongation zone, in which cells start to elongate until they attain a maximum length and start to differentiate in the differentiation zone (third stage) at the most distal part of the root with respect to the tip (Ivanov and Dubrovsky, 2012).

The role of mechanical forces in the development of *Arabidopsis* root

Auxin is the most studied phytohormone and presents a concentration gradient along the apical-basal axis of the root, with a peak at the QC, that is partially correlated with the patterns of cell division and elongation (Sabatini et al., 1999; Petersson et al., 2009). The auxin gradient is formed, among others, by the efflux carriers PIN-FORMED (PIN) which are proteins anisotropically located in the plasma membrane, *i.e.* polarized, and present particular patterns of polarization in the radial and longitudinal axis of root (Blilou et al., 2005; Wisniewska et al., 2006). The polarization of the PINs is a dynamic process that responds to different signals (*e.g.* light, mechanical forces). It has been previously reported that changes in the mechanical tension sensed by the plasma membrane, can regulate the rates of endo- and exocytosis and that the density and polarization of PIN carriers are also affected (Homann, 1998; Nakayama et al., 2012). Also, it was shown that the GTPase ROP6 inhibits the endocytosis of PIN2 located at the plasma membrane in epidermal cells of *Arabidopsis* roots (Chen et al., 2012).
Although some models try to explain PIN polarization dynamics, they are still unable to incorporate this dynamical richness. In this work we integrate experimental data for processes of different nature (*e.g.*, chemical, molecular and mechanical) that interact among them in non-linear ways to polarize PIN transporters. Based on experimental information about the regulation the polarization of PIN carriers, we propose a dynamical module (*sensu* Newman and Bhat, 2009) that takes into account feedbacks between biochemical and physical components. We have generated a Boolean and a continuous models for this biophysical module. Both models reproduce some essential features of PIN polarization dynamics and render some novel predictions, such as the relative role of the plasma membrane mechanical tension that we are currently testing in the laboratory. Our work integrates uncoupled information and studies the overall regulatory logic behind the complex dynamic of PIN polarization. It also contributes to uncover generic mechanisms of plant morphogenesis focusing on the role of mechanical forces in these processes.

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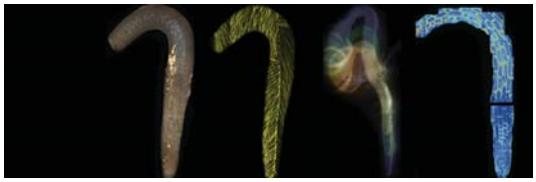
Cellular asymmetry in wall elasticity, triggers growth symmetry breaking and cell expansion rate in the Arabidopsis hypocotyl.

Alexis Peaucelle[1,2,3*], Raymond Wightman[3] and Herman Höfte[1]

[1]Institut Jean-Pierre Bourgin, UMR1318, Institut National pour la Recherche Agronomique-AgroParisTech, Saclay Plant Science, Versailles, France;
[2]Laboratoire Matières et Systèmes Complexes, Université Paris Diderot, UFR de Physique de Paris 7, 75205, France;
[3]Sainsbury Laboratory, University of Cambridge, Cambridge, United Kingdom.
*For correspondence: alexis.peaucelle@versailles.inra.fr

Complex shapes in biology depend on the ability of cells to shift from isotropic to anisotropic growth during development. In plants, this growth symmetry breaking reflects changes in the extensibility of the cell walls. The textbook view is that the direction of turgor-driven cell expansion depends on the cortical microtubule (CMT)-mediated orientation of cellulose microfibrils [1, 2]. We will present results that show that this view is incomplete at best. We used Atomic Force Microscopy (AFM) to study changes in cell wall mechanics associated with growth symmetry breaking within the hypocotyl epidermis. We show that firstly, growth symmetry breaking is preceded by an asymmetric loosening of longitudinal, as compared to transverse, anticlinal walls, in the absence of a change in CMT orientation. Secondly, this wall loosening is triggered by the selective de-methylesterification of cell wall pectin in longitudinal walls and thirdly the resultant mechanical asymmetry is required for the growth symmetry breaking. Indeed, preventing or promoting pectin de-methylesterification respectively increased or decreased the stiffness of all the cell walls but in both cases reduced the growth anisotropy. We will also show that the subsequent CMT reorientation contributes to the consolidation of the growth axis but is not required for the growth symmetry breaking.
Finaly combaning these results with chinematique mesurment and mesurment of cell turger presur using AFM we deraved the rule that links cell/tissue gowthe to its mechanicale propeurtis in pramery cell walls. We will discus the importens paradigm chifte these results chould lead to:
-Growth symmetry breaking is controlled at a cellular scale by bipolar pectin de-methylesterification, rather than by the cellulose-dependent mechanical anisotropy of the cell walls themselves. Such a cell asymmetry-driven mechanism is comparable to that underlying tip-growth in plants [3] but also anisotropic cell growth in animal cells [4].
-Cell growth rat is propionate to the elastic stretching of the cell wall (Pressure divided by the young modulus) and not plastic properties of the cell wall components. This correlation is not determent by the total cell wall length as for en elastic but to the number of cells.



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Mechanics of single plant cells

P. Durand-Smet[1], Nicolas Chastrette[1], Axel Guiroy[1], A. Richert[1], A. Berne-Dedieu[2], M. Bendahmane[2], J. M. Frachisse[4], O. Hamant[2, 3], A. Boudaoud[2, 3] and A. Asnacios[1]

[1] UMR 7057 CNRS & Université Paris Diderot, Laboratoire Matière et Systèmes Complexes, Paris, France
[2] Laboratoire de Reproduction de développement des plantes, INRA, CNRS, ENS Lyon, UCB Lyon 1, Université de Lyon, 46 Allée d'Italie, 69364 Lyon, Cedex 07, France
[3] Laboratoire Joliot Curie, CNRS, ENS Lyon, UCB Lyon 1, Université de Lyon, 46 Allée d'Italie, 69364 Lyon Cedex 07, France
[4]Institut des Sciences du Végétal - CNRS - bat 23 - avenue de la Terrasse - 91198 Gif-sur-Yvette –France
pdurand@caltech.edu

Cell mechanics is involved in many fundamental biological processes such as development and morphogenesis(Hamant & Traas 2010; Asnacios & Hamant 2012). Plant and animals have evolved different strategies for their development. Whether this is linked to major differences in their cell mechanics remains unclear, mainly because measurements on plant and animal cells relied on independent experiments and set-ups. We will present here recent measurements obtained with the same custom-made micro-rheometer to compare animal and plant cell rheology(Durand-Smet et al. 2014). This setup allowed us to determine the mechanical properties of single cells by performing dynamical oscillation tests. We found that wall-less plant cells exhibit the same weak power law rheology as animal cells, with comparable values of elastic and loss moduli. Remarkably, microtubules primarily contributed to the rheological behavior of wall-less plant cells whereas rheology of animal cells was mainly dependent on the actin network. Thus, plant and animal cells evolved different molecular strategies to reach a comparable cytoplasmic mechanical core, suggesting that evolutionary convergence could include the internal biophysical properties of cells. Besides measurements on protoplasts, we also characterized the mechanical behavior of single plant cells retaining their cell wall. The plant cell mechanical response was mainly elastic with a modulus of ~10^6 Pa. Decreasing the turgor pressure, the elastic modulus decreased accordingly before reaching a plateau value corresponding to the mechanical response of the cell-wall (plasmolysis). Comparing this behavior to that of a passive pressurized shell (soccer ball!), we reveal the way single plant cells adapt their internal pressure to changes in the osmolarity of their environment.

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Probing the role of xylan in irreversible deformation of stems of *Arabidopsis thaliana*

Darshil Shah[1*], Thomas Reynolds[1], Marta Busse-Wicher[2], Li Yu[2], Paul Dupree[2], Michael Ramage[1]

[1] Centre for Natural Material Innovation, Department of Architecture, University of Cambridge, UK
[2] Department of Biochemistry, University of Cambridge, Cambridge, UK
*dus20@cam.ac.uk

Introduction

Plant stems exhibit a combination of elastic and plastic deformation under external loading. The biomechanical processes behind the plastic component of deformation are not yet well understood. Keckes et al. (2003) proposed a stick-slip mechanism analogous to Velcro, in which a permanent shear deformation of the lignin-hemicellulose matrix around the cellulose microfibrils is possible whilst retaining the strength and elastic stiffness of the undeformed cell. Under cycles of load, this plastic behaviour may present itself as viscoelasticity, with hysteretic energy dissipation by the plastic component of the deformation. Köhler and Spatz (2002) showed the role of lignin in this viscoelasticity, using chemically modified *Aristolochia macrophylla*, noting a great increase in viscoelasticity under the first cycle of load in the delignified version of the latter. This suggests a greater irreversible slip of the microfibrils due to a given load in the absence of lignin. Busse-Wicher et al. (2014) showed the potential for xylan to form the link between the hydrophobic lignin and hydrophilic faces of the cellulose chains, making it possible that it plays a role in this stick-slip mechanism. Tests on genetically modified *Arabidopsis thaliana* have given some evidence for the influence of various cell-wall compounds on strength and stiffness of secondary cell walls (Goubet et al. 2009). These stems are of particular interest because of the similarity between the secondary cell walls of *Arabidopsis thaliana* and those in dicot trees, so that they may give some insight into the nature of viscoelasticity and plastic behaviour in wood.

Through mechanical testing of *Arabidopsis Thaliana* stems, we investigate the role of xylan in forming physical bonds between the cellulose microfibrils and the lignin, and therefore its role in the stick-slip mechanism of irreversible deformation in secondary cell walls of plants.

Methodology

Basal stem sections (approximately 30 to 50 mm in length) were obtained from wild type (WT), and xylan-deficient (*irx9*) xylem mutants and cellulose-deficient (*bah*) mutants of Col-0 ecotype of Arabidopsis plants. Plant material and growth conditions are the same as those described elsewhere (Goubet et al. 2009).

For mechanical testing, stem sections were mounted onto thick cardboard frames using a slow-setting precision Araldite epoxy resin (Fig. 1). The laser-cut frames had 1 mm wide grooves running along the centreline, within which the stems were placed, to protect specimen

ends from crushing during specimen loading. A gauge length of 10 mm was used, with an embedded length of at least 10 mm either side.

Mechanical testing involved loading the frames onto an Instron tensile testing machine, equipped with screw-action grips and a 500N load cell. A loading rate of 0.5 mm/min was used. Strain was monitored from cross-head displacement, as well as using a laser extensometer. The latter relied on measuring the relative displacement of retroflective tapes that were placed on the gauge-length section of stems (Fig. 1). To convert applied load into stress, stem cross-section areas were determined from optical microscopy of failed specimens that were cast into solid resin blocks and polished to 4000 grit sand paper (particle size of 3 μm). Image analysis was conducted on Image-J freeware.

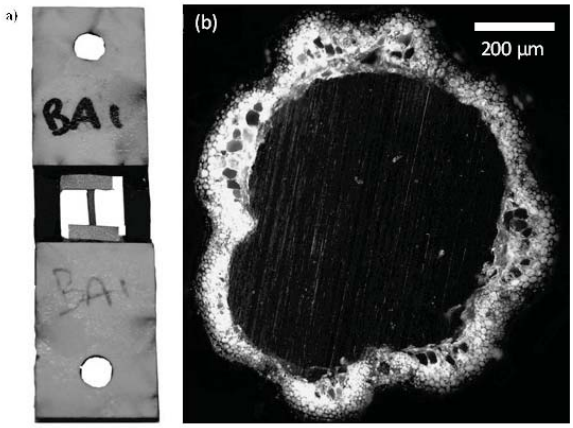

Fig. 1 : a) Specimen mounted in laser-cut cardboard frame for tensile testing. Retroflective tapes are used for laser extensometry. b) Cross-section area of the stems was determined using optical microscopy.

The test regime involved progressive tensile loading to failure, with three loading-unloading cycles each from 4 N to 1 N, 8 N to 5 N and 8 N to 1 N (Fig. 2). From the tests, first, initial modulus E_0 was determined from the gradient of the stress-strain curve in the load range of 0 to 4 N applied load. To investigate the potential contribution of cell wall constituents (based on comparison between wild type and mutant stems), particularly xylan, to the stick-slip mechanism responsible for mediating irreversible deformation, residual moduli in the three loading-unloading regions of the stress-strain curve were measured from their gradients; these are referred to as E_1 (measured from 4 N to 1 N), E_2 (measured from 8 N to 5 N), E_3 (measured from 8 N to 1 N). Secondly, the residual plastic strain from unloading was also estimated for all cycles. This provided a measure of permanent deformation in the form of plastic displacement and visco-elastic creep. Thirdly, noting a characteristic yield point in the stress-strain curve for the stems, the stress at this yield point was recorded. Finally, for samples that failed in the gauge section, tensile stress at failure was recorded. Most of these measurements are illustrated on a simplified stress-strain profile for a typical sample (Fig. 2).

