19 01

A potential role for apoplast related and membrane bound enzymes in the generation of active oxygen species during incompatible host-pathogen interactions

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Native PAGE was used to identify qualitative and quantitative changes in apoplastic enzymes extracted from bean leaves during a hypersensitive response (HR) resulting from inoculation with an avirulent race of *P. s.* pv. *phaseolicola.* A burst of apoplastic peroxidase (EC-POX) activity was identified 2 h after inoculation. There was an apparent increase in the activity of an extracellular isoenzyme of superoxide dismutase (EC-SOD 2). In addition a second extracellular SOD isoenzyme (EC-SOD 1) was detected after 5 h. Contamination of the apoplast by cytoplasmic constituents was excluded as glucose-6-phosphate dehydrogenase activity was not detectable in apoplastic fluids during this period. This represents the first report of the involvement of an apoplastic SOD in the HR. The possibility that EC-POX may generate activated oxygen species via NAD(P)H oxidation is discussed. *In vitro* studies on the generation of activated oxygen species by microsomes isolated from potato tubers identified the existence of two distinct NADH oxidase activities, with pH optima of 6 and 7.8. At pH 6, NADH oxidase activity was stimulated by Mn^{2+}, 4-hydroxy phenolics and 3,4-dihydroxy cinnamic acid and was sensitive to inhibition by SOD and catalase (CAT). It is suggested that this activity is the result of a membrane associated peroxidase.

19 02

Peculiarities of hormone formation by phytopathogenic fungi (especially by *Septoria* Sacc. species)

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Fungi of more than 100 species of various taxonomic and ecological groups have been investigated to synthetise phytohormones up to now. It is hypothesized that pathogenous fungi closely associated in the life-cycle with plants are the most perspective producents of physiologically active metabolites. Hormones were detected for 50 phytopathogenous fungi species of orders *Taphrinales, Erysiphales, Helotiales, Ustilaginales, Uredinales* and group of *Fungi Imperfecti.* These fungi are able of producing indolyl acetic acid (IAA), abscisic acid (ABA), gibberellins (GA), cytokinins and ethylene as well as growth regulators of other chemical structure. Phytohormones as metabolites of fungi are isolated from cultural filtrates mostly, nevertheless, it have been demonstrated that the content of these substances may be of the same level in mycelium. Maximum hormones formation did not correlate with period of maximum accumulation of absolute dry mycelium mass. The varieties of hormone biosynthesis activity on different onthogenesis stages of fungus was observed also. Cytokinins and ABA greatest contents are intrinsic to active vegetative growth period, IAA and cytokinins greatest contents are usual to spore-formation and germination periods. Maximum level of ABA accumulation was determined within the range 15 ng/g - 202 ng/g, IAA, GA, cytokinin ones were up to 6×10^5, 4×10^4, 1×10^5 ng/g of dry micelium mass respectively.

The phytohormone balance of *Septoria tritici* and *S.nodorum* caused dangerous wheat epiphytotic deseases has been investigated. *Septoria* species are characterized by the whole plant growth substances complex (ABA, IAA, GA, cytokinin, ethylene). It was found that the hormones accumulation dynamics and range of their biosynthesis for different *Septoria* species isolates conform to principal pecularities of phytopathogenous fungi hormone formation.

19 03

The physiological aspects of plant-pathogen interactions with special attention to resistance mechanisms

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Studies on physiological and biochemical events during plant-pathogen interactions have contributed to better understanding of the mechanisms of disease resistance and susceptibility. My presentation tries to summarize our knowledge on the role of toxins, cell wall- and membrane-degrading enzymes and plant growth regulators as general virulence mechanisms. Significance of phytoalexins, elicitors, active oxygen species, lignification and PR-proteins in resistance is also evaluated. Finally the role of the physiological state of plants in disease and stress resistance is discussed.

