

JOURNAL OF PLANT PHYSIOLOGY

(Incorporating: **Biochemie und Physiologie der Pflanzen BPP**)

Editorial Board

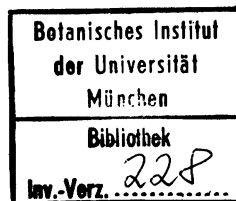
T. ap Rees, Cambridge · **Th. Boller**, Basel · **J. Carman**, Logan
F. Constabel, Saskatoon · **L. Erdei**, Szeged · **E. F. Elstner**, München
W. H. O. Ernst, Amsterdam · **C. V. Givan**, Durham, NH
P. M. Gresshoff, Knoxville · **W. Haupt**, Erlangen
D. Heß, Stuttgart-Hohenheim · **H. Kende**, East Lansing · **E. Komor**, Bayreuth
H. K. Lichtenthaler, Karlsruhe · **H. Lörz**, Hamburg · **U. Lüttge**, Darmstadt
A. Maretzki, Aiea/Hawaii · **H. Marschner**, Stuttgart-Hohenheim
Y. Masuda, Nara · **Ph. Matile**, Zürich · **R. Mendel**, Braunschweig
A. Oaks, Guelph, Ontario · **W. L. Ogren**, Urbana · **B. Parthier**, Halle
P. E. Pilet, Lausanne · **A. Pirson**, Göttingen · **L. H. W. van der Plas**, Wageningen
R. Ranjeva, Toulouse · **D. Robinson**, Göttingen · **H. Schnabl**, Bonn
P. Schopfer, Freiburg i. Br. · **H. R. Schütte**, Halle · **J. Stöckigt**, Mainz
G. H. N. Towers, Vancouver · **C. Wasternack**, Halle
E. W. Weiler, Bochum · **D. Werner**, Marburg · **H. Ziegler**, München

Editor-in-Chief

Martin Bopp, Heidelberg

Editors

K. Müntz, Gatersleben · **F. B. Salisbury**, Logan



Book-Reviews-Editor

K. H. Lichtenthaler, Karlsruhe

Volume 141



GUSTAV FISCHER VERLAG
Stuttgart · New York · 1993

Contents

All papers of this volume (141) including issue 1 have to be cited as 141. 1993.

MICROORGANISM-PLANT INTERACTIONS

- Bothe, H. 1 Symbiotic Interactions between Microorganisms and Plants
- Buscot, F. 12 Synthesis of two Types of Association between *Morchella esculenta* and *Picea abies* under Controlled Culture Conditions
- Danneberg, G., C. Latus, W. Zimmer, B. Hundeshagen, H. Schneider-Poetsch, and H. Bothe 33 Influence of Vesicular-arbuscular Mycorrhiza on Phytohormone Balances in Maize (*Zea mays* L.)
- Drüge, U. and F. Schönbeck 40 Effect of Vesicular-Arbuscular Mycorrhizal Infection on Transpiration, Photosynthesis and Growth of Flax (*Linum usitatissimum* L.) in Relation to Cytokinin Levels
- Horn, K., A. Hahn, F. Pausch, and B. Hock 28 Isolation of Pure Spore and Hyphal Fractions from Vesicular-Arbuscular Mycorrhizal Fungi
- Jording, D., P. K. Sharma, R. Schmidt, T. Engelke, C. Uhde, and A. Pühler 18 Regulatory Aspects of the C₄-Dicarboxylate Transport in *Rhizobium melliloti*: Transcriptional Activation and Dependence on Effective Symbiosis
- Kape, R., K. Wex, M. Parniske, E. Göрге, A. Wetzler, and D. Werner 54 Legume Root Metabolites and VA-Mycorrhiza Development
- Knaak, C., P. Roskothen, and G. Röbbelen 49 Symbiotic Efficiency of *Vicia faba* Genotypes after Field Inoculation with Different Strains of *Rhizobium leguminosarum* Preselected in Greenhouse Tests
- Reinhard, S., P. Martin, and H. Marschner 7 Interactions in the Tripartite Symbiosis of Pea (*Pisum sativum* L.), *Glomus* and *Rhizobium* under Non-limiting Phosphorus Supply
- Schellenbaum, L., S. Gianinazzi, and V. Gianinazzi-Pearson 2 Comparison of Acid Soluble Protein Synthesis in Roots of Endomycorrhizal Wild Type *Pisum sativum* and Corresponding Isogenic Mutants

A BIOCHEMISTRY AND METABOLISM

- Corpas, F. J., M. Gómez, J. A. Hernández, and L. A. del Río 160 Metabolism of Activated Oxygen in Peroxisomes from two *Pisum sativum* L. Cultivars with Different Sensitivity to Sodium Chloride
- Daza, M. C., L. M. Sandalio, M. Quijano-Rico, and L. A. del Río 521 Isoenzyme Pattern of Superoxide Dismutase in Coffee Leaves from Cultivars Susceptible and Resistant to the Rust *Hemileia Vastatrix*
- De Gara, L., C. Paciolla, R. Liso, A. Stefani, A. Blanco, and O. Arrigoni 405 Ascorbate Metabolism in Mature Pollen Grains of *Dasypyrum villosum* (L.) Borb. during Imbibition
- Golz, A. and H. K. Lichtenthaler 276 Isolation and Characterization of Acetyl-CoA Synthetase from Etiolated Radish Seedlings
- Gärtner, D. E. and H. Ulrich Seitz 269 Enzyme Activities in Cardenolide-Accumulating, Mixotrophic Shoot Cultures of *Digitalis purpurea* L.
- Harada, M., S. Takio, and S. Takami 527 Nitrogen-Assimilating Enzymes in Chlorophyllous Cells of the Liverwort, *Marchantia paleacea* var. *diptera*, Grown in the Dark
- Harloff, H. J. and K. Wegmann 513 Evidence for a Mannitol Cycle in *Orobanche ramosa* and *Orobanche crenata*
- Hendershot, K. L. and J. J. Volenec 129 Nitrogen Pools in Taproots of *Medicago sativa* L. After Defoliation
- Hendershot, K. L. and J. J. Volenec 68 Taproot Nitrogen Accumulation and Use in Overwintering Alfalfa (*Medicago sativa* L.)
- Konno, Y., T. Vedvick, L. Fitzmaurice, and T. E. Mirkov 385 Purification, Characterization, and Subcellular Localization of Soluble Invertase from Tomato Fruit
- Limami, A., L. Roux, J. Laville, and Y. Roux 263 Dynamics of Nitrogen Compounds in the Chicory (*Cichorium intybus* L.) Tuberised Tap Root during the Growing Season and Cold storage Period
- McClellan, D., L. Kott, W. Beversdorf, and B. E. Ellis 153 Glucosinolate Metabolism in Zygotic and Microspore-Derived Embryos of *Brassica napus* L.
- Ochoa-Alejo, N. and J. E. Gómez-Peralta 147 Activity of Enzymes Involved in Capsaicin Biosynthesis in Callus Tissue and Fruits of Chili Pepper (*Capsicum annum* L.)
- Quaas, A., M. Chiong, A. Roco, R. Bravo, D. Seelenfreund, and L. M. Pérez 393 Antibodies against Fungal Conidia and Antibiotics Inhibit Phenylalanine Ammonia-Lyase Activation in *Citrus*
- Reggiani, R., N. Aurisano, M. Mattana, and A. Bertani 136 Influence of K⁺ Ions on Polyamine Level in Wheat Seedlings
- Schmidt, B., C. Rivero, B. Thiede, and T. Schenk 641 Metabolism of 4-Nitrophenol in Soybean Excised Leaves and Cell Suspension Cultures of Soybean and Wheat
- Sowa, S., E. E. Roos, and W. S. Caughey 647 Effector Molecules to Probe Cytochrome c Oxidase Activity in Germinating *Phaseolus vulgaris* L. Seeds
- Vella, J. and L. Copeland 398 Phosphofructokinase from the Host Fraction of Soybean Nodules

- Vézina, L.-P., N. Lavoie, K. W. Joy, and H. A. Margolis
 Xu, Y. and R. B. van Huystee
 Yupsanis, T., P. Eleftheriou, A. Pantazaki, and J. G. Georgatsos
 Zheleva, D. I., V. S. Alexieva, and E. N. Karanov
- 61 The Fate of Newly Absorbed Ammonium and Nitrate Ions in Roots of Jack Pine Seedlings
 141 Association of Calcium and Calmodulin to Peroxidase Secretion and Activation
 257 Multiplicity of Metal-Independent Protein Phosphatases of Germinated Alfalfa Seeds
 281 Influence of Atrazine on free and Bound Polyamine Levels in Pea Leaves