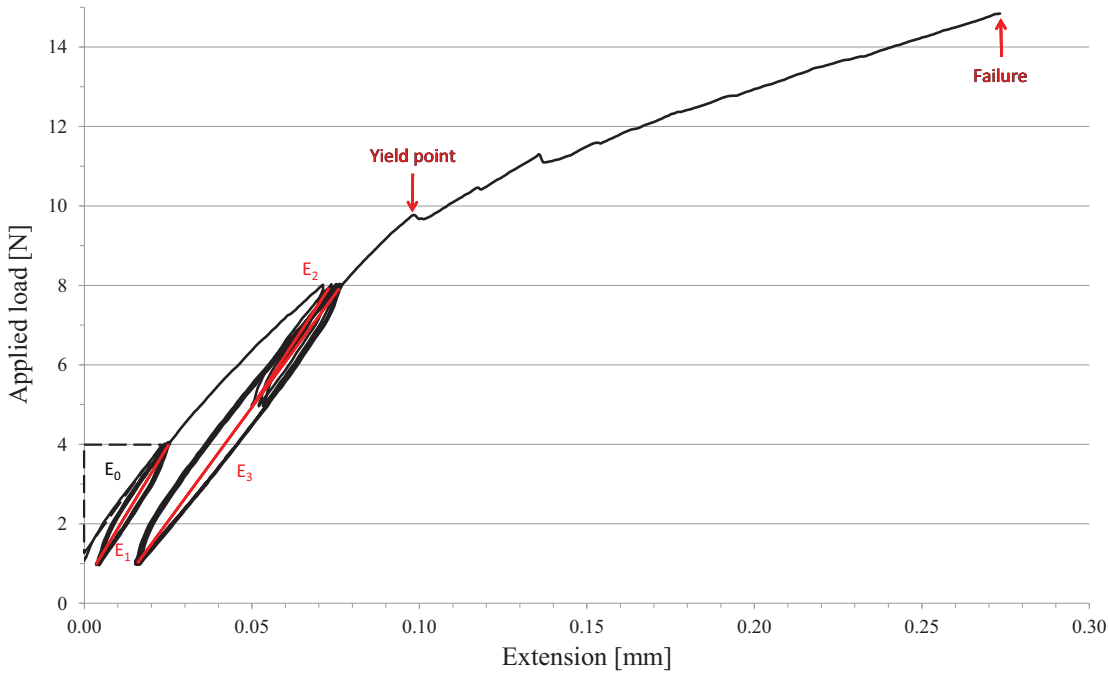

Fig. 2 : Example load-displacement curve, showing gradients measured for comparison of reversible and irreversible components of deformation, a characteristic yield point, and tensile stress at failure.

Results

Cross-section properties are key in accurately determining stem material properties such as elastic modulus and strength. The difference in the means of cross-section areas of WT stems to mutants stems was found to be statistically significant (two-tailed t-test, $p < 0.03$, $n = 8, 9$). WT stems were 22% larger in cross-section area than xylan-deficient *irx9* stems, and 57% larger than cellulose-deficient *bah* stems. The results are in accordance to visual observations that the mutants exhibit (severe, in the case of *bah*) dwarf growth phenotypes. The principal reason for this is attributed to xylem collapse and impediment of water/nutrient transport in the mutant stems.

The observed difference in cross-section area has several implications on the test results and interpretations. For example, as load-unload cycles were based on loads (e.g. 4 N to 1 N), the stem material in WT plants would experience lower stresses than the mutant plants. The higher stresses in the mutant stems (for the same load) would at least partly explain higher residual plastic strains. However, we note that while our cross-section area measurements account for the hollow nature of the stems (i.e. pith) and irregular cross-section shape, they don't account for the hollow nature of cells within stems (i.e. luminal porosity). Given that the stems of mutant plants have collapsed xylem tissue, luminal porosity may be less important in them, in comparison to stems of WT plants. A more accurate measurement technique for cross-section area of only solid cell wall material would enable more accurate interpretation of mechanical testing results.

The mechanical testing results are presented in Fig. 5. The initial stiffness E_0 of WT stems ranged between 0.45 to 0.55 GPa. This compared to *irx9* and *bah* stems with E_0 ranging between 0.38-0.60 GPa and 0.27-0.50 GPa. The unload-reload stiffness E_1 showed a similar

trend, with *irx9* stems exhibiting larger spread in properties with a mean comparable to the WT stems, and the *bah* stems having lower stiffness. The lower stiffness in *bah* was expected and was attributed to their cellulose-deficient composition, as cellulose is the principal structural polymer in the cell wall.

The comparable initial and unload-reload stiffness of *irx9* and WT stems was, however, not expected. As xylan is thought to be responsible for enabling the stick-slip mechanism in the cell wall, we had hypothesized that a reduction in xylan content and chain length (as is the case in *irx9*) would have increased the unload-reload stiffness by a lesser amount than the increase expected in a WT stem. Interestingly, we found that the ratio of E_0/E_1 was around 0.70-0.75 for the WT and the mutant plants we studied.

Cellulose-deficient *bah* mutant stems were the weakest, failing at loads lower than 8N (Fig. 3 d)), and therefore E_2 and E_3 could not be measured for them. WT stems did exhibit higher mean failure loads than *irx9* stems, however once cross-section areas were accounted for, the failure strengths were comparable if not slightly higher for *irx9* stems.

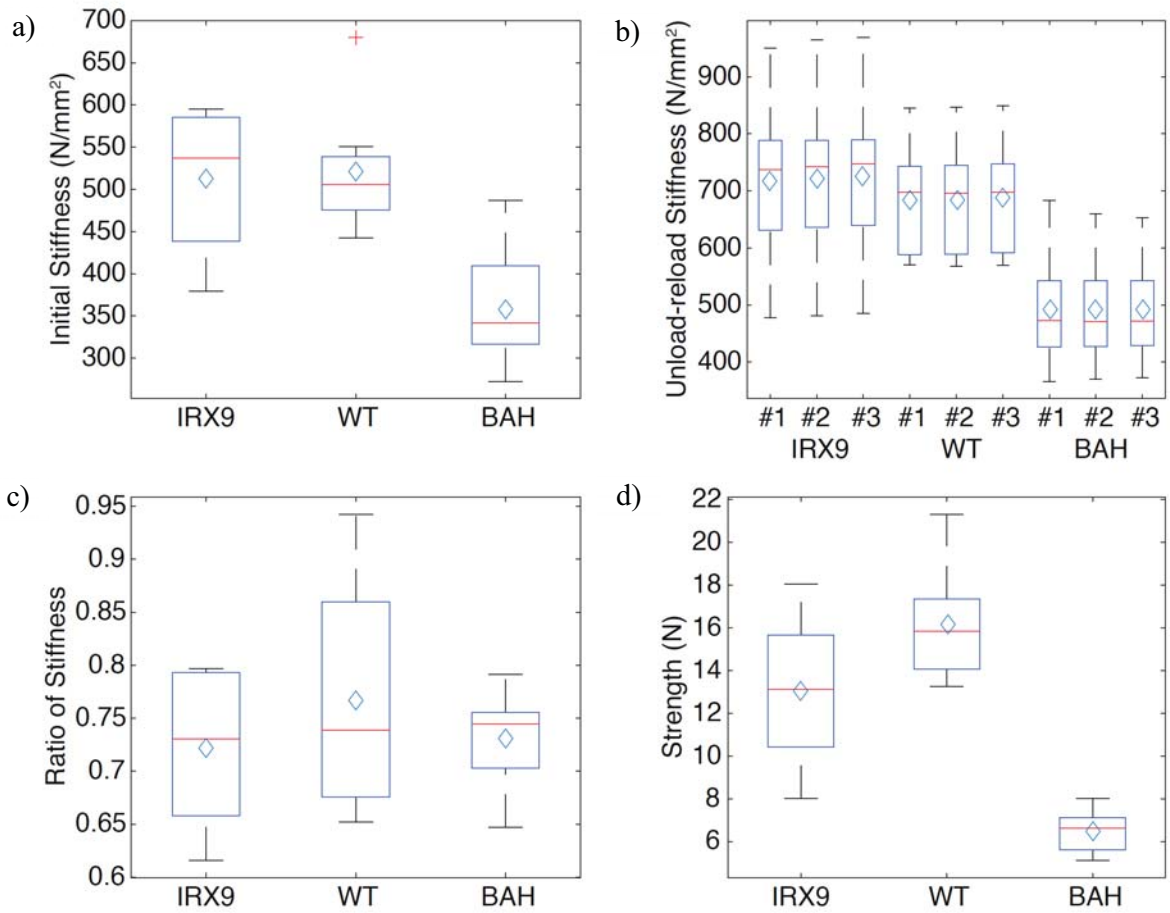

Fig. 3 : Box plots showing the variation in a) initial stiffness E_0, b) unload-reload stiffness E_2 for cycles 1, 2, and 3 (between 4 N and 1 N applied load), c) ratio of stiffness' E_0/E_1, and d) failure load, of stems from WT, *irx9* mutant and *bah* mutant plants. Box plots present the range as error bars, the upper 75% and lower 25% quartile as the blue box, the median as the red line and the diamond as the mean.

Summary and future work

We have developed a systematic testing methodology to measure the mechanical properties of plant stem materials to elucidate structure-property-function relations, ascertain the roles of the cell wall constituents (based on hypothesized molecular architecture), and to explain the behavior of wood by studying plant stems. To enable more accurate interpretation of results, we are looking into ways, such as measuring mass per unit length and cellular porosity through tomography, to appreciate the effects of luminal porosity on cross-section measurement.

Accounting for substantial differences in cross-section areas, our results clearly show that while cellulose-deficient *bah* stems have lower mechanical properties, and exhibit greater irreversibility than wild-type stems. However, no significant difference in properties of xylan-deficient *irx9* stems and wild-type stems was observed. This may be because although the content of xylan is reducing, how the xylan is interacting with the lignin and cellulosic microfibrils remains the same. For clarity on our current results, we will conduct mechanical tests on lignin mutants, as well as xylan mutants which do not exhibit severe dwarf growth phenotypes, and rather modify the way they link to the cellulose microfibrils (e.g. studying *gux-1,2* double mutants).

Encouraged by differences in residual plastic strain observed for the different stems, to further probe 'slippage' (in the stick-slip mechanism) and visco-elastic creep, we will be conducting stress relaxation tests on the stems to examine difference in fundamental material properties and creep-governing mechanisms, as measured by activation energies and volumes.

Acknowledgements

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Making the mechanics visible: Micro-indentation on a Confocal Microscope

Anne-Lise Routier-Kierzkowska[1], Alain Weber[2], Aleksandra Sapala[1], Tanja Ruetti[2], Gabriella Mosca[2], Richard S. Smith[1]

[1]Department of Comparative Development and Genetics, Max Planck Institute for Plant Breeding Research, Cologne, Germany.
[2]Institute of Plant Sciences, Bern University, Switzerland.
routier@mpipz.mpg.de

Abstract

Micro-indentation techniques have been increasingly used to assess cell mechanics, in both plant and animal systems. However plant cell mechanical measurements involve much higher forces than animal cells due to tugor pressure. To address this, we have developed Cellular Force Microscopy (CFM), an indentation method dedicated to non-invasive force measurements on plant cells. Combined with physically based simulation models of indentation experiments, we have shown that CFM can be used to measure turgor pressure in a non-invasive way.

Osmotic treatments can also be used to assess the mechanical properties in tissues and whole organs. This is done by quantifying cell or organ size change upon a change in turgor pressure. To aid in this quantification, we have developed MorphoGraphX, a custom 3D reconstruction software that can very precisely track cell size changes in time-lapse data. This has allowed us to determine the relative stretchability of cells within zones of the tomato shoot apex based on their deformation upon osmotic treatment. However, to fully determine the cell wall elasticity and turgor pressure, a combination of CFM measurements, osmotic treatments and a 3D simulation model based on the realistic cellular geometry of the tissue is required. Towards this end, we have developed MorphoRobotX, a miniaturized version of the CFM that can be plugged onto a standard Confocal Microscope. The controlling software of the CFM has been integrated into MorphoGraphX, enabling the user to select interactively the region of interest for the measurements. The technology allows near simultaneous measurement of both mechanical properties and cell wall deformation in live samples, in addition to fluorescent marker lines to monitor gene expression.

CFM can also be used to study the cellular response to very precisely applied mechanical forces. A local force application of medium intensity can simulate the physical pressure exerted by a fungus on the cell wall during pathogenic or *mycorrhizal infection*. When we apply such forces with the CFM probe, we observe similar physiological responses, such as cytoskeleton reorganization and nuclear migration. Using larger forces, the CFM can be used as a micro-ablation device to perforate the cell wall in a controlled manner, with high precision force feedback. Confocal imaging can then be used to observe the response of cells to disturbed stress patterns around the ablated cells. We propose this method as a complement to laser ablation techniques, to avoid issues such as heat damage to surrounding cells.