19 04

Effects of inoculation by ectomycorrhizal fungi on survival and growth of micropropagated trees

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In vitro produced plants planted to soil are exposed to complex stress effects which reduce their survival and growth intensity. The aim of our investigations is to prevent this "planting stress" by the means of a treatment with possible mycorrhiza partners of micropropagated fruit trees /Prunus persica, Populus sp. etc./ and Robinia pseudoacacia. The ectomycorrhiza forming fungi have been used were Terfezia terfezioides, Tuber rapaeodorum and Tuber aestivum.
Positive effects on growth and survival /30-50% and 80-100% respectively/ have been detected, although typical morphological evidences proving mycorrhiza formation /fungal mantle or Hartig net/ were not present in the root system. Any significant differences in the activity of photosynthetic apparatus of inoculated and control plants have not been measured by Pulse Amplitude Modulation method.
The effect of fungal treatment on growth and survival might be considered as a synergetic phenomenon.
This work has been supported by an OMFB grant.

19 05

Growth stage response to salinity on *Vicia faba-Rhizobium leguminosarum* bv. *viciae* symbiosis

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Information on the growth stage response to salinity within a crop is important in adopting suitable genetic and management strategies for saline soils. Legumes plants in salt affected fields are usually exposed to steady levels of salinity, however in other cases plants are exposed to changing conditions, weekly, annually or same growth season. The effect of salinity on plant growth and N_2 fixation, during changes salt periods, can sometime be more dramatic than the effect of a constant salinity level.

In this study the effect of sodium chloride (0, 50, 75 and 100 mM) when it is added immediately at the time of inoculation and in the vegetative growth season, on some process related with N_2 fixation and growth of *V. faba* has been determined. *V. faba* plants were inoculated with strain GRA19 of *R. leguminosarum*, selected like tolerant salt strain.

When the salt supply was at beginning of growth N_2 fixation was more sensitive to salinity than plant growth. Plant growth was reduced by 30% with 100 mM NaCl while the drop in the ARA was more intense. However when the salt supply was added in vegetative growth season, the nodulation was not affected, and N_2 fixation, reduced N content and NH_4 assimilation enzymes activity (GS and NADH-GOGAT) were less affected. This dual response would complicate the selection process if it is based on a single growth stage.

Financial support from INIA, Grant AGR91-0549.

19 06

Hormonal signalling associated with root growth promoting *Azospirillum* in wheat

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Azospirillum inoculated wheat seedlings showed significant elongation of the primary root and profuse lateral root differentiation. The results suggested that the response of bacteria inoculation was associated with the capacity of nitrate-grown bacteria strains to produce IAA and NO_2 particularly under low oxygen concentration in the medium. When IAA and NO_2 (10^{-7} M) were added to root segments active elongation occurred, the effect extending up to 24 h of incubation. The extent of acidification of the root medium indicated that both IAA and NO_2 caused H^+ extrusion, which was inhibited by o-vanadate solely in the IAA-treated segments. IAA promotion of root elongation was prevented by procaine, the NO_2-induced root growth being less affected. Incubation of root segments with sulphydryl agents of differential membrane permeation, iodoacetamide, n-ethyl-maleimide, diamide and p-HMB, suggested that both IAA and NO_2 growth effects would be associated with SH-proteins. IAA and NO_2 growth promotion decreased in segments treated with trifluoperazine, a Ca-calmodulin antagonist, the inhibition being more pronounced in the nitrite treatment. Our results indicated that the IAA and NO_2 effects are governed by somewhat distinct mechanisms: IAA seems to be effective on both short and long term growth responses, while NO_2 is particularly active in the long term events leading to wall loosening and matrix incorporation.

