Société Française de Phytopathologie

ORSTOM

HISTOLOGIE, ULTRASTRUCTURE ET CYTOLOGIE MOLECULAIRE DES INTERACTIONS PLANTES-MICRO-ORGANISMES

Montpellier - Le Corum
30 novembre
1 et 2 décembre 1994

Société Française de Phytopathologie

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Montpellier - Le Corum
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1 et 2 décembre 1994

ORGANISATION

Laboratoire de Phytopathologie
ORSTOM
BP 5045
34032 MONTPELLIER (France)

COMITE D'ORGANISATION

Bernard BOHER (ORSTOM)
Michel DOLLET (CIRAD)
Jean-Paul GEIGER (ORSTOM)
Vivienne GIANINAZZI-PEARSON (INRA/CNRS)
Xavier MOURICHON (CIRAD)
Michel NICOLE (ORSTOM)
Jean-Loup NOTTEGHEM (CIRAD)
Katia RUEL (CNRS)

Le comité remercie les Organismes et Sociétés qui ont participé financièrement à l'organisation de cette manifestation :

* Conseil Régional du Languedoc-Roussillon
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* ORSTOM
* CIRAD
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RESUME DU PROGRAMME

MERCREDI 30 NOVEMBRE / *WEDNESDAY, NOVEMBER 1st*

A partir de 8h / *from 8h00*
Accueil des participants; installation des affiches */welcome of all participants; poster installation*

10h30 : Séance inaugurale / *Welcoming session*

12h00 : Déjeuner / *Lunch*

14h00 : Session 1
15h45 : Pause
16h00 : Session 1 - suite / *continued*
17h30 : Affiches / *Posters* : Sessions 1 et 3
19h00 : Cocktail (Salle / *room* Antigone 2)

JEUDI 1 DECEMBRE / *THURSDAY, DECEMBER 2nd*

8h00 : Session 2
9h45 : Pause
10h00 : Session 2 - suite / *continued*

12h00 : Déjeuner / *Lunch*

14h00 : Session 3
15h45 : Pause
16h00 : Session 3 - suite / *continued*
17h45 : Affiches / *Posters* : Sessions 2 et 4
20h00 : Banquet

VENDREDI 2 DECEMBRE / *FRIDAY, DECEMBER 3rd*

8h00 : Session 4
9h45 : Pause
10h05 : Session 4 (suite / *continued*)
12h00 : Cloture / *Closing talk*

12h15 : Déjeuner / *Lunch*

PROGRAMME DETAILLE / DETAILED PROGRAMME

MERCREDI 30 NOVEMBRE / *WEDNESDAY, NOVEMBER 1st*

10:30
OUVERTURE OFFICIELLE / *WELCOMING SESSION* - **Salle ANTIGONE 1**
Allocutions de bienvenue

11:00
P. RICCI - Président de la Société Française de Phytopathologie -

12h00
Déjeuner / *Lunch*

SESSION 1 : modérateur / *chairperson* : **Dr. R. Dargent**
Salle Antigone 1

Méthodes récentes de détection et de localisation in situ de molécules chez les plantes, les microorganismes et les virus / *Recent advances for in situ detection and localization of plant, microorganism and virus molecules*

Présentations Orales / *Oral Presentations*

14h00
C. SOUCHIER. Application of image analysis in biology.

14h35
R. G. MILNE. In situ detection of viruses and mycoplasmas.

15h10
J. BRANGEON. Procedures of in situ hybridization to RNA in plant tissues.

15h45
PAUSE

16h00
S. HAMON and D. FERNANDEZ. Cytofluorometry in fungal diversity and taxonomy.

16h20
N. BENHAMOU, M. NICOLE and B. BOHER. Enzyme-gold cytochemistry : its application and relevance in plant pathology.

16h40
J. LHERMINIER and E. BOUDON-PADIEU. In situ detection and distribution of grapevine flavescence doree MLO in a host plant.

17h00
L. FIUZA, L. DRIF, N. MICHAUX-FERRIERE, R. FRUTOS and P. WAGO. Laser scanning cytometry : an alternative technique to study binding sites of Bacillus thuregensis toxins.

17h30
AFFICHES / *POSTERS* (pour les sessions 1 et 3 ; *for sessions 1 and 3*)
Salle Antigone 2

1.1 M.T. MARTIN and B. GELIE. Intracellular localization of plum pox potyvirus polypeptides by immunogold labelling.

1.2 T. RICHARD, J. GERARD, J. DEIXHEIMER and B. BOTTON. Localization of the affinity sites of a lectin produced by Rigidoporus lignosus.

1.3 J. de ALMEIDA ENGLER, W. Van Der EYCKEN, M. Van MONTAGU, G. ENGLER and G. GHEYSEN. In situ mRNA hybridization of plant and nematode transcripts in a Meloidogyne incognita infected tomato root.

3.4 K. BHATNAGAR and U. KANT. Histopathological studies in blighted cumin plant induced by A. burnsii.

3.5 B. BOHER, K. KPEMOUA, M. NICOLE, V. VERDIER and J. P. GEIGER. Histopathology of Cassava infected by Xanthomonas campestris pv. manihotis : involvement of EPS in pathogenesis.

3.6 B. BOHER, K. KPEMOUA, V. VERDIER, M. NICOLE, G. HAINNAUX and J. P. GEIGER. The use of monoclonal antibodies in studying pathogenicity of Xanthomonas campestris pv. manihotis.

3.7 C. BESTWICK, M.H. BENNETT, I. BROWN and J. MANSFIELD. Responses of lettuce to wild type and Hrp- strains of Pseudomonas syringae pv. phaseolicola.

3.8 L. CHENET, B. DUBOS and R. LE MENN. Immunocytochemical study of pectin of grape berry cell wall, sound and infected by Botrytis cinerea.

3.9 M. CHERIF, G. B. OUELLETTE and L. BERNIER. Ultrastructural and histo-cytochemical aspects of a staghorn sumac wilt disease associated with a form of Fusarium oxysporum.

3.10 C. CORDIER, V. GIANINAZZI-PEARSON, S. GIANINAZZI. Interactions between symbiotic and pathogenic fungi in roots: studies of spatial relationships by immunocytochemistry.

3.11 C. COULOMB, C. POLIAN, Y. LIZZI and PH. J. COULOMB. Ultrastructural modifications of the photosynthetic system in elicited susceptible leaves of pepper infected by Phytophthora capsici.

3.12 R. DUPONNOIS, T. K. TABULA, K. SENGHOR and P. CADET. Interactions between a vesicular arbuscular mycorrhizal fungus, Glomus sp. and the root-knot nematode, Meloidogyne javanica, on Acacia seyal and Acacia holoserica.

3.13 C. GREIF, S. CHEVALIER, P. BASS and B. WALTER. Histological evolution of grapevine Kober stem grooving symptoms on sensitive rootstock tissues.

3.14 F. LAURANS, S. CHARTIER, F. LEFEVRE, C. JAY-ALLEMAND, G. SALLE and G. PILATE. Histological and biochemical studies of rust/poplar interactions.

3.15 F. PIOLA, S. RADAWIEC and R. ROHR. In vitro mycorrhiza initiation of larch stomatic embryo plantlets with four basidiomycetes : structural and functional aspects.

3.16 J. M. PLOTNIKOVA. Host-pathogen interrelationships of Uromyces caryophyllinus and canartion cells.

3.17 C. POLIAN, C. COULOMB, Y. LIZZI and Ph. J. COULOMB. Action of capsidiol on susceptible pepper cultivar protoplasts.

3.18 M. RUZZIER-DEBOST, L. GRILLET, J. NGUEFACK and N. MICHAUX-FERRIERE. Compared histology of sound bean cortex of Theobroma cacao L. in connexion with their sensitiveness to Phytophthora megakarya.

3.19 C. VALETTE, M. NICOLE, J. L. SARAH, M. FARGETTE, M. BOISSEAU and J.P. GEIGER. Ultrastructure of banana roots infected by the burrowing nematode Radopholus similis.

3.20 L. VARVARO, A. BAFI, C. ANGELACCIO and A.R. TADDEI. Ultrastuctural changes in tomato plants infected with Clavibacter michiganensis subsp. michiganensis.

3.21 A. YLIMARTIMO, G. LAFLAMME, M. SIMARD and D. RIOUX. Ultrastructural and cytochemical results on Gremmeniella-infected red pine.

JEUDI 1 DECEMBRE / *THURSDAY, DECEMBER 2nd*

SESSION 2 : modérateur / *chairperson* : **Dr. J. Mansfield**
Salle Antigone 1
Adhésion, reconnaissance et pénétration des plantes par les microorganismes
Adhesion, recognition and host penetration by microorganisms

Présentations Orales / *Oral Presentations*

8h00
R. J. O'CONNELL, N. A. PAIN and J. R. GREEN. Use of monoclonal antibodies to study differentiation of fungal infection structures.

8h35
P. BONFANTE-FASOLO. Cellular and molecular mechanisms of plant colonization by symbiotic endomycorrhizal fungi.

9h10
H. DEISING, S. HEILER, M. HAUG, M. RAUSCHER and K. MENDGEN. Cellular aspects of rust infection structure differentiation: spore adhesion and fungal morphogenesis.

9h45
PAUSE / *BREAK*

10h00
E. W. MERCURE and R. L. NICHOLSON and H. KUNOH. Adhesion of conidia of Colletotrichum graminicola : visualization by light and scanning electron microscopy.

10h35
H.R. HOHL, H. DING, S. BALSIGER and C. GUGGENBUHL. A putative IgG-binding 65kD adhesin involved in adhesion and infection of soybean by Phytophthora megasperma f.sp. glycinea.

10h55
J. D. HIPSKIND and R.L. NICHOLSON. Lesion development and fungal ingress comparison of maize lines resistant and susceptible to Bipolaris maydis.

11h15
M. GIOVANNETTI, C. SBRANA, A. S. CITERNESI and L. AVIO. Host recognition, hyphal morphogenesis and infection structure differentiation in arbuscular mycorrhizal fungi.

11h35
M. DOLLET, S. MARCHE, D. GARGANI, E. MULLER and T. BALTZ. Virus of plant trypanosomes (Phytomonas spp).

12h00 : Déjeuner / *Lunch*

SESSION 3 : modérateur / *chairperson* : **Dr. B. Vian**
Salle Antigone 1
Altérations cellulaires chez les plantes infectées */Cellular alterations of infected plants*

Présentations Orales / Oral Presentations

14h00
K. RUEL. Ultrastructural aspects of the biodegradation of lignified plant cell walls by filamentous fungi.

14h35
W. ROBERTSON. Morphology of plant cell modifications by nematodes.

15h10
R. HONEGGER. Mycobiont-photobiont interactions in lichens: structural and functional aspects.

15h45
PAUSE / *BREAK*

16h00
B. BOHER, M. NICOLE, K. KPEMOUA, V. VERDIER, J. F. DANIEL and J. P. GEIGER. Cytological aspects of diseases caused by Xanthomonas.

16h35
K. KPEMOUA, B. BOHER, M. NICOLE, V. VERDIER, J. M. LUISETTI and J. P. GEIGER. Cytochemistry of cell wall degradation in leaves of a susceptible cassava infected by Xanthomonas campestris pv. manihotis.

16h55
I. BROWN, J. MANSFIELD and U. BONNAS. Xanthomonas : mutants under the microscope.

17h15
L. MONDOLOT-COSSON and C. ANDARY. Histochemical study of a tolerant wild species of sunflower (Helianthus resinosus), before and after infection by Sclerotinia sclerotiorum.

17h45
AFFICHES / *POSTERS* (pour les sessions 2 et 4 ; *for sessions 2 and 4*)
Salle Antigone 2

2.22 L. GEA, B. VIAN and A. JAUNEAU. Localization of homogalacturonic polymers and calcium in the ectomycorrhizal model Pinus pinaster / Hebeloma cylindrosporium.

2.23 J. LHERMINIER, D. JONES, W. J. McHARDY and D. VAUGHAN. Effect of the potentially toxic metals zinc and copper on the ultrastructure of Alnus nitrogen fixing nodules.

2.24 D. MAFFI, R. CARZANIGA and A. CARELLI. Use of anionic colloidal-gold for detection of chitosan in fungal cell by microscopy.

2.25 R.J. O'CONNELL and K. MENDGEN. Ultrastructure and cytochemistry of the Colletotrichum-bean interface after high pressure freezing.

2.26 M. POTERI and L. RYYN. Infection structures and histopathology in the interactions between birch and birch rust.

4.27 O. VIRET and L. TOTI. Adhesion and host surface recognition by the beech leaf endophyte Discula umbrinella (Berk and Br.).

4.28 Z. BENHLAL and M. LE NORMAND. Evidence and characterization of a low molecular weight polypeptide in oilseed rape-Phoma lingam relationships.

4.29 A. CLERIVET and C. EL MODAFAR. Scanning electron microscopy and histochemical studies of Ceratocystis fimbriata f. sp. platani - Platanus spp interaction.

4.30 D. DIOUF, H. GERBI, C. FRANCHE, Y. PRIN, E. DUHOUX and D. BOGUSZ. Nodulation of transgenic Casuarina glauca root.

4.31 A. GIRE, A. BEVERAGGI, J. J. MACHEIX and X. MOURICHON. Evidence of a constitutive polyphenolic component in resistance of Banana to Mycosphaerella fijiensis.

4.32 A. GOLLOTTE, M. LEMOINE, V. GIANINAZZI-PEARSON and S. GIANINAZZI. Immunocytochemical localization of ß-1,3 glucans in different interactions between plants and root-infecting fungi.

4.33 V. GRIMAULT, B. GELIE, P. PRIOR and J. SCHMIT. Compared histology of colonization of resistant and susceptible tomato cultivars by Pseudomonas solanacearum.

4.34 H. J. L. JORGENSEN, E. de NEERGAARD and V. SMEDEGAARD-PETERSEN. Mechanisms of inhibition of Drechslera teres in barley by Septoria nodorum from wheat and Bipolaris maydis from maize.

4.35 K. KPEMOUA, B. BOHER, M. NICOLE, V. VERDIER and J. P. GEIGER. Histopathology of Cassava infected by Xanthomonas campestris pv. manihotis : defense reactions.

4.36 K. KPEMOUA, B. BOHER, M. NICOLE, V. VERDIER and J. P. GEIGER. Killer tyloses from Cassava : a lethal weapon.

4.37 J. LHERMINIER, V. GIANINAZZI-PEARSON and S. GIANINAZZI. Cellular aspects of partial resistance to endomycorrhizal fungi in pea mutants.

4.38 M. LUMMERZHEIM, M. FERREIRA, D. DE OLIVEIRA, M. VAN MONTAGU and D. ROBY. Microscopic characterization of hxc-1, an Arabidopsis mutant affected in the hypersensitive response to Xanthomonas campestris pv. campestris.

4.39 P. REY, N. BENHAMOU and Y. TIRILLY. Ultrastructural and cytochemical studies of cucumber roots infected by two Pythium species.

4.40 C. SANIER, F. BRETON and J. d'AUZAC. Histochemical characterization of Hevea brasiliensis / Corynespora cassiicola interaction.

4.41 N. SEJALON-DELMAS, A. BOTTIN, F. VILLALBA, M. RICKAUER, M. T. ESQUERRE-TUGAYE and R. DARGENT. Characterization of an elicitor preparation from Phytophthora parasitica var nicotianae and assessment of its defense inducing activity on tobacco plants.

4.42 M.C. SILVA, L. RIJO and M. I. VASCONCELOS. Light and electron microscopy of the incompatible interactions Coffea spp.-Hemileia vastatrix.

4.43 S. SOYLU and J. MANSFIELD. Light and electron microscopy of interactions between Albugo candida and Arabidopsis.

4.44 J. VASSE, P. FREY and A. TRIGALET. Tomato root infection and colonization by a Pseudomonas solanacearum mutant deficient for expression of HRP genes.

4.45 P. S. WHARTON and A. M. JULIAN. Compatible and incompatible interactions between Sorghum bicolor and Colletotrichum sublineolum.

VENDREDI 2 DECEMBRE / *FRIDAY, DECEMBER 3rd*

SESSION 4 : modérateur / *chairperson* : **Dr. P. Bonfante-Fasolo**
Salle Antigone 1
Mécanismes de contrôle des infections et réactions de défense des plantes / *Control mechanisms of infection and plant defense.*

Présentations Orales / Oral Presentations

8h00
A. GOLLOTTE, V. GIANINAZZI-PEARSON and S. GIANINAZZI. Cytolocalisation of plant defence responses to fungi in root tissues during symbiotic or incompatible interactions.

8h35
D. RIOUX. Compartmentalization in trees: historic and new facts during the study of Dutch Elm Disease.

9h10
F. de BILLY, J. VASSE, F. MAILLET, M. ARDOUREL, F. DEBELLE, C. ROSENBERG, N. DERMONT, P. ROCHE, A. DEDIEU, E.P. JOURNET, D.G. BARKER, J. C. PROME, J. DENARIE and G. TRUCHET. Rhizobium-legume symbiotic interactions : nodule development and defence mechanisms.

9h45
PAUSE */BREAK*

10h05
F. DAAYF, M. NICOLE, B. BOHER, J. F. DANIEL, A. PANDO and J. P. GEIGER. Responses of cotton roots to Verticillium dahliae.

10h25
L. BIGARRE, M. GRANIER, B. REYNAUD, M. NICOLE and M. PETERSCHMITT. In situ localization of maize streak virus (MSV) in a susceptible and a resistant maize cultivar.

10h55
L. GUERIN, N. BENHAMOU and F. ROUXEL. An ultrastructural and cytochemical comparison of fungal development and host reactions in susceptible and resistant carrot roots infected by Pythium violae.

11h15
D. GARCIA, V. TROIPOUX, C. SANIER, N. NICOLIN, M. COUPE, F. RIVANO and J. d'AUZAC. Chemical and structural barriers in Hevea ssp. to Microcyclus ulei (P. Henn.) V. Arx. causing south american leaf blight.

11h35
G.H. DAI, C. ANDARY, L. M. COSSON and B. BOUBALS. Histochemical reactions of grapevines (Vitis ssp.) to infection by Plasmopora viticola.

MERCREDI 30 NOVEMBRE / *WEDNESDAY, NOVEMBER 1st*

SESSION 1

Méthodes récentes de détection et de localisation in situ de molécules chez les plantes, les microorganismes et les virus

Recent advances for in situ detection and localization of plant, microorganism and virus molecules

modérateur / *chairperson*

Dr. R. DARGENT

APPLICATIONS OF IMAGE ANALYSIS IN BIOLOGY

Catherine Souchier,

Centre Commun de Quantimétrie et laboratoire de Cytologie Analytique, 8, avenue Rockefeller, 69373 Lyon CEDEX 08, France

Image analysis[1,2] is a method that mainly makes possible geometric and densitometric measurements on images from any source. Futhermore, it provides useful tools for numerical image acquisition and enhancement. In biology, the main application field is quantitative microscopy.

Image analysis consists of a sequence of three main steps. First, image is acquired with the CCD camera mounted on the microscope and is converted to a grey or color image with 8 or 3X8 bits per pixel. Second, image is processed, segmented in order to successively identify the different objects of interest. It might be the labelled and unlabelled cells, or the nuclei and hybridization spots, or the different tissue components. Third, measurements are performed and data are stored and analysed. Such measurements might be the volume density of tissue components, the number, size and shape of cells, the DNA content and texture of nuclei, the intensity and spatial distribution of immunoenzymatic, immunofluorescent or autoradiographic labellings.

With care taken in sample preparation and in image formation, objective, reproducible and precise data are acquired and allows us to describe samples. Such data might be used to compare different cell types, vegetal species, experimental conditions and growth or kinetic times. Moreover, it allows us to automatically detect unexpected events. Microscope stage may be controlled by the image analysis sytem and a slide may be systematically scanned.

With both image analysis and stereology[3], three dimensional data may be obtained. Furthermore, new perspectives are offered by confocal laser scanning microscope (CLSM) images[4,5,6]. In CLSM, the image is illuminated and acquired pixel per pixel. Imaging may be performed in fluorescence and in reflective mode. The main advantages of CLSM are 1) reduction of contribution of out-of-focus structures, 2) improvement of X/Y/Z resolution, 3) possibility to acquire optical serial sections that makes possible three dimensional vizualisation[7] and quantification[8].

1. Russ JC. Computer-assisted microscopy, the measurement and analysis of images, Plenum Press, London, 1990.

2. Souchier C. L'analyse d'images, Techniques de l'ingénieur, traité Analyse chimique et caractérisation, 7:855-1-855-18, 1991.

3. Cruz Orive LM, Weibel ER : Recent stereological methods for cell biology : a brief review, Am J Physiol, 258:L148-L156, 1990.

4. Pawley JB, Handbook of biological confocal microscopy, Plenum Press, 1990.

5. Souchier C, Bryon PA: La microscopie confocale. in : Cours Biologie Peau (COBIP) séminaire Inserm, Eds J Thivolet, D Schmitt, 1993, 239-250.

6. Kwon YH, Wells KS, Hoch HC : Fluorescence confocal microscopy : applications in fungal cytology, Mycologia, 85, 721-733, 1993.

7. Harders J, Lukacs N, Robert-Nicoud M, Jovin TM, Riesner D : Imaging of viroids in nuclei from tomato leaf tissue by in situ hybridization and confocal laser scanning microscopy, Embo J, 8:3941-3949, 1989.

8. Montijn MB, Houtsmuller AB, Oud JL, Nanninga N : The spatial localization of 18 S rRNA genes, in relation to the descent of the cells, in the root cortex of Petunia hybrida, J Cell Sci, 457-467, 1994.

IN SITU DETECTION OF VIRUSES AND MYCOPLASMAS

Robert G. Milne

Istituto di Fitovirologia Applicata (CNR),
Strada delle Cacce 73, I-10135 Torino, Italy

In situ data for viruses and MLOs in their hosts have always been valuable but now are especially so in connection with a number of current problems. Examples of these problems are: **1** Virus transport from cell to cell and over short and long distances within the plant; **2** Presence and distribution of viruses in cultured meristems, in relation to production of virus-free propagation material; **3** Seed transmission and why virus infections are or are not eliminated at the seed phase; **4** Expression of viral genomes, messenger RNAs or proteins in transgenic plants: their location and copy number; **5** For MLOs, we know basically where they are (in the phloem) but they are often highly localised and unevenly distributed in the plant, so in situ methods are especially interesting; we also know essentially nothing about the interactions, at cell or tissue level, of different MLOs occurring in mixed infections; **6** For both viruses and MLOs, tracing infection pathways and kinetics in vectors demands critical use of in situ methods.

The classical approach to in situ detection has been simple visualization of the agents or their effects by light and electron microscopy of fresh or fixed and embedded tissue. This has been improved on both in discrimination and sensitivity by use of antibody labeling, using fluorescent or enzymatic markers for light microscopy, and gold or gold-plus-silver labeling for electron microscopy. The DAPI test for revealing the presence of MLOs by their DNA content has been particularly successful at the light microscope level, where relatively large samples of tissue can be screened; lucky for us that the phloem essentially contains no nuclei, chloroplasts or mitochondria, as these are also sources of DNA picked up by the stain.

In situ hybridization of nucleic acids is becoming a routine technique with light microscopy but has yet to be widely used at the EM level.

Also, as we pass from LM to EM, we often find that fixation and embedding methods that seemed adequate for the former are disastrous for the latter: problems arise due to poor preservation of structures, loss of antigenicity or other activity, and possible migration of target molecules. These difficulties have yet to be resolved satisfactorily and cheaply, though cryofixation and embedding are offering some solutions.

Finally, there is the problem of presenting the data: slide shows are best as a vast acreage of landscape can be quickly presented - and even absorbed by the audience. Posters also offer some scope for displays of micrographs. But when the paper comes to be published in a journal, there is tremendous pressure to reduce the size, magnification and number of Figures. What remains, especially after image degradation resulting from halftone reproduction, may be disappointing, unrepresentative and uninformative. Digitization of images and image processing of course is a whole new ball game; if *National Geographic* magazine can move the pyramids, what is to stop us from using our imaginations to put just a little more sparkle into our data?