B PHOTOSYNTHESIS, CARBOHYDRATES

- Bücker, J. and R. Guderian
 Choudhury, N. K., H. T. Choe, and R. C. Huffaker
 Diehl, S., U. Kull, and S. Diamantoglou
 Guidi, L., A. Panicucci, G. Lorenzini, and G. F. Soldatini
 Idso, S. B. and B. A. Kimball
 Ivanov, A. G. and M. I. Kicheva
 Kar, M., P. Streb, B. Hertwig, and J. Feierabend
 Mishra, R. K. and G. S. Singhal
 Nešpůrková, L., J. Lazarová, K. Janáček, and R. Rybová
- 654 Marked Increases in Raffinose in Leaves of Populus Due to Ambient Air Pollution
 551 Ascorbate Induced Zeaxanthin Formation in Wheat Leaves and Photoprotection of Pigment and Photochemical Activities During Aging of Chloroplasts in Light
 657 Incorporation of ¹⁴C-Photosynthate into Major Chemical Fractions of Leaves and Bark of *Ceratonia siliqua* L. at Different Seasons
 545 Ozone-Induced Changes in Chlorophyll Fluorescence Kinetics and CO₂ Assimilation in *Vicia faba*
 166 Effects of Atmospheric CO₂ Enrichment on Net Photosynthesis and Dark Respiration Rates of Three Australian Tree Species
 410 Chlorophyll Fluorescence Properties of Chloroplast Membranes Isolated from Jasmonic Acid-Treated Barley Seedlings
 538 Sensitivity to Photodamage Increases during Senescence in Excised Leaves
 286 Photosynthetic Activity and Peroxidation of Thylakoid Lipids during Photoinhibition and High Temperature Treatment of Isolated Wheat Chloroplasts
 533 Effects of Electron Acceptors on Anion Uptake in *Hydrodictyon reticulatum*

C CELL AND MOLECULAR BIOLOGY

- Altherr, S., S. Stirn, and H.-J. Jacobsen
 Aoshima, H., M. Yamada, N. Sauer, E. Komor, and C. Schobert
 Drory, A., S. Mayak, and W. R. Woodson
 Falk, J., A. Schmidt, and K. Krupinska
 Jung, G., B. Hahne, and W. Wernicke
 Korcz, A. and T. Twardowski
 Lopez-Gomez, R. and M. A. Gomez-Lim
 Schulz, M., D. Wolf, and H. Schnabl
 Spanu, P., T. Boller, and H. Kende
 Szopa, J., A. J. Backer, A. F. Sikorski, and A. Kozubek
 Szopa, J., J. Bode, V. Kay, A. Kozubek, and A. F. Sikorski
 Wolf, D., M. Schulz, and H. Schnabl
 Y. Gounaris
- 415 Immunobiochemical Analysis of a Nuclear Protein Marker for Regeneration Potential in Higher Plants
 293 Heterologous Expression of the H⁺/Hexose Cotransporter from *Chlorella* in *Xenopus* Oocytes and its Characterization with Respect to Sugar Specificity, pH and Membrane Potential
 663 Expression of Ethylene Biosynthetic Pathway mRNAs is Spatially Regulated within Carnation Flower Petals
 176 Characterization of Plastid DNA Transcription in Ribosome Deficient Plastids of Heat-bleached Barley Leaves
 428 Cell Cycle in Potentially Dedifferentiating Cereal Mesophyll Protoplasts Cultured *in vitro* II. Behaviour of the Cytoskeleton
 75 Lupin Ferritin: Purification and Characterization, Biosynthesis and Regulation of *in vitro* Synthesis in Plant System
 82 Changes in mRNA and Protein Synthesis during Ripening in Mango Fruit
 298 Age Dependent Appearance of Polypeptides with Immunoreactivity to Ubiquitin Antibodies in Chloroplast Membranes of *Vicia faba* L.
 557 Differential Accumulation of Transcripts of 1- Aminocyclopropane-1-Carboxylate Synthase Genes in Tomato Plants Infected with *Phytophthora infestans* and in Elicitor-Treated Tomato Cell Suspensions
 172 Does the Nuclear Matrix Endonuclease Show Specificity towards DNA Topoisomers?
 668 Does a Prototype Scaffold/Matrix-Attached Region (SAR Sequence) Affect Intrinsic Nuclear Matrix Endonuclease Specificity?
 304 Influence of Horizontal Clinostat Rotation on Plant Proteins: 1. Effects on Ubiquitinated Polypeptides in the Stroma and Thylakoid Membranes of *Vicia faba* L. Chloroplasts
 423 Comparison of Restriction Patterns of Mitochondrial DNA from Low and High Sugar Accumulating Cultivars/Selections

D MEMBRANES, UPTAKE, TRANSPORT

- Caffaro, S., S. Scaramagli, F. Antognoni, and N. Bagni
- 563 Polyamine Content and Translocation in Soybean Plants

IV

- Duarte, P. J. P. and C.-M. Larsson 182 Translocation of Nutrients in N-limited, Non-Nodulated Pea Plants
- Fromm, J. and W. Eschrich 673 Electric Signals Released from Roots of Willow (*Salix viminalis* L.) Change Transpiration and Photosynthesis
- Förster, J. C. and W. D. Jeschke 322 Effects of Potassium Withdrawal on Nitrate Transport and on the Contribution of the Root to Nitrate Reduction in the Whole Plant
- Harmens, H., G. C. P. B. Gusmão, N., P. R. Den Hartog, J. A. C. Verkleij, and W. H. O. Ernst 309 Uptake and Transport of Zinc in Zinc-sensitive and Zinc-tolerant *Silene vulgaris*
- Hoffmann-Thoma, G. and J. Willenbrink 681 Differential Sensitivities towards Inhibitors and SH-Compounds of H⁺-ATPase and H⁺-Pyrophosphatase Associated with Intact Vacuoles from Sweet Sorghum (*Sorghum bicolor* [L.] Moench) Stem Parenchyma
- Kelly, K. M. and J. Van Staden 436 A Comparison of Transport and Metabolism of [¹⁴C] Sucrose and [¹⁴C] Fructose in Summer and Winter in Guayule (*Parthenium argentatum* Gray)
- Marienfeld, S. and R. Stelzer 569 X-Ray Microanalyses in Roots of Al-Treated *Avena Sativa* Plants
- Mozafar, A. and J. J. Oertli 316 Vitamin C (Ascorbic acid): Uptake and Metabolism by Soybean
- Rensburg, L. van, G. H. J. Krüger and H. Krüger 188 Proline Accumulation as Drought-tolerance Selection Criterion: its Relationship to Membrane Integrity and Chloroplast Ultrastructure in *Nicotiana tabacum* L.

E GROWTH, DEVELOPMENT, DIFFERENTIATION

- Appenroth, K.-J. and R. Bergfeld 583 Photophysiology of Turion Germination in *Spirodela polyrhiza* (L.) Schleiden. XI. Structural Changes during Red Light Induced Responses
- Barthe, P. and M.-T. Le Page-Degivry 195 Maternal Abscisic Acid Transport in Developing Embryos of Sunflower
- Derkx, M. P. M. and C. M. Karssen 574 Variability in Light-, Gibberellin- and Nitrate Requirement of *Arabidopsis thaliana* Seeds due to Harvest Time and Conditions of Dry Storage
- Donkin, M. E., D. N. Price, and A. Watson 347 Optical Properties of the Pod Wall of the Pea (*Pisum sativum* L.) II. Varietal Differences and Species Comparisons
- Fischer, C., H. Lüthen, M. Böttger, and R. Hertel 88 Initial Transient Growth Inhibition in Maize Coleoptiles Following Auxin Application
- Grill, R. and H. Schraudolf 457 Phytochrome-Mediated Mitotic Activity and Induction of two-Dimensional Growth in Gametophytes of *Anemia phyllitidis* (L.) Sw.
- Haddad, C. R. B. and I. F. M. Valio 704 Effect of fire on flowering of *Lantana montevidensis* Briq.
- Hammatt, N. and N. J. Grant 341 Apparent Rejuvenation of Mature Wild Cherry (*Prunus avium* L.) during Micropropagation
- Horton, R. F. 690 Peroxidase, Ethylene, and Submergence-Promoted Growth of Petioles of *Ranunculus sceleratus* L.
- Jung, G. and W. Wernicke 444 Cell Cycle in Potentially Dedifferentiating Cereal Mesophyll Protoplasts Cultured *in vitro*. I. Abnormalities in Cycle Kinetics
- Kuriyama, A., F. Kawai, M. Kanamori, and W. Dathe 694 Inhibitory Effect of Jasmonic Acid on Gametophytic Growth, Initiation and Development of Sporophytic Shoots in *Equisetum arvense*
- Minkov, I. N., C. Sundqvist, and M. Ryberg 708 Protochlorophyllide and Chlorophyllide in Reformed Prolamellar Bodies and Thylakoids of Irradiated Dark-grown Wheat (*Triticum aestivum* L.)
- Mohammadi, M. and A. L. Karr 206 The use of Soybean Plantlets to Study Root Nodule Senescence
- Oosterhaven, K., K. J. Hartmans, and H. J. Huizing 463 Inhibition of Potato (*Solanum tuberosum*) Sprout Growth by the Monoterpene S-Carvone: Reduction of 3-Hydroxy-3- Methylglutaryl Coenzyme A Reductase Activity without Effect on its mRNA Level
- Pressman, E., R. Shaked, and L. Arcan 210 The Effect of Flower-Inducing Factors on Leaf Tipburn Formation in Chinese Cabbage
- Radermacher, E. and D. Klämbt 698 Auxin Dependent Growth and Auxin-Binding Proteins in Primary Roots and Root Hairs of Corn (*Zea mays* L.)
- Santos, I., J. M. Almeida, and R. Salema 450 Plants of *Zea mays* L. Developed under Enhanced UV-B Radiation. I. Some Ultrastructural and Biochemical Aspects
- Swaraj, K., J. S. Laura, and N. R. Bishnoi 202 Nitrate Induced Nodule Senescence and Changes in Activities of Enzymes Scavenging H₂O₂ in Clusterbean (*Cyamopsis tetragonaloba* Taub.)
- Villanueva, V. R. and H. Huang 336 Effect of Polyamine Inhibition on Pea Seed Germination
- Werner, O. and M. Bopp 93 The Influence of ABA and IAA on *in vivo* Phosphorylation of Proteins in *Funaria hygrometrica* Hedw.
- Woltering, E. J., D. Somhorst, and C. A. de Beer 329 Roles of Ethylene Production and Sensitivity in Senescence of Carnation Flower (*Dianthus caryophyllus*) Cultivars White Sim, Chinera and Epomeo
- Yang, S., G. Xiang, S. Zhang, and C. Lou 98 Electrical Resistance as a Measure of Graft Union