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Mechanical characterization of developing tension wood fibre wall by atomic force microscopy

M. Capron[1], M. Ramonda[2], F. Laurans[3], B. Clair[4], T. Alméras[1], O. Arnould[1]

[1] Laboratoire de Mécanique et Génie Civil (LMGC), CNRS, Université de Montpellier, France
[2] Service commun CTM « champ proche », Université de Montpellier, France
[3] INRA, UR588 Amélioration, Génétique et Physiologie Forestières, Orléans, France
[4] CNRS, UMR Ecologie des Forêts de Guyane (EcoFoG), Kourou, France
olivier.arnould@umontpellier.fr

A key element of the biomechanical design of trees is their ability to generate large mechanical stresses in wood at the stem periphery. This function is necessary for the tree to control the orientation of its axes, and therefore to grow in height, maintain its branches at an optimal angle or achieve adaptive reorientations (Fournier et al., 2013). This "maturation stress" appears in wood fibres during their cellular maturation when their secondary cell wall is thickening. In hardwoods, the stress asymmetry is generated by the formation of specific fibres with a very high tensile growth stress on the upper side of the inclined axis. The resulting tension wood has microstructural features highly different from the normal wood. Tension wood in almost all temperate species has a specific gelatinous cell wall layer, called G-layer, that is not lignified and highly mesoporous (Chang et al., 2015). It has been shown recently, at the macroscopic scale, that cellulose tension appears during maturation and is synchronous with the development of this specific layer (Clair et al., 2011). Tropical species, like simarouba, are able to generate lignified tension wood fibres. The mechanisms of stress generation in tension wood fibres are not yet well known. The aim of our study is to understand these mechanisms and to know the spatial and temporal kinetics of the different cell wall layers stiffening during maturation, in parallel with tensile stress generation.

Contact-Resonance Atomic Force Microscopy, CR-AFM, was used here to address this question on embedded samples (Arnould and Arinero, 2015) using dual resonance frequency tracking (DRFT, Rodriguez et al., 2007). Change in the contact modulus between, and within, each layer of the cell wall has been measured on several radial lines of developing fibres at different steps. In order to understand the obtained results, and to estimate the sensitivity of the AFM indentation-like technique to the different cell wall components stiffness, an elastic anisotropic indentation model was used (Vlassak et al., 2003; Jäger et al., 2011). Finally, these mechanical measurements have been compared to topochemical data obtained on the same fibres.

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Does anatomical diversity of tension wood reflect diversity of mechanisms to generate stress?

Barbara Ghislain, Julien Engel, Bruno Clair
CNRS - UMR Ecologie des Forêts de Guyane (EcoFoG), French Guiana (France)
barbara.ghislain@ecofog.gf

The angiosperms trees have the ability to produce stresses, called maturation stresses, to control their orientation when necessary. These strong tensile stresses are associated with a wood with peculiar structure and behavior, called tension wood. In most temperate species, tension wood is characterized by the presence of a particular layer in the cell wall: the gelatinous layer (G-layer), consisting of a network of cellulose microfibrils aligned with the axis of the cell embedded in a matrix of pectins and specific polysaccharides. It has been shown that microfibrils are the support of the maturation stress and that they are put under tensile stress shortly after their incorporation into the cell wall (Clair 2011) presumably due to the swelling of the mesoporous matrix of the gel pushing on the cellulose microfibrils network (Chang et al. 2015). However, considering the high diversity of the amazonian forest, the usual unlignified G-layer was observed only in a minor number of species (Onaka 1956, Fisher 1982, Ruelle et al. 2006).
The aim of our work was to revisit the high diversity with a special attention to the maturation process in order to make clear the diversity of mechanisms of tensile stress generation.

We collected 321 trees with a diameter at breast height above 10 cm and a tilting angle over 10°. Most of the trees were collected randomly in the tropical rainforest in French Guiana, in order to screen tension wood diversity. Tension wood (TW) and opposite wood (OW) were sampled. Sections (20 µm thick) of each sample were colored with safranin / blue astra and observed under a microscope. UV microscopy was also used on thinner sections (2 µm thick) to highlight the presence or absence of lignin.

Our observations show the presence of a G-layer in numerous species. This G-layer can be lignified during the maturation process (Fig. 1). Lignification hides the expected unlignified and swollen aspect of the G-layer. A classification is proposed to organize the different features of tension wood fibers, depending on the kinetic and the final stage of lignin deposition during the maturation process. Our observations allow to better understand numerous unexplained observations described in the literature of tension wood.

Thereby, a single mechanism, same as in G-layer species, could occur in most non-G-layer species. So far, the role of lignification in the tensile stress generation remains unclear especially regarding its production cost.

Besides, another mechanism seems to prevail for a few number of non-G-layer species and has yet to be understood.

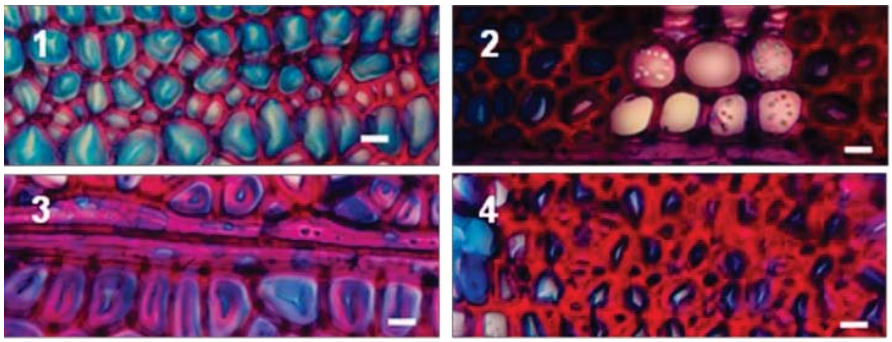

Fig. 1: Tension wood with Safranin/Blue Astra coloration of 1) *Brosimum parinarioides*: G-layer; 2) *Perebea rubra*: lignified G-layer; 3) *Caryocar glabrum*: partly lignified G-layer; 4) *Brosimum rubescens*: partly lignified G-layer. Scalebar = 10 µm

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The Influence of Tissue and Morphology on Stalk Bending Stresses

Douglas Cook[1], Daniel Robertson[1], Shien Yang Lee[1], and Gregory Von Forell[2]

[1]Plant Biomechanics Laboratory, New York University Abu Dhabi, United Arab Emirates.
[2] NextStep Arthropedix, 3916 S Arlington Rd #708 Uniontown, OH 44685
douglascook@nyu.edu

Introduction

Mechanical stresses in plant tissues are determined by three factors: loading, material properties, and morphology (Niklas, 1992). However, the influences of individual parameters on mechanical stresses in plant stems are poorly understood, in part because they are difficult to control experimentally. The purpose of this study was utilize an *in silico* approach to quantify the influence of these factors on mechanical bending stresses of plants in the *Poacea* family.

Methods

Because of its prevalence and economic importance, maize was chosen as a model system. A finite element model of the maize stalk was developed (Fig. 1A) based on geometric data acquired from quantitative computed tomography (QCT) scans of the fourth above-ground node and adjacent internodes of corn stalks.

Sensitivity analysis was performed to investigate relationships between model inputs (geometry, material properties) and model outputs (stress). In natural conditions, failure of the maize stalk typically occurs when compressive stresses cause the rind tissue to buckle (Robertson et al., in press). Parametric variation of geometric and material parameters resulted in a total of 17 unique finite element models. Each model was subjected to four bending loads of 25 Nm each. Figure 1 depicts one model with and without applied loading.

Results

In total, 68 finite element simulations were performed. Natural asymmetries in the stalk geometry produced stress distributions which were unique for each loading case. The locations of maximum stresses predicted by the finite element models correlated well with field observations of naturally lodged stalks which typically break just apical of the node line (Robertson et al., in press).

Comparisons of sensitivity values (Figure 2) revealed that geometric parameters have a much greater influence on stress levels than material properties. All material parameters exhibited normalized sensitivity values less than 0.3. Changes in geometry had (on average) 18 times more influence on stalk stresses than material properties.

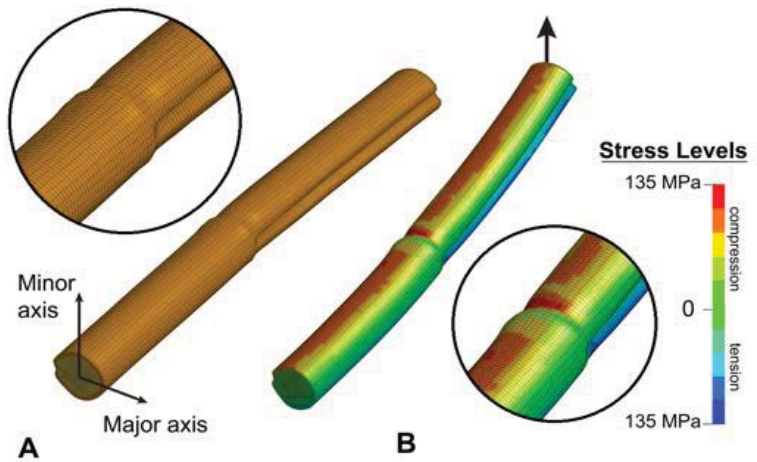

Fig. 1: A finite element model of maize stalk. A – unstressed and B – under constant bending load. The arrow depicts the direction of displacement of the corresponding face. Colors represent stress levels (B only).

This finding is corroborated by recent 3-point bending experiments in our laboratory in which geometric variables account for the majority of inter-hybrid

variation in stalk strength. In a set of tests involving 1000 maize stalks from 5 different hybrid varieties and grown at 5 different planting densities, geometric factors alone explained up to 78% of all variation in strength (see Figure 3).

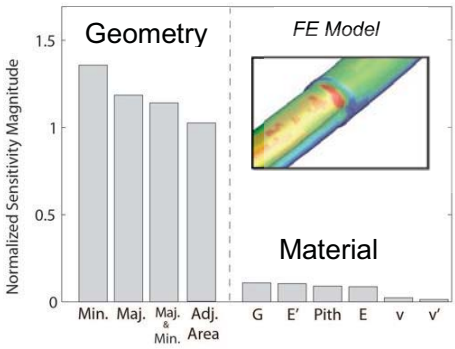

Fig. 2: The average magnitude of normalized sensitivity values for each type of variation, illustrating the general difference in sensitivity between geometric and material parameters.

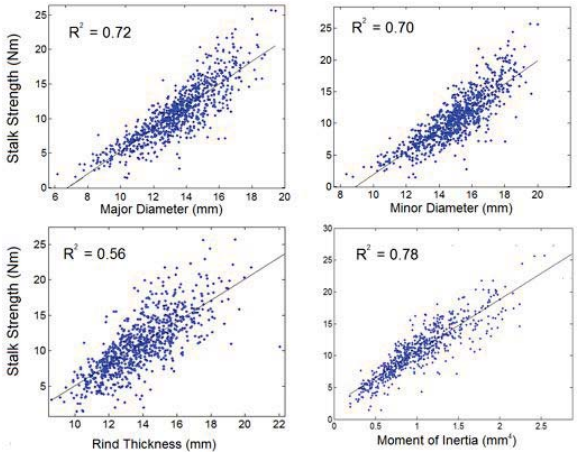

Figure 3: Linear regression and coefficient of determination (R^2) value between stalk strength and various geometric features.

Conclusions

Bending stresses in maize are most strongly influenced by stalk geometry. This is consistent with similar findings in human biomechanics in which stresses in the vertebrae are sensitive to geometry (REF). While material properties did exhibit an influence on bending stresses, the influence of material properties was approximately 18 times smaller than the average influence of geometric features. While indicating the lesser influence of material properties, this study also provided information on which material properties were most influential. This information is valuable for guiding experimental measurements (i.e. the greatest benefit will be attained by measuring material properties in order of their influence).

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Using MRI for analyzing the anatomy and biomechanics of monocotyledons

Tom Masselter[1,2,3], Linnea Hesse[1,2], Jochen Leupold[4], Nils Spengler[5], Jan Gerrit Korvink[5], Thomas Speck[1,2,3]

[1]Plant Biomechanics Group and Botanic Garden, University of Freiburg, Germany
[2]Freiburg Centre for Interactive Materials and Bioinspired Technologies (FIT), Germany
[3]Competence Network Biomimetics, Germany,
[4]Medical Physics, Department of Radiology, University Medical Center Freiburg
[5]Institute of Microstructure Technology, Karlsruhe Institute of Technology (KIT), Germany
tom.masselter@biologie.uni-freiburg.de

Introduction

In the past decades, replacing a reliable and proven technical component by novel lightweight materials and structures has often met skepticism concerning their stability, load-bearing capacity, durability and producibility. The most straightforward approach was a simple substitution of heavy, traditionally used engineering material such as steel by a material with low density such as lightweight alloys or fiber reinforced composites. This approach did not allow for really innovative insights gained by a radical rethinking of established design rules. A biomimetic method using biological concept-generators as a source of inspiration may help to overcome this shortcoming and to develop novel alternative design concepts that make full use of the potential of the new lightweight materials and structures.

The outer shape and internal structure of plant ramifications can be an inspiration to develop biomimetic concepts for improving existing branched fiber reinforced technical components and for developing novel biomimetic lightweight nodal elements for different applications as e.g. in space- and aircrafts, automobiles and other motorized transportation means, as well as in prosthetics, sports equipment, and especially in a novel bio-inspired architecture. However, this implies a detailed understanding of the underlying form-structure-function principles of the plant structures that are used as role models. Thus, deeper insights as to the functional outer shaping (Schwager et al. 2010, 2013), and especially into the inner three-dimensional structure of the plants used as concept generators, have to be gained and quantitative analyses of the hierarchical inner structure has to be performed (Haushahn et al. 2014).