Procedures of *in situ* Hybridization to RNA in Plant Tissues

Judy Brangeon

Institut de Biotechnologie des Plantes,
Université de Paris-Sud, Bât. 630, 91405 Orsay

The *in situ* hybridization (ISH) technique makes possible the detection and localization of specific nucleic acid sequences within tissue sections or whole mount preparations of single cells, organelles, or chromosomes. It is based on the principle that a labelled single-stranded fragment of DNA or RNA (probe), exogenously applied, will hybridize to a complementary sequence on the cellular DNA or RNA (target), forming stable hybrids. The hybrids are then located at a particular morphological site by using a system of detection, (visible marker) which can be coupled to the labelled probe and visualized in the microscope.

If the probe is labelled with a radioisotope, the hybrid is detected by autoradiographic procedures, whereas nonradioactive labels such as biotin, bromo-deoxyuridine, and digoxigenin are invariably detected by histochemical techniques (enzyme reactions, affinity, or immunocytochemical (ICC) methods).

The kinetics of *in situ* hybridization depends on probe to tissue penetration and diffusion, accessibility of targets to probe, and the hybridization reaction itself. These requirements, in turn, depend on fixation conditions which should give acceptable structural preservation and good nucleic acid retention while permitting probe diffusion throughout the tissue.

An *in situ* hybridization protocol adheres to the following general outline:

- fixation and embedding of material—compromise between conservation of morphology and targets.
- tissue sectioning.
- pretreatments of material—permeabilization to permit access of probe to target
- preparation of probe—labelling, denaturation.
- *in situ* hybridization—conditions for formation of probe/target duplex.
- post-hybridization washes—removal of noncomplexed probes.
- revelation of labelled probe / target—immunocytochemistry, affinity reactions
- microscopy.

Plant material presents drawbacks in that I) most tissue is composed of a highly heterogeneous cell population which necessitates a compromise in fixation/permeabilization procedures 2) the cell wall constitutes a barrier to probe penetration adding to those of cell and organelle boundary membranes and 3) the vacuole, a water-filled membrane-bound compartment, must be maintained during preparatory steps- e.g. disruption can lead to leakage/diffusion of targets. Certain modifications of standard *in situ* protocols to overcome these drawbacks will be evoked.

We have chosen to use paraformaldehyde-fixed, resin-embedded material in that our goal was to use the same tissue preparation for both EM and LM studies. Biotinylated and digoxigenylated DNA probing was used, both of which offer several advantages I) rapid detection with either affinity or immunocytochemical methods 2) clear visualization of markers 3) non-radioactive probes avoid risks associated with radioisotopes and can be stored for extended periods 4) same system can be used at the light microscope level by cutting thicker sections and amplifying the colloidal gold markers with silver enhancement.

Two approaches were tried I) pre-embedding labelling of tissue pieces and 2) post-embedding labelling on sectioned material. Pre-embedding using paraformaldahyde-fixed, protease-digested leaves has successfully been used in conjunction with biotin labelled DNA probes. Bound probe was detected with an avidin/ferritin conjugate. We have also developed procedures for post-embedding labelling of plant material embedded in LR White resin— a biotin or digoxigenin labelled probe was hybridized to sections and then detected with immunogold and/or silver enhanced markers. Using these methods, we have visualized LSU and SSU mRNAs for ribulose bisphosphate carboxylase in tobacco leaves and protoplasts, two subunit mRNAs for ADGppase detected in developing maize kernels and seedlings, and ß- ATPase mRNAs in pollen grains of tobacco wild type and mutant anthers.

CYTOFLUOROMETRY IN FUNGAL DIVERSITY AND TAXONOMY

S. Hamon (1) and D. Fernandez (2)

(1) ORSTOM, Laboratoire de Ressources Génétiques et d'Amélioration des plantes, BP 5045, Montpellier, France; (2) ORSTOM, Laboratoire de Phytopathologie, BP 5045, Montpellier, France.

The purpose of this lecture is to provide some basic information on the Flow Cytometric analysis of nuclear DNA content and then a small review of the current status of the developing area in fungi.

Quantitative cytofluorometry, based on the application of DNA specific fluorochromes was introduced by Ruch (1966). By the late 1970s, Flow CytoMetry (FCM) was established as a more powerful technique for DNA content analysis. The major advantages over microdensitometry and static cytofluorometry are convenience, precision and rapidity. Tens of thousands of cells can be analysed within several minutes. The flow cytometer may be also equipped with a sorting facility allowing the possibility of selecting subpopulations of cells for further analysis or even culture. Such an instrument is often called a Fluorescence Activated Cell Sorter (FACS). During the early 1980s, FCM was introduced into the field of plant sciences and is now routinely used in many laboratories (Dolezel 1991, Marie and Brown 1993) and also by ORSTOM (Cros et al.1994).

FCM analysis of nuclear DNA content is based on the use of DNA-specific fluorochromes and on the analysis of the relative fluorescence intensity of stained nuclei. A typical flow cytometer consists of several basic components: a light source, a flow chamber and optical assembly, photodetectors and processors to convert light signals into analog electrical impulses and a computer system for the analysis and storage of digitalized data.

Fluorochromes such as DAPI (4',6 diamidino-2 phenylindole) and PI (Propidium Iodine) have many attractive properties as nuclear stain. DAPI binds selectively to AT-rich double-strand DNA. The fluorescence of DAPI-DNA is nearly proportional to the DNA quantity and only needs an UV light. PI is an intercalent dye and is fully proportional but needs a laser exitation. This dye also binds with double-stranded RNA and consequently needs a ribonuclease pretreatment. The determination of nuclear DNA content by FCM requires comparison with a reference standard.

For fungi, 3 majors uses were identified : 1. Ploidy level identification ; 2. Nuclear DNA content estimation; 3. Species identification and subpopulation separation.

1. Ploidy level identification. Martegani and Trezzi (1979) first mentionned the successful staining of nuclei in conidia and mycelium *of Neurospora crassa*. Staining with DAPI was investigated as an alternative to Feulgen staining procedure for zoospores of *Phytophthora infestans*. Isolates with approximately 4C values were shown to be tetraploid (Whittaker et al. 1991). The relative DNA content of different species and discussed ploidy levels were studied within the genus *Armillaria* (Motta et al. 1986). Three levels of ploidy were determined within *Pleurotus* (Bresinsky et al. 1987) and for thirteen species of *Coniophoraceae* (Meixner and Brezinski 1988).

2. Nuclear DNA content estimation. FCM and IP were used to estimate the relative nuclear DNA content of pycniospores of 85 collections of 13 species of rust fungi (Eilam et al. 1994). For each sample 10,240 fluorescent events were measured and the peaks were of good quality (CV<10%). DNA content, relative *to Puccinia graminis*, gave a large level of diversity : *P. lagenophorae* (53% - 67 Mbp), *P. recondita* (105%), *P. allii* (164%) and *Uromyces appendiculatus* (346% - 418 Mpb). It is also important to note that DNA content genetically

diverge witin the same species according to the host species (i.e. *P. hordeum* on different species of the genus *Hordeum*).

Fungi have been shown to contain some forms of repetitive DNA sequences other than ribosomal repeats. For example, the genome of *Bremia lactucae* and obligate fungal pathogen has a genome size of about 50 Mbp of DNA and 65% of the nuclear DNA is repeated (Francis et al. 1990).

3. Species identification and subpopulation separation. The light scattering properties in addition to the DNA contents of spores could also be useful. With the spores of five basidiomycetes, the forward and wide angle light scatter and the DNA amount are enough for discrimination. In addition sub-populations were evident in samples of *Megacollybia platyphylla* and *Fuligo septica* which would not have been detected using conventional techniques (Allman 1992). Bianciatto and Bonfante (1992) shown that *Glomus versiforme* and *Gigaspora margarita*, two arbuscular mycorrhizal fungi possess nuclei with significantly different diameters and DNA contents.

It seems that the FCM technology, when used in conjunction with standards of DNA, will provide an efficient method genetic analysis and systematics of fungi.

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ENZYME-GOLD CYTOCHEMISTRY : ITS APPLICATION AND RELEVANCE IN PLANT PATHOLOGY

N. Benhamou*, M. Nicole and B. Boher.

*Université Laval, Recherches en Sciences de la Vie et de la Santé, Québec, G1K 7P4, (Canada). ORSTOM, BP 5045, 34032 Montpellier (France).

In the last decade, it has become increasingly apparent that in situ localization of plant molecules could lead to a better understanding of the functional activity of the plant cell during various biological processes. One of the most recent advances in plant biology has been the development of highly specific cytochemical coloidal gold-probes in electron microscopy. With this respect, advantages of the biological properties of enzymes have been taken for in situ cytolocalization of their substrates. In plant pathology, the enzyme-gold affinity approach has been shown to provide useful and often unique information on various topics including (a) spatio-temporal distribution of newly-synthesized molecules during the time course of infection (b) vulnerability of plant cell wall polymers to microbial enzymes (c) vulnerability of microbial cell walls to plant enzymes (d) accumulation of new macromolecules in the plant cell wall (e) chemical composition of newly-formed barriers such as wall appositions and (f) activation of the phenylpropanoid pathway.

Because polysaccharides are major plant and parasite cell wall components, their involvement in recognition, adhesion, and attachment was of key importance in the outcome of a given interaction. Consequently, their precise localization has been the subject of extensive studies and a number of hydrolases conjugated to gold such as ß-1,4-exoglucanase, pectinase, chitinase and glucosidase have been proved useful for studying cell surface interactions during the infection process. Another facet that has been widely investigated concerns cellular changes induced in response to infection and thought to contribute to plant resistance. Among the molecules involved in defense against pathogens, callose, a structural polymer of ß-1,3-glucans, and phenolic compounds have been successfully targeted by means of two newly-introduced gold-complexed enzymes : a plant ß-1,3-glucanase and a fungal paradiphenoloxydase, the laccase.

In conjunction with biochemistry and molecular biology, enzyme-gold cytochemistry of plant tissues, associated with immunocytochemistry has proved powerful in elucidating some aspects of relationships between plants and pathogens. Thus it is clear that molecular and traditional approaches in plant pathology will continue to benefit from exciting, new findings generated by the in situ localization of plant and microbe molecules.

" IN SITU" DETECTION AND DISTRIBUTION OF GRAPEVINE FLAVESCENCE DOREE MLO IN A HOST PLANT

J. Lherminier* and E. Boudon-Padieu**
*Service Commun de Microscopie Electronique, **Station de Recherches sur les Mycoplasmes et Arbovirus des Plantes, INRA, 21034 Dijon Cedex, France.

A pathogenic Mycoplasma-like organism (MLO), recently called "Phytoplasma", is the aetiological agent of Flavescence dorée (FD), an important grapevine yellows disease. Like other MLOs, this FD-MLO strain has not been axenically cultured; it is experimentally maintained in broadbean plants (*Vicia faba*) to which it is transmitted by leafhoppers (*Euscelidius variegatus*). As "in situ" detection methods of the MLO cells were not specifc and sensitive, we developed a post-embedding colloidal gold indirect immunolabelling 1) to provide information about the morphology of MLO cells during the course of infection of the plant after inoculation with infectious leafhoppers and 2) to determine their cellular and histological distribution in the plant tissues before symptom expression. In addition DAS-ELISA was used to monitor the movement of FD-MLOs during the process of plant infection. Both ELISA and immunolocalization first detected MLOs in roots 17 days after inoculation. Some amoeboid MLO cells but also small vesicular (80-120 nm in diameter) and filamentous bodies were, for the first time, unequivocally identified as MLOs in the cytoplasm of root phloem cells. Furthermore, 17-24 days after inoculation, numerous similar structures and budding MLOs could be accurately detected in the four groups of root phloem suggesting active multiplication of the pathogen in root tissues. This conclusion was supported by ELISA results indicating that roots were the primary site of multiplication of FD-MLOs. In the same period a basal axillary shoot had emerged and ELISA indicated that MLOs then preferentially multiplied in the upper part of this symptomless growing shoot. Histological immunolabelling performed on the stem apices and leaf petioles of the axillary shoot allowed us to locate MLOs in non functional phloem cells, in mature sieve tubes of the metaphloem and in early necrotic phloem cells. Furthermore, electron dense and distorded elongated bodies were labelled and identified as MLO cells in these collapsed cells. Terminal buds without disease symptoms exhibited a necrosis of recently differentiated sieve tubes which were nevertheless infected.

The pattern of systemic colonization of FD-MLOs has been established and described. This study underlined the important role of the root system in the multiplication of MLOs during the infection process. Moreover, it provided useful information on morphology of MLO cells associated with different stages in the infection process.

LASER SCANNING CYTOMETRY: AN ALTERNATIVE TECHNIQUE TO STUDY BINDING SITES OF *BACILLUS THURINGIENSIS* TOXINS

Lidia FIUZA[1,2], Latifa DRIF[1], Nicole MICHAUX-FERRIERE[1], Roger FRUTOS[1], François LECLANT[2] and Philippe VAGO[3]

[1] BIOTROP-CIRAD, B.P. 5035, 34032 Montpellier, France
[2] ENSA-M, Chaire Ecol. Anim. et Zool. Agric., Place Viala, 34060 Montpellier Cedex 1, France
[3] CRIC-INSERM-U 254, CHU St Charles, 34295 Montpellier cedex 5, France

Bacillus thuringiensis is the most widely used micro-organisms for insect biocontrol. This gram-positive spore-forming bacterium characteristically produces parasporal crystal proteins. The crystals dissolve in the larval midgut, releasing one or more protoxins. Which are proteolytically converted into smaller polypeptides: the toxins (Höfte and Whiteley, 1989).

To be active, these toxins must recognize, in a first step, binding sites in the insect midgut. These specific binding sites are considered as key elements in the specificity of toxins and therefore in the specificity of *B. thuringiensis* strains (Denolf et al., 1993). *In vitro* studies are of interest for screening these binding sites and therefore better understand the mechanism of action of toxins in insect pests.

To this aim, we propose Laser Scanning Cytometery (LSC). This technique allows quantitative *in situ* studies of fluorescence in adherent cells or tissue sections (Métézeau, 1993a, Métézeau *et al.*, 1993b). The X-Y scanning motorised stage of an inverted microscope moves under a laser beam thereby exposing in turn each point of the preparation. The emitted fluorescent signals are selected by an optical circuit comprising mirrors and filters and converged to photomultipliers where each signal is amplified, converted into electrical signals and directed to the computer of the instrument which establishes pseudocolor images from the intensities of fluorescence.

Longitudinal sections of wax-embedded gut tissue were incubated with a solution of either native or biotinylated toxins. Bound native toxins were visualised by incubating the sections first with rabbit polyclonal antibodies, then with fluorescent conjugated goat-anti-rabbit antibodies. Sections incubated with biotinylated toxins were revealed with fluorescent streptavidin conjugates. Samples used as controls for both types of experimentations were prepared by omission of either toxins, primary antibodies, fluorescent conjugated antibodies, biotinylated toxins, or fluorescent streptavidin conjugates.

Histological sections were analysed using a Meridian ACAS-570 cytometer equipped with a coherent INNOVA 90-5 argon ion laser and attached to an Olympus IMT-2 inverted microscope. The laser was tuned to 488 nm of wavelength and operated at 200 mW in a light regulation mode. The analyses were performed through a 100x oil objective and with a final power of the pulsed laser beam comprised between 0.1 and 1.5 mW. The images were produced by X-Y scanning of the field of interest with a step of either 0.2, 0.6 or 1 µm. Green (520 nm) fluorescence of fluorescein and/or red (560 nm) fluorescence of phycoerythrin were recorded.

This LSC approach has allowed a precise *in situ* localization and quantification of binding sites of *B. thuringiensis* toxins on histological sections of lepidopteran larvae midgut. Reported informations assess the following aspects.

The toxin binding sites are specifically localized to the microvilli of midgut epithelial cells (Fiuza *et al.*, 1994a).

The distribution of binding sites varies depending on the toxin. Some toxins are present uniformily throughout the whole length of the midgut, whereas the distribution of others is not uniform (Fiuza *et al.*, 1994b).

Moreover, dual fluorescence investigations and cross competition experiments have suggested that binding sites are not specific for a toxin and that their affinity vary depending on the couple of toxins simultaneously investigated.

In conclusion, LSC is a sensitive and rapid technique for investigating toxin binding sites. It allows easy screening which can be used for studies of the susceptibility of individual insects to different toxins in a pest population and for monitoring genetically induced resistance to *B. thuringiensis* .

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INTRACELLULAR LOCALIZATION OF PLUM POX POTYVIRUS POLYPEPTIDES BY IMMUNOGOLD LABELLING.

M.T. Martin (*) and B. Gélie (*).

(*) INRA, route de St Cyr, 78026 Versailles cedex, France.

Plum pox potyvirus (PPV) is the causal agent of sharka. This severe disease, widespread throughout Europe, affects stone-fruit trees of the genus Prunus. PPV belongs to the potyvirus group. Its flexuous particles are about 700nm long and 12 nm wide. It induces in infected plant cells characteristic cytoplasmic inclusions known as pinwheels (transverse section) or laminated aggregates (longitudinal section). The virus is transmitted by aphids.

The nucleic acid is a single-stranded messenger RNA. The RNA genome is expressed as a large polyprotein precursor which is proteolytically processed to yield the mature proteins P1, HC, P3, CI, NIa, NIb, CP and two putative polypeptides 6K1 and 6K2 located on either side of the CI protein. The role of P1 and P3 have not yet been clearly defined. HC (helper component) is required for transmission by aphids. CI (cytoplasmic or cylindrical inclusion) acts as an RNA helicase-ATPase. NIa (nuclear inclusion) is a VPg-protease fusion product. NIb (nuclear inclusion) contains the typical sequence of RNA-polymerases. CP (coat protein) is the structural protein that encapsidates the viral RNA.

It has been previously described that PPV CI and NIa aggregate in infected cells to form distinctive inclusions. CI protein is associated with pinwheel inclusions and laminated aggregates, which are frequently distributed perpendicular to the cell wall. NIa protein forms crystalline inclusions, present both in the nucleus and in the cytoplasm (1).

In the present study, we expressed P3, 6K2 and NIb polypeptides in transformed *Escherichia. coli.* Polyclonal antibodies to P3, K2 and NIb were then used to localize these proteins on leaves of *Nicotiana clevelandii* ten days after inoculation with PPV Rankovic isolate.

As expected, NIb antiserum labelled crystalline inclusions encountered both in the nucleus and in the cytoplasm. No labelling occurred on pinwheels. P3 and 6K2 polypeptides were

associated to the crystalline inclusions which have until now been labelled with NIa and NIb only. None of these polypeptides were found associated with pinwheels or laminated aggregates. Opposite results, stating that P3 is associated to the cylindrical inclusions in the tobacco vein mottling virus, have been reported (2).

We showed that at least four proteins were structurally related to the nuclear and cytoplasmic crystalline inclusions induced by PPV infection on *Nicotiana clevandii* leaves. We suggest that these structures may represent remnants of replication and transcription complexes.

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LOCALIZATION OF THE AFFINITY SITES OF A LECTIN PRODUCED BY *RIGIDOPORUS LIGNOSUS* , PARASITE OF THE RUBBER TREE *HEVEA BRASILIENSIS*.

T. Richard, J. Gérard, J. Dexheimer and B. Botton

Laboratoire de Biologie Forestière associé à l'INRA, Université H. Poincaré, BP 239, 54506 Vandoeuvre-les-Nancy Cédex, France.

Rigidoporus lignosus [(Kl.) Imazeki (*Fomes lignosus* (Kl.) Bres.] is a polyporaceous Basidiomycete which can attack the roots of many tropical trees causing serious damage especially in rubber-tree plantations. The fungus differentiates mycelial strands which grow rapidly and play a significant role in the infection of healthy trees.

The young vegetative mycelium exhibited glycogen-containing cells and cell walls highly reactive to the PATAg test. This carbohydrate disappeared in the old cultures. Mycelial strands were composed of glycogen-free aggregated hyphae with highly sinuous cell walls.

A lectin with high specificity for L-fucose has been purified from the fungus. This lectin was mainly present in the mycelial strands and may play a role in the differentiating process of the aggregated organs. Complexation of the lectin with colloidal gold particles allowed localizing its affinity sites which were preferentially distributed on the cell walls of the hyphae. In addition, septa and dolipores were also found as potential affinity sites for the lectin.

JEUDI 1 DECEMBRE / *THURSDAY, DECEMBER 1st*

SESSION 2

Adhésion, reconnaissance et pénétration des plantes par les microorganismes

Adhesion, recognition and host penetration by microorganisms

modérateur / *chairperson*

Dr. J. MANSFIELD

USE OF MONOCLONAL ANTIBODIES TO STUDY DIFFERENTIATION OF FUNGAL INFECTION STRUCTURES

O'CONNELL R.J.[1], N.A. PAIN[2], J.R.GREEN[2].

[1]Department of Agriculture, University of Bristol, IACR Long Ashton Research Station, Bristol BS18 9AF, UK. [2]School of Biological Sciences, University of Birmingham, Birmingham B15 2TT, UK.

Successful infection of *Phaseolus vulgaris* by the hemibiotrophic fungus, *Colletotrichum lindemuthianum*, involves differentiation of a series of specialised infection structures. Conidial germ-tubes form appressoria, which penetrate the epidermis directly to form infection vesicles and primary hyphae within living host cells. These intracellular hyphae (IH) invaginate the host plasma membrane, from which they are separated by a matrix layer. Monoclonal antibody (MAb) techniques have been used to study the cell surfaces of these various infection structures.

MAbs specific for particular cell types were obtained using a co-immunisation procedure with fungal structures isolated from infected bean leaves. MAb UB25 identifies a protein epitope in a set of *N*-linked glycoproteins (40-245 kDa) specific to the cell wall and interfacial matrix of IH. These glycoproteins are expressed at an early stage of intracellular development, suggesting a possible role in biotrophy or recognition. UB25 was used to affinity-purify IH from homogenates of infected leaves using an immunomagnetic technique.

MAb UB26 recognises a protein epitope in two *N*-linked glycoproteins (140 and

155 kDa) which are restricted to the fibrillar sheath around germ-tubes and appressoria and could be involved in adhesion.

MAb UB27 recognises a protein epitope of a 48-50 kDa glycoprotein specific to the plasma membrane of appressoria. The glycoprotein is restricted to the domed region of the appressorium and is absent from the basal region around the penetration pore. The UB27 antigen thus defines two domains within the appressorial plasma membrane and is a marker for cell differentiation and polarisation.

CELLULAR AND MOLECULAR MECHANISMS OF PLANT COLONIZATION BY SYMBIOTIC ENDOMYCORRHIZAL FUNGI

Paola Bonfante
Dipartimento di Biologia Vegetale dell'Università and Centro di Studio sulla Micologia del Terreno del CNR
Viale Mattioli 25 10125 Torino Italy

In natural conditions, soil fungi join the root apparatus of 90% of the land plants creating different types of mycorrhizas, i.e. the symbiotic associations which play a central role in the capture of nutrients from soil in almost all the ecosystems.
Arbuscular mycorrhizas (AMs) are the most common type, occurring in about 80% of the plants, and -in the mean time- they are the most appealing for developmental programs based on low-input agriculture. The fungal partners belong to a small group of Zygomycetes, origin of which has been dated 353-452 Ma ago, suggesting that they were instrumental in the colonization of lands by ancient plants. They are characterized by an obligatory biotrophic status, since the completion of their life cycle depends on their ability to colonize a host plant developing an intraradical mycelium. On the basis of their topological relationships with the host root, AM fungi are identified as endomycorrhizal fungi: they accomplish in fact a complex morphogenesis with the development of intracellular structures inside specific tissues of the host root. AMs represent therefore a complex experimental model where the fungus and the host require a regulated balance for their harmonious development.