F CELL AND TISSUE CULTURE

- | | | |
|--|-----|---|
| Berglund, T., A. B. Ohlsson, and J. Rydström | 596 | Nicotinamide Increases Glutathione and Anthocyanin in Tissue Culture of <i>Catharanthus roseus</i> |
| Chupeau, M.-C., M. Lemoine, and Y. Chupeau | 601 | Requirement of Thidiazuron for Healthy Protoplast Development to Efficient Tree Regeneration of a Hybrid Poplar (<i>Populus tremula</i> × <i>p. alba</i>) |
| Egertsdotter, U. and S. von Arnold | 222 | Classification of Embryogenic Cell-lines of <i>Picea abies</i> as Regards Protoplast Isolation and Culture |
| Ghosh Biswas, G. C. and F. J. Zapata | 470 | High-Frequency Plant Regeneration from Protoplasts of Indica Rice (<i>Oryza sativa</i> L.) Using Maltose |
| Hadrami, I. El, F. Housti, N. Michaux-Ferrière, M. P. Carron, and J. d'Auzac | 230 | Effects of Gelling Agents and Liquid Medium on Embryogenic Potential, Polyamines and Enzymatic Factors in Browning in <i>Hevea brasiliensis</i> Calli |
| Koetje, D. S., H. Kononowicz, and T. K. Hodges | 215 | Polyamine Metabolism Associated with Growth and Embryogenic Potential of Rice |
| M. C. Liu | 714 | Factors Affecting Induction, Somatic Embryogenesis and Plant Regeneration of Callus from Cultured Immature Inflorescences of Sugarcane |
| Marassi, M. A., O. A. Bovo, G. L. Lavia, and L. A. Mroginski | 610 | Regeneration of Rice Double Haploids Using a one Step Culture Procedure |
| Martinelli, L., A. Scienza, P. Villa, P. De Ponti, and E. Gianazza | 476 | Enzyme Markers for Somatic Embryogenesis in <i>Vitis</i> |
| Millam, S. and D. Davidson | 353 | Evidence for an Interactive Response of Flax (<i>Linum usitatissimum</i>) Hypocotyl Tissue Cultures to Auxin and Carbohydrates |
| Nakano, M. and M. Mii | 721 | Antibiotics Stimulate Somatic Embryogenesis without Plant Growth Regulators in Several <i>Dianthus</i> Cultivars |
| Nielsen, K. Annette and E. Knudsen | 589 | Regeneration of Green Plants from Embryogenic Suspension Cultures of Kentucky Blue Grass (<i>Poa pratensis</i> L.) |
| Palmer, C. E. and M. Oelck | 105 | The Relationship of Phosphinothricin to Growth and Metabolism in Cell Cultures of <i>Brassica napus</i> L. |
| Wang, X.-H., P. A. Lazzeri, and H. Lörz | 726 | Regeneration of Haploid, Dihaploid and Diploid Plants from Anther- and Embryo-Derived Cell Suspensions of Wild barley (<i>Hordeum murinum</i> L.) |
| Watanabe, M., Y. Watanabe, and N. Shimada | 111 | Colorimetric Estimation of Leaf-Protoplast Potential-to-Divide by Use of 2,3,5-Triphenyl Tetrazolium Chloride |

G STRESS, TOLERANCE AND ECOPHYSIOLOGY

- | | | |
|---|-----|---|
| Collier, D. E. and W. R. Cummins | 745 | Sensitivity of the Cytochrome and Alternative Pathways to Osmotic Stress in Leaf-slices of <i>Saxifraga cernua</i> L. |
| Donatz, M. and B. M. Eller | 750 | Plant Water Status and Water Translocation in the Drought Deciduous CAM-Succulent <i>Senecio medley-woodii</i> |
| Gulati, A. and P. K. Jaiwal | 120 | Comparative Salt Responses of Callus Cultures of <i>Vigna radiata</i> (L.) Wilczek to Various Osmotic and Ionic Stresses |
| Hariyadi, P. and K. L. Parkin | 733 | Chilling-induced Oxidative Stress in Cucumber (<i>Cucumis sativus</i> L. cv. Calypso) Seedlings |
| Johnson-Flanagan, A. M. and J. Singh | 487 | A Method to Study Seed Degreening Using Haploid Embryos of <i>Brassica napus</i> cv. Topas |
| Lu, G., C. Li, and Z. Lu | 115 | Wound-induced Respiration in Thin Slice of Chinese Jujube Fruit |
| Manderscheid, R. and H.-J. Jäger | 494 | Seasonal Changes in Nitrogen Metabolism of Spruce Needles (<i>Picea abies</i> (L.) Karst.) as Affected by Water Stress and Ambient Air Pollutants |
| Rensburg, L. van and G. H. J. Krüger | 357 | Differential Inhibition of Photosynthesis (<i>in vivo</i> and <i>in vitro</i>), and Changes in Chlorophyll a Fluorescence Induction Kinetics of four Tobacco Cultivars under Drought Stress |
| Rütten, D. and K. A. Santarius | 739 | Osmotic Potentials of Water-Saturated Mosses |
| Sudhakar, C., P. S. Reddy, and K. Veeranjanyulu | 621 | Effect of Salt Stress on the Enzymes of Proline Synthesis and Oxidation in Greengram (<i>Phaseolus aureus</i> Roxb.) Seedlings |
| Tischler, C. R., B. L. Burson, and W. R. Jordan | 482 | Physiological Variation in Tissue Culture Regenerated Apomictic <i>Paspalum dilatatum</i> |
| Walker, M. A. and B. D. McKersie | 234 | Role of the Ascorbate-Glutathione Antioxidant System in Chilling Resistance of Tomato |
| Wen, C.-S., M.-J. Tseng, and C.-H. Lin | 240 | Effect of LAB 173711, an ABA Analogue, on Low Temperature Resistance of Suspension Cultured Sugarcane Cells |
| Wessler, A. and A. Wild | 615 | Investigations on the Content of Indole-3-acetic Acid in Spruce Needles of Healthy and Damaged Trees of Various Sites |

SHORT COMMUNICATIONS

- Atzmon, N. and J. van Staden
Baldy, P.
Bannister, A., A. J. Maule, and S. N. Covey
Baset, A., E. C. Cocking, and R. P. Finch
Clauss, M., A. Motel, and H. K. Lichtenthaler
Davidonis, G.
Doorn, W. G. van, C. Pak, and
C. J. J. Buddendorf
Fauconnier, M.-L., M. Jaziri, M. Marlier,
J. Roggemans, J.-P. Wathelet, G. Lognay,
M. Severin, J. Homes, and K. Shimomura
Kutschera, U. and K. Köhler
Lefrançois, C. and Y. Chupeau
Martin, C. E. and F. S. Harris
Park, H.-Y. and W. Wernicke
Reddy, K. R. K., A. H. Rao, B. Karuna Sree, and
A. R. Reddy
Sauter, J. J. and B. van Cleve
Staden, J. van and F. E. Drewes
T. Górski
Taylor, M. A., L. R. Burch, and H. V. Davies
Yang, C., A. Wessler, and A. Wild
- 366 The Effect of Zeatin and *iso*-Pentenyladenine on Root Growth of *Pinus pinea* L.
633 Corn Phosphoglycolate Phosphatase: Immunodetection in Kranz Cells
502 Cauliflower Mosaic Virus Particles Alter the Sensitivity of *Arabidopsis thaliana* Seedlings to 2,4-D
245 Regeneration of Fertile Plants from Protoplasts of the Wild Rice Species *Oryza granulata*
508 Studies on the Plant Methylcrotonyl-CoA Carboxylase
505 A Comparison of Cotton Ovule and Cotton Cell Suspension Cultures: Response to Gibberellic Acid and 2-Chloroethylphosphonic Acid
251 Effects of Surfactants on the Vascular Occlusion Induced by Exposure to Air in Cut Flowering Stems of Astilbe, Bouvardia, and Rose
759 Essential Oil Production by *Anthemis nobilis* L. Tissue Culture
757 Turgor Pressure and Elongation Growth in Developing Sunflower Hypocotyls
629 Standard Conditions for Plant Regeneration from Leaf Protoplasts of Several *Lycopersicon* Species
762 Nocturnal Respiration Rates and Malic Acid Accumulation in Five Species of *Talinum* (Portulacaceae) during CAM- Cycling
376 Improved Mesophyll Protoplast Culture and Plant Regeneration in *Arabidopsis thaliana* (L.) Heynh., Genotype Landsberg Erecta
373 Water Stress-induced 23 kDa Polypeptide in Cell Suspension Cultures of Rice (*Oryza sativa* L.) is Immunologically similar to that of Seedlings
248 Occurrence of a Maltose Pool and of Maltase in Poplar Wood (*Populus × canadensis robusta*) during Fall
380 Incorporation of [14 C]Adenine into Cytokinins in Pea Fruits
627 Improvement of Lettuce Seedling Vigour after Far Red Irradiation of Aged Achenes
370 Changes in Polyamine Biosynthesis during the Initial Stages of Tuberisation in Potato (*Solanum tuberosum* L.)
624 Studies on the Diurnal Courses of the Contents of Abscisic Acid, 1-Aminocyclopropane Carboxylic Acid and Its Malonyl Conjugate in Needles of Damaged and Undamaged Spruce Trees