19 07

Activation of defence reactions in *Allium cepa*

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An example of rapid defence responses to pathogen attack is observed in the interaction of onion with *Botrytis* spp. Among the rapidly activated genes are those encoding hydrolytic enzymes whereas the genes encoding phytoalexins, such as tsibulins 1d and 2d, respond more slowly. Analysis of bulb scales tissues by SDS-PAGE revealed that several pathogenesis-related (PR)-proteins accumulated earlier in incompatible interactions than in compatible ones. Two of the accumulating PR-proteins were purified by ion-exchange chromatography and used to raise polyclonal antibodies. One of the proteins (M_r 27 kD) showed chitinase activity, while the other (M_r 33 kD) showed 1,3-β-glucanase activity. The early accumulation incompatible interactions of both chitinase and glucanase was also visualised by Western blotting. The rapid gene expression and accumulation of these enzymes, potentially capable of degrading the hyphal walls of *B. cinerea* at the site of penetration, might play an important role as plant defence reactions.

19 08

Interaction of protein inhibitors from the seeds of buckwheat and proteases secreted by micromycetes *Alternaria alternata* and *Fusarium oxysporum*

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The interrelation between higher plants and pathogenic fungi is very complex and involves different chemical compounds from both sides. In dry buckwheat *Fagopyrum esculentum* seeds we found a group of trypsin inhibitors capable of suppressing the germination of spores and the growth of mycelium of micromycetes *A.alternata* and *F.oxysporum* which infect buckwheat. The inhibitors were purified to homogeneity and identified as proteins with molecular masses of 7.7-9.2 kDa. None of the inhibitors suppressed the activities of papain, elastase, pepsin or subtilisin. At the same time all of them inhibited the activities of proteases secreted by the mentioned fungi. The study of the secretion of the proteases by the micromycetes revealed that it was initiated only in the presence of protein substrates in the culture medium. Thus addition of such substrates to a medium devoid of protein may serve as a good model for an investigation of the interrelation between the pathogen and the plant-host. The effect of the cultivation conditions on the synthesis and secretion of proteases by fungi *A.alternata* and *F.oxysporum* was also studied.

19 09

Transformation studies with English elm

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In vitro grown shoot cultures of English elm (*Ulmus procera*) have been transformed using biolistics and *Agrobacterium* protocols. Transient expression of the GUS reporter gene has been demonstrated histochemically in elm leaf tissue following bombardment using gunpowder, electrical discharge and helium driven biolistic devices. A helium driven particle inflow gun has been constructed and is being used to compare promoter effects in elm tissues. Various *Agrobacterium tumefaciens* strains have been used to produce tumours, from some of which shoots have been induced to develop. These *U. procera* shoots, which have successfully been rooted, have been shown to be transgenic by nopaline testing. The establishment of reproducible transformation and regeneration systems for elm trees will be beneficial in studying tree growth, development, and in combatting Dutch elm disease.

19 10

Drought effects on nitrogen and carbon metabolism in soybean nodules

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Symbiotic nitrogen fixation by legumes depends on an interaction between plants and bacteria and is affected by changes in both the aerial and the underground environments. Drought may affect nodule activity through a decrease in photosynthetic activity. However, as nitrogen fixation declines faster than photosynthesis, a direct effect on nodule oxygen diffusion has been suggested. However, little research has been conducted on the effects of drought on nodule biochemistry. Here, we report the effects of drought stress on enzymatic activities related to carbon and nitrogen metabolism in soybean nodules. Western blots of nitrogenase components showed that component I was virtually insensitive to water stress, whilst only a slight decline could be observed in component II. No significant changes could be detected on aspartate amino transferase, phosphoenolpyruvate carboxylase, glutamine synthetase and alkaline invertase activities nor in the amounts of lehaemoglobin or total protein. However, sucrose synthase, one of the enzymes involved in sucrose metabolism in legume nodules, declined dramatically during drought. This enzyme may play a key role in the regulation of nodule metabolism and, therefore, of nitrogen fixation.

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19 11

A relationship between protein body degradation and defence response in pine megagametophytes

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Defence reactions of megagametophytes of *Pinus nigra Arn.* against a tester, *Phaeolus schweinitzii (Fr.) Pat.* basidiomycete, were studied by a simple method of dual culture. In vitro cultivation was carried out on the agar medium B-25 with 5 mg/l naphthaleneacetic acid (NAA) and 0.1 mg/l benzylaminopurine(BAP).The megagametophyte showed a strong defence reaction mainly when directly contacted with the basidiomycete mycelium. The growth of fungal mycelium is inhibited by L-glutamine which, in megagametophytes, is a component of protein bodies and is released by their degradation. This process was studied during cultivation of megagametophyte with the tester by freeze-etching.