The aim of the presentation is to review the more recent progresses spawned in the area of cell and molecular biology and focussed to the understanding of the molecular strategies followed by AM fungi, when they - as obligate biotrophs- establish a successful symbiosis with their host plants. Four topics will be analyzed:
i) the parameters controlling the fungal growth both in the absence and in the presence of the host root, and in particular timing and location of fungal cell cycle activation;

ii) the mechanical forces and/or enzymic mechanisms thanks to which the fungus may extensively colonize the root tissues, keeping them viable. Comparisons will be made with another group of endomycorrhizal fungi, the ericoid ones;

iii) the large range of morphological changes induced by AM fungi in the host cells and in particular the formation of a new compartment, the so called interface, and

iv) the modifications of gene expression induced in the plant hosts following fungal colonization.

Cellular aspects of rust infection structure differentiation: Spore adhesion and fungal morphogenesis

H. Deising, S. Heiler, M. Haug, M. Rauscher and K. Mendgen
Universität Konstanz, Fakultät für Biologie - Phytopathologie, Universitätsstr. 10, D-78434 Konstanz, FRG.

Rust infection structures derived from dikaryotic spores (uredospore and aeciospore) usually penetrate the leaf of the host plant not directly, but through the stomatal openings [1-3]. In order to understand morphogenesis of rust fungi and events related to establishment of a stable host-parasite system, infection structure differentiation of the broad bean rust fungus *Uromyces viciae-fabae* was studied by biochemical and molecular techniques. A surface signal corresponding to the lips of the stomatal guard cells is sufficient to induce infection structure differentiation [4]. Scratched polyethylene has been shown to provide the surface features needed to induce differentiation up to the stage of haustorial mother cells in the absence of its host plant [5] and was used in our studies on rust infection structure specificities. Since fungal differentiation on inductive polyethylene membranes occurs synchronously, differentiation-specific formation of enzymes or mRNA species can be related to the respective infection structure formed.

Questions important to understanding the complex interplay between rust fungi and their hosts are which genes or gene products are regulated in a way to allow the pathogen to grow in the mesophyll of the leaf, to breach the plant cell wall and to form a haustorium without destroying cell integrity and elicitation of plant defense. To answer these questions, we investigated enzymes potentially important to establish the fungus on its host plant. In order to study genes which are turned on at late stages of infection structure differentiation, i.e. when the rust fungus prepares penetration of the mesophyll cell wall, we constructed a cDNA library representing transcripts of fully differentiated infection structures. The library was screened with cDNA from 7 and 20 h old infection structures (appressoria and haustorial mother cells), and differentially hybridizing clones have been used to study kinetics of transcript formation.

Enzyme formation during infection structure differentiation

When rust fungi invade the host leaf through the stomata they grow in an environment which contains plant defensive enzymes such as β-1,3-glucanases and chitinases. The question thus is how rust fungi can avoid lysis of infection structures and generation of elicitor-active molecules. One of the most distinct changes occurring during fungal morphogenesis is that

chitin, the dominating polymer on structures growing on the plant surface is not detectable on structures formed in the intercellular space [6], implying that chitin may be not accessable to plant chitinases. Infection structures of *Puccinia striiformis* formed in the leaf were specifically labelled by antibodies raised against chitosan [7], suggesting that enzymatic alteration of chitin localized on the infection structures starts with stomatal penetration. Deacetylation of chitin may be of importance since wheat *endo* -chitinase activity has been shown to depend on the degrees of acetylation of the substrate [8]. *U. viciae-fabae* begins to secrete four chitin deacetylase forms when it grows through the stomatal pore (Deising & Siegrist, unpublished results). We speculate that enzymatic deacetylation of chitin represents a mechanism to encounter lysis of infection structures and formation of elicitors.

Cell wall-degrading enzymes of fungal plant pathogens are thought of as important factors in many host-parasite systems [9]. To prove the importance of these enzymes by inactivating or deleting genes encoding such enzymes [10, 11] is difficult, due to several isoforms contributing to a specific activity. In addition, in many fungi both *exo* - and *endo* -cleaving enzymes are present, so that even after successfully deleting genes encoding *endo* -cleaving enzymes interpretation of the results is difficult, due to remaining *exo* -activities [10]. For obligately biotrophic rust fungi only methods allowing transient DNA-mediated transformation have been described [12]. Therefore, rust mutants deficient in one specific enzyme can currently not be obtained.

However, curcumstantial evidence exists suggesting that these enzymes are important for wall degradation by rusts. In *U. viciae-fabae* all cell wall-degrading enzymes analyzed are formed after induction of infection structure differentiation. Heiler et al. [13] have shown that a minimum of seven enzymes contribute to total cellulase activity of the broad bean rust fungus. Interestingly, the onset of enzyme synthesis co-incides with appressorium differentiation. The neutral *endo* -cellulases only contribute some 5% of the extracellular cellulase fraction of 24 h old infection structures, and possibly are secreted very locally when cell wall penetration is initiated. It would be of interest to investigate if factors which induce formation of haustoria on artificial membranes [14] are also able to induce the secretion of *endo* -cellulases. When substomatal vesicles are formed, pectin methylesterase activity becomes detectable [15]. The existance of four isoforms differing in kinetics of formation and physico-chemical properties has been demonstrated. These enzymes, according to their affinities to pectins with differing degrees of esterification, are thought to be important to the preparation of the infection court since polygalacturonate lyase of *U. viciae-fabae* requires largely deesterified substrate. The latter enzyme is formed when haustorial mother cells are differentiated. While cellulases and pectin methlyesterases are regulated strictly differentiation-specifically and are formed in the absence of their substrates, polygalacturonate lyase induction requires the presence of polygalacturonate. Sugars present in the apoplast of broad bean leafs (sucrose, glucose and

fructose) do not significantly repress formation of these cell wall-degrading enzymes at physiological concentrations.

Recent work demonstrated that, apart from carbohydrate components, fibrous proteins play an important structural role in plant cell walls [16]. Preformed extensin molecules have been shown to get immobilized in the cell wall, and also *de novo* synthesis of the protein in response to challenge by elicitors or plant pathogenic fungi has been reported [16]. Hammerschmidt et al. [17] have shown that the level of hydroxyproline-rich glycoproteins of different cucumber varieties correlates with resistance to *Cladosporium cucumerinum* infection. In *U. viciae-fabae* a complex pattern of extracellular proteases has been observed after SDS-PAGE using gels containing gelatin as substrate. Almost no activity can be isolated from spores and germ tubes, but increased activity occurs with appressorium formation. Extracellular proteases, in contrast to the intracellular enzymes, preferentially degrade fibrous proteins such as collagen or its denatured form, gelatin. These proteins, like plant cell wall extensin, contain high proportions of hydroxyproline and can be used as a protease substrate modelling structural plant cell wall proteins. The kinetics of protease formation and the substrate specificity suggest that these enzymes may be involved in cell wall penetration or/and degradation of antifungal plant proteins.

Differentiation-specific mRNA transcripts

Differential screening of DNA libraries is widely used to clone cDNAs corresponding to differentially regulated genes. Using this technique, differentially expressed genes have been cloned from several plant pathogenic fungi such as *Magnaporthe grisea*, *Bremia lactucae*, *Puccinia graminis* and *U. appendiculatus* [18-22].

In order to clone cDNAs representing transcripts formed at late stages of infection structure differentiation of *U. viciae-fabae*, a cDNA library was constructed in λZAPII using mRNA isolated from 20 h old differentiated structures which had formed haustorial mother cells. Probes used for differential screening were cDNAs from 7h (appressoria) and 20h old infection structures (haustorial mother cells). Three pBluescript phagemids harboring cDNA differentially hybridizing to cDNA probes from 7 and 20 h old rust infection structures were used as probes in Northern blots. *Uromyces* genes corresponding to these cDNAs were designated *rif32*, *rif21* and *rif16* (*r*ust *i*nfection). While *rif32* mRNA was only slightly increased in total RNA fron 24h old differentiated (haustorial mother cells) as compared with 24h old germ tubes or 7h old appressoria, *rif16* and *rif21* transcripts were significantly increased in 24h old induced as compared with equivalent non-induced and 7h old induced rust

structures. Detailed analyses have shown that *rif16* induction is first detectable in 18 and 24 h old induced infection structures.

The function of the differentiation-specific genes of *rif16* and *rif21* remains to be determined. However, transcript formation at late stages of infection structure differentiation suggests that the gene products may be of importance in host-pathogen interaction.

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Adhesion of conidia of *Colletotrichum graminicola:* visualization by light and scanning electron microscopy

Eric W. Mercure and Ralph L. Nicholson , Department of Botany and Plant Pathology, Purdue University, West Lafayette, IN 47907, USA

Hitoshi Kunoh, Laboratory of Plant Pathology, Mie University, Tsu, Japan

Ungerminated conidia of *Collectotrichum graminicola* , the cause of anthracnose leaf blight of corn, adhere to leaves within minutes of contact. Scanning electron microscopy and micromanipulation revealed both the alteration of the leaf surface to which conidia had adhered and the presence of an apparent adhesive on the contact surface of conidia. Conidia adhere to hydrophobic, but not hydrophilic, surfaces. Adhesion was not affected by temperature but was affected by inhibitors of glycoprotein transport and protein synthesis. Together, these results suggest that glycoprotein synthesis is involved in adhesion. Conidia that had adhered began to release materials immediately upon contact. The materials could be visualized by scanning electron microscopy (SEM) or by light microscopy after staining for protein or carbohydrate. SEM and micromanipulation revealed that material had been released onto the substratum surface where conidia had made contact with the surface. Gold/silver staining revealed patterns of stain that indicated that proteinaceous materials had been released from the tip of the conidium and at the contact interface of the conidium with the substratum. When adhered conidia were stained with fluorescein (FITC)-conjugated lectins, only FITC-ConA labeled carbohydrates at the contact interface. ConA also bound materials that surrounded conidia. Different patterns of labeling by other FITC-conjugated lectins were also observed, indicating that the material released from a conidium has a specific spatial arrangement around the conidium. Both ConA and the *Lens culnaris* agglutinin lectin blocked adhesion, indicating that the material released from conidia contained glucose and/or mannose. Together, the results suggest that the material associated with

adhesion is composed of glycoprotein. The work emphasizes the importance of adhesion to the infection process and to the phenomena that establish the pathogen at the infection court.

A putative IgG-binding 65 kDa adhesin involved in adhesion and infection of soybeans by Phytophthora megasperma f. sp. glycinea

Hans R. Hohl, Hua Ding, Sylvia Balsiger, and Chantal Guggenbühl

Institute of Plant Biology, University of Zürich, Zollikerstr. 107, CH-8008 Zürich, Switzerland

Using an in vivo adhesion test and a newly developed leaf-disc infection assay we demonstrate that antibodies against mammalian substrate adhesion molecules (SAM), and also preimmune IgG, and D-mannose, inhibit (1) adhesion of the pathogen Phythophthora megasperma f. sp. glycinea (Kuan and Erwin) to the walls of soybean mesophyll cells, and (2) infection and colonization of the host through open wounds. ConA inhibited adhesion and infection in the infection assay but not adhesion in the adhesion test described previously. In control experiments, these compounds had no significant influence on cyst germination rates, or on germ tube growth of the pathogen. D-glucose was able to partially reverse the inhibitory effect of ConA and IgG. Intercellular washing fluids of soybean leaf tissue and surface (glyco)proteins from the pathogen were screened for putative IgG-binding adhesins. From both sources a major 65kDa IgG-binding band was localized on SDS-PAGE gels. Based on ConA and IgG binding, the putative adhesins are (manno)glycoproteins. The study shows that adhesion of the fungal pathogen to the host cell wall is a prerequisite for colonization of the host tissue and that an IgG-binding 65 kDa glycoprotein appears to be instrumental in adhesion.

LESION DEVELOPMENT AND FUNGAL INGRESS: COMPARISON OF MAIZE LINES RESISTANT AND SUSCEPTIBLE TO *BIPOLARIS MAYDIS*

John D. Hipskind and Ralph L. Nicholson, Department of Botany and Plant Pathology, Purdue University, West Lafayette, IN 47907, USA

A significant question in plant disease interactions is to ask when the development of a fungal pathogen in a plant is inhibited. The problem becomes somewhat more complicated when one attempts to relate the cessation of fungal development to the biochemical events associated with the expression of host resistance. We have used microscopy to address this issue in the southern corn leaf blight disease. Maize cultivars that express differential resistance (B73*Ht rhm*) or susceptibility (B73*Ht*) to *Bipolaris maydis* were used to determine the biochemical events associated with resistance expression. Maize does not produce unique phytoalexin-like secondary products as a response to infection. Rather, leaves respond by an augmented synthesis of phenylpropanoid phenols. It appears that the same events that occur in the resistant cultivar occur in the susceptible cultivar, but more slowly or to a lesser extent. The first response is the formation of a lignified papilla in an epidermal cell immediately beneath the site at which an appressorium has formed. If penetration is successful, three phenomena involving phenylpropanoid synthesis and distribution occur. The first is the accumulation of two caffeic acid esters, one of which is caffeoyl glucose, which begin to increase by 16 h in the resistant cultivar. Next, ferulic and *p*-coumaric acids become esterified to the host cell wall. This begins at about 20 h after inoculation in both cultivars, but eventually reaches greater levels in the resistant cultivar. The next step involving phenylpropanoid dissemination is the formation of a "stress" lignin at the margin of the developing lesion in both resistant and susceptible cultivars. This begins at about 72 h after inoculation. The apparent last event in resistance expression is the synthesis of an anthocyanin pigment (cyanidin 3-dimalonylglucoside) in uninfected cells that surround lesions on

the resistant cultivar. We suggest that the synthesis of the pigment serves to remove excess amounts of potentially toxic phenylpropanoids from the leaf tissue. Because the biochemical events in both the resistant and susceptible cultivars are essentially identical, we asked when the pathogen is actually inhibited in the resistant cultivar. Leaf tissue was inoculated and the events in fungal morphogenesis were monitored by light microscopy. Morphogenesis through the time of penetration was the same on both the susceptible and the resistant host. Analysis of leaf tissue after penetration was carried out to determine when growth of the fungus was inhibited. Assays were done by staining the tissue with calcofluor, observing specimens by fluorescence microscopy, and finally determining the apparent volume of the fungus in the tissue by image analysis. The results demonstrated that, in the resistant cultivar, hyphal ingress was arrested by 18 h post-inoculation while lesion enlargement continued for an additional 24 to 36 h. In contrast, in the susceptible interaction hyphal growth and lesion enlargement progressed linearly.

HOST RECOGNITION, HYPHAL MORPHOGENESIS AND INFECTION STRUCTURE DIFFERENTIATION IN ARBUSCULAR MYCORRHIZAL FUNGI

M. Giovannetti, C. Sbrana, A.S. Citernesi and L. Avio.

Istituto di Microbiologia Agraria, Centro di Studio per la Microbiologia del Suolo C.N.R., Via del Borghetto 80, 56124 Pisa, Italy.

In arbuscular mycorrhizae (AM), the infection process is characterized by distinct stages which involve complex changes in both symbiotic organisms, leading to their morphological and physiological integration. Spore germination of AM fungi can occur in the absence of the host, and it seems to be controlled by dormancy and environmental factors. The presence of the host affects the further events of the infection process. The dialogue between the two partners of the symbiosis starts early, after spore germination, by fungal discrimination of arbuscular mycorrhizal hosts from all the other plant species. The recognition process operates through the perception of host signals which promote a morphogenetical response in fungal hyphae. Physiological, cytochemical and molecular studies were carried out with the aim of better understanding the nature of this hyphal differentiation. Factors such as plant or fungal age, nutrient availability and mycorrhizal state did not affect the morphogenetical response in the fungus. The activity of some enzymes such as succinate dehydrogenase, alkaline phosphatase and esterases did not change with plant or fungal age. The length of vital mycelium increased with time up to the sixth day after the beginning of the interaction. The morphogenetical nature of the fungal response suggested that cytoskeletal elements could be involved. Indirect immunofluorescent microscopy and immunoblotting experiments showed differences in tubulin and actin in differentiated hyphae, compared with undifferentiated ones.

VIRUS OF PLANT TRYPANOSOMES (*Phytomonas spp*)

Michel DOLLET[1], Sylvie MARCHE[2], Daniel GARGANI[1], Emmanuelle MULLER[1], Théo BALTZ[2].

1. Unité de Recherche Virologie CIRAD. LPRC CIRAD-ORSTOM - BP 5035-34032 Montpellier Cedex, France.
2. Laboratoire d'Immunologie et Parasitologie Moléculaire, Univ. Bordeaux II, Bordeaux, France.

Trypanosomes have been found in several plant families. Given their general ultrastructural characteristics, they can all be classified in the family Trypanosomatidae. As soon as they were discovered in plants, a new genus, "*Phytomonas*", was proposed. But it seems that this genus is extremely heterogeneous. This has been demonstrated for instance by isoenzyme analysis, or biochemical properties of the minicircles of the mitochondrial DNA.

Cytological localization of plant trypanosomes is already a criterion for distinguishing them. Most of them live in the laticiferous tubes of latex bearing plants. Some have been found in fruits. The last group on which we are particularly concentrating, are located in the sieve tubes of phloem vessels, and are specifically associated with pathological syndromes of cultivated plants (oil palm, coconut, coffee tree, *Alpinia purpurata*) in South America and in the Caribbean. The ultrastructure of different isolates can reveal some differences in the endoplasmic reticulum, the glycosome and sometimes the mitochondrial DNA network. But, it is impossible to be exhaustive (we are currently working with more than 60 isolates) and differences could originate from the age of the culture, or modifications of the basic medium, etc...

A double stranded RNA (ds RNA) was found in 6 phloem restricted isolates associated with pathological syndromes. It was not detected in 10 isolates originating from laticiferous plants or insect trypanosomatids. Analysis by electron microscopy revealed virus like particles by negative staining. Ultrastructural studies of phloem restricted isolates revealed similar virus particles. We intend to use different molecular probes we now possess in order to study virus multiplication *in situ*.

LOCALIZATION OF HOMOGALACTURONIC POLYMERS AND CALCIUM IONS IN THE ECTOMYCORRHIZAL MODEL PINUS PINASTER / HEBELOMA CYLINDROSPORUM

L. Gea (1), B. Vian (1), A. Jauneau (2).

(1) Laboratoire de Pathologie végétale INA P-G,16 rue Claude Bernard 75231 Paris Cedex 05 France. (2) Laboratoire des Processus Ioniques Cellulaires, CNRS URA 203, Université des Sciences et Techniques de Rouen, 76821 Mont-Saint-Aignan, France.

The model used in the present work was an ectomycorrhiza formed by *Pinus pinaster* and *Hebeloma cylindrosporum*. Some IAA-overproducer mutant strains of *H. cylindrosporum* have been isolated (1). When they were associated with their usual host plant, they produce more mycorrhizas than the wild strain from which they were produced.

A morphological approach showed the characteristics of mycorrhizas established with the mutant strain 331 at the ultrastructural level:1) a thick and organized mantle with a thick interhyphal cement in its innermost part; 2) an overdeveloped Hartig net , which can form an actual "fungal tissue" reaching the endodermis; 3) the occurrence of intracellular hyphae in living cortical cells (2).

Among the attempts performed in order to characterize the cell surfaces of both partners (by means of affinity cytochemistry, subtractive cytochemistry, detection of elements), the attention will focus on the detection of pectic compounds and associated calcium. Immunolabeling was performed using a monoclonal antibody JIM 5 which recognizes unesterified homogalacturonic sequences. The labeling is weak on the host cell walls in the Hartig net region, though it is strong in the meristematic region of the root. Moreover, the labeling increases following a chemical demethylation on grids. The localization of calcium was carried out using Secondary Ions Mass Spectrometry (S.I.M.S.) and Energy Dispersive X Ray Analysis. The results show an accumulation of calcium in the overdeveloped Hartig net, probably associated to the fungal material (3).

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EFFECTS OF THE POTENTIALLY TOXIC METALS ZINC AND COPPER ON THE ULTRA STRUCTURE OF *ALNUS* NITROGEN FIXING NODULES

Jeannine Lherminier[1], D. Jones[2], W. J. McHardy[2] and D. Vaughan[2]

[1]INRA, Service Commun de Microscopie Electronique, 21 034 Dijon, Cedex, France
[2]The Macaulay Land Use Research Institute, Craigiebuckler, Aberdeen AB9 2QJ Scotland

Although trees have always been an important crop, current trends in agriculture, particularly in the EU and N. America, have resulted in the diversification towards the growth of more trees on land previously used for cereal production. We are examining roots from trees grown on such land at Lower Affleck farm, Grampian Region, Scotland. A number of contrasting tree species ranging from Sitka spruce (a coniferous evergreen) to sycamore (a deciduous broadleaf) were planted in May 1990. Another species is the non-leguminous N_2 fixer *Alnus glutinosa* and its symbiont *Frankia* present in the root nodules. Some heavy metals are potentially harmful to the N_2 fixing capacity of this actinomycete. This is particularly important as the disposal of heavy metal-contaminated sewage sludge to the forest land in Scotland will increase because disposal of this waste at sea will cease by 1998 under EU regulations. Hence the rationale for examining the effects of heavy metals on the N_2 fixing capacity of *Alnus* nodules.

As part of this study we are examining nodule structure in both the scanning (SEM) and transmission electron microscopes to note any abnormal features that may arise on application of zinc and copper sulphates to pots of sand containing living saplings of *Alnus glutinosa*. The fine roots with associated ectomycorrhizal fungus are being examined in addition to the actinorrhizal symbiont. Cryo-fixed as well as chemically fixed samples were observed in the SEM but the former is less likely to cause artifacts such as shrinkage. Root growth was considerably reduced in pots to which copper and zinc was applied and leaves were fewer in number and wrinkled when compared with those on untreated trees. Data on chemical analyses of leaves and nodules for copper and zinc will be presented.

In addition to the observations on alder saplings grown in sand in pots, we have made observations on the ultrastructural details of nodules from alder taken from a field plot to which zinc-rich sewage sludges had been added.

USE OF ANIONIC COLLOIDAL-GOLD FOR DETECTION OF CHITOSAN IN FUNGAL CELL BY MICROSCOPY.

Dario Maffi, Raffaella Carzaniga and Angelina Carelli

Istituto di Patologia Vegetale, Università degli Studi di Milano, via Celoria, 2, 20133 MILANO (Italy).

Chitosan, a polymer of β-(1-4)-linked D-glucosamine, derived from N-deacetylation of chitin, is a component of fungal cell wall of some fungi (Bartnicki Garcia, 1968). Evidence has been reported that this polycation plays an important role in the host-pathogen interaction, such as elicitation of phytoalexin synthesis, lignification (Hadwinger *et al.*, 1994) and synthesis of callose (Kauss *et al.*, 1989).

The localization of this polymer entails difficulties in obtaining suitable probes. Immunocytochemical (Hadwinger *et al.*, 1981) and cytochemical (Grenier *et al*, 1991) procedures have been developed for the labelling of chitosan; both are time consuming.

The property of chitosan of presenting a net positive charge at pH lower than 6.5 has been exploited to develop a less expensive and quicker procedure.

PROTOGOLD® is a colloidal gold negatively charged produced from Biocell (Cardiff, U.K.) especially for the staining of proteins blotted onto membranes by binding positively charged aminoacid residues. Therefore, we have extended the same principle for the localization of chitosan in fungal cell samples by optical and transmission electron microscopy.

With the aim of avoiding interference by other positive charges it has been necessary to explore a variety of different conditions. These involved a range of buffer pHs, embedding media and concentrations of colloidal gold. Preliminary enzymatic digestion of proteins was also carried out to avoid their possible interference.

Results on different samples derived from *Ustilago maydis* and *Erysiphe graminis* f.sp. *hordei*, are presented . A quantitative evaluation of the labelling was also attempted compared with the chemical method for chitosan detection.

The application of this procedure to the study of plant-microorganism interactions could gain new insights into the role of chitosan during pathogenesis .

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ULTRASTRUCTURE AND CYTOCHEMISTRY OF THE *COLLETOTRICHUM*-BEAN INTERFACE AFTER HIGH PRESSURE FREEZING

O'CONNELL R.J.[1], K. MENDGEN[2].

[1]Department of Agriculture, University of Bristol, IACR Long Ashton Research Station, Bristol BS18 9AF, UK.

[2]Fakultät für Biologie, Universität Konstanz, D-78434 Konstanz, Germany.