The arborescent monocot *Dracaena marginata* (Dragon tree, Asparagaceae) is a suitable concept generator for optimizing technical fiber reinforced ramifications. The vascular bundles which consist of conduction tissues (xylem and phloem) and sclerenchymatous fiber strands represent the mechanically relevant tissue and are arranged comparably to how the fibers or fiber bundles of a technical lightweight component would be organized. The vascular bundles are clearly separated from each other and embedded in a matrix of parenchyma tissue (Haushahn et al. 2014, Masselter et al. 2011, Zimmermann and Tomlinson 1969, 1970).

Effects of mechanical loading on the branching region analyzed by photoelasticity tests

In a first approach, photoelasticity tests were performed on isotropic casts of ramifications of *Dracaena marginata* to understand how mechanical loading of the branch affects the branch-stem-transition region (Fig. 1). The casts were produced after performing a 3D-scan of the ramification surface of the real plant. Subsequently, a 1:1-mold of the ramification was created using the 3D-surface scan and the open-source 3D computer graphics software "Blender" (www.blender.org), and 3D-printed with a HP Designjet 3D. Then isotropic casts

of the ramifications were fabricated by using gelatin as a photoactive polymer. The observed isochromatics represent the shear-stress distribution in the mechanically loaded sample, two regions of high shear-stress are indicated by arrow 1 and 2 (Fig. 1), These regions were congruent to experimentally measured strain concentrations in the branching region of *Dracaena marginata* by Haushahn et al. (2012). Subsequently magnetic resonance images (MRI) of the ramification of tested *D. marginata* were used to analyze the course and arrangement of the mechanical relevant tissue, the vascular bundles, and to compare their arrangement in the branching region to the results of the photoelasticity tests (Fig 1c). The analyses show that in the upper part of the branching region domains of high shear-stress are located in the main stem, whereas in the lower region they are found in the side branch and in the main stem (Fig. 1B). By overlaying an MR-image of the arrangement of the vascular bundles it could be observed that the vascular bundles in the upper part of branch are directed nearly perpendicular towards the domain of high shear-stressin the main stem (Fig. 1, arrow 1). The vascular bundles of the lower region of the ramification are arranged in a nearly parallel manner along the isochromatics in this region (Fig 1, arrow 2).

These findings lead to the assumption that the vascular bundles are arranged in a load adapted manner during the development of a branch and probably help for decreasing stress peaks during loading of the branch. Thus they may also reduce the risk of potentially lethal fracture of the entire stem-branch-attachment.

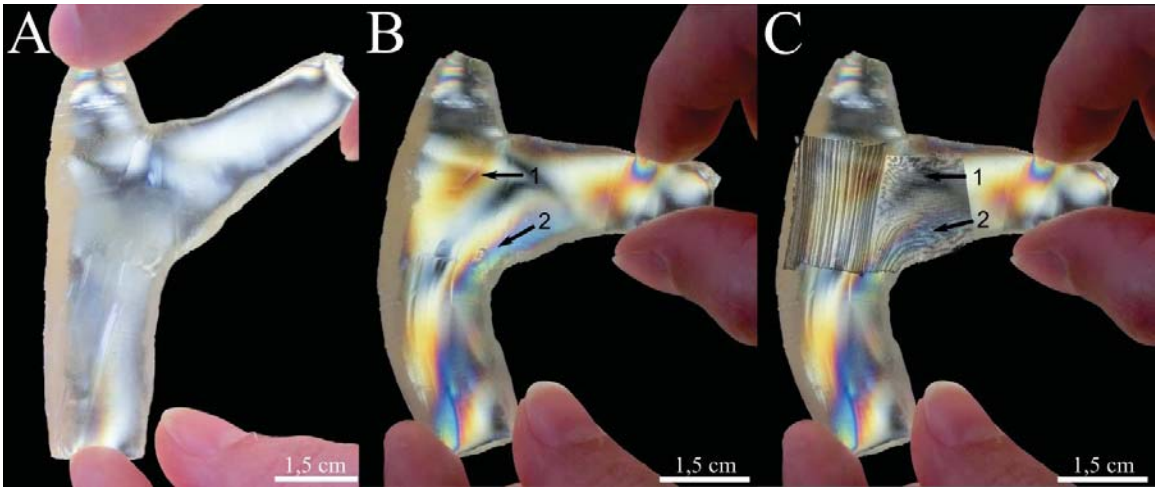

Figure 1: Photoelasticity tests of an isotropic cast of a ramification of *Dracaena marginata*. A: Isotropic cast of the ramification without mechanical load. B: Isotropic cast under mechanical load. The main shear-stresses are visible as colored areas (isochromatics) within the ramification. C: Overlay of a magnetic resonance image showing the arrangement, course and connections of the vascular bundles (dark regions) within the branch-stem-region of *D. marginata*.

Identifying a load adapted orientation of vascular bundles via magnetic resonance imaging (MRI)

For validating and quantifying the qualitative findings of the photoelasticity tests the anatomy of loaded and unloaded branch-stem attachment regions of *D. marginata* need to be studied in vivo and the arrangement of the vascular bundles with their fiber caps have to be analyzed quantitatively. To the best knowledge of the authors, such a study has never been performed before. Common imaging techniques using serial microtome slides (see Haushahn et al. 2014) or µ-CT analyses prohibit stress-strain analyses in unloaded and loaded situations of the same

sample since they are invasive and the sample has to be destroyed for the analysis (in the case of serial microtome slides) or is severely damaged due to the long exposure time in hard x-rays for acquisition of a good enough spatial resolution (µ-CT). Additionally these two methods require a time consuming preparation of the samples and an extensive image post-processing. Magnetic resonance imaging (MRI) can help to overcome these shortcomings as this method allows for non-invasive, non-destructive and repetitive imaging of various plant tissues in living plants. For the underlying principles of Magnetic Resonance Imaging (MRI) technology we refer for example to Hornak (2008).

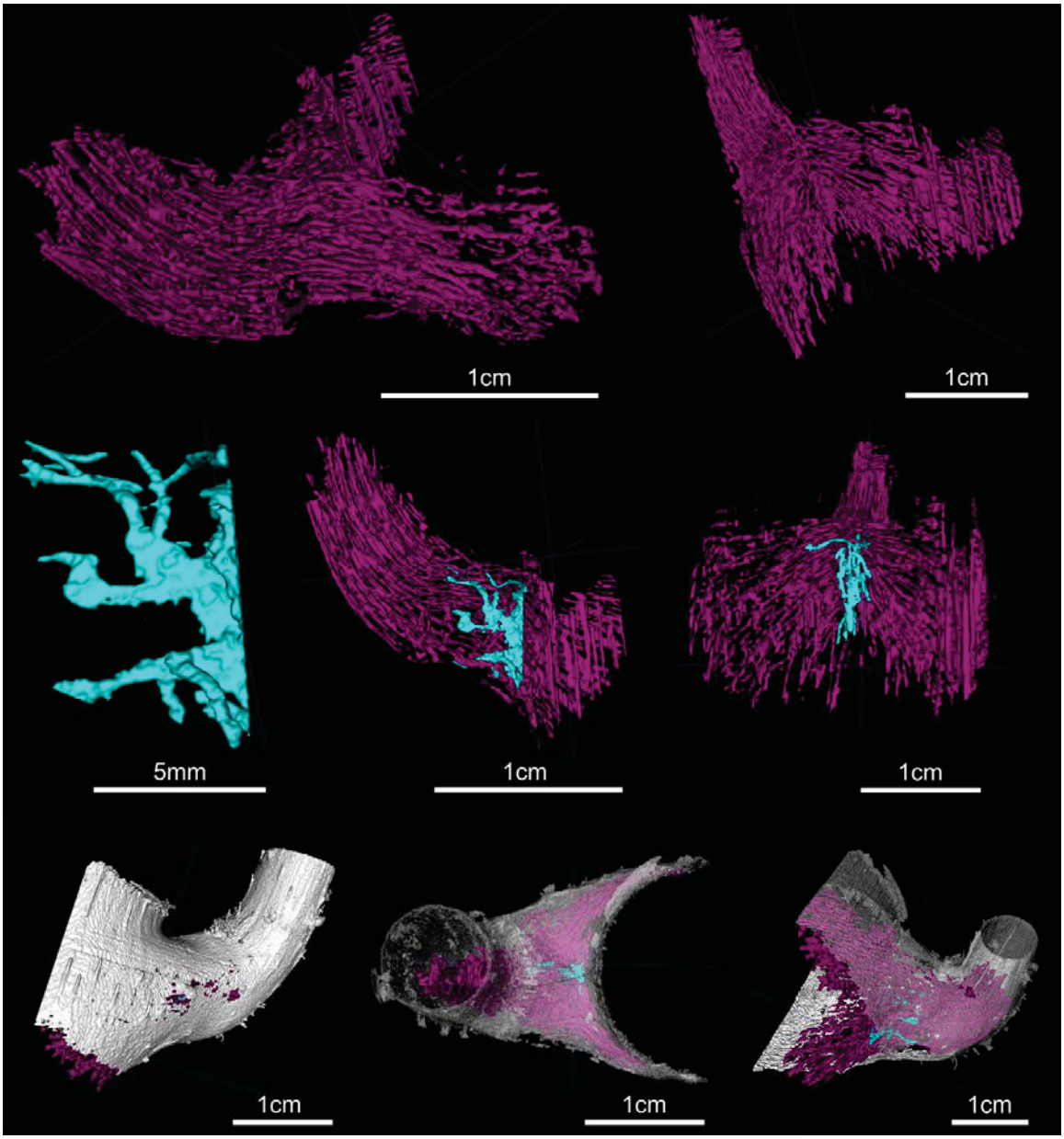

Figure 2: Image segmentation of magnetic resonance images and 3D reconstruction of the vascular system (magenta) as well as of a single vascular bundle (cyan) and of the meristematic tissue (white) of an unloaded branch-stem attachment region of *Dracaena marginata*.

Although MRI has been used in the past for clarifying various problems in plant biology (Borisjuk et al. 2012, Clearwater and Clark 2003, MacFall and Johnson 1994, Williamson et al. 1994), the methodical approach of the imaging process always needs to be optimized according to its specific application. Additionally, an experimental setup has to be developed that allows for imaging plant ramifications that are being mechanically loaded during image acquisition. This also calls for the solution of entirely new imaging challenges that arise when imaging plant structures in the moment of mechanical loading with the aim to analyse the biomechanically relevant structural parameters, e.g. in this case the arrangement of vascular bundles in unloaded and loaded conditions. In a joint collaboration of biologists from the Plant Biomechanics Group Freiburg with physicists from the Department of Radiology (Medical Physics, University Medical Center Freiburg) and engineers from the IMT (Institute of Microstructure Technology, Karlsruhe Institute of Technology), a step-by-step approach was chosen to develop a new and innovative imaging method based on magnetic resonance imaging. MRI allows to gather image data in a comparatively short period of time making it possible to live-record the images of ramifications in an unloaded and a loaded state, i.e. before and during which a force acts on the branch. Moreover, the three-dimensional representation of the tissues within the ramification, especially of the vascular bundles as the mechanically relevant tissue, and the strains occurring in stressed stem-branch attachments can thus be acquired. The simultaneous observation of morphological/structural and biomechanical parameters in 'real time' is of high interest for a multitude of analyses that require an understanding of structural anatomy and biomechanics 'inside' a plant's body. First results gained with this method are very promising and will be further optimized for analyzing changes of fiber/fiber bundles arrangement and stress-strain fields in unloaded and loaded branching regions of a variety of plant species.

Acknowledgements
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From biomechanics to material behavior of *Buxus sempervirens* L. - *An angiosperm forming compression wood*

Pierre Cabrolier, Iris Brémaud

WOOD Team, Laboratory of Mechanics and Civil Engineering (LMGC), CNRS, Université de Montpellier, France

pcabrolier@hotmail.fr

In general, in the gravitropic reorientation process, compression wood is associated to gymnosperms and tension wood to angiosperm. Except for very few hardwood species where compression wood have been observed, such as for example *Pseudowintera colorata* (Kubera and Philipson 1978; Meylan 1981), *Hebe salicifolia* G. *Forst*. (Kojima et al. 2012), *Viburnum odoratissimum* var. (Wang et al. 2010), or *Buxus sempervirens* (Baillères et al. 1997) and *Buxus microphylla* var. *insularis* (Yoshizawa et al. 1999).

The presented research focused on the properties of *Buxus sempervirens* from biomechanical aspects on the tree to the mechanical and physical behaviour of the material. Indeed, there are virtually no scientific publications about its physical-mechanical properties of Boxwood although it is an interesting study case for uncommon re-orientation process.