19 12

Forms of nodule cytosol glutamine synthetase and the efficiency of *Rhizobium*-legumes symbiosis

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It has been established that in the cytosol of plant tissues of root nodules formed at the symbiosis of effective and uneffective *Rhizobium* with the host plant from the *Leguminaceae* family (soybean, alfalfa and pea) 3 forms of glutamine synthetase (GS) are found during the phase of the highest intensity of nitrogen fixation. However, both the total activity and the activity of each of the forms in ineffective nodules is twice as low as in effective ones. Three forms of GS from soybean nodule cytosol were obtained and purified, some of their physico-chemical properties were studied, the contribution of the GS forms in the NH_4^+ assimilation during root nodule development was shown.

19 13

Monitoring of the early stage of mycorrhiza formation in *in vitro* cultures

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The typical ectomycorrhiza of deciduous trees is generally a slowly developing form, appearing relatively late after infection. The detection of fully developed ectomycorrhiza in natural symbiont systems /wood - fungi/ is demonstrated by the presence of a fungal mantle, or the Hartig net, a characteristic interface of the fungus and host.
Our investigations to optimize the conditions of in vitro mycorrhization need a rapid checking of the state of fungal colonization in host roots before the creation of a mantle or the Hartig net.
A combined staining of root segments to follow the establishment of an artificial ectomycorrhiza is presented. The usefulness of the method is demonstrated by the linden tree and Tuber rapaeodorum symbiosis monitoring.
This work was supported by a Mecenatura grant of OMFB.

19 14

Host-plant lectin as a cell-recognition molecule in the *Rhizobium*-lupine symbiosis

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The recognition between rhizobia and legume host-plant root in the process of formation of symbiotic system involves a binding of components of contacting surfaces of partners-plant lectin and unique carbohydrates of bacterial symbiont. It has been found that lupine root lectin has several properties which define it as a cell-recognition molecule in the *Rhizobium*-lupine symbiosis. This lectin is located on plant root in grater amount within the zone of the development of root hairs where the infection penetrates. *Bradyrhizobium* sp. (*Lupinus*) are found and agglutinated by the hapten of lupine lectin-D-galactose. Lupine root lectin binds not only bacterial cells, but also cellular exopolysaccharides. However, functional activity of exopolysaccharide-lectin interactions is determined by the degree of homologicality of symbiotic pairs.

19 15

Formation of Indole-3-acetic Acid (IAA) and Abscisic Acid (ABA) in *Pisum sativum* damaged by *Aphis fabae*.

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The role of IAA and ABA on the resistance of garden pea plants (*Pisum sativum*) to bean aphid (*Aphis fabae*) has been studied. The endogenous phytohormones were assayed by means of high-performance liquid chromatography (HPLC). It was found that the damaging effect of insects on leaves caused changes in the levels of phytohormones in plants. The increasing of damaging effect of insects on plants caused increase of ABA and decrease of the IAA in leaves of plants. Changes in ABA and IAA levels in the leaves of pea were correlated with the number of feeding insects. Changes in IAA contents in the roots of infested plants have been decreased. The damaging effect of insects on bean caused increase of ABA both in the leaves and in the roots. It has been suggested that the resistance of plants against insect pests may be determined by different abilities to form and accumulate ABA and IAA.

19 16

Defence reactions of strawberry plants after infection with *Botrytis cinerea* and salicylic acid treatment.