BOOK REVIEWS

125, 254, 383, 512, 636, 765

Auteurs Index

- Alexieva, V. S., 281
Almeida, J. M., 450
Altherr, S., 415
Antognoni, F., 563
Aoshima, H., 293
Appenroth, K.-J., 583
Arcan, L., 210
Arrigoni, O., 405
Atzmon, N., 366
Aurisano, N., 136
- Backer, A. J., 172
Bagni, N., 563
Baldy, P., 633
Bannister, A., 502
Barthe, P., 195
Baset, A., 245
Bergfeld, R., 583
Berglund, T., 596
Bertani, A., 136
Beverdors, W., 153
Bishnoi, N. R., 202
Blanco, A., 405
Bode, J., 668
Böttger, M., 88
Boller, T., 557
Bopp, M., 93
Bothe, H., 1, 33
Bovo, O. A., 610
Bravo, R., 393
Buddendorf, C. J. J., 251
Bücker, J., 654
Burch, L. R., 370
Burson, B. L., 482
Buscot, F., 12
- Caffaro, S., 563
Carron, M. P., 230
Caughey, W. S., 647
Chiong, M., 393
Choe, H. T., 551
Choudhury, N. K., 551
Chupeau, M.-C., 601
Chupeau, Y., 601, 629
Clauss, M., 508
Cocking, E. C., 245
Collier, D. E., 745
Copeland, L., 398
Corpas, F. J., 160
Covey, S. N., 502
Cummins, W. R., 745
- Danneberg, G., 33
Dathe, W., 694
d'Auzac, J., 230
Davidonis, G., 505
Davidson, D., 353
Davies, H. V., 370
Daza, M. C., 521
de Beer, C. A., 329
De Gara, L., 405
- De Ponti, P., 476
Derkx, M. P. M., 574
Diamantoglou, S., 657
Diehl, S., 657
Donatz, M., 750
Donkin, M. E., 347
Doorn, W. G. van, 251
Drewes, F. E., 380
Drory, A., 663
Drüge, U., 40
Duarte, P. J. P., 182
- Egertsdotter, U., 222
Eleftheriou, P., 257
Eller, B. M., 750
Ellis, B. E., 153
Engelke, T., 18
Ernst, W. H. O., 309
Eschrich, W., 673
- Falk, J., 176
Fauconnier, M.-L., 759
Feierabend, J., 538
Finch, R. P., 245
Fischer, C., 88
Fitzmaurice, L., 385
Förster, J. C., 322
Fromm, J., 673
- Gärtner, D. E., 269
Georgatsos, J. G., 257
Ghosh Biswas, G. C., 470
Gianazza, E., 476
Gianinazzi, S., 2
Gianinazzi-Pearson, V., 2
Görge, E., 54
Golz, A., 276
Gómez, M., 160
Gomez-Lim, M. A., 82
Gómez-Peralta, J. E., 147
Górski, T., 627
Gounaris, Y., 423
Grant, N. J., 341
Grill, R., 457
Guderian, R., 654
Guidi, L., 545
Gulati, A., 120
Gusmão, G. C. P. B., 309
- Haddad, C. R. B., 704
Hadrami, I. El, 230
Hahn, A., 28
Hahne, B., 428
Hammatt, N., 341
Harada, M., 527
Hariyadi, P., 733
Harloff, H. J., 513
Harmens, H., 309
Harris, F. S., 762
Hartmans, K. J., 463
Hartog, P. R. D., 309
- Hendershot, K. L., 68, 129
Hernández, J. A., 160
Hertel, R., 88
Hertwig, B., 538
Hock, B., 28
Hodges, T. K., 215
Hoffmann-Thoma, G., 681
Homes, J., 759
Horn, K., 28
Horton, R. F., 690
Housti, F., 230
Huang, H., 336
Huffaker, R. C., 551
Huizing, H. J., 463
Hundeshagen, B., 33
- Idso, S. B., 166
Ivanov, A. G., 410
- Jacobsen, H.-J., 415
Jäger, H.-J., 494
Jaiwal, P. K., 120
Janáček, K., 533
Jaziri, M., 759
Jeschke, W. D., 322
Johnson-Flanagan, A. M., 487
Jordan, W. R., 482
Jording, D., 18
Joy, K. W., 61
Jung, G., 428, 444
- Kanamori, M., 694
Kape, R., 54
Kar, M., 538
Karanov, E. N., 281
Karr, A. L., 206
Karszen, C. M., 574
Kawai, F., 694
Kay, V., 668
Kelly, K. M., 436
Kende, H., 557
Kicheva, M. I., 410
Kimball, B. A., 166
Klämbt, D., 698
Knaak, C., 49
Knudsen, E., 589
Köhler, K., 757
Koetje, D. S., 215
Komor, E., 293
Konno, Y., 385
Kononowicz, H., 215
Korc, A., 75
Kott, L., 153
Kozubek, A., 172, 668
Krüger, G. H. J., 188, 357
Krüger, H., 188
Krupinska, K., 176
Kull, U., 657
Kuriyama, A., 694
Kutschera, U., 757
- Larsson, C.-M., 182
Latus, C., 33
Laura, J. S., 202
Lavia, G. L., 610
Laville, J., 263
Lavoie, N., 61
Lazarová, J., 533
Lazzeri, P. A., 726
Le Page-Degivry, M.-T., 195
Lefrançois, C., 629
Lemoine, M., 601
Li, C., 115
Lichtenthaler, H. K., 276, 508
Limami, A., 263
Lin, C.-H., 240
Liso, R., 405
Liu, M. C., 714
Lörz, H., 726
Lognay, G., 759
Lopez-Gomez, R., 82
Lorenzini, G., 545
Lou, C., 98
Lu, G., 115
Lu, Z., 115
Lüthen, H., 88
- Manderscheid, R., 494
Marassi, M. A., 610
Margolis, H. A., 61
Marienfeld, S., 569
Marlier, M., 759
Marschner, H., 7
Martin, C. E., 762
Martin, P., 7
Martinelli, L., 476
Mattana, M., 136
Maule, A. J., 502
Mayak, S., 663
McClellan, D., 153
McKersie, B. D., 234
Michaux-Ferrière, N., 230
Mii, M., 721
Millam, S., 353
Minkov, I. N., 708
Mirkov, T. E., 385
Mishra, R. K., 286
Mohammadi, M., 206
Motel, A., 508
Mozafar, A., 316
Mroginski, L. A., 610
- Nakano, M., 721
Nešpůrková, L., 533
Nielsen, K. Annette, 589
- Ochoa-Alejo, N., 147
Oelck, M., 105
Oertli, J. J., 316
Ohlsson, A. B., 596
Oosterhaven, K., 463

- Paciolla, C., 405
Pak, C., 251
Palmer, C. E., 105
Panicucci, A., 545
Pantazaki, A., 257
Park, H.-Y., 376
Parkin, K. L., 733
Parniske, M., 54
Pausch, F., 28
Pérez, L. M., 393
Pressman, E., 210
Price, D. N., 347
Pühler, A., 18
- Quaas, A., 393
Quijano-Rico, M., 521
- Radermacher, E., 698
Rao, A. H., 373
Reddy, A. R., 373
Reddy, K. R. K., 373
Reddy, P. S., 621
Reggiani, R., 136
Reinhard, S., 7
Rensburg, L. van, 188, 357
Río, L. A. del, 160, 521
Rivero, C., 641
Roco, A., 393
Röbbelen, G., 49
Roggemans, J., 759
Roos, E. E., 647
Roskothen, P., 49
Roux, L., 263
Roux, Y., 263
Rütten, D., 739
- Ryberg, M., 708
Rybová, R., 533
Rydström, J., 596
- Salema, R., 450
Sandalio, L. M., 521
Santarius, K. A., 739
Santos, I., 450
Sauer, N., 293
Sauter, J. J., 248
Scaramagli, S., 563
Schellenbaum, L., 2
Schenk, T., 641
Schmidt, A., 176
Schmidt, B., 641
Schmidt, R., 18
Schnabl, H., 298, 304
Schneider-Poetsch, H., 33
Schobert, C., 293
Schraudolf, H., 457
Schulz, M., 298, 304
Schönbeck, F., 40
Scienza, A., 476
Seelenfreund, D., 393
Seitz, H. U., 269
Severin, M., 759
Shaked, R., 210
Sharma, P. K., 18
Shimada, N., 111
Shimomura, K., 759
Sikorski, A. F., 172, 668
Singh, J., 487
Singhal, G. S., 286
Soldatini, G. F., 545
Somhorst, D., 329
- Sowa, S., 647
Spanu, P., 557
Sree, B. K., 373
Stefani, A., 405
Stelzer, R., 569
Stirn, S., 415
Streb, P., 538
Sudhakar, C., 621
Sundqvist, C., 708
Swaraj, K., 202
Szopa, J., 172, 668
- Takami, S., 527
Takio, S., 527
Taylor, M. A., 370
Thiede, B., 641
Tischler, C. R., 482
Tseng, M.-J., 240
Twardowski, T., 75
- Uhde, C., 18
- Valio, I. F. M., 704
van Cleve, B., 248
van Huystee, R. B., 141
van Staden, J., 366, 380, 436
Vedvick, T., 385
Veeranjaneyulu, K., 621
Vella, J., 398
Verkleij, J. A. C., 309
Vézina, L.-P., 61
Villa, P., 476
Villanueva, V. R., 336
Volenec, J. J., 68, 129
von Arnold, S., 222
- Walker, M. A., 234
Wang, X.-H., 726
Watanabe, M., 111
Watanabe, Y., 111
Wathelet, J.-P., 759
Watson, A., 347
Wegmann, K., 513
Wen, C.-S., 240
Werner, D., 54
Werner, O., 93
Wernicke, W., 376, 428, 444
Wessler, A., 615
Wetzel, A., 54
Wex, K., 54
Wild, A., 615, 624
Willenbrink, J., 681
Wolf, D., 298, 304
Woltering, E. J., 329
Woodson, W. R., 663
- Xiang, G., 98
Xu, Y., 141
- Yamada, M., 293
Yang, C., 624
Yang, S., 98
Yupsanis, T., 257
- Zapata, F. J., 470
Zhang, S., 98
Zheleva, D. I., 281
Zimmer, W., 33

The articles published in this journal are protected by copyright. All rights are reserved, especially the right to translate into foreign languages. No part of the journal may be reproduced in any form – through photocopying, microfilming or other processes – or converted to a machine language, especially for data processing equipment – without the written permission of the publisher. The rights of reproduction by lecture, radio and television transmission, magnetic sound recording or similar means are also reserved.