The ultrastructure of the interface between cells of bean (*Phaseolus vulgaris*) and biotrophic hyphae of *Colletotrichum lindemuthianum* was studied using high pressure freezing (HPF) and freeze-substitution (FS). The cytoplasm of fungal infection vesicles and primary hyphae was preserved much better by HPF/FS than by conventional fixation and dehydration. Membranes were smooth in profile and organelles had a turgid appearance. Golgi equivalents, endoplasmic reticulum, microbodies and multivesicular bodies were all well-preserved. Golgi equivalents were closely appressed to mitochondria, microbodies and vacuoles.

The fungal cell wall was separated from the host plasma membrane by a finely granular matrix layer of variable thickness. Immunogold labelling with monoclonal antibody JIM7 showed that electron-opaque granules in the matrix contained methylated pectin. The host plasma membrane invaginated around hyphae contained many coated pits, some of which were associated with pectin granules in the matrix. The cytoplasm of infected cells contained abundant vesicles, both small coated vesicles (37-50 nm diameter) and large smooth vesicles (75-100 nm

diameter). The latter appeared to fuse with the host plasma membrane, releasing their contents into the matrix. Host cortical microtubules were most abundant near the invaginated host plasma membrane and closely followed the contours of fungal hyphae.

Our data indicate that intense host exo- and endocytosis at the interface with *C. lindemuthianum* hyphae are associated with secretion of cell wall components from host Golgi into the matrix.

INFECTION STRUCTURES AND HISTOPATHOLOGY IN THE INTERACTIONS BETWEEN BIRCH AND BIRCH RUST.

M. Poteri (1) and L. Ryynanen (2).

(1) Department of Plant Biology, Forest Pathology Section, P.O. Box 28, FIN-00014 University of Helsinki, Finland; (2) Finnish Forest Research Institute, Punkaharju Research Station, FIN-58450, Punkaharju 2, Finland.

The compatible and incompatible interactions between the urediniospores of Melampsoridium betulinum and the two birch species Betula pendula and B. pubescens, were studied by using micropropagated pot plantlets and sterile shoot cultures. Pre-penetrational events on mature and on sterile in vitro leaves were followed with SEM. Light microscopy was used to detect the responses of the mature leaves to rust infections. Both compatible and incompatible urediniospores could germinate on mature and on sterile in vitro leaf surfaces. The germ tubes and specially the growing hyphal tips were attached on the leaf surfaces by adhesive material visible in SEM. This extracellular matrix, which was also secreted by the urediniospores, in some cases formed thicker reticulate layers over the germ hypha and spores. The germ hypha differentiated to appressoria in both compatible and incompatible interactions, although the fungal failings to locate stomata were also common; 27-62% of the appressoria formed did not locate over stomata. The chemical adherence of the germ tubes was weaker on the leaf surfaces of in vitro leaves than on the mature leaves, probably because of the undeveloped epicuticular wax layers on the surfaces of sterile leaves. However, according to the preliminary results, the frequencies for appressoria formations and locations on in vitro leaves were in the range of the frequencies obtained with mature leaves. In light microscopy safranin stained paraffin sections indicated in the incompatible interactions hypersensitive reactions in the palisade and spongy mesophyll cells of inoculated birch leaves.

ADHESION AND HOST SURFACE RECOGNITION BY THE BEECH LEAF ENDOPHYTE *DISCULA UMBRINELLA* (BERK. & BR.)

O. Viret (1) and L. Toti (2).

1) Station fédérale de recherches agronomiques de Changins, 1260 Nyon, Switzerland; (2) Mikrobiologisches Institut, ETH-Zentrum, 8092 Zürich, Switzerland.

The role of the conidial extracellular sheath of the beech leaf endophyte *Discula umbrinella* in recognition and attachment to the host surface has been investigated. After treatment of conidia with different lectins the adhesion of conidia to the host surface was effectively inhibited. A strong fluorescence was observed after treatment with TRITC-labelled concanavalin A. Fluorescence with TRITC-labelled wheat germ agglutinin was observed only after enzymatic digestion with proteases. Observations with the transmission electron microscope (TEM), after labelling sections with colloidal gold conjugated lectins, confirmed the presence of mannose and/or glucose in the extracellular sheath, while chitin was present only in the conidial cell wall but not in the extracellular sheath. Enzymatic treatment of the conidial sheath resulted in strongly reduced attachment and changes in the binding of fluorescence-labelled lectins. TEM studies of partially digested conidia revealed that snail enzyme modified only slightly the structure of the sheath, while proteases completely dissolved the fibrillar sheath leaving a comparatively smooth cell wall. We conclude that a proteinaceous sheath is responsible for the adhesion of *D. umbrinella* conidia to the beech leaf surface and that glycoproteins are involved in the recognition and attachment process.

JEUDI 1 DECEMBRE / *THURSDAY, DECEMBER 1st*

SESSION 3

Altérations cellulaires chez les plantes infectées

Cellular alterations of infected plants

modérateur / *chairperson*

Dr. B. VIAN

ULTRASTRUCTURAL ASPECTS OF THE BIODEGRADATION OF LIGNIFIED PLANT CELL WALLS BY FILAMENTOUS FUNGI

RUEL Katia

Centre de Recherches sur les Macromolécules Végétales (CERMAV.CNRS)
BP 53,38041 GRENOBLE cédex 09.

Among filamentous fungi,White Rotters are the most powerful lignin biodegrading organisms.Due to variations in their complex enzymatic equipment of cellulose and hemicellulose hydrolases together with several oxidizing enzymes involved in lignin degradation,fungal strains do not degrade the wood cell walls with the same efficacy.This results in various patterns of degradation as visualized at the ultrastructural level in Transmission Electron Microscopy.

Using different strains and mutants from Phanerochaete chrysosporium,Phlebia radiata and Dichomitus squalens,we tried to establish a correlation between the observed degradation pattern and the lignin degrading enzymes excreted by the fungus.Thus,typical patterns of defibrillation could be ascribed to the action of manganese peroxidase (MnP).Moreover,biomimetic studies with manganic chelates reproduced the patterns observed for MnP.A combination of cytochemical staining of the polymeric substrates from the wood cell walls,and of immuno-gold labeling of the enzymes from the fungi gave indications on the sequence of intervention of the enzymes into the degradation process.The results suggest that xylanases are involved at a very early stage of the degradation and therefore are key-enzymes in the penetration of fungi in the lignified cell walls.

MORPHOLOGY OF PLANT CELL MODIFICATIONS BY NEMATODES

W.M. Robertson

Scottish Crop Research Institute,
Invergowrie, Dundee DD2 5DA, Scotland, UK

Plant parasitic nematodes cause a variety of modifications to host plant cells dependent on the level of control that the nematode exerts by means of its feeding behaviour. These effects range from simple removal of the cell contents as found with several nematode species to sophisticated levels of control of cell metabolism by *Meloidogyne* species. In between these two extremes there exists a progression of different levels of parasitism. For example an improvement for nematodes which simply remove cell contents consists of entering the root and feeding on the cortical cells. This change provides the nematodes (e.g. *Pratylenchus* species) with an enclosed, protected environment. In contrast, *Heterodera* and *Globodera* have slightly less control of the cells they modify when compared to *Meloidogyne* because they are not able to stimulate nuclear division. However, their modified feeding cells become multinucleate by virtue of the dissolution of adjacent cell walls and fusion of the cell cytoplasm.

Some nematode species have developed the ability to produce feeding tube structures which permit them to remove cytosol without damaging the modified cell cytoplasm. It is these modified feeding cells which attract the greatest interest and this talk will describe and compare the changes induced by endoparasitic (viz. *Meloidogyne* and *Heterodera* spp.) and ectoparasitic nematodes (viz. *Longidorus* and *Xiphinema* spp.).

MYCOBIONT - PHOTOBIONT INTERACTIONS IN LICHENS: STRUCTURAL AND FUNCTIONAL ASPECTS

Rosmarie Honegger
Institute of Plant Biology, University of Zürich, Zollikerstr. 107, CH-8008 Zürich

Lichen-forming fungi are, like biotrophic plant pathogens or mycorrhizal fungi, a polyphyletic group of **nutritional specialists** which acquire fixed carbon from a living photoautotroph partner (usually referred to as the photobiont). In the lichen symbiosis the photobiont is not a multicellular structure as in plants but a population of minute green algal or cyanobacterial cells which are housed and controlled within the thallus of the quantitatively predominant fungal partner (the mycobiont). Lichenization is a successful nutritional strategy, one out of five fungal species being lichenized. The majority of lichen-forming fungi produce relatively simple, often quite inconspicuous crustose or microfilamentous thalli (often termed **microlichens**) by overgrowing or ensheathing compatible photobiont cells on or within the substratum. In about 25% of lichens (often termed **macrolichens**) the fungal partner enters the 3rd dimension by developing morphologically complex, internally stratified, foliose or fruticose thalli in which the photobiont cells are carried along by growth processes and kept in an optimal position with regard to illumination. Water and dissolved nutrients are made available by the mycobiont. It is the fungal partner of these symbioses which competes for space above ground and secures the gas exchange and illumination of the photobiont cell population, an unique situation in the fungal kingdom. Main building blocks of such thalli are hydrophilic, conglutinate pseudoparenchyma, usually as peripheral cortical layers, and a system of loosely interwoven aerial hyphae with hydrophobic wall surfaces in the gas-filled thalline interior. Some of these aerial hyphae are in close contact with the photobiont cells. Their wall surface hydrophobicity is primarily due to a thin, semicrystalline protein layer which reveals a distinct rodlet pattern in freeze-fracturing preparations at the TEM level. In large numbers of lichen species, especially in the Lecanorales, the water-repellency of this proteinaceous wall surface coat is enhanced by mycobiont-derived secondary metabolites which crystallize in and on this hydrophobic rodlet layer. Foliose and fruticose macrolichens are the most complex vegetative structures among fungi. These highly specialized symbiotic phenotypes can be interpreted as the fungal adaptation to the cohabitation with a population of minute photobiont cells; they are impressive examples of the innovative force of the symbiotic lifestyle.

A variety of mycobiont-photobiont interactions have been described in lichens, depending on 1) the taxonomic identity of the partners involved, 2) the structure and composition of the algal cell wall, and 3) the degree of morphological complexity of the thallus. **Haustoria** (transparietal fungal structures) are typically found in morphologically more primitive microlichens with either green algae with cellulosic walls or with cyanobacterial photobionts. **Wall-to-wall apposition** predominates in micro- and macrolichens with green algal photobionts with a peripheral trilaminar wall layer either with sporopollenin-like biopolymers as in the genera *Coccomyxa* and *Elliptochloris*, or without such hydrolysis-resistant compounds as in the genera *Dictyochloropsis* and *Myrmecia*. **Intraparietal haustoria** are formed at the mycobiont-photobiont interface of lecanoralean and teloschistalean macrolichens with unicellular green algae of the genus *Trebouxia* (Microthamniales). The peculiarities of intraparietal haustoria are summarized as follows:

- The cellulosic walls of *Trebouxia* spp. resemble the walls of parenchymatous plant cells with regard to structure and composition. Mature intraparietal haustoria are the product of a co-ordinated development of growing hyphal tips of the fungal partner and juvenile *Trebouxia* autospores.
- The algal wall is not pierced by the fungal infection peg. No attempts are made by the mycobiont to form a large exchange surface in close contact with the plasma membrane of the photoautotroph partner as it is seen in many interactions of biotrophic fungi and plant cells (e.g. in arbuscules of arbuscular mycorrhizae or in the highly specialized, branched or lobate haustoria of rusts or powdery mildews).
- At the very first contact of the growing hyphal tip with the wall surface of the juvenile *Trebouxia* cell the very thin, proteinaceous fungal wall surface layer spreads over the algal wall surface, thus sealing the apoplastic continuum between the partners of the symbiosis with a water-repellent coat. This is an elegant mode of getting access to apoplastic solutes of the algal partner, especially during drought stress periods when the membranes of both partners are getting leaky and a considerable portion of total soluble carbohydrates are passively released into the apoplast (see below).
- Clusters of juvenile autospores are separated from each others and shifted over short distances by intercalary growth processes within the haustorial complex.

Water and dissolved nutrients are passively taken up by the congutinate cortical layers at the thalline periphery, then passively translocated towards the algal layer in the fungal apoplast right underneath the hydrophobic wall surface layer. As **pokilohydrous microorganisms** lichen mycobionts and photobionts are subjected to continuous wetting and drying cycles, *i.e.* to often quite dramatic fluctuations in cellular water contents between saturation and desiccation ($< 20\%$ water $\cdot$ dw^{-1}).

During desiccation the drought-stressed cells of lichen mycobionts and photobionts shrivel dramatically and their cytoplasm is getting strongly condensed but their cellular membrane systems are perfectly well preserved (as seen in ultrathin sections of cryofixed, freeze-substituted samples [1]), pre-requisite for the impressively rapid recovery of the metabolic activity after rehydration. So far it was not possible to visualize drought stress-induced structural alterations in the plasmamembrane of either symbiont, but analyses of leachage fluids of desiccated lichens, collected during the rewetting phase, indicate that the protoplasts of both partners are leaky during stress events; a high percentage of mobile, soluble carbohydrates leaks out into the apoplast during drought stress events. During rehydration the plasmamembranes re-establish, in a yet unknown manner, their structural configuration.

Plant pathologists or experts in mycorrhizal research are likely to consider the lichen symbiosis as a rather exotic case with little or no relations to the fungus/plant interactions they are investigating. Beside all differences there is, however, at least one quite important feature which might be widespread and occur in large numbers of fungal interactions with photoautotrophs: the spreading of the hydrophobic fungal wall surface coat over the wall surface of the photoautotroph partner for adhesion and for directing apoplastic solutes towards the heterotroph. Semicrystalline, proteinaceous rodlet layers are likely to be an ubiquitous feature of aerial fungal structures (conidia, hyphae etc.); they have been structurally identified in large numbers of asco-, basidio- and deuteromycetes [2, 3]. **Hydrophobins**, a group of small, cysteine-rich proteins have been characterized in a range of non-lichenized fungi [2, 3]. In the non-lichenized *Schizophyllum commune* they have been shown 1) to self-assemble at the liquid/air interface into a semicrystalline membrane with a hydrophilic and a hydrophobic side, the latter with a distinct rodlet pattern, and 2) to adhere to hydrophilic surfaces with their hydrophilic side and to hydrophobic layers with their hydrophobic side [2, 4]. It remains to be seen how the proteins of the rodlet layer of lichen-forming fungi are chemically related to the hydrophobins of non-lichenized taxa.

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CYTOLOGICAL ASPECTS OF DISEASES CAUSED BY XANTHOMONAS

B. Boher, M. Nicole, K. Kpémoua, V. Verdier, J.F. Daniel and J. P. Geiger.

ORSTOM, Laboratoire de Phytopathologie, BP 5045, 34032 Montpellier, France.

Plant pathogenic Xanthomonas are gram-negative motile bacteria with single polar flagella. Among the six species of Xanthomonas, X. campestris (XC) includes more than 140 pathovars (pvs) that cause severe damages to economical plants such as cassava, citrus, cotton, crucifer plants, rice and sugarcane. The various symptoms appearing on host plants result either from the intercellular progression of the pathogen within leaf parenchyma or/and from a systemic colonization of all organ tissues.

Numerous XC pvs are found as foliar epiphytes without causing any symptoms. Although this epiphytic stage is poorly documented, scanning electron microscopy and immunohistochemistry have shown the organization of bacterial colonies on the leaf surface, pointing out the preferential localization of the pathogen close to stomata. Attachement of X.c. pv. hyacynthi fimbrial proteins on the stomatal cell surface has been demonstrated by means of immunofluorescence (1). For other XC pvs, it has been noticed that the extracellular polysaccharidic sheath (EPS) surrounding bacterial cells plays an important role in adhesion of colonies to the leaf surface (2).

Direct leaf penetration through stomata and hydathodes was clearly observed and relation of these structures with resistance was established for some host plants. Wounds also increase the number of penetration sites used by XC pvs. for infecting plants, but healing processes characterized by callose deposition, lignification, cell hypertrophy and hyperplasia are able to prevent this way of infection (3). After successful penetration, the bacteria rapidly spread in mesophyll intercellular spaces.

In the incompatible interaction, many plant species has been shown to develop an hypersensitive reaction (HR) in response to XC infection. Contacts between the parasite and host cell walls (HCW) not only induced aspecific plant responses (attachment of bacterial cells to HCW and encapsulation of the pathogen) which do not prevent the multiplication of the pathogen, but may also trigger the HR or other early induced resistance (4). Molecular genetics has greatly contributed to the identification of different sets of Xanthomonas genes involved in XC pathogenesis and elicitation of the HR. Extension of these works opened new research avenues for cytological studies of plant resistance. In this respect, the protein encoded by the gene *AvrBs3* of X.c. pv. vesicatoria was cytolocalized during the infection process in pepper (5).

In compatible interactions between Xanthomonas and susceptible or tolerant host plants, close contacts between the pathogen and HCW were seldom observed because of abundant EPS produced in intercellular spaces. Although the origin of EPS are controversely discussed, immunocytochemistry using of specific monoclonal antibodies raised against (a) EPS and (b) HCW epitopes, strongly demonstrates the bacterial origin of the sheath. During bacterial ingress in the leaf mesophyll, ultrastructural cell modifications were noticed such as damages of primary and secondary HCW and middle lamellae (6), cell vacuolation, bursting of plasma membrane and disorganization of organelles. Numerous Xanthomonas pvs penetrate within xylem vessels of infected plants; however, a few only are able to spread into vessels for infecting areas located at a distance from the initial infection point.

Generally, during compatible interactions, defense-like responses consist in reinforcement of cell barriers such as formation of wall appositions, callose deposition and lignification of HCW evidenced in the parenchyma, phloem and xylem of leaves and stems. Cell hyperplasia lead to the reduction of intercellular spaces thus compartmentalizing infected areas in the aim to stop host tissue colonization by bacteria. Accumulation of electron-dense compounds, fluorescent under UV illumination, was also reported in vacuoles, the cytoplasm and HCW of parenchyma cells. These fluorescent coumpounds were also found to occur within the bacterial EPS and cells. In xylem vessels, the bacterial progession is blocked by lumen and pit occlusions that result in accumulation of pectin- and lignin-like molecules, differentiation of tyloses and formation of HCW coatings.

Besides the HR elicited during the incompatible interactions, the host responses reported here occurred in both susceptible and tolerant plants. These reactions are differentiated early in the time course of infection with a greater intensity in tolerant lants, thus resulting in limitation of the pathogen spread to restricted areas.

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CYTOCHEMISTRY OF PLANT CELL WALL DEGRADATION IN LEAVES OF A SUSCEPTIBLE CASSAVA INFECTED BY XANTHOMONAS CAMPESTRIS PV. MANIHOTIS.

K. Kpémoua, B. Boher, M. Nicole, V. Verdier, J. M. Luisetti and J. P. Geiger.

ORSTOM, Laboratoire de Phytopathologie, BP 5045, 34032 Montpellier, France.

A cytochemical investigation was carried out on the development of an aggressive strain of Xanthomonas campestris pv. manihotis (XCM), responsible for the cassava bacterial blight, to gain better insights into molecular and cellular mechanisms involved in leaf cell wall degradation by this pathogen.

The use of anti-pectin monoclonal antibodies revealed that plant middle lamellae were damaged during the infection process, from the epiphytic stage on the leaf surface to invasion of vascular bundles. In parallel, application of an ß-1,4-exoglucanase-gold probe to healthy and infected tissues indicated that primary and secondary cell walls were also altered. Quantitation of gold labelling confirmed that pectin were more severely degraded than cellulose.

Sheaths (EPS) surrounding bacteria were routinely observed during pathogenesis. They had a dense or loosened fibrillar appearance and were differentiated from the pathogen cell wall, early after leaf inoculation. Close association occurred between extracellular fibrils and leaf cell walls, both at early and advanced stages of wall degradation. Bacterial EPS were often seen deep in altered host cell walls, sometimes enclosing residual plant cell wall fragments.

Our cytochemical data demonstrated that cell wall degradation of cassava by XCM plays an important role in host tissue colonization.

XANTHOMONAS: MUTANTS UNDER THE MICROSCOPE

Ian Brown, John Mansfield and Ulla Bonas*

Biological Sciences Department, Wye College, University of London, Wye, Ashford, Kent, TN25 5AH, U.K. and *C.N.R.S., Institut des Sciences Végétales, 91198 Gif sur Yvette, France.

The ultrastructure of interactions between pepper leaves and *Xanthomonas campestris* pv. *vesicatoria* have been examined. Bacterial development and the plants response were compared using EPS$^-$, Hrp$^-$ and wild-type strains. Immunogold staining was used to locate xanthan and the AvrBs3 protein. "Flag" epitope technology was also applied to the localizatoin of AvrBs3. Strains with mutations in each of the *hrp* loci A, B, C, D, E and F did not cause macroscopically visible symptoms but induced papilla deposition in cells adjacent to bacteria. Each *hrp*$^-$ mutant caused a similar response. Cells of wild-type strain 85-10 were rarely associated with papillae. If wild-type cells were killed in the plant by antibiotic treatment they did induce papilla formation. Results obtained provide evidence that Hrp functions are associated with the suppression of early responses by the plant.

HISTOCHEMICAL STUDY OF A TOLERANT WILD SPECIES OF SUNFLOWER (*HELIANTHUS RESINOSUS*) BEFORE AND AFTER INFECTION BY *SCLEROTINIA SCLEROTIORUM*

L. MONDOLOT-COSSON and C. ANDARY

Laboratoire de Botanique, Phytochimie et Mycologie, Faculté de Pharmacie, Université de Montpellier I, 34060 Montpellier cedex, France

Histochemical observations and chemical analysis of healthy leaves and bracts of *H. resinosus* proved that this tolerant species to *S. sclerotiorum* possesses pre-existent defense factors (cortical fiber cells,sclerenchyma, glandular hairs, epidermic flavonoids and high level of caffeic derivatives).
After inoculation by the pathogen,the infection remained very limited. Caffeic derivatives accumulated just near necrosis and in stomatal cells; this fact suggested that these molecules may play a part in resistance mechanisms. Glandular hair content and epidermic flavonoids are modified as well.
H. annuus does not present these structures and it would be an interesting solution to select high tolerance hybrids using simple histochemical analysis methods.

IN SITU LOCALIZATION OF PLANT AND NEMATODE TRANSCRIPTS IN A MELOIDOGYNE INCOGNITA INFECTED TOMATO ROOT

De Almeida Engler J., Van der Eycken W., Uan Montagu M., Engler G. and Gheysen G.

Universiteit Gent, Laboratorium Genetika, Ledeganckstraat, 35 Gent

Root knot nematodes are highly specialized sedentary endoparasites of plants. One of the major responses to nematode feeding is the induction of giant cells. These cells are multinucleated, show high metabolic activity and are specialized in the uptake of solutes from the vascular system. The maintenance of these giant cells depends on the continuous feeding by the nematode. Simultaneously, plant tissue that surrounds the nematode proliferates giving rise to a swelling known as a "gall".
We have used the technique of mRNA in situ hybridization (ISH) to localize the transcripts of a collagen gene, Lemmi 5 in the root knot nematode Meloidogyne incognita and to localize in the plant roots the expression of a highly induced tomato gene probably involved in protection against osmotic stress, named Lemmi 9. The c-DNA clones used as templates for the synthesis of RNA probes were obtained by the differential screening of a c-DNA library from tomato roots infected with M. incognita. ISH experiments perfomed with the Lemmi 5 probe showed a strong hybridization signal only in the hypodermis and ovaries of adult females. No detectable signal was observed in younger stages of nematode development. On the other hand Lemmi 9 transcripts were detected mainly inside the giant cells of the tomato roots.
A molecular analysis is now carried out in order to characterize the Lemmi 9 promoter in greater detail. The use of this giant cell specific gene for the control of root knot nematode infection is being tested.

HISTOPATHOLOGICAL STUDIES IN BLIGHTED CUMIN PLANT INDUCED BY A. BURNSII.