A wide experimental plan (approx. 150 specimens for various types of tests) have been realized on a living « archetypal » tree of 110 years, which restored verticality in a steep mountain slope of South of France. External residual growth strains have been measured at 4 positions along the circumference and 2 heights. After growth stress measurement, hygrothermal recovery test were performed. Then sampling of specimens have been realized regarding to those positions in order to compare the mechanical and physical behaviour of reaction wood, opposite wood and « normal » wood. Thus measurement have been performed, such as isotherms of sorption and associated shrinkage/swelling, ultrasonic velocities in the 3 principal directions, and dynamic Young's modulus and damping at audio-frequencies (for green wood and dry wood conditions). Concurrently Young's modulus and damping coefficient of air-dry wood have been measured on more than 100 samples from 3 different origin (6 trees in total) at different radial position in the stem cross section. Samples for determination of microfibril angle were also cut along the radial position for the different studied trees.

Growth stress measurement confirmed high level of compression in the lower side of the curvature of the tree. Microfibril angle measurements also clearly highlighted the presence of compression wood. Similarities where found with the mechanical properties of compression wood in gymnosperms, such as, lower Young's modulus and higher longitudinal shrinkage. However it is interesting to note that, in this species with high wood specific gravity (around 1) the anisotropy was rather moderate and the difference in physical/mechanical properties between compression and normal wood were less extreme than in gymnosperms species. In the case of sorption isotherm the differences were surprisingly small. On the contrary, compression wood and "normal" wood reacted very differently to hygrothermal recovery tests.

Generally the results confirmed that the reaction wood of *Buxus sempervirens* is indeed compression wood. They also asked the question of amplitude in difference of wood properties in the case of a wood which has normally a very high density and a moderate stiffness.

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Relationships between growth stress and hygrothermal recovery of compression wood

Miyuki Matsuo[1], Kumiko Nomi[1], Yoshihito Suzuki[2], Noboru Yamashita[2], Ginji Niimi[3], Masato Yoshida[1], Hiroyuki Yamamoto[1]

[1]Laboratory of biomaterial physics, Nagoya University, Japan
[2]Aichi forestry research institute, Japan
[3]Ojimateria co., ltd., Japan
miyuki@agr.nagoya-u.ac.jp

Introduction

Steaming or boiling of wood boards induces dimensional changes of wood. The dimensional changes have been considered as the results of relaxation of growth stress in wood, called the hygrothermal recovery (HTR) [1, 2]. As reaction wood in a tilted tree especially generates large growth stress to support its body, larger HTR is expected to be observed [3]. This study investigated the relationships between the intensity of HTR and the original growth stress of standing trees and the temperature dependency of HTR to evaluate its mechanism.

Materials and method

Japanese cedar (*Cryptomeria japonica* D. Don) and Japanese cypress (*Chamaecyparis obtusa* Endl.) trees with tilted stems that were approximately 20-30 years old were used to measure growth stress and HTR. Fig. 1 shows the sampling and experimental setting. The release strain of surface growth stress (RS) was measured by using the foil strain gauge. Specimens with the size of 30mm (longitudinal direction) × 15mm (tangential direction) × 5mm (radial direction) were cut from xylem where the strain gauges were pasted, immediately after tree felling to keep green. Specimens were treated in water at the temperature of 20°C, 40°C, 60°C, 80°C, and 100°C for Japanese cedar and 100°C for Japanese cypress. Treatment durations for higher temperature were shorter and those for lower temperature were longer. The treated specimens were cooled in iced water just after treatment. The longitudinal dimensions of specimens were measured before and after treatment to measure dimensional change based on untreated specimens.

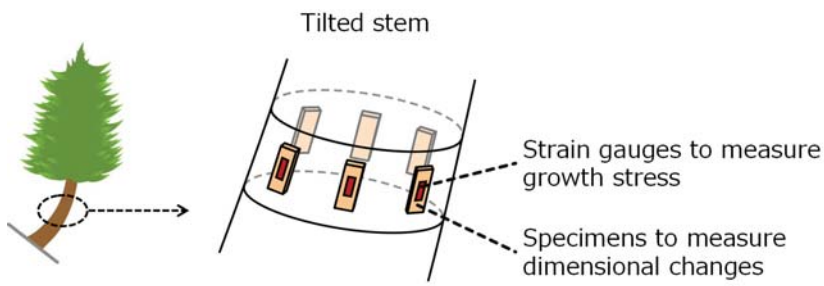

Fig. 1 : Measurement of released strain of surface growth stress and specimen preparation.

Results and Discussion

The maximum value of longitudinal RS was 0.41% for compression wood, which express the formation of intense compression wood xylem. The values for opposite and lateral (normal) wood were small, ranging from -0.049% to 0.040%.

Longitudinal dimension increased by up to 0.73% after hygrothermal treatment for the specimens from compression wood. The maximum increase was observed at 100°C. While the dimensional changes of normal wood were rather slight ranging from -0.31% to 0.14%. The values of dimensional changes showed the positive relationship with the values of longitudinal RS.
The higher treatment temperature induced the more rapid and larger dimensional increase of compression wood (Fig. 1), which indicated that the intensity of dimensional change had time and temperature dependency.

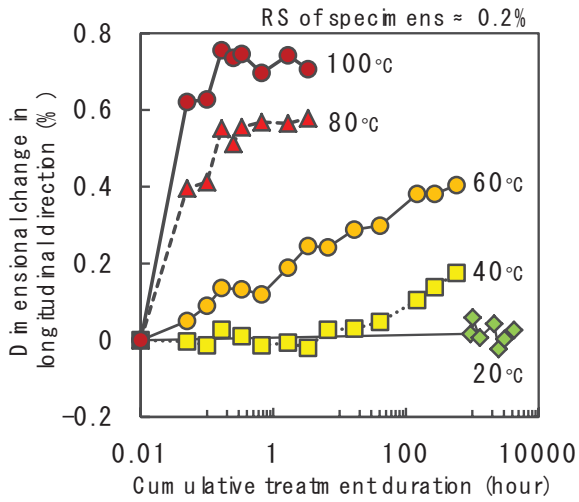

Fig. 1 : Dimensional change in logitudinal direction at 20°C, 40°C, 60°C, 80°C, and 100°C. Example of Japanese cypress specimens with approximately 0.2% of release strain of longitudinal growth stress (RS).

These results indicated that the HTR had an intense relationship with the growth stress or xylogenesis of compression wood, and implied that the HTR relied on physicochemical reaction for its mechanism.

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Are old beech poles able to react to heavy thinning? And how does growth response affects pole biomechanics?

NOYER Estelle[1, 2], DLOUHA Jana[1, 2], COLLET Catherine[1, 2], FOURNIER Meriem[1, 2]

[1] INRA, UMR 1092 Laboratoire d'Etude des ressources Forêt Bois (LERFoB), Centre INRA de Nancy-Lorraine, 54280 Champenoux, France
[2] AgroParisTech, UMR 1092, Laboratoire d'Etude des ressources Forêt Bois (LERFoB), ENGREF, 14 rue Girardet, 54000 Nancy, France
enoyer@nancy.inra.fr

Abstract

Canopy opening increases resource availability around trees. It also induces an increase of mechanical loads applied on newly isolated tree, and an increase of soil and air temperatures (Aussenac 2000). Changes in foliar and wood traits (Caquet *et al.* 2009) are generally observed and seen as an adaptation to the new microclimatic conditions.

In the present work, we investigated the growth response of 60 to 100-years-old beech poles to heavy thinning. We studied 42 poles; half were thinned and half were not thinned. Six years later, all poles were cut for stem and wood analysis. Axial and radial growth in the past twelve years were recorded. Biomechanical performances were described, as defined by Fournier *et al*. (2013) *i.e*. the safety against buckling, safety against wind, motion velocity and posture control. These integrative traits combine effect of the shape, size and wood material properties. Shape and size factors are described by morphological tree measurements (crown projection, taper, biomass distribution, tree straightness), and axial and radial growth by annual ring width and axial growth units measurements. Wood properties measured are the growth stress indicators, reaction wood distribution and density.

First results demonstrated an increase in radial growth but no clear impact of thinning on axial growth. Safety against buckling increased after thinning (*p-value* < 0.01) mainly due to an increase in diameter while shape factors involved (*i.e*. biomass distribution along the height and taper) were not modified by thinning. It is interesting to note that values of safety against buckling in poles were low compared to values usually observed in dominant beech trees. Safety against wind also increased after thinning (*p-value* < 0.1). The most notable change in biomechanical traits was the increase in motion velocity (*p-value* < 0.01). The relationship between increase in motion velocity and posture control will be performed when the axial growth unit measurements are available.

Financial support

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Simulation of tree deformation of Japanese black pine caused by temporally varied wind

Kazuki Nanko[1], Satoru Suzuki[1], Hironori Noguchi[1], Hiroaki Hagino[1], Akira Ogura[2], Yoji Ishida[2], Hiroshi Matsumoto[2], Hiromi Takimoto[2], Tomoki Sakamoto[3]

[1]Department of Meteorological Environment, Forestry and Forest Products Research Institute, Japan
[2]Ishikawa-ken Forest Experiment Station, Ishikawa Agriculture and Forestry Research Center, Japan
[3]Tohoku Research Center, Forestry and Forest Products Research Institute, Japan
knanko@affrc.go.jp

Abstract
Forests reduce wind force, whereas forest trees are broken down by the wind with extremely huge force. It is difficult to estimate their interactive relationship between wind and trees because wind load deform tree architecture and deformed tree changes wind field simultaneously. For the dynamic clarification of tree deformation and wind behavior, fluid-structure interaction analysis are necessary. For the initial step, we have developed dynamic simulation of tree sway and breakage caused by temporally changing wind based on a vibrating system with multiple degrees of freedom.

The target specie of the simulation was Japanese black pine (*pinus thunbergii*), which is the most major specie for coastal forest in Japan to secure livelihood area from the damage by blown sand and salt. Uprooting resistance for the Japanese black pines was measured by tree-pulling test according to Nicoll et al. (2005). Modulus of elasticity (MOE) and modulus of rupture (MOR) for the Japanese black pines were measured by three-point bending test using cross cut stems of the pulled trees. For the simulation, a tree was segmented into 0.2 m long circular truncated cones. Turning moment induced by wind load and self-weight was calculated at each segment bottom. Tree deformation was computed on multi-degree-of-freedom vibration equation. Tree sway was simulated by iterative calculation of the tree deformation with time step 0.05 second with temporally varied wind speed. From the calculation of bending stress and turning moment at tree base, we estimated critical wind speed to occur tree breakage.

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Physiology and biomechanics of extinct plants

Jonathan P. Wilson[1]

[1]Department of Biology
Haverford College
Haverford, Pennsylvania, United States
jwilson@haverford.edu

Abstract

The fossil record of terrestrial plants contains anatomical structures whose functional properties can be interpreted using novel techniques, including mathematical modeling and geochemical analysis. When these methods are applied to plant fossils, the physiological, biomechanical, and hydraulic properties of extinct plants can be understood in a quantitative way. Over the last 30 years, a variety of techniques applied to plant fossils have documented novel anatomical arrangements not found among living plants, changes in biomechanical structure through plant ontogeny, and new ecophysiological interpretations derived from the functional consequences of cortical and xylem tissues.

In this talk, I will discuss the investigative tools and resulting patterns and processes that have been observed in the fossil record, focusing on the unusual combinations of physiological and biomechanical features found within Paleozoic Era plants. Many early land plants–including extinct lycophytes, sphenopsids, and stem group seed plants–developed low-resistance xylem at the cost of increased embolism risk and reduced structural support. Multiple lines of evidence, from the geochemical to the anatomical, suggest that a variety of self-supporting, non-self-supporting, climbing, and scandent habits evolved soon after plant terrestrialization, which, in turn, frequently facilitated efficient long-distance water transport. These results highlight the convergent evolution of high conductivity xylem in step with biomechanical novelty across terrestrial ecosystems in space and time.

Arabinose-rich polymers as an evolutionary strategy to plasticize resurrection plant cell walls against desiccation: A case study of the woody angiosperm *Myorthamnus flabellifolia*

John P. Moore[1], Eric Nguema-Ona[2], Mäite Vicré-Gibouin[2], Iben Sørensen[34], William G.T. Willats[3], Azeddine Driouich[2], Jill Farrant[5]

[1]Institute for Wine Biotechnology, Department of Viticulture and Oenology, Stellenbosch University, South Africa
[2]Glyco-MEV Laboratoire, Université de Rouen, Mont Saint Aignan, France
[3]Department of Plant and Environmental Sciences, University of Copenhagen, Denmark
[4]Department of Plant Biology, Cornell University, NY, USA
[5]Department of Molecular and Cell Biology, University of Cape Town, South Africa

moorejp@sun.ac.za

Introduction

A variety of desiccation-tolerant resurrection plants from Southern Africa were surveyed using high-throughput cell wall profiling tools (Moore et al. 2009, Nguema-Ona et al. 2012). Hydrated and desiccated leaf (frond) material, were analysed using monosaccharide analysis, CoMPP and FT-IR spectroscopy coupled with chemometrics (Moore et al. 2013). Arabinan-rich pectin and an abundance of arabinogalactan proteins were found associated with the resurrection fern *Mohria caffrorum* (Farrant et al. 2009) and the woody angiosperm *Myrothamnus flabellifolia* (Moore et al. 2006). In contrast, *Craterostigma plantagineum* up-regulated wall proteins and osmoprotectants (Moore et al. 2013). The hemicellulose-rich walls of the grass-like *Xerophyta* spp. and the resurrection grass *Eragrostis nindensis* possess highly arabinosylated xylans and arabinogalactan proteins for protection (Moore et al. 2013).