Legume Root Metabolites and VA-Mycorrhiza Development

RÜDIGER KAPE, KERSTIN WEX, MARTIN PARNISKE, ELISABETH GÖRGE, ASTRID WETZEL,
and DIETRICH WERNER

Fachbereich Biologie, Botanisches Institut der Philipps-Universität Marburg, Karl-von-Frisch-Straße, D-3550 Marburg,
Germany

Received April 21, 1992 · Accepted August 25, 1992

Summary

The communication by phenylpropane-metabolites between symbiotic microorganisms and their legume host plants was further studied by the elucidation of root segment-specific exudation of aromatic compounds. The excreted flavonoids were collected by blotting seedling roots directly onto cellulose acetate filters. The flavonoids were eluted from filter segments and subsequently analysed by capillary electrophoresis.

Effects of various flavonoids on VA-mycorrhiza (VAM) development were studied. *Glomus mosseae* and *Glomus intraradix* (Schenck and Perez, 1990) were used in these studies. Daidzein (2 to 5 μM) increased germination of spores in a period between 15 to 30 days. Myricetin increased hyphal growth of germinated spores at a concentration of 2 μM between 20 and 50 days. Quercetin had a similar effect in a concentration range of 0.8 to 2.0 μM , whereas 5 μM slightly inhibited the growth of hyphae from the germinating spores.

Phytoalexin production in VA-mycorrhiza infected legume roots was studied with *Vicia faba* by wyerone concentration to quantify the reaction of a non-aromatic secondary root metabolite. VAM infected roots increased wyerone concentration by a factor of 3 to 5 compared with the control, however, at a very low absolute level.

A phenylpropane-communication concept for *Rhizobium* infected legumes is discussed in relation to a still incomplete similar scheme for VAM.

Key words: Flavonoids, wyerone, root development, VA-mycorrhiza.

Abbreviations: MES = 2-(N-morpholino)ethane sulfonic acid; MGD = 6''-O-malonyl-7-O- β -D-glucosyl daidzein; VAM = vesicular-arbuscular mycorrhiza.

Introduction

On the basis of chemotaxis, *nod* gene inducing activity (Kape et al., 1991), phytoalexin production (Parniske et al., 1991 a; Schmidt et al., 1992) and isoflavonoid inducible resistance in rhizobia (Parniske et al., 1991 b), a new communication concept for the *Bradyrhizobium*-legume symbiosis was proposed. The basic of this concept is that metabolites of the phenylpropane pathway have very different functions: precursors such as cinnamic acid and p-coumaric acid have

mainly a chemotactic effect, whereas intermediates such as isoliquiritigenin and daidzein have *nod* gene inducing activity as well as glyceollin resistance inducing activity. Endo-products such as glycinol and glyceollin still have this function, but are not so efficient (Kape et al., 1992).

In the present publication our aim was to contribute to a further development of the concept in two directions: the identification of root segment-specific aromatic compounds with signalling functions and the study of the effect of some of the identified compounds on the first stages of VA-my-

corrhiza development (spore germination and hyphal growth from spores). Some data on VA-mycorrhiza development in the legume species used are also included.

Materials and Methods

Production of soybean seed and root exudate

The production of soybean (*Glycine max* (L.) Merr. cv. Maple Arrow) root exudate is described by Kape et al. (1992). It made use of the ability of flavonoids to absorb to cellulose acetate filters (Re-court et al., 1991).

Soybean (cv. Maple Arrow) seed exudate was produced with a similar procedure. Seeds were sterilized as described in Kape et al. (1992). The seeds were subsequently placed on a sterile cellulose acetate filter and imbibed for 24 h in 2-(N-morpholino)ethane sulfonic acid buffer (5 mM, pH 6.2) plus 1 mM CaCl_2 (MES- CaCl_2) on the filter. The flavonoids were extracted from the filter and analysed by HPLC as recently described (Kape et al., 1992).

Analysis of local differences in the exudation of flavonoids along the root of soybean seedlings

Soybean seeds (cv. Preston) were surface sterilized for 10 min in 30% hydrogen peroxide. After sterilization, seeds were washed thoroughly and imbibed for 2.5 h in sterile tap water. Afterwards, seeds were placed on agar (1.5%) prepared with MES- CaCl_2 . Seedlings were grown in a growth cabinet (25 °C; relative humidity, 75%, 16–8 h light-dark period; irradiance 13 W m^{-2} , white light).

The roots of 4-day-old seedlings were placed between two cellulose acetate filters moistened with MES- CaCl_2 . Layers of moist tissue papers were used to wrap the seedlings plus the filters. The moist tissue papers assured close contact between the seedling root and the filters, and prevented desiccation of the seedlings. After 1 hour the seedlings were removed and the filters dried at room temperature.

Both filters originating from one seedling root were cut into sections of 4 mm. Flavonoids were extracted with methanol from the corresponding filter sections belonging to identical segments of a root.

The methanolic solution was dried in a stream of nitrogen at room temperature. The residue was dissolved in 100 μL electrophoresis buffer (50 mM boric acid, 10 mM sodium tetraborate, pH 8.5). The samples were analysed with capillary zone electrophoresis (Dionex CES 1, Sunnyvale, California/USA) supplied with a fused silica column (inside diameter 75 μm , length 60 cm); 45 nL of the sample was loaded into the column. The separation was performed at a voltage of 20,000 V with positive polarity for the sample. The electrophoresis buffer used for flavonoid separation was adjusted to pH 9. Compounds were detected with a variable-wavelength detector at 250 nm.

Flavonoids were identified by their retention time and by coelectrophoresis with authentic standards. Daidzein and genistein were purchased from Serva (Heidelberg, Germany), coumestrol from Kodak-Eastman (Rochester, USA).

VA-mycorrhiza-methods

Isolation of spores: Spores were isolated by wet-sieving according to Gerdemann and Nicolson (1963). The isolated spores were sterilized (modified from Mosse, 1959) in the following solution: 2% (w/v) chloramine T, 0.02% (w/v) streptomycin sulphate and 0.05% (w/v) tween 40. For sterilization, the spores were placed on a paper filter and a permanent flow of sterilizing solution through the

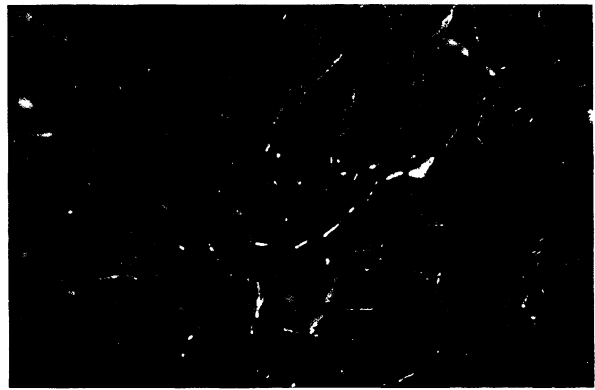


Fig. 1: Fluorescence photograph of soybean root exuded compounds absorbed to a cellulose acetate filter in UV light (254 nm). The fluorescent compounds were exuded by seedling roots of *Glycine max* cv. Maple Arrow growing on the surface of the filter. The arrow points at a side root emerging from the main root. The origin of the side root is characterized by increased fluorescence.

filter was applied for 20 min. Spores were then carefully rinsed several times with sterile tap water.

Axenic spore germination and hyphal growth from spores were tested on buffered water agar (MES 10 mM, 8.76 mM NaOH, 11.24 mM NaCl, pH 7.0).

To discriminate living from dead VA-mycorrhiza spores equal volumes of a spore suspension and a 0.05% (w/v) nitroblue tetrazolium solution were mixed at room temperature and incubated for 40 h. Living spores produced a brilliant red colour, whereas dead spores were uncoloured or became blue after prolonged incubation periods.

Other methods

Cultivation and origin of *Rhizobium*, *Bradyrhizobium* and host plants (*Phaseolus vulgaris*, *Glycine max*, *Vicia faba*) were as previously described (Wolff et al., 1991; Buttery et al., 1990; Kinnback and Werner, 1991). For VA-mycorrhiza infection of legume host plants, the following preparations were used: 1. *Glomus intraradix* infected roots of *Allium schoenoprasum*, cut into 1 cm pieces, and 2. *Glomus mosseae* in a soil inoculum produced with *Helianthus annuus* or *Allium* sp.