Kalpna Bhatnagar* and U. Kant.**

* Asstt. Professor, Deptt. of Plant Pathology, Agricultural Research Station, Durgapura, Jaipur, INDIA.

**Professor & Head, Deptt. of Botany, University of Rajasthan, Jaipur, INDIA.

Cumin, belonging to family Umbelliferae, is an important condiment and occupies a significant place among the non-food crops grown in India. *Alternaria burnsii* (Uppal, Patel and Kamat) causes blight disease of cumin, resulting in heavy losses to the crop. The conidia of *A. burnsii* germinate and produce germ tubes on the host surface that penetrate directly through stomata during infection. Histopathological studies of infected seed revealed the presence of mycelium inside the pericarp and endospermic cells, on the dorsal and commissural side of the mericarp. T.S. of infected stem showed a highly disintegrated epidermis and septate mycelium in adjacent cells of vascular bundle. Discoloured, disorganized mesophyll cells were characteristic of infected leaf.

Histochemical studies of various metabolites in the normal and infected tissues, were carried out. Intense localization of insoluble polysaccharides, proteins, lipids and tannins was observed in the diseased stem and seed, as compared to the healthy counterpart. However starch and lignin were less in the diseased material. This study revealed a distrubance in the histochemical profile of the normal healthy plant, due to the impact of the pathogen *Alternaria burnsii.*

HISTOPATHOLOGY OF CASSAVA INFECTED BY XANTHOMONAS CAMPESTRIS PV. MANIHOTIS : INVOLVEMENT OF BACTERIAL EPS IN PATHOGENESIS

B. Boher, K. Kpémoua, M. Nicole, V. Verdier and J. P. Geiger.

ORSTOM, Laboratoire de Phytopathologie, BP 5045, 34032 Montpellier, France.

Xanthomonas campestris pv. manihotis (XCM) is the causal agent of Cassava (Manihot esculenta Crantz) Bacterial Blight. During the infection process, bacteria were surrounded by a fibrillar material that embbeded microcolonies on the leaf surface at the epiphytic stage and filled the intercellular spaces in the parenchyma and xylem vessels of leaves and stems. To further investigate the chemical nature and roles of this bacterial fibrillar matrix in pathogenesis, histological and cytochemical studies were conducted on a susceptible cassava infected with a virulent strain of XCM.

The pink toluidine blue extracellular material was positively stained after the PATAg reaction, indicating the polysaccharidic nature of this structure. Immunogold and immunofluorescence performed in planta revealed an even labelling of the matrix after using a murine monoclonal antibody (mAb) specific for the xanthan exopolysaccharide side chain (EPS) of XC pv. campestris. This matrix was locally labelled when mAbs raised against XCM surface extracellular components were used (see poster 6). In the opposite, no labelling was detected after the use of a polyclonal antibody (pAbs) raised against XCM whole killed cells. Gold probes targeting plant cell wall compounds (mAbs anti-pectin JIM 5 and JIM7, pAbs anti-ß-1,3-glucan, pAbs anti-xylopyranose and a gold conjugated-exoglucanase) did not yield significant labelling when tested on the bacterial structures. These data strongly suggest the bacterial origin of this extracellular fibrillar matrix.

During the infection process, fibrils of the bacterial EPS were always found closely associated with altered areas of host middle lamellae and wall components, indicating that they could be involved in mechanisms of host cell wall degradation. The presence of eroded wall fragments within the matrix suggested the occurence of bacterial cell wall-degrading enzymes within the EPS. Close contacts between the bacterial EPS and host cell surface were also indicative of involvement of XCM EPS in adhesion and recognition. Also, accumulation of EPS within xylem vessels led to occlusions that may partially explain plant wilting.

From our observations, it appears that XCM EPS likely play important roles in Cassava pathogenesis in contributing to adhesion of bacteria on cell surfaces, recognition of plant cell wall structures, plant cell wall degradation and leaf colonization. This suggest that XCM EPS, early and abundantly produced in host tissues, reach healthy tissue areas before the expression of defense genes are visible, thus favouring bacterial multiplication and rapid propagation in the whole plant.

THE USE OF MONOCLONAL ANTIBODIES IN STUDYING PATHOGENICITY OF XANTHOMONAS CAMPESTRIS PV. MANIHOTIS.

B. Boher, K. Kpémoua, V. Verdier, M. Nicole, G. Hainnaux and J.P. Geiger.
Laboratoire de Phytopathologie, Centre ORSTOM, B.P.5045, Montpellier.

Murine hybridomas secreting monoclonal antibodies (mAbs) against surface compounds of Xanthomonas campestris pv. manihotis (XCM) have been prepared after fusing myeloma and spleen cells from Balb/c mice immunized with exopolysaccharides produced in vitro by the pathogen. Six mAbs were obtained and two of them (Bor 9H8 and Bor 18B8) were selected for pathovar specificity.

An indirect immunofluorescence staining procedure was used to test these two mAbs on 70 XCM isolates and 28 different pathovars of X. campestris (XC). Among the 28 pvs. of XC only 2 (pv. vasculorum and pv. euphorbiae) were immunolabelled. All of the XCM isolates reacted positively, except 6 isolates which are non or weakly pathogenic on cassava.

Immunofluorescence and immunogold labelling were performed in planta on fixed, resin embedded tissues from healthy or inoculated leaves and stems. No significant labelling was observed over healthy tissue sections. In infected tissues, a strong fluorescence or an even gold labelling was only observed over the bacterial outer membrane and extracellular matrix close to the bacterial cells; the remaining matrix was devoid of any significant labellin. In highly contaminated tissues, labelling was also observed on the middle lamellae close to bacterial structures.

Controls, including incubation of Bor 9H8 and Bor 18B8 mAbs, with XCM EPS did not yield any significant labelling. In the opposite, labelling was not inhibited after antibodies were incubated with various plant cell wall molecules.

RESPONSES OF LETTUCE TO WILD TYPE AND Hrp$^-$ STRAINS OF *PSEUDOMONAS SYRINGAE* PV. *PHASEOLICOLA.*

Charles Bestwick, Mark H. Bennett, Ian Brown and John Mansfield

Biological Sciences Department, Wye College, University of London, Wye, Ashford, Kent, TN25 5AH, U.K.

A rapid hypersensitive reaction (HR) is caused in lettuce leaves by wild-type strain S21 of *P.s.* pv. *phaseolicola*. Although no macroscopical symptoms developed after inoculation with the *hrpD*$^-$ isolate S21-533 microscopical studies revealed dramatic responses in cells adjacent to bacteria. Localized appositions developed incorporating callose, hydroxyproline rich glycoproteins and phenolic materials. Secretion of deposits was associated with the proliferation of smooth vesicles and multi-vesicular bodies at reaction sites. Increases in peroxidase activity were also detected in plant cell walls close to bacterial colonies. Massive cell wall alterations appeared to lead to the death and collapse of a few mesophyll cells. The presence of isolated dead cells was associated with accumulation of low levels of the phytoalexin lettucenin A. Much greater accumulation of phytoalexins occurred during the HR. It is clear that the *hrpD*$^-$ strain does elicit a number of cellular reactions but fails to cause rapid and irreversible membrane damage which is the characteristic feature of the HR in the non-host.

IMMUNOCYTOCHEMICAL STUDY OF PECTIN OF GRAPE BERRY CELL WALL, SOUND AND INFECTED BY BOTRYTIS CINEREA

I. Chenet (1), B. Dubos (2) and R. Le Menn (1)

(1) Département de Microscopie Électronique, Université Bordeaux I, 351 cours de la Libération, 33405 Talence; (2) Station de pathologie végétale, INRA, BP 81, 33883 Villenave d'Ornon, France.

A preliminary microscopic study of sound berry epidermis cell walls, incubated with Botrytis cinerea pectolytic enzymes, has shown that pectins hydrolysis occured more rapidly at the mature stage of the berry than at the early stage. At the mature stage, the reaction was more obvious in a B. cinerea sensitive grapevine variety (Sauvignon) than in a tolerant one (Arriloba): this result suggests that pectins quality plays a role in this difference of sensitivity.

Therefore we tried to characterize pectins in the grape berry cell walls of sound and infected berries of the grapevine varieties previously mentionned, using two monoclonal antibodies, JIM 5 and JIM 7. JIM 7 recognizes pectins esterified from 35 to 90%, JIM 5 pectins esterified from 0 to 50%. The immunolabelling was performed on ultrathin sections (Roy et al, 1992).

Ultrastructural investigations showed that at the early stage of development, JIM 7 and JIM 5 immunogold labelling was intense throughout the grape berry epidermal cell walls.
At the mature stage, the intensity of JIM 7 and JIM 5 labelling was greatly reduced. In the inferior epidermal cell wall, gold particles were distributed throughout the thickness of the cell wall, whereas in the superior epidermal cell wall, a zone remained devoid of labelling near the plasma membrane.
When the berry has been invaded by B. cinerea, the epidermal cell walls appeared to be completely degraded. But JIM 7 and JIM 5 epitopes remained located in the intercellular spaces and in the transitional zone of the cutine of the superior epidermal cell wall.
At any stage of maturation, before or after B. cinerea infection, the labelling intensity and localisation observed were the same in both varieties.

The JIM 5 epitopes localization was identical to the JIM 7 one : no difference in pectin esterification was perceptible in the cell walls at the same stage, nor during the grape berry development, as if the grape berry pectins were either 35 to 50%, or 0 to 90% esterified. The pectin solubilisation during the fruit maturation explains the pectin labelling reduction at the mature stage.
After B. cinerea invasion of the berry and degradation of the greatest part of the pectins, some remained, probably protected by the cutine in the transitional zone of the superior epidermal cell wall and by the complex structure of the cell wall in the intercellular spaces.

This study was performed on sensitive and tolerant grapevine varieties and unexpectedly the same pectins localizations were observed. So it can be assumed that the pectins esterification probably played no role in the variety's sensitivity.

Acknoledgements. The authors are grateful to Dr. Knox for the generous gift of the JIM 7 and JIM 5 monoclonal antibodies.
This study was supported by the Conseil Interprofessionnel des Vins de Bordeaux.

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ULTRASTRUCUTAL AND HISTO-CYTOCHEMICAL ASPECTS OF A STAGHORN SUMAC WILT DISEASE ASSOCIATED WITH A FORM OF *FUSARIUM OXYSPORUM*

M. Chérif (1), G.B. Ouelette (2) and L. Bernier (2)

(1) Laboratoire de Microbiologie et Biochimie, ESMISAB, Technopôle Brest-Iroise, 29280 Plouzané, France ; (2) Canadian Forest Service, Quebec Region, P.O. Box 3800, Sainte-Foy, Quebec GIV 4C7, Canada

Staghorn sumac plants (*Rhus typhina* L.) showing typical wilt disease symptoms, including xylem discoloration, were collected from several open areas in the surroundings of Quebec city (Quebec, Canada). Culturing from surface sterilized stems and petioles yielded constantly pure cultures of a *Fusarium oxysporum* fungus. Inoculation of healthy plants resulted in the development of disease symptoms identical to those observed in naturally infested plants, including xylem discoloration. Stem and petiole samples obtained form healthy plants and from plants that showed a range of mild to more severe wilting symptoms were examined by light and electron microscopy, and the distribution of the main plant and fungal cell wall components, namely cellulose and chitin, was studied by using specific gold-conjugated probes. At incipient stages of disease development, the fungus was present in only a few xylem vessel elements and little damage was observed in neighbouring tissues. At apparently more advanced stages of disease development, plant tissues were heavily colonized comprising not only xylem vessels, but also neighbouring stelar parenchyma cells, cambial cells and the cortical area. This massive colonization was always associated with marked alteration and distorsion of plant tissues. Ingress of the pathogen through plant tissues was mainly achieved by direct host primary and secondary wall penetration accompanied by intracellular as well as intercellular and intraparietal growth. Fungal cells and microhyphae were often found to be linked with pervading opaque material as well as fibrils or sheath-like structures that seemed to extend freely through host walls resulting in pronounced chiselling and chipping of

secondary walls. A variety of cell reactions were observed in sumac that may be indicative of defense mechanisms, such as papilla formation and suberin deposition. Occlusion of vessels by tyloses and gels was rarely encountered indicating that necroses of parenchyma and cortical cells may be more determinant than vessel occlusion in producing wilt symptoms. The role of cell wall damage in disease expression and anatomical defense mechanisms in this plant disease interaction are discussed in comparison with other fungal wilt diseases the authors have studied.

INTERACTIONS BETWEEN SYMBIOTIC AND PATHOGENIC FUNGI IN ROOTS : STUDIES OF SPATIAL RELATIONSHIPS BY IMMUNOCYTOCHEMISTRY

CORDIER C., V. GIANINAZZI-PEARSON, S. GIANINAZZI.
Laboratoire de Phytoparasitologie INRA/CNRS, SGAP, INRA, BV 1540, 21034 DIJON cédex, France.

The role of arbuscular mycorrhiza in decreasing root damage caused by fungal pathogens has often been studied, but the mechanisms of plant pathogen control are not well understood.

Ultrastructural studies provide an approach for studying tissular and cellular changes which occur in roots as a result of colonization by symbiotic and/or pathogenic fungi, and for understanding the role of arbuscular mycorrhizal fungi in resistance or tolerance to pathogens.

Our research is aimed at analysing interactions between the mycorrhizal fungus *Glomus mosseae* and the pathogen *Phytophthora parasitica var. nicotianae* in roots of tomato (*Lycopersicon esculentum*). A first requisite for cytological investigations for interactions between these two fungi was to be able to discriminate between their hyphae within root tissues. In this study, we adopted an immunocytochemical approach to resolve this problem. Using polyclonal antibodies and light microscope immunocytochemistry, performed on roots infected by each of these fungi, it has been possible to distinguish between the mycorrhizal and the pathogenic fungus within root tissues.

Comparative studies of plant reactions to colonization of root tissues by the symbiotic or the pathogenic fungus have been initiated using fluorescence and cytochemical techniques. In sections of roots infected by *P. parasitica*, necrosis of cortical parenchyma cells invaded by the pathogen was observed. This was associated with a strong fluorescence under blue light, indicating the accumulation of phenolic components. No increase in fluorescence was observed in cells colonized by the mycorrhizal fungus. Likewise, callose detected using β 1,3 glucan antibodies accumulated around intraradicular hyphae of *P. parasitica* but this was not observed during infection by *G. mosseae*.

These results show that antibodies are very useful for distinguishing between *G. mosseae* and *P. parasitica* in root tissues, in order to precisely define cellular events which are associated with plant reaction to the pathogen in mycorrhizal roots.

ULTRASTRUCTURAL MODIFICATIONS OF THE PHOTOSYNTHETIC SYSTEM IN ELICITED SUSCEPTIBLE LEAVES OF PEPPER INFECTED BY PHYTOPHTHORA CAPSICI.

Claude COULOMB, Claude POLIAN, Yves LIZZI et Ph.J. COULOMB
Laboratoire de Cytologie et Pathologie Végétales, Université d'Avignon;
Faculté des Sciences, 33, rue Louis Pasteur; 84 000 Avignon.

Elicitation of susceptible sweet pepper (Capsicum annuum) roots by a fungus derivative elicitor, induces resistance in leaves infected by Phytophthora capsici.Most of infected host cells are intact and develop wall appositions with an heterogenous content and a lytic function (1).

In induced infected leaves, numerous peroxisomes, with a well developped crystal, desert their close association with plastids then surround wall appositions probably to assume a detoxication function (2).In healthy leaves, peroxisomes rarely possess crystals and their association with chloroplasts priviligiates the photorespiration processes.

After a short infection time (48h), no chlorosis symptoms were observed. Elicitation brakes the damages caused by infection. Fungus penetration provokes an acidification of the host-plant tissues with many resulting perturbations as pheophitinisation and degradation of chlorophylls (3).Inside the chloroplasts the grana structures are disorganized and disappear and the stroma saccules increase in number. Consequently, photosynthesis is affected principally by the PSII -grana thylakoïd dissociation.Then, the saccules, associated in parallel structures, produce pseudo-crystallin formations resulting probably of the membrane plastidial protein recombination. In infected leaves, plasto-quinone-globuli increase in number, still more in previously induced ones, go through the plastid enveloppe towards apposition-wall to participate in defence mechanisms.

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2 - Trans-cinnamate 4-hydroxylase activity in host-parasite interaction: Capsicum annuum-Phytophthora capsici. I. SAIMMAIME, C. COULOMB et Ph.J. COULOMB,Plant Physiol. Biochem.,29, p481-487, 1991.

3 - Comparative effects of a fungal and viral infection with the action of an elicitor on the photosynthetic pigments of the leaves of susceptible and resistant varieties of Capsicum annuum. M. BOUNIAS, C. COULOMB, Y. LIZZI and Ph.J. COULOMB. Indian J. agric. Biochem., 1, 1-10, 1988.

INTERACTIONS BETWEEN A VESICULAR ARBUSCULAR MYCORRHIZAL FUNGUS, *GLOMUS* SP. AND THE ROOT - KNOT NEMATODE, *MELOIDOGYNE JAVANICA*, ON *ACACIA SEYAL* AND *ACACIA HOLOSERICEA*

Duponnois, R.(1), Tabula, T.K. (2), Senghor, K. (3) & Cadet, P. (1)

(1) ORSTOM, Laboratoire de Nématologie, B.P. 1386, Dakar, Sénégal. (2) Centre Africain de Recherches et de Formation Phytosanitaire, B.P. 409, Dschang, Cameroun. (3) Université Cheik Anta Diop, Faculté des Sciences et Techniques, Dakar, Sénégal

Vesicular and arbuscular mycorrhizal fungi (VAM) had potential as biocontrol agents when both groups of microorganisms (plant - parasitic nematodes and VAM) occured simultaneously in the roots or rhizosphere of the same plant. Our study investigated the interactions between *Meloidogyne javanica* and an endomycorrhizal fungus, *Glomus* sp. on *Acacia seyal* and *Acacia holosericea* , a Sahelian and an Australian species with considerable potential for use in reafforestation in Sahelian areas but very susceptibles to *M. javanica* .

In pots, mycorrhizal and non mycorrhizal seedlings have been inoculated with different nematode inoculum densities (100, 250 and 2000 J2s per pot). One month after the nematode inoculation, the biomass and the nematodes development were measured. An histological study was carried out on the development of *M. javanica* in the roots of both acacias.

All the mycorrhizal plants with or without *M.javanica* grew significantly better than non mycorrhizal plants infected or not by *M. javanica* . The mycorrhizal application did not reduce nematode population per plant but significant differences have been measured between nematode populations from mycorrhizal and non mycorrhizal roots of each plant. Mycorrhizae markedly influence plant health by altering plant reactions to nematodes. The mechanisms which could be involved in these interactions are discussed and the possibilities to improve this mycorrhizal effect are exposed.

HISTOLOGICAL EVOLUTION OF GRAPEVINE KOBER STEM GROOVING SYMPTOMS IN SENSITIVE ROOTSTOCK TISSUES

C. Greif, S. Chevalier*, P. Bass and B. Walter

INRA, Station de Recherches Vigne et Vin, BP 507, 68021 Colmar, France
* SANOFI, La ballastière, BP 126, 33501 Libourne, France

Rugose wood of grapevine is a complex of at least four diseases, all thought to be of viral origin, which are distinguished by the specific reaction they induce on different grapevine rootstock species: Rupestris stem pitting (RSP), Kober stem grooving (KSG), corky bark (CB) and LN33 stem grooving. Recently two closterolike viruses, grapevine virus A and B, have been associated respectively with KSG and CB. In natural conditions rugose wood symptoms are expressed in 2-3 years after grafting on sensitive rootstock and extend basipetally from the graft point. We have developped a green cutting-grafting technique allowing to see the appearence of clear KSG and RSP symptoms by observation of coarse sections of the rootstock stem under a microscope 6 to 8 months after grafting. To follow the development of the first anatomical disorders of KSG, in parallel with the spread and the multiplication of GVA in the infected tissues, pieces of Kober 5BB stem just below the graft point were included in LR white resin every month from the grafting date. Fine sections were analysed after colouration with orthotoluidin blue or after immunolabelling with IgG gold conjugates and silver enhancement. At 2 months post-grafting the first abnormalities appeared as parenchimatoses in the xylem rays and squashing of pericyclic fibres. GVA could be detected in few zones within phloem parenchima cells and in some adjacent phloem ray cells. After 4 months symptoms progressed to extended phloem and xylem disorders (in which GVA became difficult to detect) surrounded by healthy looking tissues apparently free of virus.
RSP and KSG are also characterized by their latency in *Vitis vinifera* cultivars as in sensitive rootstock species unless these are grafted together. GVA was back-inoculated to Kober 5BB cuttings by "heterografting" on infected *Nicotiana benthamiana*. Immunolabelled stem sections of GVA-infected Kober 5BB were observed before and after grafting with healthy *V. vinifera* to study the virus behaviour in latent and in symptomatological KSG context.

HISTOLOGICAL AND BIOCHEMICAL STUDIES OF RUSTS/POPLAR INTERACTIONS. (*Melampsora larici-populina/Populus*).

F. LAURANS[1], S. CHARTIER[1], F. LEFEVRE[1], C. JAY-ALLEMAND[1], G. SALLE[2] and G. PILATE[1].

[1]INRA, Station d'Amélioration des Arbres Forestiers. F 45160 Ardon, France.

[2]Université Pierre et Marie Curie, Laboratoire de Cytologie et Morphogenèse Végétales, F 75230 Paris Cedex 05, France.

Melampsora leaf rust is the most widespread and serious foliar disease for poplar species and their hybrids. Severe damage has been attributed to these pathogens including growth reduction the following year or even death of the plant (1).

Our objective is to characterize histological and biochemical level the response of poplar to rust infection. Toward this end, we use an *in vitro* test consisting of leaf disks of *Populus nigra x P. deltoides* clones inoculated with different physiological races of *Melampsora larici-populina*. Depending on the poplar clone or on the physiological race used, the response will be compatible or incompatible. In compatible interaction, the first infection symptoms appear 8 to 9 days after inoculation. Sampling are performed daily during the week following inoculation.

Pathogenesis development of the fungus was studied by light microscopy and scanning electron microscopy. We describe the early stages of infection for both interactions (compatible, incompatible): spore germination on leaf epidermis, penetration of the developing hyphae through the stomata.

A lot of studies have outlined the role of phenolic compounds in defense response (phytoalexines). In order to establish correlations between phenolic content and biological response of the poplar leaf to pathogen attack, we performed kinetic analysis of leaf phenolic compounds after inoculation using high performance liquid chromatography and thin layer chromatography. Biochemical data will be discussed in comparison with histological observations.

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IN VITRO MYCORRHIZA INITIATION OF LARCH SOMATIC EMBRYO PLANTLETS WITH FOUR BASIDIOMYCETES : structural and functional aspects.

Piola F., Radawiec S. and Rohr R.

Laboratoire Interactions Plantes-Champignons et Micropropagation, Université Claude Bernard Lyon I, Bât. 405, 69622 Villeurbanne Cedex.

Somatic embryos of an hybrid larch (*Larix x eurolepis*) were grown under aseptic conditions in Petri dishes and were inoculated with four ectomycorrhizal fungi i.e. *Laccaria laccata* (Scop ex Fr.) Berk. et Br., *Hebeloma cylindrosporum* Romagnesi, *Suillus grevillei* (Klotzsch) Sing., (= *Boletus elegans* (Schum. ex Fr.) Goldrohrling) and *Pisolithus tinctorius* (Pers.) Coker and Couch.

The mycelia of *L. laccata*, *H. cylindrosporum* and *S. grevillei* grew first around the root system of the plantlets whereas the mycelium of *P. tinctorius* grew all over the surface of the agar medium.

After one month, only 25% of the control plantlets exhibited root growth. Furthermore no lateral roots were produced, even after six months of subculture.

The roots of 64.5% of the plantlets associated with *L. laccata* elongated and ramified within one month after inoculation. During the same period of time, only root elongation was highly stimulated in the case of plantlets inoculated with *H. cylindrosporum* (80%) and *P. tinctorius* (70%). Root ramification occurred less frequently i.e., 27% and 17% after one month respectively, but it reached 70% and 36% of the plantlets 3 months later.