The woody resurrection plant *Myrothamnus flabellifolia*

Of these species, only the resurrection bush *Myrothamnus flabellifolia* (Moore et al. 2007) (see Figure 1), is a true woody perennial, displaying the remarkable capacity to be able survive extensive periods (i.e. years) in the desiccated (quiescent) state. Recent research has highlighted a number of anatomical (xylem modification), physiological (photosynthetic adjustment), chemical (antioxidant load), biochemical (cell wall flexibility and membrane stabilizers) and molecular (sugar transport) properties which facilitate survival of this plant under adverse environmental conditions (Moore et al. 2007). This presentation will review the current hypotheses and data that support a general evolutionary mechanism of 'plasticising'

the cell walls of resurrection plants (Moore et al. 2006; 2007) to desiccation and implicate arabinose-rich polymers (pectin-arabinans, arabinogalactan-proteins and arabino-xylans) (Moore et al. 2013) as the major contributors in ensuring flexibility is maintained and rehydration is facilitated in these remarkable plants.

Fig. 1: The resurrection plant *Myorthamnus flabellifolia*; (desiccated – left; hydrated – right)

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A novel method quantifying stiffness of epidermis and mesophyll layers reveals leaves have a highly efficient sandwich structure

Yusuke Onoda[1], Feike Schieving[2], Niels P R Anten[3]

[1] Department of Agriculture, Kyoto University, Kyoto, Japan
[2] Institute of Environmental Sciences, Utrecht University, Utrecht, The Netherlands (NL)
[3] Centre for Crop Systems Analysis, Wageningen University, Wageningen, NL
yusuke.onoda@gmail.com

Introduction

Plant leaves commonly exhibit a thin, flat structure that facilitates a high light interception per unit mass, but may increase risks of mechanical failure when subjected to gravity, wind and herbivory as well as other stresses. Leaf laminas are composed of thin epidermis layers and thicker intervening mesophyll layers (Fig. 1), which resemble a composite material, i.e. sandwich structure used in engineering constructions (e.g. airplane wings) where high bending stiffness with minimum weight is important. Yet, to what extent leaf laminas are mechanically designed and behave as a sandwich structure remains unclear.

Materials & Methods

We developed a novel method to estimate the Young's moduli of epidermis- and mesophyll layers without separating the layers (described below), and applied this method to diverse 36 plant species.

The Young's modulus (E) of leaf lamina can be expressed with E of mesophyll layer (E_c) and E of epidermis layers (E_f) weighted by thickness of each layer (Fig. 1). In tensile tests, E of leaf lamina can be expressed as follows:

$$E_T = (1-\alpha)E_f + \alpha E_c \qquad \text{Eq.1}$$

where α is the ratio of mesophyll thickness to lamina thickness. In bending tests, E of leaf lamina can be expressed as follows:

$$E_B = (1-\alpha^3)E_f + \alpha^3 E_c \qquad \text{Eq.2}$$

From Eq.1 and 2, E_B/E_T can be expressed as

$$\frac{E_B}{E_T} = \frac{1-\alpha^3(1-\beta)}{1-\alpha(1-\beta)} \qquad \text{Eq.3}$$

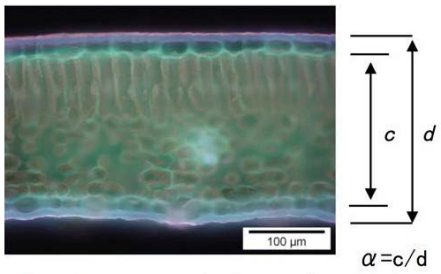

Fig. 1. Representative images (UV fluorescence) of leaf lamina cross-sections (*Helleborus orientalis*). The mesophyll fraction in leaf thickness is expressed as α.

where $E_c/E_f = \beta$. When the face layers are exclusively stiffer than the core as expected for efficient sandwich structure ($\beta \approx 0$), Eq. 3 can be expressed as follows;

$$\frac{E_B}{E_T} \approx 1 + \alpha + \alpha^2 \qquad \text{Eq.4}$$

This equation shows that the theoretical maximum of E_B/E_T for an ideal sandwich structure with an extremely thin and stiff face layers ($\alpha \approx 1$, $\beta \approx 0$), the E_B/E_T ratio can increase up to 3. Furthermore, In Eq. 1 and 2, E_c and E_f cannot be directly measured, however because there are two equations and only two unknowns, these two variables can be solved analytically;

$$E_f = \frac{E_B - \alpha^2 E_T}{1-\alpha^2} \qquad \text{Eq.5}$$

$$E_c = \frac{(1+\alpha+\alpha^2)E_T - E_B}{\alpha(1+\alpha)} \qquad \text{Eq.6}$$

Results & Discussion

Across a phylogenetically diverse range of 36 angiosperm species, a strong corelation between E_B and E_T was found, and the relationship was clearly different from the 1:1 relationship (Fig.2a, $E_B/E_T = 2.59 \pm 0.48$, n=36) and close to the theoretical maximum ($E_B/E_T = 3$), meaning that leaf laminas of these plant species behaved as nearly ideal sandwich structures. The estimated Young's moduli of the epidermis layers (calculated from Eq.5) were from 11 MPa in *Arabidopsis thaliana* to 463 MPa in *Eucalyptus pauciflora*, while the estimated Young's moduli of the mesophyll layers were much lower (0.61 ± 9.61 MPa, n=36). The Young's modulus of epidermis layers was tightly associated with cuticle thickness, which was higher in evergreen species than in deciduous species (Fig. 2b). The ubiquitous nature of sandwich structures in leaves across studied species suggests that the sandwich structure has evolutionary advantages as it enables leaves to be simultaneously thin and flat, efficiently capturing light and maintain mechanical stability under various stresses.

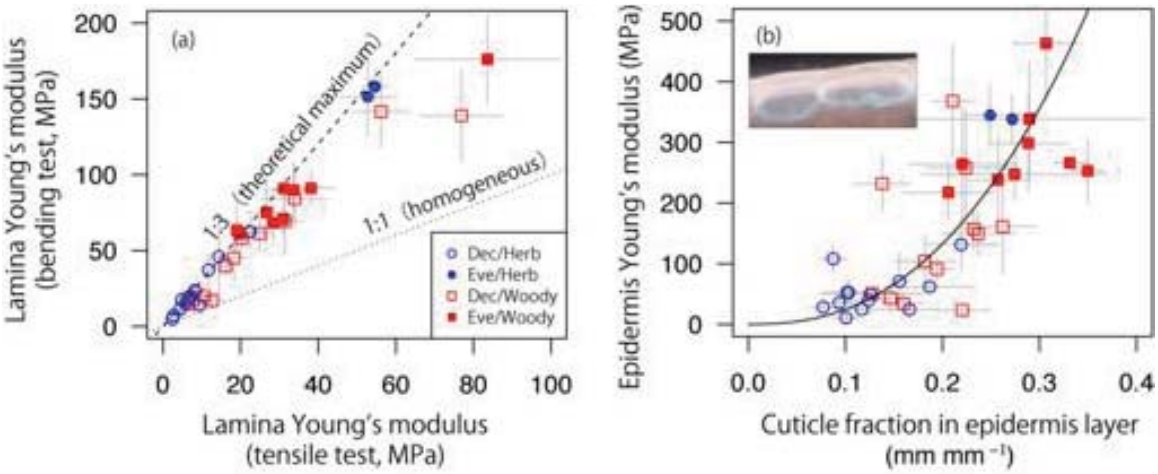

Fig. 2. (a) Relationship between lamina Young's modulus measured by bending and tensile tests aross 36 species. (b) Estimated epidermis Young's moduli are plotted against cuticle fraction in the epidermis layer.

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Diversity of Gymnosperm Reaction Wood Anatomy and Growth Stress

Tatsuya Shirai [1], Miyuki Matsuo[1], Lahjie Abubakar Mhd[2], Masato Yoshida[1], Hiroyuki Yamamoto[1]
[1]School of Bioagricultural Sciences, Nagoya University, Japan,
[2]Faculty of Forestry, University of Mulawarman, Indonesia
hiro@agr.nagoya-u.ac.jp

Intoroduction

Conifer species forms the reaction wood (RW) along the lower side of the secondary xylem in an inclined stem or a branch, called compression wood (CW), where a large compressive growth stress is generated. In the meantime, angiosperm generally forms the tension wood (TW) along the upper side of the secondary xylem in the inclined stem, where a large tension growth stress is generated. Those stresses help inclined woody stems or branches to rise upward, which is called the negative-gravitropism.

This presentation seeks for a biomechanical reason why the pattern of the RW formation differs between gymnosperm and angiosperm, based on the properties of xylem structure and growth stresses, especially focusing the key material species, *i.e.*, *Ginkgo biloba* (Ginkgoopsida) and *Gnetum gnemon* (Gnetopsida), both belonging to gymnosperm.

Material and Method

Two mature stands of *Ginkgo biloba*, grown in Aichi prefecture, Japan, and five mature stands of *Gnetum gnemon*, grown in Tenggarong, Kalimantan Timur, Indonesia, were tested. Some had grown vertically, while the others had inclined stems. Released strains of the surface growth stress were measured around the periphery of the stem at breast height in each tested tree. After measuring the released strains, wood blocks were sampled, and processed into the specimen for microscopic observation.

Result and Discussion

(1) *G. biloba* (Ginkgo) (Shirai *et al*. in press)

Ginkgo had a thick, lignified secondary xylem, consisting of many tracheids and uniseriate rays as in conifers, and secondary growth was promoted on the lower half of the inclined stem, where CW-like RW tissue was formed. In the RW region, a large compressive growth stress (CGS) was generated. The CGS was closely related to increases in the cellulose microfibril angle (MFA) in the secondary cell wall of tracheid

and in lignin content as well as a decrease in cellulose content. The generation mechanism of growth stress of ginkgo is quite similar to that of conifers.

(2) *G. gnemon* (Gnemon) (Shirai *et al*. 2015)
Gnemon had a thick, lignified secondary xylem, consisting of wood fibers, vessel elements, and multi-seriate rays as in a typical hardwood. Its secondary growth was promoted on the upper half of the inclined stem. However, no difference could be observed in xylem anatomy between the upper and the lower sides (see Fig.2), excepting the fact that the MFA becomes lower on the upper half side but higher on the lower half side in the inclined stem. Tensile growth stress increased on the upper side and decreased on the lower side of inclined stems. The MFA partially controls the increase and/or decrease of the tensile growth stress, whereas cellulose content does not participate. These findings are partly similar to negative gravitropism in Magnoliaceae, in which the typical G-fibers are absent in TW. However, in Magnoliaceae, cellulose content does influence the magnitude of tensile growth stress in concert with MFA.

(3) Why the pattern of the RW differs between two plant phyla? - A possible reason
Some primitive angiosperm species, *e.g.*, Amborellaceae, some Chloranthaceae, and some Winteraceae, do not form vessel element in their secondary xylem. Aiso et al (2013) reported that *Sarcandra glabra* (Chloranthaceae) forms the CW-like reaction wood and generates compressive growth stress on the lower side of inclined stems. Similar result was reported for *Pseudowintera colorata* (Winteraceae) by Meylan (1981). Considering our results with Aiso et al (2013) and Meylan (1981), appearance and development of vessel element in the secondary xylem is possibly concerned with origin of TW type reaction wood fiber in angiosperm.

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Root tropisms for plant adaptation to terrestrial environment: gravitropism *vs.* hydrotropism

Hideyuki Takahashi[1]

[1]Graduate School of Life Sciences, Tohoku University, Japan
hideyuki@ige.tohoku.ac.jp

Introduction

Water is a requisite for all organisms to maintain their lives. Land plants evolved various genuine abilities to survive terrestrial environment in which water availability differs depending on locations and seasonal changes on Earth. Plants obtain water by roots that are able to maximize water acquisition via regulation of growth orientation. It is well known that gravitropic responses of primary and lateral roots govern the development of root system architecture. Root downward growth is thus essential for obtaining water that normally accumulates in deeper soil. Another ability plant developed is hydrotropism; roots bend toward the moistened side in response to moisture gradients. It has long been implicated that plant roots possess the ability of hydrotropism. However, its demonstration and studies for the mechanistic aspects of hydrotropism in roots began relatively recently.