Both inocula were placed together with the plant seed into the growth substrate.

Wyerone (phytoalexin of faba beans) was isolated and analysed as described by Görges and Werner (1991).

Results and Discussion

Spatial pattern of flavonoid root exudation

Figure 1 visualizes the exudation of fluorescent phenolic compounds by roots of soybean (cv. Maple Arrow). The roots growing on the cellulose acetate filters leave traces of fluorescent phenolic compounds behind (e.g. flavonoids). Differences in the intensity of fluorescence in Fig. 1 may reflect some differences in the amounts and composition of the exuded compounds, although no quantitative interpretation is possible. Quantitative studies ascertain a close contact between the root and the cellulose acetate filter. Such a close

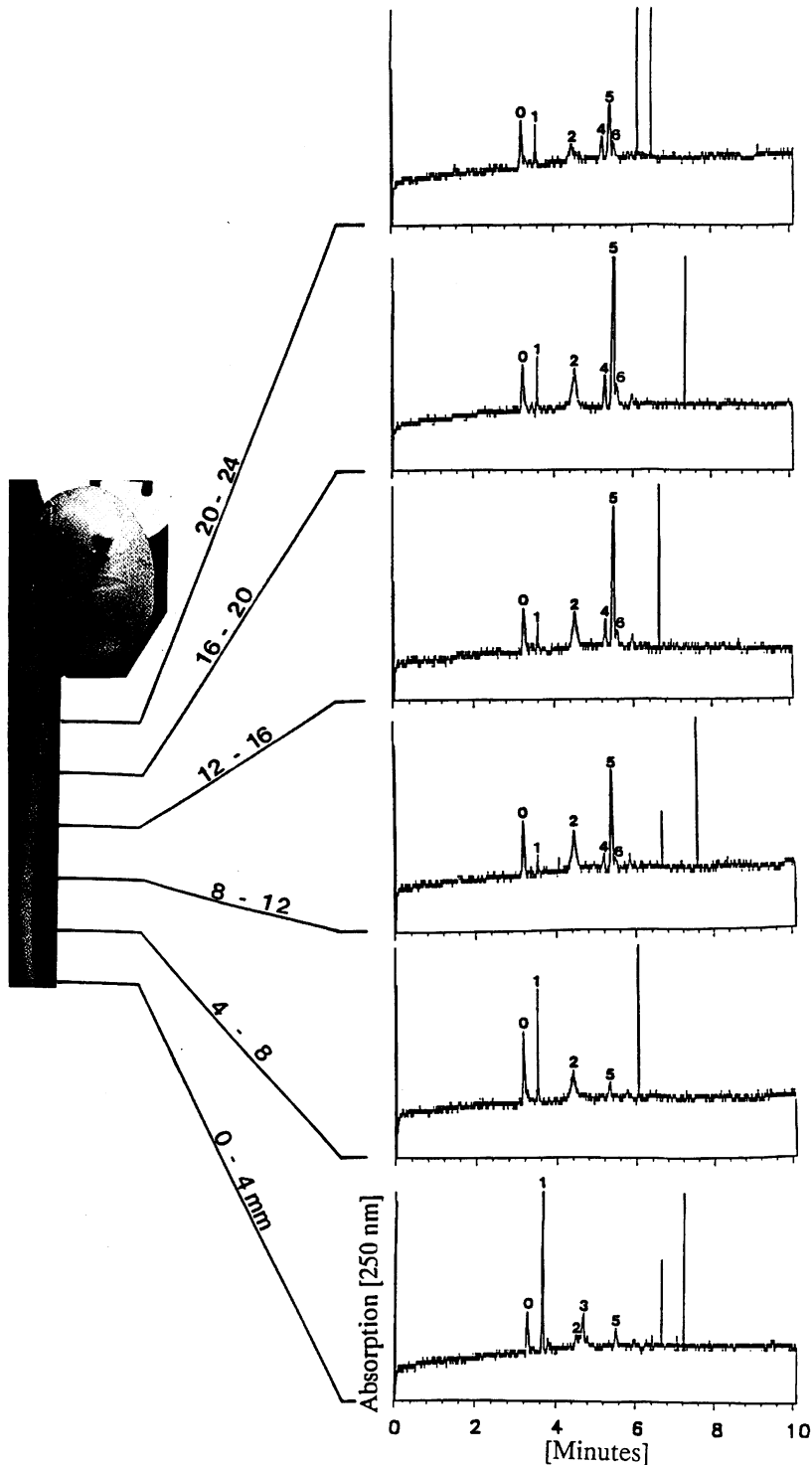


Fig. 2: Local differences in the root exudation of soybean seedlings. A 4-day-old seedling of *Glycine max* (cv. Preston) was used. The root exudates of individual segments (4 mm long) of this seedling were collected and analysed by capillary zone electrophoresis. The electropherograms of these exuded fractions are shown on the right side of the figure. UV absorbing compounds were detected at 250 nm. Peaks with identical retention times are labeled with the same numbers. Peak 5 was identified as daidzein and peak 6 as genistein (Peak O is an unknown contamination present in all samples).

contact was guaranteed in the analysis of the local root exudation. This quantitative analysis of flavonoids exuded by different soybean (cv. Preston) root areas is depicted in Fig. 2. The flavonoids exuded by different areas were analysed by capillary electrophoresis. The ratio of the five major com-

pounds separated differs significantly in the root hair zone, the elongation zone and the root tip. Peak 5 was identified as daidzein, peak 6 as genistein. Especially the ratio of peak 1 (not identified) to daidzein (peak 5) changed to a large extent. Graham (1991) also found root segment specific flavonoid

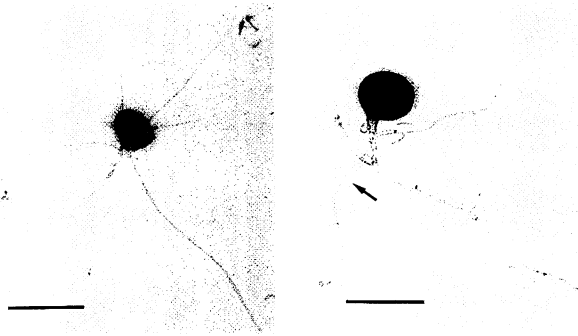


Fig. 3: Axenically germinated spores of *Glomus intraradix* (a) and *Glomus mosseae* (b) on water agar (bar, 125 μ m). Only *G. mosseae*, but not *G. intraradix*, produces small vesicles (arrow).

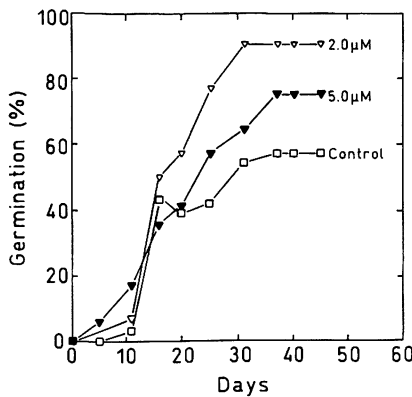


Fig. 4: Spore germination of *Glomus mosseae* on water agar with different concentrations of daidzein.

expression in soybean with another method of isolation of the root exudate. He found high concentrations of daidzein at the root tip and only small concentrations of MGD (6''-O-malonyl-7-O- β -D-glucosyldaidzein). Graham collected exudates with the help of cotton wicks. By heating the wick immediately after sampling, the ratio of daidzein to MGD changed significantly. This difference was explained by heat inactivation of β -glucosidase in the root exudate. In our method using a cellulose acetate filter, which was immediately dried after contact with the seedling and extracted by methanol, a possibly present β -glucosidase is unlikely to become active.

Differences between seed and root exudates

Differences in the flavonoid compositions of seed and root exudates of alfalfa were found by Phillips et al. (1990). The different flavonoids in the seed and root exudates had divergent effects on the microsymbiont of alfalfa, *Rhizobium meliloti* (Hartwig et al., 1990). To describe these effects on the microbial population in the areas around the seed and the root Phillips et al. (1990) used the term «ecochemical zones».

Also for soybean (cv. Maple Arrow), differences in the composition of seed and root exudates were found.

Coumestrol and isoliquiritigenin were missing in the seed exudate, but were present in the root exudate (Kape et al., 1992). The isoflavonoids daidzein and genistein were found in different ratios in the root exudate (daidzein:genistein, 14:1) and in the seed exudate (3.2:1) as determined by HPLC analysis. In *B. japonicum*, the microsymbiont of *Glycine max*, these four flavonoids induce *nod* gene activity and glyceollin resistance to different extents. These data sup-

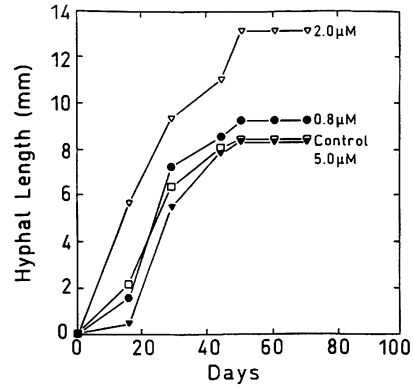


Fig. 5: Average hyphal length of *Glomus mosseae* per germinated spore on water agar with different concentrations of myricetin.