Root system stimulation due to the plantlet/ectomycorrhiza association was concomitantly accompanied with an enhanced growth of the aerial parts of the plantlet.

A conspicuous Hartig net was noticed in growing roots inoculated with *L. laccata* and *H. cylindrosporum*. In the case of *P. tinctorius*, Hartig net was not observed. Nevertheless isolated living hyphae were recorded in the intercellular spaces of the cortical root parenchyma. The presence of mycelium of *S. grevillei* in the root of inoculated plantlets remains to be demonstrated.

Our study shows that a direct correlation exists between the efficiency of the plantlet/fungus association and the presence of the Hartig net in the root cortex.

HOST-PATHOGEN INTERRELATIONSHIPS OF UROMYCES CARYOPHYLLINUS AND CARNATION CELLS

J.M. Plotnikova

Department of Plant Protection, Main Botanical Garden Russian Academy of Scienses. Moscow 127276. Russia.

Cytological study of host-pathogen interrelationships of Uromyces caryophyllinus and carnation cells was carried out at various stages of infection development starting from uredospore germination to formation of new uredospores. Differentiation of U. caryophyllinus infection structures started at 32°C. Variation of heat shock temperature led to decrease in percentage of full infection structure formation. The pathogen developed slowly, its incubation period was equal to 21 days. U. caryophyllinus formed dicaryotic intercellular mycelium and haustoria at its parasitic stage. The most evident alterations of host cells concerned the host nucleus. We found that volume of infected leaf mesophyll cells at mass sporulation enhanced considerably in comparison with healthy cells. At the same time IAA content in infected leaves was two times higher than in healthy leaves. Cytokinin concentration increased in rust-infected leaves during incubation period. Abscisic acid concentration lowered in infected leaves in comparison with healthy leaves. U. caryophyllinus is seEmed to effect synthesis of physiologically active substances accompanied with host cell hypertrophy to attract host metabolites.

ACTION OF CAPSIDIOL ON SUSCEPTIBLE PEPPER CULTIVAR PROTOPLASTS.

Claude POLIAN, Claude COULOMB, Yves LIZZI et Ph.J. COULOMB.Laboratoire de Cytolologie et Pathologie Végétales, Université d'Avignon, Faculté des Sciences, 33,rue Louis Pasteur, 84 000 Avignon.

Numerous works report that plants react to fungal infections by synthesis of phytoalexins which stop the pathogen growth. In pepper leaves, infected by Phytophthora capsici, cells produce a bicyclic sesquiterpene: the capsidiol.

Capsidiol at 1000 $\mu g/ml^{-1}$caused total lysis of protoplasts isolated from pepper leaves of susceptible and resistant strains to Phytophthora capsici (1).At lower capsidiol concentrations, the ultrastructural examination shows very important alterations of cell plasmalemma and all the endomembranous system is also highly affected: endoplasmic reticulum is dilated and vesiculated, Golgi cisternae present a typical circular degenerated form, mitochondria are swelling and their cristae dilated, vacuoles become smaller and increase in number.

All these ultrastructural alterations confirm previous observations realized on in situ infected leaves (2).and on fungus isolated membranes incubated with 250 μg of capsidiol per millilitre showing 50% and 33% decrease respectively of the protein and the phospholipid content (3)

Observations with electron microscopy revealed dramatic perturbations caused by capsidiol affecting the chloroplasts of purified protoplasts. These organelles are swollen, their grana disorganized disappear and crystalline-like formations are observed in the stroma as in situ in induced infected leaves. The chemical characterization of crystals was realized by enzyme digestions and immunological test for Rubisco detection.

1 - Apprécіation du pouvoir phytotoxique du Capsidiol et d'un inducteur de résistance au Phytophtora capsici sur des protoplastes de piment. C.POLIAN et Ph.J.COULOMB C.R.Acad.Sc.Paris,298,p237-242,1984.

2 - Etude cytologique du mode d'action du Capsidiol sur les hyphes de P.capsici. M. TURELLI, C.COULOMB, S. MUTAFTSCHIEV et Ph.J.COULOMB. Can. J. Bot., 64, p701-709, 1986.

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COMPARED HISTOLOGY OF SOUND BEANS CORTEX OF *THEOBROMA CACAO L.* IN CONNEXION WITH THEIR SENSITIVENESS TO *PHYTOPHTHORA MEGAKARYA* .

RUZZIER-DEBOST M.*,GRILLET L.*,NGUEFACK J.*,
MICHAUX-FERRIERE N.**.
* Université de Provence , Laboratoire de Physiologie cellulaire végétale , 3 Place Victor Hugo 13331 MARSEILLE Cedex 3 .
**CIRAD,Laboratoire de Cytologie BIOTROP,BP 5035, 34032 MONTPELLIER cedex .

Phytophthora megakarya is , in Cameroon , responsible for the appearing of necroses typical of the *Phytophthora* pod rot on the cortex of Theobroma cacao L. fruit . A disease whose economical consequences can be very important . As the chemical fight against *Phytophthora_megakarya* appears to be expensive and difficult to put to use, pathogene resisting clones have been researched .
We wanted to check the hypothesis according to which the soluble phenolic compounds present within the cortex of sound fruit would be biochemical markers of resistance to *phytophthora megakarya* .
Our studies were focused on the cortex of two clones sound fruit mesocarpes SNK 10, very sensitive to *P.megakarya*,and SNK 413 ,hardly sensitive to the pathogene.
The microscopic observation of a transversal section showed the presence of compounds - which happened to be phenolic compounds - under the shape of small intra cellular drops displayed in spans towards the vascular strands or scattered within the cells .
The size and the number of phenolic inclusions varied according to the sensitiveness of the clone towards the parasite . The parenchyma of the very sensitive SNK10 clone fruit cortex showed few phenol spans and also few vascular strands. The transversal section of the SNK 10 clone fruit cortex showed a single span of phenols towards one vascular strand . The parenchyma of the hardly sensitive SNK 413 clone fruit cortex showed several spans of phenol towards vascular strands. These observations are to be improved and precised by the use of histochemical reactions of phenolic acids or flavonoids .
All these observations corroborate the results of our biochemical analysis realized by H.P.L.C. and by the method of Folin-Ciocalteu on the sound fruit cortex of SNK 10 and SNK 413 clones : The rate of total phenols and of flavanols is much weaker with SNK10 than with SNK 413 .
As a conclusion , we can say that the presence of phenols within the fruit mesocarpi can be considered :
- either as the evidence of the existence of natural "fongitoxic" substances whose more or less important rate can be used to select clones less sensitive to the pathogene agent, *Phytophthora megakarya* ,
- or as a way to research on mechanisms of increased synthesis responding to fongic aggression .

ULTRASTRUCTURAL CHANGES IN TOMATO PLANTS INFECTED WITH *CLAVIBACTER MICHIGANENSIS* SUBSP. *MICHIGANENSIS.*

L. Varvaro (1), A. Fabi (1), C. Angelaccio (2) and A. R. Taddei (3).

Università della Tuscia, 01100 Viterbo, Italy: (1) Dipartimento di Protezione delle Piante; (2) Dipartimento di Scienze dell'ambiente forestale e delle sue risorse; (3) Dipartimento di scienze ambientali.

Three different genotypes of tomato were infected with *Clavibacter michiganensis* subsp. *michiganensis,* the etiological agent of bacterial canker of tomato, then ultrastructural changes were followed by transmission electron microscopy (TEM). Tomato plants were characterized by a different susceptibility to the pathogen: *Lycopersicon esculentum* cv. ISI (susceptible), *L. esculentum* cv. CORDEOK (partially resistant) and *L. peruvianum* accession 1573 (resistant).

After inoculation with a bacterial suspention, samples of tomato stems were taken until 20 days, prepared (fixation, dehydration, infiltration and embedding, microtomy) and observed to the TEM.

Up to three days from the inoculation, no bacteria were detected inside tomato tissues. Afterwards, bacteria were found in all three genotypes, at first in the intercellular spaces of parenchymatous tissues and then in xylem vessels, too. Their number increased in time as observed by quantitative assayes.

Necrosis of plant cells were noted only 10 days after inoculation. On 'ISI' more cells were damaged and necrotic areas appeared larger than on 'CORDEOK' and *L. peruvianum.*

Our data indicate a good correlation between the degree of susceptibility and the bacterial colonization of tomato tissues. Since the values of bacterial cells are similar in all three genotypes, further studies will be necessary to explain the different susceptibility to bacterial canker.

It needs to answer the following questions concerning the resistant genotype *L. peruvianum*: a) Are bacteria confined in a few cell layers? b) Are bacteria limited in producing high quantity of wilt inducing toxin? c) Is the toxin unable to carry out its pathogenetic role?

ULTRASTRUCTURE OF BANANA ROOTS INFECTED BY THE BURROWING NEMATODE RADOPHOLUS SIMILIS.

C. Valette*, M. Nicole, J.L. Sarah*, M. Fargette*, M. Boisseau* and J.P. Geiger.

* Laboratoire ORSTOM/CIRAD de Nématologie, CIRAD, BP 5035, Montpellier (France).
ORSTOM, Laboratoire de Phytopathologie BP 5045, Montpellier (France).

The burrowing nematode Radopholus similis (Rs) (Cobb) Thorne is an important root pathogen of banana plants. The infection reduced plant vigour as well as bunch size and weight. Although it is known that the nematode population is low in some banana cultivars, defense to Rs is poorly documented. Histological and ultrastructural investigations were undertaken on in vitro plantlets for studying the infection process at the cellular level, both on susceptible and resistant banana cultivars (cv).

In the susceptible cv, the nematodes penetrated the plant after degradation of the root surface. Pathogen ingress within host tissues was characterized by the occurrence of large tunnels, resulting in heavy host cell wall alterations. Host cells close to the nematode displayed various cytoplasm modifications seen in the cortex and the vascular bundle. Hypertrophied cells close to the endodermis were also observed.

Root penetration appeared to be less important in the resistant cv. Nematodes were only found to be localized in the root cortex. Plant cell damages were less important and hypertrophic cells were not observed in infected roots. However, numerous cells, both in the cortex and the vascular bundle, were seen to accumulate electron-dense compounds in the vacuole, early after root inoculation. These compounds that are blue-stained by the toluidine blue could be from phenolic origin.

In light of our data, resistance of banana root to Rs may result from :

- a limitation mechanism to root penetration by Rs,
- a reinforcement of host cell walls during Rs ingress in root tissues,
- and/or the production of phenolic compounds.

ULTRASTRUCTURAL AND CYTOCHEMICAL RESULTS ON *GREMMENIELLA* INFECTED RED PINE

A. Ylimartimo[1], G. Laflamme[2], M. Simard[2], and D. Rioux[2]

[1] Dept. of Forest Ecology, P.O. Box 24, FIN-00014 University of Helsinki, Finland

[2] Canadian Forest Service-Quebec Region, P.O. Box 3800, Sainte-Foy, G1V 4C7, Canada

The development of the infection by *Gremmeniella abietina* at the cellular level of the host is largely unknown. An ultrastructural and cytochemical study based on colloidal gold techniques was undertaken to elucidate the infection processes of this pathogen in *Pinus resinosa* seedlings.

Hyphae of the pathogen were observed along middle lamellas, in cell walls and inside the cells of the bract and short shoot tissues of the infected seedlings. Obviously, the pathogen cells in those tissues penetrate into host cells by enzymatic degradation of the host cell walls. An extracellular fungal sheath (EFS) surrounded the pathogen on the host surface as well as inside the host. The fibrillar material of EFS appeared at times spread along the host surface, presumably contributing to attaching the hyphae to the surface. Inside the host cell, the fibrillar material of EFS seemed to be connected to the remnants of host cytoplasm and sometimes the fibrils seemed to extend and intersperse into the host cell wall material.

The labelling result with exoglucanase-gold complex suggest that the fungal wall and cytoplasm of *G. abietina*, even inside the host, do not contain cellulose. However, EFS and its fibrils interspersing into the surrounding (labelled) host cell wall appeared partly labelled, indicating a close contact between the fungal sheath and the host cell wall components.

Our study was the first attempt to use JIM-5 and JIM-7 antibodies to localize pectin

in gymnosperm tissue. Only sparse labelling of middle lamellas or host cell walls or only inner cell wall appositions were observed in *Gremmeniella* infected red pine. Possibly, the chemical nature of pectin in gymnosperms may be somewhat different from that of angiosperms or chemical links of pectin with other wall constituents such as lignin may mask the epitopes of pectin molecules in the studied tissues.

Our preliminary results suggest that EFS of *G. abietina* may be implicated in host-pathogen interactions, such as attachment of hyphae to the host surface and degradation of host cell walls inside host tissues.

VENDREDI 2 DECEMBRE / *FRIDAY, DECEMBER 2nd*

SESSION 4

Mécanismes de contrôle des infections et réactions de défense des plantes

Control mechanisms of infection and plant defense.

modérateur / *chairperson*

Dr. P. BONFANTE-FASOLO

CYTOLOCALISATION OF PLANT DEFENCE RESPONSES TO FUNGI IN ROOT TISSUES DURING SYMBIOTIC AND INCOMPATIBLE INTERACTIONS

GOLLOTTE A., V. GIANINAZZI-PEARSON, S. GIANINAZZI.
Laboratoire de Phytoparasitologie INRA/CNRS, SGAP, INRA, BV 1540, 21034 Dijon Cédex, France.

In nature, plants are continually confronted with a wide range of micro-organisms, amongst which are a certain number of root-colonizing pathogenic or symbiotic soil-borne fungi. Whilst root pathogens generally show a limited host range, the large majority of terrestrial plants show a remarkably widespread susceptibility to fungi forming symbiotic arbuscular mycorrhizal associations. This means that most plants must have evolved systems to distinguish between harmful and beneficial root-infecting fungi and that these must be based on widely occurring molecular and genetic determinants. The cellular processes essential to such discrimination can only be fully appreciated through analyses of the spatio-temporal nature of events at the tissue and cellular level. Comparative *in planta* investigations of defence responses in tobacco and pea roots forming compatible or incompatible interactions with symbiotic or pathogenic fungi show that distinct structural and molecular modifications are associated with each type of infection.

Defence-associated reactions like wall thickening, phenolics accumulation, callose deposition or PR-protein synthesis are only weakly activated and are limited to very localized domains within individual root cells colonized by the arbuscular mycorrhizal fungus *Glomus mosseae* (1,2), whilst such cell responses show a strong, more generalized elicitation throughout infected root tissues during development of resistance to a pathogenic fungus like *Chalara elegans* (3,4). Single locus mutations have been induced in pea which do not change the infection phenotype nor cell responses to pathogens, but which completely alter plant behaviour towards arbuscular mycorrhizal fungi. Specific resistance develops towards the symbiotic fungi in the mutant plants (myc^{-1}) and this incompatibility is characterized by inhibition of hyphal penetration at the root surface (5). Events associated with mutant resistance result in the formation of wall thickenings containing phenolic compounds, callose and PR-protein in root cells adjacent to hyphae (6). These observations provide evidence that arbuscular mycorrhizal fungi do possess elicitors of plant defence responses and that these

must be somehow repressed or suppressed during symbiotic interactions with host plants. The fact that repression of defence responses to the symbiotic fungi is inactivated by mutation of a single plant gene means that it must be under specific gene control. Furthermore, since at least four independently mutated loci give the same resistant phenotype (5), such control must be multigenic. We speculate that activation of specific symbiosis-related plant genes by arbuscular mycorrhizal fungi suppress or diminish defence gene expression in host roots so that the outcome of root fungal interactions is determined at an early stage.

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COMPARTMENTALIZATION IN TREES: HISTORIC AND NEW FINDINGS DURING THE STUDY OF DUTCH ELM DISEASE.

Rioux, D.
Natural Resources Canada, Canadian Forest Service-Quebec Region, 1055 du P.E.P.S., P.O. Box 3800, Sainte-Foy, Quebec G1V 4C7, Canada.

Compartmentalization processes have been reported many times to explain tree resistance to various diseases. They are based on the formation of anatomical barriers that limit the extent of affected tissues in the xylem as well as in the bark. Although the first observations of these barriers were made in 1887, their importance was recognized only around 1977 when the CODIT (Compartmentalization Of Decay In Trees) model was proposed to explain how the tree limits the colonization of decay-causing fungi. This model involves four walls, the first three called reaction zones associated with cells present at the moment of infection and the wall 4, or barrier zone (BZ), formed by the cambium in response to the infection. Some of these historical aspects also deal with recent findings which suggest that compartmentalization happens when the water content becomes too low in different tree tissues.

In studying Dutch elm disease, we found that BZ formation occurs early after inoculation and is continuous around infected xylem tissues in two nonhosts, Prunus pensylvanica and Populus balsamifera. In the American elm, the BZ was usually absent or discontinuous; in the latter case, twice as much time was necessary for its formation as compared to the nonhosts. Histochemical tests revealed that lignin and suberin impregnate the walls of BZ cells whereas phenols were detected within these cells.

The wall 3 impeding the tangential colonization of microorganisms has been reported of being formed by rays and thus would be discontinuous. In our nonhosts, this reaction zone was efficient but gels and tyloses were also important constituents of this zone. Cytochemical tests revealed that gels and tyloses are related through pectin secretion mechanisms.

P. balsamifera also responded to inoculation by forming a suberized layer between the pith and the invaded xylem. This new zone was quite similar to suberized bands described by other workers in herbaceous plant species infected by pathogens causing wilt diseases.

Wall layers were identified as being suberized in light microscopy by intense autofluorescence but did not always present a clear lamellar structure when examined in transmission electron microscopy. Such unlamellar suberized layers having a whitish appearance might even be interpreted as being a separation between wall layers. The use of an exoglucanase complexed to colloidal gold demonstrated frequently that cellulose is closely associated with various suberized layers.

Finally, preliminary results on the physiology of compartmentalization are presented, in particular the role that abscisic acid may play during these processes.

RHIZOBIUM-LEGUME SYMBIOTIC INTERACTIONS: NODULE DEVELOPMENT AND DEFENCE MECHANISMS

F. de Billy, J. Vasse, F. Maillet, M. Ardourel, F. Debellé, C. Rosenberg, N. Demont*, P. Roche*, M. Pichon, A. Dedieu, E-P. Journet, D. G. Barker, J-C. Promé*, J. Dénarié and G. Truchet
Laboratoire de Biologie Moléculaire des Relations Plantes-Micro-organismes, CNRS-INRA, BP 27, 31326 Castanet-Tolosan Cédex, France.
*Laboratoire de Pharmacologie et de Toxicologie Expérimentales, CNRS, 205 route de Narbonne, 31077 Toulouse Cédex, France.

The symbiotic interaction between leguminous plants and rhizobia, soil bacteria currently classified into three genera *Rhizobium*, *Bradyrhizobium* and *Azorhizobium*, results in the formation of nodules, novel root organs in which atmospheric nitrogen is converted into ammonia, thereby providing the plant with an alternative to soil-based inorganic nitrogen. Nodule induction is specific and a given rhizobial strain can infect a limited number of hosts. Symbiotic nodule formation depends on two finely balanced processes: a controlled infection and the development of the nodule. In temperate legumes such as alfalfa, the bacteria invade the plant by means of infection threads which are initiated from curled root hairs and which grow towards the plant cortex (13). Simultaneously, inner cortical cells which are often located in front of growing threads, dedifferentiate and a nodule primordium is formed (13). The activity of the nodule meristem, which is subsequently initiated on the outer side of the primordium, accounts for the consequent growth of the nodule over several weeks. At maturity, nodules developing on most of the temperate legumes are of the indeterminate type (15). Longitudinal sections reveal the presence of central zones including the apical meristem and the central nitrogen-fixing zone and peripheral tissues (32). In this type of nodule, nodule growth and functioning occur simultaneously and all intermediates in differentiation can be observed within a single longitudinal section of a nodule (32). Another type of nodule, the so-called determinate type, develop generally on tropical legumes. In these plants, infection occurs either via root hairs or intercellularly, the meristem is induced in the outer cortex, meristematic activity is restricted to a short period of time and meristematic cells differentiate simultaneously to form the nitrogen-fixing tissue (15, 26). As a result, determinate nodule growth and functioning are dissociated, and only a single stage of plant and bacterial differentiation can be observed at any particular moment in time.

Current interest in *Rhizobium*-legume interactions has focused on the discovery that there is an exchange of signals between the two

organisms during very early stages of the interaction. The molecular dialogue between symbionts is initiated when root-secreted plant flavonoids and NodD proteins act in conjunction as transcriptional activators of a set of nodulation (*nod*) genes required for infection, nodule formation and the control of host specificity (7, 10). Proteins encoded by the activated *nod* genes contribute to the synthesis of extracellular lipo-oligosaccharide molecules known as Nod factors (7, 14, 20, 23, 24, 25). The Nod factors produced by different species of rhizobia share a common basic structure; they are β-1,4-linked tetramers or pentamers of N-acetyl-D-glucosamine with a partially unsaturated fatty acid chain N-acyl linked to the terminal non-reducing sugar residue. Nod factors differ in terms of the substituents linked to the chitin oligomer backbone. For example, the *Rhizobium meliloti* nodulation (NodRm) factors are O-sulfated on the C6 of the terminal reducing sugar (14), O-acetylated on the C6 of the terminal non-reducing sugar (1, 20), and mono-N acylated by unsatured C16 acyl groups and a series of hydroxylated fatty acids (3, 6, 7, 20).

The role of the various structural *nod* genes in the control of Nod factor biosynthesis is currently under study. The so-called common *nodABC* genes are involved in the synthesis of the lipo-oligosaccharide core (2, 5, 25), while the species-specific *nod* genes determine the different substituents linked to the chitin oligomer. For example, the species-specific NodPQ and NodH proteins control the O-sulfation of the reducing sugar of the *R. meliloti* NodRm factors, a modification that confers alfalfa specificity (20). The species-specific *nodFE* and *nodL* genes are involved in the decoration of the non-reducing end of the chitooligosaccharidic backbone. NodFE proteins are involved in the synthesis of the particular polyunsaturated fatty acids that N-acylate the Nod factors of both *R. leguminosarum* bv. viciae (with C18:4 acyl chains; 25) and *R. meliloti* (C16:2 and C16:3; 6). NodL is required for the O-acetylation of the nonreducing terminal glucosamine residue (1, 25). It is now well established that both the length of the oligosaccharide backbone and the nature of the substituents linked to the terminal sugars of the Nod factors determine the molecular basis of symbiotic host specificity in the various *Rhizobium*-legume interactions.

Purified Nod factors specifically elicit a series of morphogenic responses on the roots of legumes, that are similar to those induced by the bacteria such as deformation of root hairs (14, 20, 25), and mitotic activity in the root cortex (25, 28). In the *R. meliloti*-alfalfa interaction, the hair-deformation response is induced at concentrations as low as 10^{-12}M (28). At higher doses (19^{-9}-10^{-7}M) the same factors induce the formation of non-nitrogen fixing nodular structures (28). Evidence exists that Nod factors are also involved in the formation of infection threads: (a) In *Vicia*, the exogenous supply of NodRlv factors

elicits the formation of radially-aligned cytoplasmic strands similar to the preinfection thread structures induced by the invading bacteria (29); (b) A double *nodF/nodL* mutant of *R. meliloti* is unable to penetrate into its specific hosts and to form infection threads, although it induces generalised cell wall tip growth and elicits cortical cell activation (1). This result shows that different structural requirements of Nod factors are necessary for bacterial entry into target root hair cells and for the induction of plant symbiotic developmental responses; (c) Finally, purified NodRIv and NodRm factors induce the transcription of plant early nodulin genes *ENOD*5 and *ENOD*12 which encode putative proline-rich proteins and which are strongly expressed during the infection process both in the cortical cells traversed by the infection thread and in the infection zone of the nodule (11, 12, 19, 22).