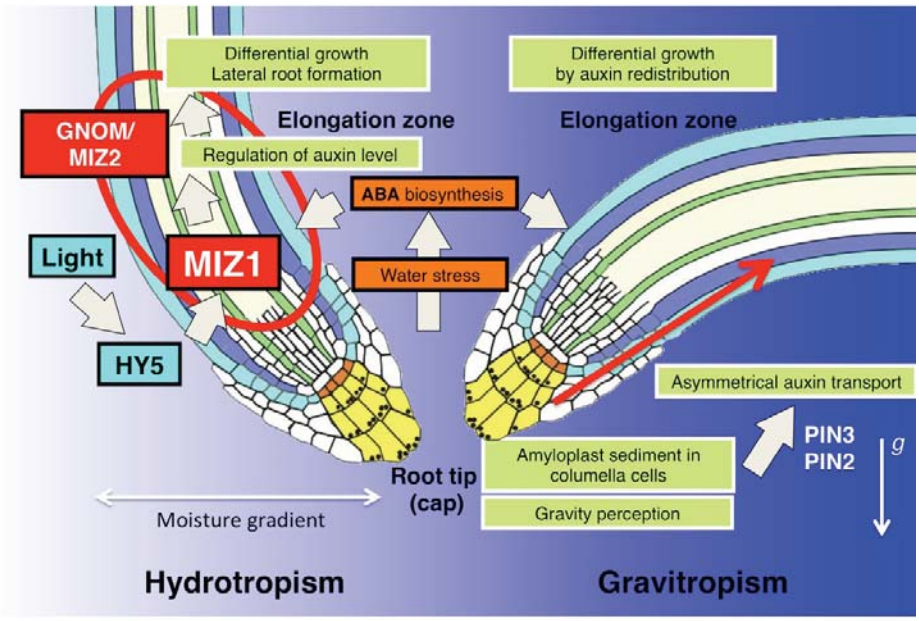

Fig. 1 : Models for hydrotropism and gravitropism in Arabidopsis roots
(Modified from Moriwaki et al., 2013)

Regulatory mechanisms unique to hydrotropism in roots

We identified two genes essential for the hydrotropic response in Arabidopsis roots, *MIZU-KUSSEI1* (*MIZ1*) and *MIZ2*. *MIZ1* encodes an uncharacterized protein containing a domain of unknown function (DUF617), which is termed the MIZ domain. *MIZ1*-overexpressor dramatically accelerates the hydrotropic response, and the overexpression of *MIZ1* leads to an increase in plant growth and survival under water-limited conditions. Thus, MIZ1 plays a crucial role in root hydrotropic response and contributes to plant drought avoidance. *miz2* mutant that is defective in hydrotropic response but shows normal morphology is a novel allele of *gnom* isolated as mutants of pattern formation. *GNOM/MIZ2* encodes a guanine

exchange factor for ADP-ribosylation factor-type G protein (ARF-GEF), which plays an important role in vesicular trafficking. The *gnom/miz2* mutation inhibits the phenotype of *MIZ1*-overexpressor, suggesting that MIZ2 functions downstream of MIZ1. MIZ1 and MIZ2 are indispensable for hydrotropism but not graviropism, indicating an existence of molecular mechanism unique to hydrotropism in Arabidopsis roots.

Tissues functional for gravitropism and hydrotropism in roots

In roots, gravisensing cells are considered to be columella cells in which amyloplasts sediment. For an example, laser ablation of columella cells results in a substantial reduction of gravitropic response in Arabidopsis roots. Although mechanisms for perception and transduction of gravity signal remain obscure, it could be responsible for auxin transport that causes auxin redistribution and thereby differential growth in the elongation zone. Apparatus for sensing moisture gradients were also considered to reside in the root cap in earlier studies. In Arabidopsis roots, however, we recently revealed that neither the root cap nor meristimatic tissue was required for the induction of hydrotropism. Likewise, it was found that tissues required for MIZ1 function resided in the elongation zone but not in the root cap or meristem. Thus, the sensory mechanism for hydrotropism differs from that of gravitropism, at least in Arabidopsis roots.

Roles of auxin in gravitropism and hydrotropism in roots

During the gravitropic response of roots, auxin is asymmetrically transported from the root cap to the lower flank of the elongation zone. This auxin dynamics is mediated by re-localization of PIN-FORMED (PIN) proteins, which ultimately leads to asymmetrical auxin accumulation and differential growth in the elongation zone. However, it has been shown that manner of auxin involvement in root hydrotropism differs depending on plant species.

I will compare and discuss the models for mechanisms of gravitropism and hydrotropism in roots. Particular interests in future studies are how roots sense moisture gradients and integrate multiple signals of environmental cues for the regulation of growth orientation. We also need to clarify the species differences in the regulatory mechanisms responsible for hydrotropic responses.

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Hygroscopically Coiling Cells in a Grass – *Stipa capensis*

Yael Abraham[1], Sunil Kumar[2], Yaron Silberberg[2], Rivka Elbaum[1]

[1] The RH Smith Institute for Plant Sciences and Genetics in Agriculture, The Hebrew University of Jerusalem, Rehovot, Israel
[2] Departement of Physics of Complex Systems, The Weizmann Institute of Science, Rehovot, Israel
rivka.elbaum@mail.huji.ac.il

Hygroscopic movement is present in many seed dispersal mechanisms: As the seed dispersal unit disconnects from the mother plant and dies, its drying induces a directed movement that promotes seed dispersing and sowing. In *Stipa capensis*, a wild grass, each seed is decorated by a long thin hygroscopic awn that twists and bends as it dries. Many awns entangle and create bundles that are blown by winds. Once on the ground, the awn may push the seed across the surface and into the soil. Hygroscopic movement mechanisms are usually based on a bilayer, where one layer contracts in a specific direction while the other one resists that contraction and supplies the device with mechanical stability. However, the awns of *S. capensis* are built out of a single tissue layer made of coiling cells. The cells have thick cell walls, with high microfibril angle. Apparently, the cell wall density and cellulose crystallinity are similar around the cells. Our X-ray diffraction, Raman, polarized light, and second harmonic microscopy analyses indicate that the microfibril angles change along the cells' circumference, thus forcing the cells to coil as they dry. Curiously, the cell lumen is not centered. Confocal microscopy indicates that the cells form an ellipsoid coil, probably as a result of the shifted lumen. Our results show that hygroscopically coiling cells exist in the grass family, providing plants with a versatile tool for constructing hygroscopic moving devices.

Vascular structure of leaf petioles

Derek G. Gray

Department of Chemistry, McGill University, Montreal, Canada
derek.gray@mcgill.ca

Summary
Leaf petioles are critical components of the vascular system that conducts water from the roots to the photosynthetic apparatus of most green plants. In previous work, we observed and isolated left-handed helical cellulosic coils from the tracheary elements of a range of plants and trees (Gray 2014). The ubiquitous presence of coiled thickenings in petiole vascular elements indicates their importance in plant functions. The main role of the spiral thickening may be to reinforce the walls of the tracheary elements, which have to withstand the negative pressure within the plant vascular system during transpiration. Examination of the fossil records of primitive vascular plants also shows evidence of coiled structures; particularly clear examples are shown by the 20 cm tall early land plant *Asteroxylon mackiei,* preserved in the 400m year old Rhynie Chert.

The fibrils that form the coils, if isolated by mild procedures from non-woody tissues, invariable appear to have cross-sections that are almost circular. The presence of cylindrical elements in the plant vascular system suggests that the capillary properties may play a role. For example, the capillary rise of a wetting liquid between two cylindrical rods depends approximately inversely on their spacing, with a very high value when the rods are touching (Liu et al. 2007). A liquid that wets the surface of the cylinders is drawn into the space close to the contact line of the cylinders. This volume is increased by winding the rod into a helix, as sketched in Fig. 1. These helical coils may provide both a transport mechanism and a reservoir of water within embolized cells, even if the source of the water is under negative pressure. It is not clear how this type of wicking could play any role in refilling embolized cells, but at least small amounts of capillary water would be trapped in the coiled structure. Capillary forces in cylinders with non-uniform cross-sections may also be involved in protection against embolism by contact line pinning.

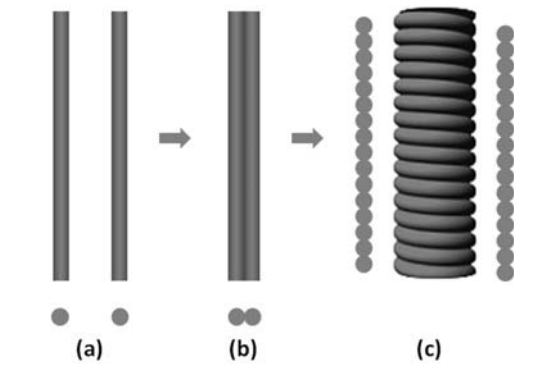

Fig. 1 Schematic of (a) two cylindrical rods with circular cross-sections (b) the two rods in contact, and (c) a helical coil formed by a cylindrical rod.

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Evolution of Herbs and Woodiness

Nick Rowe

[1]Botanique et bioinformatique de l'architecture des plantes (AMAP), CNRS 5120 Montpellier, France

nrowe@cirad.fr

The appearance of woodiness was one of the key developmental innovations during the evolution of land plants and conferred a range of biomechanical advantages to vascular plants. A secondary vascular cambium evolved independently in several plant lineages during the Palaeozoic and contributed significantly to a widespread diversification in form, size and mechanical complexity.

However, the evolutionary history of woodiness is not only about the "appearance" or "increase" or "optimisation" of wood and secondary phloem but also about its loss and reduction. Some lineages have conserved secondary growth as a permanent fixture; others have lost it. Of course, the implications of losing secondary growth actually depend on what we mean by "losing". Numerous lineages of angiosperms have completely lost the bifacial vascular cambium such as the Nymphaeales, Hydatellaceae and the monocotyledons. Such groups have remained aquatic or strictly herbaceous and small-bodied or evolved novel ways to grow as big tree-sized plants. In other lineages, expression of the bifacial cambium is merely limited or just not expressed rather than entirely lost. In several early angiosperm lineages, early diverging species are represented by herbs with little or no bifacial cambial growth but later evolved species develop significant bifacial cambial growth and mechanically significant amounts of wood.

Instances of so called secondary woodiness have fascinated botanists, evolutionists and functional biologists since the time of Darwin. The phenomenon is of particular interest for understanding how plant lineages can survive under environmental conditions that favour herbaceous growth forms but then "re-express" wood development in later-evolved species under different environmental conditions.

There are probably two main factors why vascular plant lineages can develop widely differing body sizes such as herbs and woody growth forms relatively easily and relatively frequently.

1) The, modular, indeterminate, branched, hierarchical organisation of the stem and branch systems of plants, places peculiar constraints–and opportunities–on the development and indeed evolution of mechanics, size and form. In general, *younger* distal bits of the plant body are mechanically and hydraulically supported by *older* bits of the plant. This contrasts with many other bauplans that evolved alongside vascular plants e.g. vertebrates, insects, molluscs.

2) In terms of stem mechanics, plant stems generally show a gradient of mechanical properties during growth and differentiation, from soft parenchymatous tissues at the young apex through collenchymatous and increasingly stiffer, lignified tissues and woody stems.

One might argue that these two factors can explain why plant size, form and shape can be modified by relatively simple changes in developmental rate and developmental gene expression.

In evolutionary contexts such changes have been termed "heterochronic" changes that involve changes in developmental rate between ancestor and descendent. At the risk of oversimplification, slowing down and/or early curtailing stem development will result in a short, narrow stem with little or no wood, whereas rapid development of lignified tissues and

continued indeterminate growth will result in tall, thick stems with a lot of wood. Such changes in development rate of the plant stem can also play important roles beyond direct evolutionary contexts such as adapting to different ecological constraints and engendering high or low levels of phenotypic plasticity.

The difference between herbaceous and woody plants and the mechanisms underlying the transitions between them might be explained by relatively simple changes in development. If this is true, then it is probably not surprising that switches between herbs and woody plants have occurred often during plant evolution.