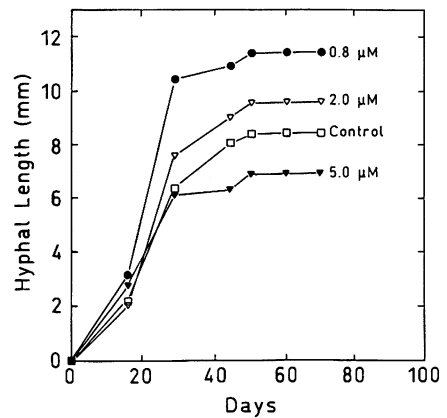


Fig. 6a

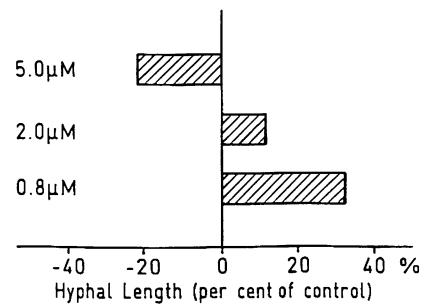


Fig. 6b

Fig. 6: Effect of different concentrations of quercetin on hyphal growth of *Glomus mosseae*; (a) Time course of hyphal growth; (b) Hyphal growth as per cent of control without quercetin after 70 days.

port the concept of «ecochemical zones» postulated by Phillips et al. (1990).

Beneficial effects of flavonoids on VAM fungi development

The germination of spores of *Glomus mosseae* and *Glomus intraradix* without addition of flavonoids is documented in Fig. 3 (a, b). An interesting difference between the two species of *Glomus* is the production of small vesicles by *Glomus mosseae*, which are absent on the hyphae of *Glomus intraradix* on the germination medium used.

In the concentration range of 2.0 to 5.0 μM , daidzein stimulates spore germination (percentage of spores germinating) between day 15 and 30 (Fig. 4). The growth of the hyphae from germinating spores is stimulated by myricetin (2 μM) whereas lower and higher concentrations had no significant effect (Fig. 5). A rather similar result was found with quercetin (Fig. 6a), with a maximal hyphal length of 11–13 mm per spore at a concentration of 0.8 μM . A concentration of



Fig. 7: Structures of the VA-mycorrhizal fungus *Glomus intraradix* in the root cortex of *Phaseolus vulgaris* cv. *saxa*. Stained with trypan blue. (a) arbuscules (bar, 50 μm), (b) vesicles (bar, 50 μm), (c) spores (bar, 30 μm).

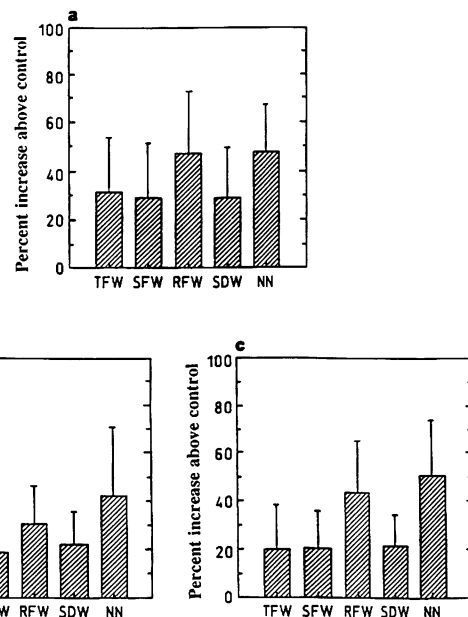


Fig. 8: Stimulation (percent increase above control) of the development of three different cultivars of *Phaseolus vulgaris* nodulated by *Rhizobium leguminosarum* bv. *phaseoli* through inoculation with *Glomus intraradix* after 8 weeks (a) Fori, (b) Saxa, (c) Hinrichs Riesen. Data are mean values of 14 plants. TFW = fresh weight of total plant, SFW = shoot fresh weight, RFW = root fresh weight, SDW = shoot dry weight, NN = nodule number.

5 μM quercetin already had a significant inhibitory effect on hyphal growth (Fig. 6b). After about 40 to 50 days hyphal growth stopped in all cases.

Also with other *Glomus* species and some other flavonoids, stimulation of hyphal growth was recently found. Apigenin (4',5,7-trihydroxyflavone) stimulated germination and hyphal growth of *Gigaspora margarita* (Gianinazzi-Pearson et al., 1989). Liquiritigenin stimulated the germination of *Glomus etunicatum* by about 20% (Tsai and Phillips, 1991) and Chrysin (5,7-dihydroxyflavone) the colonization of plant roots by an unidentified *Glomus* species at a concentration of 20 μM (Siqueira et al., 1991). Quercetin significantly (10 μM) prolonged hyphal growth from germinated spores of *Gigaspora margarita* and *Glomus etunicatum* (Bécard et al., 1992). In all cases so far, the mechanisms of the stimulation of germination and hyphal growth remain unexplained.

From a larger screening programme of combinations of various *Glomus* species with a number of legumes, we found the most reliable mycorrhization for the *Phaseolus vulgaris* cv. *Saxa* – *Glomus intraradix*-system (Fig. 7 a–c), where arbuscules, vesicles and spores were regularly formed at a high density. Root growth and *Rhizobium* nodule formation were more stimulated by VAM inoculation than stem and leaf growth (Fig. 8). These data were confirmed with two other cultivars of *Phaseolus vulgaris*.

Phytoalexin production after VA-mycorrhiza infection

To compare the production of aromatic compounds with non-aromatic secondary plant metabolites, we chose *Vicia*

Table 1: Weyerone concentrations in roots of *Vicia faba* with or without VA mycorrhiza (*Glomus aggregatum*, *Glomus macrocarpum* and *Glomus mosseae*) in dependence of the host cultivar 8 weeks after infection.

designation of cultivar	wyerone concentration (µg/g FW)	
	without infection	with infection
Alfred	0.8	1.7
Kristall	0.4	3.2
TF	0.5	3.1

faba as a host plant. *Vicia faba* produces wyerone, the phytoalexin of faba beans (Mansfield and Hargreaves, 1974). With a mixture of three different *Glomus* species (*Glomus aggregatum*, *Glomus macrocarpum* and *Glomus mosseae*) we received the best mycorrhization. Using three different cultivars of *Vicia faba*, we found a wyerone concentration between 1.7 and 3.2 µg per g fresh weight in the roots

(Table 1). The controls (without mycorrhization) were in the range of 0.4 to 0.8 µg per g fresh weight. This means that the roots react towards mycorrhiza infection with a detectable increase in this phytoalexin. Similar data were found by Morandi (Morandi et al., 1984; Morandi, 1990) with the phytoalexin glyceollin and soybean roots infected with VAM. These authors emphasized the differences between infected and noninfected roots. We interpret our data somewhat differently. We consider the very low concentration of 1.7–3.2 µg phytoalexin per g fresh weight as an indication of the almost perfect protection of the host root from the fungus by the VAM specific compartmentation. Similarly, we previously found very low glyceollin concentrations in wild-type combinations (fully effective) of *Rhizobium* and legume plants with 2 to 10 µg per g nodule fresh weight. However, in combination with symbiosis defective mutants of *Bradyrhizobium*, affecting the stability of the symbiosome (peribacteroid) membrane, the glyceollin concentration range increased by a factor of about 100, similar to a range in-