It is generally assumed that one of the key conditions for the establishment of a successful symbiosis between *Rhizobium* and legumes, is that the bacterium does not elicit the defence reaction of the plant. However, the fact that nodulation is autoregulated (4) indicates that mechanisms involved in feedback control of nodulation are elicited during the symbiotic interaction. Ultrastructural, cytochemical and biochemical studies have shown that plant defence mechanisms can occur in rhizobia-legume heterologous associations (21). Also, reactions featuring pathogenic-like and/or hypersensitive-like responses characterize developmental stages of non-nitrogen fixing (Fix^-) nodules elicited on legumes by homologous bacterial mutant strains (8, 9, 16, 17, 18, 30, 31). More recently, it has been demonstrated that alfalfa can react to infection by its wild-type symbiont *R. meliloti* by eliciting a defence mechanism displaying the cytological and biochemical features of the hypersensitive reaction (HR), characteristic of incompatible plant-pathogen interactions (33). The elicitation of an HR might be part of the mechanism by which the plant controls infection and, therefore, regulates nodulation. Finally, it has been shown that acidic chitinases, which are selectively elicited during abortive infection in the *R. meliloti*-alfalfa association (33), are able to degrade purified NodRm factors (27). These two last results emphasize the dual role that the rhizobial extracellular factors might play during symbiotic interactions, both as a potential morphogen able to induce a developmental program and as an elicitor of mechanisms resulting in defence reactions. Evidently, more studies are needed to substantiate this working hypothesis.

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RESPONSES OF COTTON ROOTS TO VERTICILLIUM DAHLIAE.

F. Daayf, M. Nicole, B. Boher, J. F. Daniel, A. Pando and J. P. Geiger.

ORSTOM, Laboratoire de Phytopathologie, BP 5045, 34032 Montpellier, France.

Verticillium dahliae (VD) is a fungal pathogen that causes vascular wilt disease of cotton (Gossypium sp.). Although numerous works deal with cotton defense mechanisms against wilt parasites, little is known about cytological aspects of root responses after infection with VD. The objective of this study was to get better insights into the early reactions that cotton roots differentiate against VD. Ultrastructural and cytochemical experiments were carried out on resistant and susceptible cotton cultivars (cv) infected by dipping with an aggressive strain of VD. The present investigation emphasized on host cell wall modifications, the production of electron-dense compounds by vascular parenchyma cells and the disorganization of infecting hyphae.

One day after inoculation, host cell wall modifications were observed in resistant roots whereas they were seen only 2 and 3 days after infection in roots of the susceptible cv. The use of gold probes (enzymes, lectins and antibodies conjugated to colloidal gold) indicated that polysaccharides such as cellulose, pectin and overall callose were present in wall appositions localized in parenchyma and phloem cells. Fluorescence and histochemical tests revealed that phenolis also accumulated in xylem vessels.

Another reaction of root cells to VD consisted in the production of electron-dense compounds (EDC) both in the susceptible and the resistant cv. In the resistant cv, EDC were detected as soon as 1 day after infection, while in the susceptible one they were observed later. The EDC appeared in parenchyma companion cells associated with xylem vessels, first in the cytoplasm before being seen within paramural spaces. EDC were also described later in various root parenchyma cells.

Observations of infected roots between 3 and 4 days after inoculation have shown damaged or dead hyphae within vessels. Hyphae were found to be trapped by structures that could be from plant origin. The apparent integrity of the fungal cell wall associated with the disorganization of the fungal cell suggested a toxic effect of plant molecules on the pathogen. Histochemical tests and biochemical studies revealed that cotton terpenoids and flavanes were produced as the first day after infection in the resistant cv.

Active defense of cotton roots to VD occurred early in the resistant cv; it mainly results from the reinforcement of physical barriers associated with phytoalexin synthesis.

IN SITU LOCALISATION OF MAIZE STREAK VIRUS IN A SUSCEPTIBLE CULTIVAR AND A RESISTANT LINE.

Bigarré L., Granier M., Reynaud B. (1), Nicole M. (2) and Peterschmitt M.

CIRAD-CA BP 5035, 34032 Montpellier cedex 1; (1) CIRAD-CA, 7 chemin de l'IRAT, 97410 St Pierre, La réunion, France; (2) ORSTOM, laboratoire de phytopathologie BP 5045, 34000 Montpellier (France).

Distribution of maize streak virus (MSV), a DNA virus from the geminivirus I group, was studied in leaf tissues of a susceptible cultivar (cv) and a resistant line of maize, using light and electron microscopy and immunocytochemistry. In both susceptible and resistant plants, infected tissues from chlorotic leaves showed stricking changes in the number and the structure of organelles. Chloroplasts were disorganized, showing a reduction in starch content associated with a loosening of thylakoids, explaining the chlorotic symptoms. In the nuclei, the chromatin seemed to be distributed only at the periphery and sometimes they present fibrillar rings. The nucleolus, generally voluminous, displayed a fibrillar and granular aspect, indicating a high transcription activity. were also observed within these nuclei.

Immunogold labelling of capsid antigens showed that virions were organized in cristalline arrays, forming large nuclear and somtimes cytoplasmic inclusions in all the leaf cellular types, except xylem vessels. The wide distribution of MSV in the mesophyll cells may explain why the insect vector, *Cicadulina mbila*, becomes infective in few second feeding only. Viral inclusions were mainly found in chlorotic tissues and occasionnely in the surrounding non-chlorotic zones.

The presence of large viral inclusions was observed on both the susceptible cultivar and the resistant line. However, immunogold labelling followed by silver enhancement made on semi-thin sections of chlorotic tissues, revealed that the average density of viral inclusions was lower in the resistant plants than in the susceptible plants.

Since MSV is not restricted to certain cell types in resistant leaves, this suggests that resistance to the pathogen may result from a limitation of virus multiplication rather than to its spread. These observations may explain (1) the delay of streaks apparition in the resistant plants and (2) the low number of streaks in resistant leaves, comparatively to the susceptible cv, although streak distribution is **qualitatively similar.**

AN ULTRASTRUCTURAL AND CYTOCHEMICAL COMPARISON OF FUNGAL DEVELOPMENT AND HOST REACTIONS IN SUSCEPTIBLE AND RESISTANT CARROT ROOTS INFECTED BY *PYTHIUM VIOLAE*

L. Guérin (1), N. Benhamou (2), F. Rouxel (3).

(1) Société Les Graines Caillard, route de Pouillé, 49130 les Ponts de Cé;
(2) Département de Phytologie, Université Laval, Sainte-Foy, Canada G1K7P4
(3) INRA, station de Pathologie Végétale, domaine de la Motte, 35650 Le Rheu.

The purpose of this work was to study interaction between carrot roots and *Pythium violae*, in order to gain greater insight on the mechanisms involved in resistance to cavity spot.

An ultrastuctural examination was carried out on susceptible and resistant carrot roots at different times after inoculation by the pathogen. In susceptible tissues, rapid intercellular and intracellular colonization by the fungus was accompanied by breakdown of host cell walls . At 72 h after inoculation, the *Pythium* growth was associated with only some accumulation of vesicular or granular material in the lumen of host cells. In resistant tissues, a more restricted fungal colonization was accompanied by a drastic modification of host cells with cytoplasm agregation, electron-opaque material in the lumen and walls of host cells, and advanced alteration of the mycelium. At 72 h after inoculation, the plugging of pericyclic cells by amorphous material was noticeable.

The chemical nature of the host and pathogen cell walls and new material formed during infection was investigated by two specific gold probes. The labelling of pectin with the complex *Aplysia* lectine-gold was found to be associated with the primary wall and the middle lamella matrice in uninoculated carrot plant tissue. In susceptible root infected by *P. violae*, a significant decrease in the number of gold particles was associated with the wall breakdown in close contact with invading hyphae. In resistant ones, fibrillar material was labelled in lumen. Labelling of cellulose with the gold complex exoglucanase was considerable in uninoculated host and fungus walls. Susceptible host wall penetration by fungus, which resulted in host breakdown, was also accompanied by a great reduction in labelling intensity. In contrast, resistant cell walls were not as altered and labelling was equally visible even 72 h after inoculation. Pectin and b-1,4 glucans were not detected within the amorphous plugging material of infected resistant cells.

In conclusion, the resistant host was characterised by more intense and precocious root cell alterations in relationship to infection. The chemical nature of electron opaque material and plugging material remains to be investigated; additionally, the ultrastructural and chemical nature of resistant host walls could be further studied.

CHEMICAL AND STRUCTURAL BARRIERS IN *Hevea spp.* to *Microcyclus ulei* (P. Henn.) V. ARX. CAUSING SOUTH AMERICAN LEAF BLIGHT

GARCIA D.*, TROIPOUX V.**, SANIER C.*, NICOLIN N.*, COUPE M.*, RIVANO F.** and d'AUZAC J.*

* Laboratoire de Physiologie Végétale Appliquée, Université Montpellier II, 34095 Montpellier cedex 5, France. ** CIRAD-CP-programme hévéa, Guyane Française, BP701, 97387 France.

South American Leaf Blight is one of the most important rubber tree disease particularly in the Amazon basin, where *Hevea brasiliensis* is originated and also in other Latin American countries. This disease is also a threat to the others rubber growing areas in the world as most of the high yielding clones bred in Asia which are extremely susceptible.

Investigating several resistant characters to combin them in horizontal resistance, histochemical studies have been undertaken to reveal phenolics compounds in leaves treated with spores of *M. ulei.* One early response to infection is the occurence of a blue fluorescent and toluidine blue reactive compound (bfc) within 6 hours after inoculation. The fluorescence is localised around the contact areas between fungus and plant, and seems to concern just sub-epidermal cells. The ability of the host tissue to arrest or to limit the spread of the pathogen depends on the speed with which the bfc is produced. Chemical analyses of the bfc reveal just a coumarine, scopoletin, yet identified in *Hevea* by Gieseman and *all.* (1986). The presence of scopoletin in germ tube and spores is in accordance with a fungitoxic action. Following, these histochemical data, thirty six clones have been tested on their scopoletine producing capacity and their resistance. Results are in accordance, for mainly clones, with histochemical observations.

A later host reaction (2 days after inoculation) of resistant and intermediate resistant clones is the production of a yellow material throughout areas where fungus hyphae has invaded the tissues. This compound is uncommon on highly susceptible clones. Its chemical nature is under investigation. Lignin, firstly reported in *Hevea-Microcyclus* interactions, is detected histochemicaly later (4 days after inoculation), with strong accumulation around fungus proliferating areas in intermediate resistant leaves, 12 days after inoculation. No hyphae and no stromatic structure are observed over lignin barrier. Fields data confirm these observations.

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HISTOCHEMICAL REACTIONS OF GRAPEVINES (*VITIS SPP.*) TO INFECTION BY *PLASMOPARA VITICOLA*

G. H. Dai[+§#], C. Andary[+], L. M. Cosson[+], and D. Boubals[§]

[+] Laboratoire de Botanique, Phytochimie et Mycologie, Faculté de Pharmacie, Université de Montpellier I, 34060 Montpellier Cedex, France

[§] Laboratoire de Viticulture, Ecole Nationale Supérieure d'Agronomie, Place Viala, 34060 Montpellier, France

[#] Permanent adresse: Laboratory of Plant Stress Physiology, Hebei Academy of Agricultural and Forestry Science, 050051 Shijiazhuang, Hebei China

Leaves of *Vitis vinifera* cv. Grenache (susceptible), of *V. rupestris* cv. rupestris du Lot (intermediate resistant), and of *V. rotundifolia* cv. Carlos (resistant) were inoculated with *Plasmopara viticola* and examined microscopically after staining with a series of reagents. These three species showed, respectively, necrotic spots without sporulation (resistant), diffuse necrosis with limited sporulation (intermediate resistant), and no visible necrosis with heavy sporulation of the parasite (susceptible). In the resistant variety, flavonoid compounds were detected in the stomatal cells and the cells around the stomata 2 days after inoculation. In the intermediate cultivar, resveratrol and peroxidase activity were detected 5 days after inoculation, flavonoid compounds and lignin was formed in tissue surrounding the necrosis at a late stage of infection (8 and 15 days after inoculation). In the susceptible cultivar, only a small amount of yellow autofluorescence was observed in the stomatal cells 8 days after inoculation. These data suggest that the rapidity of flavonoid formation plays an important role in the resistance of *V. rotundifolia* to *P. viticola*. The formation of resveratrol, flavonoids and lignin in the intermediate cultivar probably restricts the development of this pathogen.

EVIDENCE AND CHARACTERISATION OF A LOW MOLECULAR WEIGHT POLYPEPTIDE IN OILSEED RAPE-*PHOMA LINGAM* RELATIONSHIPS

Z.Benhlal, F.Val, M. Le Normand

Laboratoire de Pathologie Végétale, ENSA-Rennes. 35042 - Rennes Cec

Infection of oilseed rape (*Brassica napus*) by pathogen fungi (*Phoma lingam*) is followed by a production of plant proteins, a part of those are pathogenesis related-proteins (PR proteins).
The aim of research is to improve a secure and precise method of early detection for some oilseed rape diseases.
First, protein extracts from leaves of inoculated and non-inoculated plants, collected from16 hours to 21 days after inoculation, were analysed by electrophoresis (SDS-PAGE).
Four days after inoculation, a low molecular weight acidosoluble polypeptide is found only in the inoculated plants. It is used to immunize female mice. Immunisations are run with different dilutions series of antigen.
The antisera obtained against this acidosoluble polypeptide were used to analyze extracts of leaves of inoculated and non-inoculated plants. Samples were separated on SDS-PAGE or two-dimensional gel, and analysed by western-blot.
-Antisera obtained with higher immunisation doses of antigen revealed one polypeptide (≈7 KD) only in the infected plants.
-In contrast, antisera obtained with the lower doses of antigen, revealed completely different patterns.
Thus, we can detect early production of a low molecular weight polypeptide after stress induction.
The antisera could be used to localize the polypeptide "*in situ*".

SCANNING ELECTRON MICROSCOPY AND HISTOCHEMICAL STUDIES OF CERATOCYSTIS FIMBRIATA f.sp PLATANI - PLATANUS spp INTERACTION.

Clérivet.A and C.El Modafar. Biotechnology and Applied Plant Physiology Laboratory. University Montpellier2. 34095 Montpellier cedex 5. France

Canker stain disease (CSD) of plane tree is caused by a specific parasitic fungus *Ceratocystis fimbriata* f.sp *platani* (CFP) invading the host using mainly the vascular system. The European plan tree, *Platanus acerifolia* (P.a) is always susceptible to CSD. A source of resistance has been shown in the USA in the American species *Platanus occidentalis* (P.o) but it cannot be directly exploited since the species is not acclimatized in Europe. Also in the genetic improvment programme leading to the development of a resistant hybrid (VIGOUROUX,1992) our purpose is the characterization of resistant and susceptible plants through a study of their defence responses.

In slightly lignified P.a seedlings (10 leaves stage)relatively refractory to CFP infection (VIGOUROUX and ROUHANI,1987) tylose and gel accumulation, scabby formation and numerous warts on vessel walls and pit borders were showed by SEM methods. Using varied specific reagents,phenolic compounds accumulation is revealed at the inoculation site: flavans in thylose and gel and fluorescent blue compounds in vessel lumina, probably coumarins which appeared during infection (EL MODAFAR *et al*,1993).In inoculated leaves of resistant (P.o) plants, stomatal penetration is accompagnied by a strong blue fluorescence of stomatic cells, intercellular space of epiderm and mesophyll allowed by the restriction of CFP development. In susceptible (P.a) plants the fluorescence is lower and limited to stomatic cells,the CFP progression very extensive. The same phenolic compounds that in infected stems (scopoletine,umbelliferone) were isolated from leaf extracts and linked to fluorescent reaction. Induction of these phytoalexin accumulation is early, fast and great only in P.o plants.

Thus, host reactions like obstructive structures, phenolic impregnation and phytoalexin accumulation in the inoculation site are some defence responses leading to the restriction of CFP development. If plane tree phytoalexins are certainly implicated as the cause or one of the cause in the resistance of P.o to CFP, the involvment of vascular modifications in this interaction could be now a very interesting subject.

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EVIDENCE OF A CONSTITUTIVE POLYPHENOLIC COMPONENT IN RESISTANCE OF BANANA TO *MYCOSPHAERELLA FIJIENSIS*

A.Gire (1), A.Beveraggi (2), J.J.Macheix (1) and X.Mourichon (2)

(1) Université Montpellier II, Lab.Biotechnologie et Physiologie Végétales Appliquées, 34095 Montpellier Cedex 5, France; (2) CIRAD-FLHOR, Lab.Pathologie Végétale, BP 5035, 34032 Montpellier Cedex, France.

Black Leaf Streak Disease caused by *Mycosphaerella fijiensis* (*Cercospora fijiensis*) is a serious threat to AAA bananas, AAB plantains, and other cooking bananas. Three kind of reactions - susceptibility,S, partial resistance,PR, high resistance,HR - characterise all wild and cultivated banana varieties (AA,AAA,AAB,ABB). Host-parasite interaction studies were conducted under controlled conditions involving artificial inoculations of susceptible (Grande naine,AAA), partially (Pisang awak,ABB) and highly resistant host (Yangambi Km5, AAA). Biochemical and cytological studies (photonic and electronic microscopy) display two distinct interactions. In the highly resistant host, a defense mechanism is displayed just after the stomatal penetration. The cultivars have similar leaf tissue structure. The partially resistant Fougamou, however, is distinguishable from other two hosts by the presence of numerous specialized polyphenol-storing cells in parenchyma. During the infection, the material is released into the intercellular spaces and present a high affinity for host cell and parasite walls. It is demonstrated that this polyphenolic material may not have a crucial role in the early infection stages but it has a definite effect once necrosis begins.

To determine the degree of involvement of this constitutive component in partial resistance, the leaf soluble phenolic content was studied for eleven banana varieties displaying different resistance levels to *M.fijiensis*. Phenolic compounds were separated by chromatography and analysed by HPLC, TLC and spectophotometry. The soluble phenolic pool is represented in each banana variety by flavonol glycosides, hydroxycinnamic derivatives (HCDs) and polymeric flavan-3-ols (condensed tannins). No significant difference has been shown between sensitive and partially resistant varieties banana concerning flavonols. Depending on varieties, HCDs constitute only 5 to 10% of total phenolics and some partially resistant varieties contain two to three-fold more HCDs than sensitive one's. Flavan-3-ols contents (mg eq.catechin/g MS) may be significantly strongly different among the partially resistant hosts and a clear relation has been established between these contents and the level of partial resistance.

NODULATION OF TRANSGENIC *CASUARINA GLAUCA* ROOT

D. Diouf, H. Gherbi, C. Franche, Y. Prin, E. Duhoux and D. Bogusz

BSFT (ORSTOM/CIRAD-Forêt), 45 bis avenue de la Belle Gabrielle, 94736 Nogent sur Marne, France.

The development of a gene transfer system into actinorhizal plants provide tools to study the symbiotic process between actinorhizal trees and the actinomycete *Frankia.* The ability of *Agrobacterium rhizogenes* to transfer Ri plasmid to*Casuarina verticillata* has been demonstrated in our laboratory (1). We are currently developing a short cut protocol to induce transformed hairy roots on *Casuarina glauca* and to nodulate those transgenic roots. The goal of our work is to study the expression of actinorhizal symbiotic genes in transgenic nodules.

Hypocotyls of four weeks old *Casuarina glauca* seedlings were wounded with a needle dipped in a colony of *A. rhizogenes* A4RS carrying a binary vector. The plasmid vector pBin19 contains the 35S promoter and the GUSINT as reporter gene to monitor transformation events (2). About 70% of the inoculated plants developed hairy roots one week after wounding. After 3 weeks, the seedlings were washed for two days with cefotaxim in liquid medium. Then the original root system was cut off and the seedlings were transfered to growth cabinets or green-house. The analysis of GUS activity in transgenic roots and nodules will be presented.

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IMMUNOCYTOCHEMICAL LOCALIZATION OF ß-1,3 GLUCANS IN DIFFERENT INTERACTIONS BETWEEN PLANTS AND ROOT-INFECTING FUNGI

GOLLOTTE A., LEMOINE M. C., GIANINAZZI-PEARSON V., GIANINAZZI S.

Laboratoire de Pytoparasitologie, INRA/CNRS, Station de Génétique et d'Amélioration des Plantes, INRA, BV 1540, 21034 Dijon Cédex, France.

Pea mutants have been described that are resistant to symbiotic microorganisms *Rhizobium* and arbuscular mycorrhizal fungi (AMF) (1). In the presence of the latter, the mutants develop wall appositions containing phenolics and callose in epidermal cells in contact with appressoria, suggesting a defence reaction, and the fungus cannot penetrate further into the root (2). In order to know whether these mutants could also be resistant to other fungi, we have inoculated them and their parent cv. Frisson with the pathogenic fungi *Chalara elegans*, *Rhizoctonia* sp. and *Aphanomyces euteiches*, and a symbiotic fungus (*Hymenoscyphus ericae*) of ericaceous endomycorrhizae which can develop saprophytically in non host plants. To test the elicitation of defence reactions in each interaction, we chose to study callose (ß-1,3 glucans) accumulation. The results indicate that the infection phenotype for each fungus, contrary to what is observed with AMF, is the same in both myc$^+$ and myc$^-$ plants. *C. elegans* induced a hypersensitive response in pea roots with the formation of necroses in cortical parenchyma cells where wall appositions clearly contained callose. *Rhizoctonia* sp. infected epidermal and hypodermal cells without triggering any apparent defence reaction. *A. euteiches* also entered the root and developed in the cortical parenchyma but no callose accumulation could be observed. However, *H. ericae* sometimes elicited the formation of callose containing wall deposits but fungal development was not inhibited. In each case, cellular interactions are the same in myc$^+$ and myc$^-$ plants, confirming that the resistance in nod$^-$ myc$^-$ mutants is specifically directed towards symbiotic microorganisms.

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COMPARED HISTOLOGY OF COLONIZATION OF RESISTANT AND SUSCEPTIBLE TOMATO CULTIVARS BY *PSEUDOMONAS SOLANACEARUM.*

Grimault V. [1], Gelie, B. [1], Prior P. [2] and Schmit J. [1].

[1]: INRA, Station de Pathologie Végétale, Laboratoire de Bactériologie, 78026 Versailles Cedex. France.

[2]: INRA, Station de Pathologie Végétale, de Phytoécologie et de Malherbologie, BP 1232, 97185 Pointe-à-Pitre Cedex.

Resistance of tomato to bacterial wilt, the vascular disease caused by *P. solanacearum*, is characterized by the limitation of bacterial spread in vascular tissues of resistant cultivars. In contrast, susceptible cultivars are colonized up to the apex.

We studied the colonization of two tomato cultivars, Floradel (susceptible) and Caraïbo (resistant) by light and transmission electron microscopy. Inoculated and non inoculated plants of both cultivars were sampled 15 days after inoculation when Floradel was in the process of wilting whereas Caraïbo and control plants were symptomless.

We observed different reactions of plants to colonization by *P. solanacearum* and tyloses production appeared the more significant reaction to limit bacterial spread in xylem vessels. In the resistant cultivar, tyloses occluded the colonized vessels and the contiguous ones, limiting bacterial spread. In the wilting susceptible cultivar, no tyloses were observed in colonized vessels and bacterial spread was not limited. In the susceptible cultivar, tyloses production seemed delayed and less focused than in

the resistant cultivar because numerous non-colonized vessels were occluded by tyloses. Tyloses therefore contribute to wilting in the susceptible cultivar.

Other reactions involved in resistance or susceptibility were observed such as gums, cell wall breakdown and modifications of the primary cell wall.

The limitation of bacterial spread associated with the resistance of tomato to bacterial wilt was thus mainly attributed to an induced, non-specific, physical barrier. This study showed the importance of the spatio-temporal relationship between bacterial spread and plant reaction. It is then important to know if resistant plants are more able to produce tyloses in response to infection by other pathogens or by wounds, or in contrast, if a more specific response related to host-pathogen recognition is involved.

MECHANISMS OF INHIBITION OF *DRECHSLERA TERES* IN BARLEY BY *SEPTORIA NODORUM* FROM WHEAT AND *BIPOLARIS MAYDIS* FROM MAIZE

H.J. Lyngs Jørgensen, E. de Neergaard and V. Smedegaard-Petersen

Department of Plant Biology, The Royal Veterinary and Agricultural University, 40 Thorvaldsensvej, DK-1871 Frederiksberg C, Copenhagen, Denmark.