PBM8 Nagoya 2015 - List of participants				
Family Name	**1st name**	**Institution**	**coun**	**email (updated 2021 if possible)**
Abe	Kentaro	Kyoto U.	JP	abe.kentaro.3a@kyoto-u.ac.jp
Aida	Mitsuhiro	Nara IST	JP	m-aida@bs.naist.jp
Almeras	Tancrede	LMGC U. Montpellier	FR	tancrede.almeras@umontpellier.fr
Ando	Kosei	Nagoya U.	JP	musica@agr.nagoya-u.ac.jp
Anten	Niels	Wageningen U.	NL	niels.anten@wur.nl
Arnould	Olivier	LMGC U. Montpellier	FR	olivier.arnould@umontpellier.fr
Awano	Tatsuya	Kyoto U.	JP	awano.tatsuya.7z@kyoto-u.ac.jp
Baley	Christophe	LIMatB Lorient	FR	christophe.baley@univ-ubs.fr
Bardet	Sandrine	LMGC U. Montpellier	FR	sandrine.bardet@umontpellier.fr
Baskin	Tobias	U. Massachusetts	US	baskin@umass.edu
Bauer	Georg	U. Freiburg	DE	georg.bauer@biologie.uni-freiburg.de
Bohn	Holger	U. Freiburg	DE	holger.bohn@biologie.uni-freiburg.de
Bohr	Tomas	T.U. Denmark, Copenhag	DK	tbohr@fysik.dtu.dk
Bonnesoeur	Vivien	LERFoB Inra Nancy	FR	bonnesoeur.vivien@gmail.com
Boudaher	Firas	U. Cambridge	UK	firas.boudaher@slcu.cam.ac.uk
Bourmaud	Alain	LIMatB Lorient	FR	alain.bourmaud@univ-ubs.fr
Bremaud	Iris	LMGC U. Montpellier	FR	iris.bremaud@umontpellier.fr
Cabrolier	Pierre	LMGC U. Montpellier	FR	pcabrolier@hotmail.fr
Capron	Marie	Nagoya U.	JP	olivier.arnould@umontpellier.fr
Chabbert	Brigitte	FARE Inra Reims	FR	brigitte.chabbert@inrae.fr
Chauvet	Hugo	IUSTI Marseille	FR	hugo.chauvet@gmail.com
Cheddadi	Ibrahim	VirtualPlant Inria Montpell	FR	ibrahim.cheddadi@inria.fr
Cho	HyungTaeg	Seoul Nat. U.	KR	
Clair	Bruno	EcoFoG Kourou	GF	bruno.clair@cnrs.fr
Constant	Thiery	LERFoB Inra Nancy	FR	constant@nancy.inra.fr
Cook	Douglas	New York U. Abu Dhabi	AE	douglascook@nyu.edu
Davies	Nicholas	U. Canterbury	NZ	ntd14@uclive.ac.nz
Derome	Dominique	EMPA Zurich	CH	Dominique.Derome@empa.ch
Derr	Julien	LMSC U. Paris 7	FR	julien.derr@univ-paris-diderot.fr
Dumond	Mathilde	LRDP ENS Lyon	FR	mathilde.dumond@ens-lyon.fr
Dupuy	Lionel	James Hutton Inst.	UK	Lionel.Dupuy@hutton.ac.uk
DurandSmet	Pauline	CalTech, California	US	pauline.durand@univ-paris-diderot.fr
Dyson	Rosemary	U. Birmingham	UK	r.j.dyson@bham.ac.uk
Elbaum	Rivka	Hebrew U. Jerusalem	IL	rivka.elbaum@mail.huji.ac.il
Fakih	Mahmoud	AMAP Montpellier	FR	mahmoud.fakih@cirad.fr
Forterre	Yoel	IUSTI Marseille	FR	yoel.forterre@univ-amu.fr
Fourcaud	Thierry	AMAP Montpellier	FR	fourcaud@cirad.fr
Funada	Ryo	Tokyo U. Agr Tech	JP	funada@cc.tuat.ac.jp
Furukawa	Toshiko	Tokyo Metropolitan U.	JP	furukawa-toshiko@tmu.ac.jp
Gardiner	Barry	ISPA Inra Bordeaux	FR	barry.gardiner@inrae.fr
Geitmann	Anja	U. Montreal	CA	anja.geitmann@umontreal.ca
Ghislain	Barbara	EcoFoG Kourou	GF	barbara.ghislain@gmail.com
Gray	Derek	McGill U., Montreal	CA	derek.gray@mcgill.ca
Gril	Joseph	LMGC U. Montpellier	FR	joseph.gril@cnrs.fr
Guena	Geoffroy	IUSTI Marseille	FR	geoffroy.guena@univ-amu.fr
Hamant	Olivier	LRDP ENS Lyon	FR	olivier.hamant@ens-lyon.fr
Hanipah	SuhaizaHanim	U. Putra Malaysia, Serdang	MY	afandi@upm.edu.my
Hartmann	Felix	LERFoB Inra Nancy	FR	felix.hartmann@inrae.fr
Hashimoto	Takashi		JP	
Hayashi	Takahisa	Tokyo U. Agric.	JP	t4hayash@nodai.ac.jp
Hernandez	Valeria	U. Nat. Auton. Mexico	MX	valeriahhdez@gmail.com
Hervieux	Nathan	LRDP ENS Lyon	FR	nathan.hervieux@ens-lyon.fr
Hesse	Linea	U. Freiburg	DE	tom.masselter@biologie.uni-freiburg.de
Hiraide	Hideto	Nagoya U.	JP	i061030b@yahoo.co.jp
Huang	YanSan	Nat. Chung Hsing U.	TW	yansanhuang@ntu.edu.tw
Hwang	Youra	Seoul Nat. U.	KR	youra.h90@gmail.com
Iida	Hidetoshi	Tokyo Gakugei U.	JP	iida@u-gakugei.ac.jp
Iimura	Takuya	Tokyo Metropolitan U.	JP	t.iimura@tmu.ac.jp
Itaya	Tomotaka	Nagoya U.	JP	itaya.tomotaka@d.mbox.nagoya-u.ac.jp

PBM8 Nagoya 2015 - List of participants				
Family Name	**1st name**	**Institution**	**coun**	**email (updated 2021 if possible)**
Ito	Koji	Kanazawa U.	JP	mritkj@stu.kanazawa-u.ac.jp
Jackson	Toby	U. Oxford	UK	tobias.jackson@linacre.ox.ac.uk
Julias	Margaret	New York U. Abu Dhabi	AE	margaret.julias@nyu.edu
Jullien	Delphine	LMGC U. Montpellier	FR	delphine.jullien@umontpellier.fr
Kamimura	Kana	Shinshu U.	JP	
Kappel	Roland	Karlsruhe Ins.Tech.	DE	roland.kappel@kit.edu
Kawai	Kiyosada	Kyoto U.	JP	k.kawaikx@gmail.com
Ki	Daeeun	Seoul Nat. U.	KR	kidaeeun55@gmail.com
Kim	Yoon Soo	Chonnam Nat. U.	KR	
Kobayashi	Hidetoshi	Osaka U.	JP	hkoba@me.es.osaka-u.ac.jp
Kojima	Miho	FFPRI Tsukuba	JP	mikoji98@affrc.go.jp
Kwiatkowska	Dorota	U. Silesia Katowice	PL	dorota.kwiatkowska@us.edu.pl
Lachenbruch	Barbara	Oregon State U.	US	barb.lachenbruch@oregonstate.edu
Leblanc-Fournier	Nathalie	PIAF Inra Clermont	FR	Nathalie.Leblanc@uca.fr
LeDiguou	Antoine	LIMatB Lorient	FR	Antoine.le-duigou@univ-ubs.fr
Lee	Hyodong	Seoul Nat. U.	KR	lbdt87@naver.com
Legue	Valerie	PIAF Inra Clermont	FR	valerie.legue@uca.fr
Lipowczan	Marcin	U. Silesia Katowice	PL	marcin.lipowczan@us.edu.pl
Lucas	Peter	Kuwait U.	KU	peterwlucas@gmail.com
Mabille	Frederic	IATE Montpellier	FR	frederic.mabille@inrae.fr
Matsumoto	Daichi	Ritsumeikan U.	JP	
Matsuo	Miyuki	Nagoya U.	JP	miyuki@agr.nagoya-u.ac.jp
Meroz	Yasmine	Harvard U.	US	ymeroz@seas.harvard.edu
Moore	John	SCION Rotorua	NZ	John.Moore@scionresearch.com
Moore	John P.	Stellenbosch U.	ZA	moorejp@sun.ac.za
Morita	Miyo Terao	NIBB	JP	mimorita@nibb.ac.jp
Moulia	Bruno	PIAF Inra Clermont	FR	bruno.moulia@inrae.fr
Nagaoka	Takako	Shinshu U.	JP	
Nakada	Ryogo	FTBC-FFPRI Hokkaido	JP	ryogo@affrc.go.jp
Nakamura	Aki	Tokyo Gakugei U.	JP	m141819g@st.u-gakugei.ac.jp
Nakayama	Naomi	U. Edinburgh	UK	naomi.nakayama@ed.ac.uk
Nanko	Kazuki	FFPRI Tsukuba	JP	knanko@affrc.go.jp
Naramoto	Satoshi	Tohoku U.	JP	satoshi.naramoto.d6@tohoku.ac.jp
Natonik	Sandra	U. Silesia Katowice	PL	sandra.natonik@us.edu.pl
Nishitani	Kazuhiko	Tohoku U.	JP	nishitan@m.tohoku.ac.jp
Nonda	Junji	Yamaguchi U.	JP	nodaj@yamaguchi-u.ac.jp
Notaguchi	Michitaka	Nagoya U.	JP	notaguchi.michitaka@b.mbox.nagoya-u.ac.jp
Noyer	Estelle	LERFoB Inra Nancy	FR	estelle.noyer@inrae.fr
Ogasawara	Kimi		JP	
Ogawa	Kazuharu	Nagoya U.	JP	kazogawa@agr.nagoya-u.ac.jp
Olivier-Santos	Olivier		JP	
Onoda	Yusuke	Kyoto U.	JP	yusuke.onoda@gmail.com
Ozparpucu	Merve	IFB Zürich	CH	merveo@ethz.ch
Peaucelle	Alexis	LMSC U. Paris 7	FR	Alexis.Peaucelle@inrae.fr
Pilate	Gilles	AGPF Inra Orléans	FR	Gilles.Pilate@inrae.fr
Poppinga	Simon	U. Freiburg	DE	simon.poppinga@biologie.uni-freiburg.de
Pouliquen	Olivier	IUSTI Marseille	FR	olivier.pouliquen@univ-amu.fr
Ptashnyk	Mariya	U. Dundee	UK	m.ptashnyk@dundee.ac.uk
Rademaker	Hanna	T.U. Denmark, Copenhagen	DK	hanra@fysik.dtu.dk
Riviere	Mathieu	LMSC U. Paris 7	FR	mathieu.riviere@univ-paris-diderot.fr
Robertson	Daniel	New York U. Abu Dhabi	AE	djr7@nyu.edu
Robinson	Sarah	U. Bern	CH	sarah.robinson@ips.unibe.ch
RoutierKierzkowska	AnneLise	MPI Cologne	DE	smith@mpipz.mpg.de
Rowe	Nick	AMAP Montpellier	FR	nrowe@cirad.fr
Rueggeberg	Markus	IFB Zürich	CH	mrueggeberg@ethz.ch

PBM8 Nagoya 2015 - List of participants				
Family Name	**1st name**	**Institution**	**coun**	**email (updated 2021 if possible)**
Ruiz	Thierry	IATE U. Montpellier	FR	thierry.ruiz@umontpellier.fr
Sahaf	Michal	Hebrew U. Jerusalem	IL	michal.sahaf@gmail.com
Sakamoto	Jiro	Kanazawa U.	JP	sakamoto@se.kanazawa-u.ac.jp
Salmen	Lennart	Innventia Stockholm	SE	lennart.salmen@innventia.com
Sato	Saori		JP	
Sebera	Vaklav	Mendel U. Brno	CZ	vaclav.sebera@mendelu.cz
Shah	Darshil	U. Cambridge	UK	tpsr2@cam.ac.uk
Sharon	Eran	Hebrew U. Jerusalem	IL	erans@mail.huji.ac.il
Speck O.	Olga	U. Freiburg	DE	olga.speck@biologie.uni-freiburg.de
Speck T.	Thomas	U. Freiburg	DE	thomas.speck@biologie.uni-freiburg.de
Sugimoto	Mimi	Nagoya U.	JP	mimi@agr.nagoya-u.ac.jp
Takahashi	Hideyuki	Tohoku U.	JP	hideyuki@ige.tohoku.ac.jp
Telewski	Frank	Michigan State U.	US	telewski@msu.edu
Terwagne	Denis	Fac. Sciences Brussels	BE	Denis.Terwagne@ulb.ac.be
Thibaut	Bernard	LMGC U. Montpellier	FR	bernard.thibaut@umontpellier.fr
Thielen	Marc	U. Freiburg	DE	marc.thielen@biologie.uni-freiburg.de
Tippner	Jan	Mendel U. Brno	CZ	jan.tippner@mendelu.cz
Toba	Keisuke	FFPRI Tsukuba	JP	tobak@ffpri.affrc.go.jp
Tokita	Katsuhiro	Nara I. Sci Tech	JP	
Tsuchiya	Kei	Bridgestone Corp.	JP	kei.tsuchiya@bridgestone.com
Tyree	Melvin	Northwest A&F U. Yangling Shaanxi	CN	mtyree@hotmail.com
Ueda	Haruko	Kyoto U.	JP	hueda@gr.bot.kyoto-u.ac.jp
Wada	Hirofumi	Kyoto U.	JP	hwada@fc.ritsumei.ac.jp
Wang	BC	Chongqing U.	CN	wangbc2000@126.com
Watanabe	Ugai	Chiba IT	JP	ugai.watanabe@p.chibakoudai.jp
Wilson	Johnathan	Haverford Coll.	US	jwilson@haverford.edu
Woolfenden	Hugh	John Innes C. Norwich	UK	Hugh.Woolfenden@jic.ac.uk
Yamamoto	Hiroyuki	Nagoya U.	JP	hiro@agr.nagoya-u.ac.jp
Yamashita	Kana	FFPRI Tsukuba	JP	zaikana@ffpri.affrc.go.jp
Yan	Jie	Chongqing U.	CN	yanjie185@sina.com, wangbc2000@126.com
Yamasaki	Mariko	Nagoya U.	JP	marikoy@agr.nagoya-u.ac.jp
Yoshida	Masato	Nagoya U.	JP	yoshida@agr.nagoya-u.ac.jp
Yoshinaga	Arata	Kyoto U.	JP	yoshinaga.arata.5a@kyoto-u.ac.jp