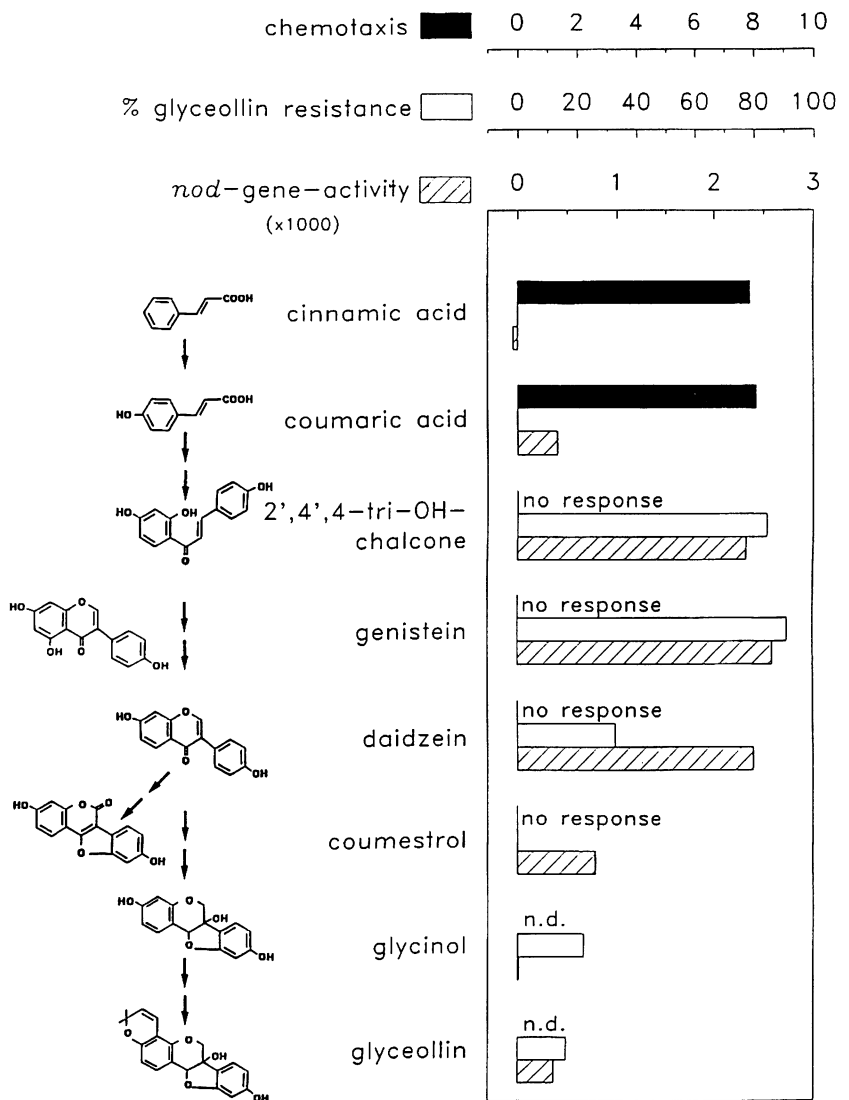


Fig. 9: Differential inductions of symbiotic activities in *Bradyrhizobium japonicum* by intermediates of the pterocarpan and the coumestan biosynthetic pathways. Test compounds were applied at a concentration of 10 µM. *Nod* gene activity is expressed in β-galactosidase units (in thousands) according to Miller (1972). Glyceollin resistance is expressed as the percentage of cells that survived a 3-h incubation in 300 µM glyceollin. The chemotaxis data are derived from Kape et al. (1991 and 1992) and are expressed as the chemotactic ratio at the concentration that elicited the chemotactic peak response. n.d., not determined (modified from Parniske, 1991).

duced by phytopathogenic fungi (Werner et al., 1985; Parniske et al., 1991a).

The symbiotic communication by phenylpropane metabolites

The thus far established results on chemotaxis, *nod* gene inducing activity and phytoalexin inducing activity of metabolites of the phenylpropane pathway in the *Rhizobium/Bradyrhizobium* – legume interaction are summarized in Fig. 9 (references are given in the figure legend). The results shown in Fig. 2 indicate that for the different root zones the concentrations of these compounds vary to a large extent. A similar scheme for the VA-mycorrhiza interaction is not available up to now, since only some effects of phenylpropane metabolites on spore germination and hyphal growth are known. Results are not yet available on compounds that influence the attraction of infecting hyphae to the rhizodermis cells. Also, there is no sufficient data on defence reactions of the host during the first stages of infection and effects of flavonoids on fungal differentiation into arbuscules, vesicles, and spores.

Acknowledgements

We thank the BMFT (Bonn) for support in the project «Symbiotische Wechselwirkungen Pflanzen-Mikroorganismen» (Förderungszeichen 0318966B). We also thank Dr. J. E. Cooper, Dr. J. Rao, and A. Bjourson, Queen's University Belfast, for experimental cooperation in the Anglo-German Academic Research Collaboration Scheme.

References

- BÉCARD, G., D. D. DOUDS, and P. E. PFEFFER: Extensive *in vitro* hyphal growth of vesicular-arbuscular mycorrhizal fungi in the presence of CO₂ and flavonols. *Appl. Environ. Microbiol.* **58**, 821–825 (1992).
- BUTTERY, B. R., S. BERNARD, W. STREIT, S. J. PARK, and D. WERNER: Effects of *Rhizobium* inoculum concentration, strain and combined nitrogen on growth and nodulation of a supernodulating common bean and its parent line. *Can. J. Plant Sci.* **70**, 987–996 (1990).
- GERDEMAN, J. W. and T. H. NICOLSON: Spores of mycorrhizal *Endogone* species extracted from soil by wet sieving and decanting. *Trans. Br. Mycol. Soc.* **46**, 235–244 (1963).
- GIANINAZZI-PEARSON, V., B. BRANZANTI, and S. GIANINAZZI: *In vitro* enhancement of spore germination and early hyphal growth of a vesicular-arbuscular mycorrhizal fungus by root exudates and plant flavonoids. *Symbiosis* **7**, 243–256 (1989).
- GÖRGE, E. and D. WERNER: Degradation of wyerone, the phytoalexin of faba beans by *Rhizobium leguminosarum*. *Cur. Microbiol.* **23**, 153–157 (1991).
- GRAHAM, T. L.: Flavonoid and isoflavonoid distribution in developing soybean seedling tissues and in seed and root exudates. *Plant Physiol.* **95**, 594–603 (1991).
- HARTWIG, U. A., C. A. MAXWELL, C. M. JOSEPH, and D. A. PHILLIPS: Effects of alfalfa *nod* gene-inducing flavonoids on *nodABC* transcription in *Rhizobium meliloti* strains containing different *nodD* genes. *J. Bacteriol.* **172**, 2769–2773 (1990).
- KAPE, R., M. PARNISKE, and D. WERNER: Chemotaxis and *nod* gene activity of *Bradyrhizobium japonicum* in response to hydroxycinnamic acids and isoflavonoids. *Appl. Environ. Microbiol.* **57**, 316–319 (1991).
- KAPE, R., M. PARNISKE, S. BRANDT, and D. WERNER: Isoliquiritigenin, a strong *nod* gene and glyceollin resistance inducing flavonoid from soybean root exudate. *Appl. Environ. Microbiol.* **58**, 1705–1710 (1992).
- KINNBAC, A. and D. WERNER: Glucosidases (α , β) and trehalase (α) in the peribacteroid space and the bacteroid periplasm of *Glycine max* root nodules. *Plant Sci.* **77**, 47–55 (1991).
- MANSFIELD, J. W. and J. A. HARGREAVES: Phytoalexin production by live cells in broad bean leaves infected with *Botrytis cinerea*. *Nature* **252**, 316 (1974).
- MILLER, J. H.: Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y. (1972).
- MORANDI, D.: Phytoalexins, isoflavonoids, and flavonoids in the plant-VAM fungus interaction. In: The Role of VA-mycorrhizae in Transformation of Matter in the Soil and their Importance for Plant Nutrition and Plant Health. Abstracts of the First Conference of Cost Action 8.10, Einsiedeln (1990).
- MORANDI, D., J. A. BAILEY, and V. GIANINAZZI-PEARSON: Isoflavonoid accumulation in soybean roots infected with vesicular-arbuscular mycorrhizal fungi. *Physiol. Plant Pathol.* **24**, 357–364 (1984).
- MOSSE, B.: The regular germination of resting spores and some observations on the growth requirements of an *Endogone* sp. causing vesicular-arbuscular mycorrhiza. *Trans. Br. Mycol. Soc.* **42**, 273–286 (1959).
- PARNISKE, M.: Flavonoide als Signal- und Abwehrstoffe in der *Glycine/Bradyrhizobium* Symbiose. Ph. D. Thesis, Philipps-Universität Marburg (1991).
- PARNISKE, M., H.-M. FISCHER, H. HENNECKE, and D. WERNER: Accumulation of the phytoalexin glyceollin I in soybean nodules infected by a *Bradyrhizobium japonicum nifA* mutant. *Z. Naturforsch.* **46c**, 318–320 (1991a).
- PARNISKE, M., B. AHLBORN, and D. WERNER: Isoflavonoid-inducible resistance to the phytoalexin glyceollin in soybean rhizobia. *J. Bacteriol.* **173**, 3432–3439 (1991b).
- PHILLIPS, D. A., U. A. HARTWIG, C. A. MAXWELL, C. M. JOSEPH, J. WERY, M. HUNGRIA, and S. M. TSAI: Host legume control of nodulation by flavonoids. In: Nitrogen Fixation: Achievements and Objectives (GRESSHOFF, P. M., ROTH, L. E., STACEY, G., and NEWTON, W. E., eds.). Chapman and Hall, New York, London, pp. 331–338 (1990).
- RECOURT, K., J. SCHRIPSEMA, J. W. KIJNE, A. A. N. VAN BRUSSEL, and B. J. J. LUGTENBERG: Inoculation of *Vicia sativa* subsp. *nigra* roots with *Rhizobium leguminosarum* biovar *viciae* results in release of *nod* gene activating flavanones and chalcones. *Plant Mol. Biol.* **16**, 841–852 (1991).
- SCHENCK, N. C. and Y. PEREZ: Manual for the Identification of VA-Mycorrhizal Fungi. 3rd ed. Synergistic Publications, Gainesville, USA (1990).
- SCHMIDT, P. E., M. PARNISKE, and D. WERNER: Production of the phytoalexin glyceollin I by soybean roots in response to symbiotic and pathogenic infection. *Bot. Acta* **105**, 18–25 (1992).
- SIQUEIRA, J. O., G. R. SAFIR, and M. G. NAIR: Stimulation of vesicular-arbuscular mycorrhiza formation and growth of white clover by flavonoid compounds. *New Phytol.* **118**, 87–93 (1991).
- TSAI, S. M. and D. A. PHILLIPS: Flavonoids released naturally from alfalfa promote development of symbiotic *Glomus* spores *in vitro*. *Appl. Environ. Microbiol.* **57**, 1485–1488 (1991).
- WERNER, D., R. B. MELLOR, M. G. HAHN, and H. GRIEBACH: Soybean root response to symbiotic infection. Glyceollin I accumulation in an ineffective type of soybean nodules with an early loss of the peribacteroid membrane. *Z. Naturforsch.* **40c**, 179–181 (1985).
- WOLFF, A., W. STREIT, J. A. KIPE-NOLT, H. VARGAS, and D. WERNER: Competitiveness of *Rhizobium leguminosarum* bv. *phaseoli* strains in relation to environmental stress factors: and plant defence mechanism. *Biol. Fertil. Soils* **12**, 170–176 (1991).