Screening for potential inducers of resistance against various cereal pathogens in the CAMAR-project "Crop Protection Using Diversification and Induced Resistance in Low-Input Cereal/Legume Cropping Systems" revealed that pre-inoculation of barley with *Septoria nodorum* from wheat or *Bipolaris maydis* from maize very effectively reduced subsequent infection by *Drechslera teres*. Hence, pre-inoculation with *S. nodorum* and *B. maydis* reduced the necrotic leaf area produced by *D. teres* by up to 69 and 75 pct, respectively and the size of individual lesions by up to 59 and 54 pct., respectively.

The results indicate that a large part of the disease reduction is due to decreased size of the individual lesions and that only a smaller part results from a reduced number of lesions (i.e. penetration frequency was only slightly reduced). For *S. nodorum* as inducer, the latter conclusion has been supported by results from a time course study examining the early stages of infection of *D. teres*. Preliminary statistical analyses have revealed that the penetration efficiency of appressoria was not reduced by inducer application. However, establishment of intracellular vesicles from the penetrating hyphae was significantly reduced on *S. nodorum* treated leaves. Furthermore, inducer application did reduce certain stages of pre-penetration growth of *D. teres*, i.e. number and branching of germ tubes and number of appressoria. Germination frequency of conidia and lenght of individual germ tubes were not significantly affected.

The mechanism(s) responsible for the observed disease reduction are currently being further investigated. This is done by scanning electron microscopy as well as light microscopy of thin stained sections of plastic embedded leaf pieces and of whole, cleared and stained leaf segments.

HISTOPATHOLOGY OF CASSAVA INFECTED BY XANTHOMONAS CAMPESTRIS PV. MANIHOTIS : DEFENSE REACTIONS.

K. Kpémoua, B. Boher, M. Nicole, V. Verdier and J. P. Geiger.

ORSTOM, Laboratoire de Phytopathologie, BP 5045, 34032 Montpellier, France.

Histological and cytochemical studies of cassava - Xanthomonas campestris pv. manihotis (XCM) interactions revealed different plant reactions localized within leaf and stem tissues. The epiphytic development of the pathogen and the bacterial growth throughout the mesophyll were quantitatively similar both in resistant (R) and susceptible (S) cultivars (cv). However, the bacterial progression was limited in the vascular stele by defense reactions that were studied at the cytological level.

In infected plants, the major host cell responses consisted in reinforcement of structural barriers. Additional callose depositions were localized in phloem cells by immunofluorescence and by immmunogold labelling. Lignification of walls was evidenced by histochemical tests in cortex and phloem cells. Phenol-like compounds were detected by autofluorescence in cells of infected zones; such autofluorescence was also observed in the exopolysaccharide matrix and the outer membrane of some bacteria. Phenol accumulation was confirmed by the toluidine blue staining and by the use of a p-diphenoloxidase, a laccase, conjugated to gold. Numerous tyloses were seen to occlude xylem vessels, as well as gels constituted with pectin-like material. Cell hyperplasia appears close to infected tissues.

Most of these reactions against XCM were observed in the S and R cultivars. Nevertheless, these responses were quantitatively higher and occurred early in R plants, suggesting that these mechanisms may slow down bacterial progression in intercellular spaces and in xylem vessels.

CASSAVA KILLER TYLOSES : A LETHAL WEAPON.

K. Kpémoua, B. Boher, M. Nicole, V. Verdier and J. P. Geiger.

ORSTOM, Laboratoire de Phytopathologie, BP 5045, 34032 Montpellier, France.

Tylose in xylem vessels results from an outgrowth of parenchyma cells through a pit cavity. In Cassava, tyloses occurred as a defense response to Xanthomonas campestris pv. manihotis (XCM) in susceptible and resistant cultivars. However, variations in morphological aspects of tyloses were only observed in the infected xylem of resistant plants, gradually ranging from normal globular tyloses to those showing a digit-like aspect. Such variations were associated with an increase of electron opacity of the cytoplasm. Also, electron-dense compounds were seen to be localized within the paramural space and the wall of the digit-like tyloses, and close to them in the vessel lumen. Bacteria cells located in the vicinity of these digit-like tyloses and close to these electron-dense compounds appeared to be collapsed.
These ultrastructural and cytochemical observations suggest that digit-like tyloses atc as killer structures which secrete toxic molecules that may contribute to limit the pathogen progression in the resistant plant.

CELLULAR ASPECTS OF PARTIAL RESISTANCE TO ENDOMYCORRHIZAL FUNGI IN PEA MUTANTS

LHERMINIER J.*, V. GIANINAZZI-PEARSON**, S. GIANINAZZI**.
*Service Commun de Microscopie Electronique, **Laboratoire de Phytoparasitologie INRA/CNRS, SGAP, INRA, 21034 Dijon Cédex, France.

A chemically induced pea mutant (DK24) of *Pisum sativum* cv. Finale exhibits an abnormal endomycorrhizal infection when inoculated with the endomycorrhizal fungus *Glomus intraradices*(1). The aim of this histological and cellular study was to describe in detail the infection pattern of this fungus in the DK24 mutant and to examine plant responses.

When young hyphae of *G. intraradices* came into contact with the root surface of the pea mutant, enlarged appressorium-like structures induced a fibrillar wall deposit in the adjacent epidermal cells, suggesting an early plant defence reaction. Moreover, the essential structure of symbiosis, the intracellular arbuscules, did not develop in parenchyma cells of the root cortex. Instead, aborted intracellular structures or incomplete arbuscules were observed. A thick layer of plant wall material surrounded hyphae as they penetrated parenchyma cells and, unlike the situation in the normal symbiosis, this persisted around incomplete arbuscule branches. The nucleus of infected parenchyma cells of the pea mutant exhibited a condensed chromatin pattern, as in uninfected cells, which contrasts with the decondensed chromatin of nuclei when arbuscules fully developed in host cells of cv. Finale. The fungal enzyme activities succinate dehydrogenase and alkaline phosphatase were detected in intercellular and intracellular branches of the incomplete arbuscules, suggesting that fungal metabolism is unaffected within mutant roots. ATPase activity which is normally present on the plasma membrane of the fungus and along the perisymbiotic membrane around arbuscules, and which is an indicator of nutritional exchange between the two partners, was detected on the plasma membrane of intercellular hyphae but not on that of branches of incomplete arbuscules in pea mutant roots. ATPase activity was also absent from the plant membrane surrounding the incomplete arbuscules suggesting that nutrient exchange between the two partners is reduced. These observations lead us to hypothesize that loss by the DK24 mutant of its potential to form a mutualistic symbiosis with the arbuscular mycorrhizal fungus results from modifications in host-fungus recognition events.

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MICROSCOPIC CHARACTERIZATION OF *hxc-1*, AN *ARABIDOPSIS* MUTANT AFFECTED IN THE HYPERSENSITIVE RESPONSE TO *XANTHOMONAS CAMPESTRIS* PV.*CAMPESTRIS*.

LUMMERZHEIM Marie[1,2], FERREIRA Marcio[3], DE OLIVEIRA Dulce[3], VAN MONTAGU Mark[1], ROBY Dominique[2].
1: Laboratorium voor Genetika, Gent, Belgium.
2: Laboratoire de Biologie Moléculaire des Relations Plantes-Microorganismes CNRS-INRA Castanet-Tolosan, France.
3: Laboratorio de Genetica Molecular Vegetal, Rio de Janeiro, Brasil.

Compatible, incompatible, and null interactions have been determined on *Arabidopsis thaliana* (Columbia (Col-O)) after inoculation with an avirulent (147), virulent (8004), and control strain (8420) of *Xanthomonas campestris* pv. *campestris* (*X.c.*pv.*campestris.*)(*), suggesting a specific recognition between host and pathogen. *X.c.*pv.*campestris* 147 induced small necrotic lesions over the leaf surface typical of an HR.The HR observed in Col-O leaves has extensively been characterised at microscopic level. Scanning electron microscopy (SEM) allowed a detailed screening of the infected leaf epidermis and an evaluation of the spatial and temporal distribution of the avirulent pathogen following spray inoculation. Fluorescent microscopy analysis revealed temporal and quantitative differences in fluorescence between leaves infiltrated with the virulent or the avirulent isolate and specific deposition of callose plugs on the cell walls at the site of the necrotic lesion was visible. Confocal microscopy demonstrated that ,when the *Arabidopsis* leaves are spray-inoculated with the strain 147 at low bacterial density ($<10^6$cfu/ml), the bright spots seen under the fluorescent microscope correspond to single cells undergoing an HR that do not coalesce to form necrotic lesions visible to the naked eye.

For the identification of genes involved in the HR, a genetic approach based on a mutational analysis has been initiated. 20700 EMS (ethylmethylsulfonate) mutagenised seeds were screened in order to identify *Arabidopsis* mutants altered in their ability to develop an HR when infected with the avirulent strain of *X.c.*pv.*campestris.*(strain 147). After several screening rounds using two different inoculation protocols (spray and infiltration) and 3 generations of selfing, 3 classes of mutants could be distinguished: 1) "HR-" mutants showing a very weak or no HR after 24 h, and no symptom development a few days later, 2) "hyper HR mutants" in which a very strong HR could be observed 24 h post-inoculation, followed by the complete collapse of leaves, 3) "Susceptibility mutants" characterized after 24 h by a stronger HR than the one of the wild-type, evolving to a disease-like phenotype several days later. The mutant *hxc-1*, for hypersensitivity to *Xanthomonas campestris*, is representative of the "Susceptibility mutants" class.

An extensive microscopic analysis has been undertaken of the mutant *hxc-1* in order to confirm and detail at a cellular level the observed mutant phenotype versus the wild-type (Col-O). When analysing under SEM, *hxc-1* is responding earlier to the presence of *X.c.*pv.*campestris* 147 than Col-O but the lesions have unsharpened borders compared to the wild-type. Transmission electron microscopy allowed us to analyse the intra-cellular responses and bacterial localization when infiltrating both mutant and wildtype with the*Xanthomonas* strains inducing the different types of interactions.The preliminary results obtained under fluorescent microscope of *hxc-1* , infiltrated with strains 147, 8004, and 8420 will also be presented.

* Lummerzheim *et al.* 1993, MPMI vol.6, 532-544.

ULTRASTRUCTURAL AND CYTOCHEMICAL STUDIES OF CUCUMBER ROOTS INFECTED BY TWO *PYTHIUM* SPECIES

P. Rey (1), N. Benhamou (2) and Y. Tirilly (1)

(1) Laboratoire de Microbiologie, ESMISAB,Technopôle Brest-Iroise, F-29280 Plouzané. (2) Laboratoire de Cytologie Moléculaire, Pav. Marchand, Univ. Laval, Sainte Foy, Quebec, Canada G1K 7P4.

Recent epidemiological studies performed in tomato and cucumber soilless cultures had revealed the frequency and abundance of *Pythium* F, which were usually considered as fungi with variable necrogenic ability in hydroponic conditions. To get a better understanding of the *Cucumis sativus/Pythium* interaction, cytochemical and ultrastructural studies were achieved. Cucumber roots were either infected by *Pythium F* or by *P. aphanidermatum*, a highly pathogenic fungus. In the case of both pathogens, ingress through the roots was mainly intracellular, but the extent of fungal colonization and host responses were different.

Pythium F colonization was associated with marked damage, only in epidermal and cortical cells. Host cell wall labeling with specific gold probes revealed a pronounced pectin breakdown but a weak cellulose alteration, which was generally restricted to sites of fungal penetration. *Pythium F* invasion was associated with various host reactions, including papillae formation and wall appositions lining the host cell wall. This last deposit material was regularly labeled with the exoglucanase-gold complex, in contrast a low labeling was obtained with a specific galactose-gold probe. Plugging of the intercellular spaces was also frequent. In the inner cortex, numerous cells were completely filled with amorphous material, electron dense aggregates or polymorphic opaque flecks. *Pythium F* hyphae growing in such occluded cells were often moribund.

Inoculation by *P. aphanidermatum* resulted in a massive colonization of all root tissues, including the stele. This colonization was associated with marked cell alterations, and high cellulose and pectin breakdown even at a distance from the point of fungal location. One of the main host reactions was the occlusion of some cortical and stelar cells with an amorphous material, similar to that observed in the presence of *Pythium F*. Nevertheless, this accumulation occurred too late, after the complete invasion of roots by the pathogen.

Another typical reaction to root invasion by the two fungi was the coating of secondary walls and pit membranes of xylem vessels by a thick layer of an osmiophilic opaque material.

However, vessel penetration by *P. aphanidermatum* through these thickened pit membranes was frequently observed.

Consistent root alteration by *Pythium F* are observed for the first time, which might explain yield losses attributed to these fungi in hydroponic cultures, even in the absence of severe root necrosis. These results would be discussed in terms of *Pythium* pathogenicity and host/pahogen relationship.

Histochemical characterization of *Hevea brasiliensis* / *Corynespora cassiicola* interaction

C. SANIER, F. BRETON and J. d'AUZAC

Laboratoire de Biotechnologie et Physiologie Végétale Appliquée, EA 728, Université Montpellier II, Place Eugène Bataillon, 34095 Montpellier cedex 5, France

Hevea brasiliensis, a lignous euphorbiaceae, is the main natural rubber source and is of a great economic importance for producing countries. However, in South East Asia as in Africa the rubber-trees young leaves are attacked by a fungus *Corynespora cassiicola* causing *Hevea* leaf fall. Many epidemiological and biological kwnoledges about this pathogen are accumulated (1) but nothing is known about defence reactions of the few resistant *Hevea* clones. Thus, the identification of the mechanisms leading to an efficient level of horizontal resistance appears essential in the perspective of genetic improvement of this culture, especially for early selection tests of resistant genotypes toward this pathogen.

The aim of our preliminar study is to differentiate the response of tested clone by kinetics of appearance of necrosis and their histochemical aspects in the young *Hevea* leaves after artificial inoculation by *C. cassiicola* spores suspensions. Excised young leaves of *Hevea* clones caracterized by different resistance and sensitivity levels, were tested under continuous light and saturated humidity.

UV visualization on the inoculated leaves, of the 10 µl conidial suspensions droplets, showed an intensive blue fluorescence. A major phenolic compound, responsible of this fluorescence has been identified unambiguously as the phytoalexin scopoletin (2).

Microscopic observations of necrosis at the sites of conidial suspensions inoculation allowed to differentiate qualitatively the sensitivity of tested clones that we try to connect with the lignin accumulation.

Furthermore, the use of leaf blotting technique (3) allowed to see a clear increase of peroxidase activities around infected spots.

Concurrently, we have tried to connect these results with biochemical analysis of some resistant mechanisms such as appearance of pathogenesis-related proteins (chitinases and β-1,3-glucanases) and phytoalexin (scopoletin) accumulation. The aim of our work is to understand the relative importance of these mechanisms in clonal resistance.

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CHARACTERIZATION OF AN ELICITOR PREPARATION FROM *PHYTOPHTHORA PARASITICA* VAR. *NICOTIANAE* AND ASSESSMENT OF ITS DEFENSE INDUCING ACTIVITY ON TOBACCO PLANTS.

SEJALON-DELMAS N. BOTTIN A. VILLALBA F. RICKAUER M. ESQUERRE-TUGAYE M.T AND R DARGENT.
Centre de Physiologie végétale
Université Paul Sabatier
118, Route de Narbonne
31 062 Toulouse

The genus *Phytophthora* contains several species which are pathogenic on a large variety of dicotyledonous plants. *Phytophthora parasitica* var. *nicotianae*, *Ppn*, (Tucker) is the causal agent of the black shank disease of tobacco, and the interaction between *Ppn* and its host plant is highly specific. We dispose at the laboratory of two near-isogenic cultivars of tobacco which are resistant or susceptible towards race 0 of the fungus.

An elicitor was extracted from the mycelium of *Ppn* race 0 and partially purified. The elicitor preparation was composed of only few polypeptides and glycoproteins. Its activity was studied on tobacco plants by measuring two defence-related responses: the induction of lipoxygenase activity and the accumulation of hydroxyproline rich glycoproteins (HRGP).

Lipoxygenase activity was induced at high level in the resistant cultivar, whereas the response in the susceptible one was very weak. The accumulation of HRGPs was studied by immunocytochemistry, with an anti-serum raised against a melon extensin. Again, HRGPs were accumulation was observed only in the resistant plant.

A polyclonal anti-serum against the elicitor preparation was obtained. In western-blots it revealed a major antigen of 34 kDa in the elicitor preparation. The migration of the elicitor throughout the plant during the bioassay was followed and revealed to be restricted to the xylem vessels; no diffusion in the parenchyma cells was observed. The migration pattern occurred was the same in the two cultivars indicating that the specific induction of HRGPs in the resistant plant was not the fact of a difference in the mobility of the elicitor.

Our elicitor appears to induce defence reactions specifically in the resistant plant. Further studies will focus on the major antigen of 34 kDa to assess its role in the tobacco-*Phytophthora* interaction.

LIGHT AND ELECTRON MICROSCOPY OF THE INCOMPATIBLE INTERACTIONS *COFFEA* SPP. - *HEMILEIA VASTATRIX*

Mª. C. SILVA, L. RIJO and Mª I. VASCONCELOS.

Centro de Investigação das Ferrugens do Cafeeiro, Quinta do Marquês, 2780 Oeiras, Portugal.

A light and ultrastructural study of the resistant expression of *Coffea arabica* and *C. congensis* plants infected with the same avirulent culture of *Hemileia vastatrix* was made. The uredospore germination, appressoria formation and mycelial growth inside leaf tissues did not differ significantly in both resistant species. Since the 3rd day after the inoculation, dead hyphae began to be observed. In fact, in both *C. arabica* and *C. congensis* plants, the fungus ceased its growth in different stages of the infection process, with higher frequency after the formation of haustorial mother cells with 3 haustoria in *C. arabica* and 4 haustoria in *C. congensis*. Ultrastructurally, as in the light microscope observations, the first signs of incompatibility were detected by the deposition of callose containing material around the haustorium and also by the necrosis of both host cell and haustorium. Callose deposits were also observed in the plant cell walls that contact with the aborted hypha, particularly in *C. congensis* plants. Later in the infection process, it was detected lignin mainly on the thickened walls of the hypertrophied cells of the tumefaction area. In both coffee species, the fungus presented a restricted growth and elicited the formation of callose and lignin.

LIGHT AND ELECTRON MICROSCOPY OF INTERACTIONS BETWEEN *ALBUGO CANDIDA* AND ARABIDOPSIS

Soner Soylu and John Mansfield.

Department of Biological Sciences, Wye College, University of London, Wye, Ashford, Kent, TN25 5AH

Following stomatal penetration, *A. candida* produced a sub-stomatal vesicle and thin intercellular hypha. In the susceptible ecotype, columbia, after formation of the first haustorium, hyphae expanded and extended rapidly between mesophyll cells. Modification of the extrahaustorial membrane was indicated by changes in staining with phosphotungstic acid. No sporulation was observed on ecotypes Keswick 1 or Keswick 2. Resistance of Keswick 1 was associated with a rapid HR following penetration of mesophyll cells. The HR was characterized by the accumulation of autofluorescent phenolics. In Keswick 2, the HR was less frequent than deposition of a callose sheath around the haustorium. In some penetrated cells of Keswick 2 callose deposition was widespread along plant cell walls but no autofluorescence was observed. The patterns of response observed were quantified. Results obtained indicate the activation of different signal transduction pathways in Keswick 1 and Keswick 2 which possess the *RAC1* and *RAC2* genes for resistance respectively.

TOMATO ROOT INFECTION AND COLONIZATION BY A *PSEUDOMONAS SOLANACEARUM* MUTANT DEFICIENT FOR EXPRESSION OF *HRP* GENES.

VASSE Jacques, FREY Pascal and TRIGALET André.
Laboratoire de Biologie Moléculaire des Relations Plantes-Microrganismes.
CNRS-INRA. BP 27. 31326. Castanet-Tolosan. Cedex.

Pseudomonas solanacearum is the causal agent of bacterial wilt that affects numerous commercially valuable plant species in the tropics. As a number of other phylogenetically distinct plant pathogens, this microorganism possesses a cluster of *hrp* genes that is essential for the development of disease symptoms, *p*athogenicity, on susceptible host-plants and for the elicitation of the *h*ypersensitive *r*esponse on resistant or non host-plants (Boucher *et al.* 1992). Recently, it has been shown that the *hrpB* gene encodes a positive regulator controlling the expression of the majority of the *hrp* genes and of additional genes located to the left of the *hrp* gene cluster (Génin *et al.* 1992). Using cytological procedure allowing to localize *in planta* bacteria harbouring a *lacZ* gene fusion, we have compared tomato root infection process by the pathogenic strain GMI1485 and by the nonpathogenic strain GMI1485 *hrpB*::Ω . The strain GMI1485 carries a Tn*5-lacZ* gene fusion located outside the *hrp* region. The strain GMI1485 *hrpB*::Ω, which harbours the same Tn*5-lacZ* gene fusion and an omega interposon in *hrpB,* though nonpathogenic, is still able to colonize tomato plants. It appears that the *hrpB* mutant intercellularly infects the inner cortex of few root extremities and of some secondary root axils with a lower intercellular development than the pathogenic strain. From these infection sites, it penetrates into protoxylem vessels but we have not observed plant defence mechanisms such as vascular coating or signs of cell degradation, as it occurs with the pathogenic strain. At the collar level, the vascular colonization was reduced to few xylem vessels surrounded by brownish cells, while tyloses were observed frequently in uninvaded vessels. On the contrary, the pathogenic strain colonized up to twenty five per cent of xylem vessels in each of the four vascular bundles of partially wilted plants. Moreover, bacterial isolations have shown that the colonization of tomato plants by the nonpathogenic strain is restricted in height and density compared to the pathogenic strain. From these observations, it appears that *in planta* development of *hrpB* mutant bacteria is reduced whatever the stage of interaction considered. Furthermore, challenge-inoculation experiments with a pathogenic strain have shown that, among several *hrp* mutants tested, *hrpB* mutant seems to have one of the highest protective ability against bacterial wilt.

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COMPATIBLE AND INCOMPATIBLE INTERACTIONS BETWEEN *SORGHUM BICOLOR* AND *COLLETOTRICHUM SUBLINEOLUM*

P.S. Wharton (1) A. M. Julian (2)

(1) Department of Agriculture, University of Reading, Earley Gate, Reading RG6 2AT (2) Natural Resources Institute, Chatham Maritime, Kent ME4 4TB.

Colletotrichum sublineolum is an important pathogen of sorghum, causing anthracnose on leaf, stalk and panicle tissue. The infection process of *C. sublineolum* on *Sorghum bicolor* was monitored on 14 day old plants using light microscopy. Two pathogen isolates 117 and 74, were tested against 3 differential cultivars: SC748-5 (resistant to both isolates), KAD 332 (susceptible to both isolates) and TAM 428 (susceptible to 117, resistant to 74).

In the susceptible cultivar, KAD 332, both isolates formed intracellular infection vesicles in living epidermal cells around 30 hours after inoculation. These developed intracellular primary hyphae which colonised adjacent living cells. This biotrophic phase lasted about 24 hours. After approximately 66 hours both isolates became necrotrophic and produced secondary hyphae which colonised the mesophyll tissue. At this time pale orange vesicles were first observed in infected host cells, these were thought to signify the host defence response although there was little apparent effect on subsequent colonisation of host tissue.

In the resistant cultivar, although both isolates were able to penetrate the cuticle and form infection vesicles, there was no evidence for a biotrophic interaction and fungal development was restricted to single epidermal cells. The host defence response was observed earlier than for KAD 332, with the appearance of dark red inclusions distributed throughout the cells under attack after 42 hours. After 48 hours these burst, releasing their contents into the cytoplasm killing both host cell and fungus, and preventing further colonisation.

In TAM 428 a differential response was observed. The virulent 117 formed a biotrophic phase and subsequently colonised the host as in KAD 332. However, the avirulent 74, was contained within single epidermal cells, with no evidence for biotrophy or proliferation through host tissue as in the resistant reaction.

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