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Salicylic acid is considered to be an endo and exogenous inducer of resistance reactions in plants. Changes in superoxide anions content, in superoxide dismutase and peroxidase activities and phenolic compounds content are suggested to be defence reactions of a plant to pathogen infection or elicitor treatment. We studied the above resistance reactions in strawberry plants treated with salicylic acid and infected with *B. cinerea*. Our results demonstrate that the amount of superoxide anions doubled as early as 1h after salicylic acid treatment of strawberry plants. The highest level of superoxide anions was observed 3h after treatment and then their amount decreased gradually. The increase in superoxide anions content was also observed after infection with pathogen . In infected plants both treated and not treated with salicylic acid the levels of superoxide anions were similar and about two and half times higher than in control. The superoxide dismutase activity in strawberry plants did not changed after treatment with salicylic acid however the activity of this enzyme significantly increase after infection. The activity of peroxidase and phenolic compounds content also increased after infection of strawberry plants with the pathogen.

19 17

Presence of the enzyme nitric oxide synthase in root nodules of lupine plants (*Lupinus albus* cv. Multolupa)

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In mammalian cells, nitric oxide synthase (NOS) is responsible for the production of nitric oxide (NO) from the amino acid L-arginine. In this work we show, for the first time, the presence of this enzyme in a plant tissue, in the cytoplasm of the lupine root nodule. NO synthesis was measured spectrophotometrically monitoring the methemoglobin formation. The maximum NO synthesis [124 pmol mg^{-1}(protein) min^{-1}] was obtained with cytosol extracts of young nodules (22 d) decreasing with the nodule growth. NOS was Ca^{2+} independent and required NADPH. In base of the close relationship between the NADPH-diaphorase and the NOS activities in mammalian cells, an indirect histochemical method for the diaphorase activity was used. NADPH-diaphorase activity was observed mainly in the vascular bundle cells.

The results might indicate a possible role of the NO synthesis in the early events of the symbiosis or a host protecting mechanism from invading microorganisms.

19 18

Regulation of uptake hydrogenase in *Frankia* isolated from *Casuarina*

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Frankia is an actinomycete that can live in symbiosis with actinorhiztal plants, which mostly are trees or shrubs. *Frankia* can fix atmospheric nitrogen to ammonia via the enzyme nitrogenase but concomitantly protons are reduced to H_2. H_2 can then be oxidized via the enzyme uptake hydrogenase and energy is gained. With few exceptions almost all *Frankia* investigated have an uptake hydrogenase in symbios.

Cultures of a free-living *Frankia* strain KB5, isolated from *Casuarina equisetifolia*, were investigated for activity and presence of a membrane-bound uptake hydrogenase.

It has earlier been shown by Sellstedt & Smith (1990) that nickel is required for uptake hydrogenase activity in *Frankia* isolated from *Casuarina*, therefore *Frankia* strain KB5 was grown in cultures either with or without addition of 1µM nickel. Uptake hydrogenase activity could be detected when nickel was added to the the media.
Western blot analysis, using antibodies against uptake hydrogenase holoenzyme purified from *Alcaligenes latus* (Pinkwart et al, 1983, Doyle & Arp, 1987), revealed that a polypeptide corresponding to the large subunit of uptake hydrogenase was present in nickel deficient as well as nickel containing cultures.

19 19

A tomato plants breeding strategy for resistance to *Alternaria solani*

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The fungus *Alternaria solani* is a highly aggressive plant pathogen. Two culture filtrates (50-12 and 50-19) with different effects on tomato plants growth and callus tissues were studied *in vitro*. These substances showed differences in their ^{1}H-NMR structure and membrane-potential activity.

A model of breeding strategy was developed on the basis of a supposed cell membrane mechanism of the action of the culture filtrate fungus-toxins. A correlation between the breeding model for fungi resistance and the aggressiveness was observed in fifteen different cultivars of *Lycopersicon esculentum*.

19 20

Nitrate-induced ethylene biosynthesis in uninoculated and inoculated soybean roots.

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In alfalfa nitrate and inoculation stimulated ethylene production by roots and the treatment with aminoethoxyvinylglycine significantly stimulated nodulation [J. Plant Physiol. (1987), 129:461-467; Plant Physiol. (1991), 97:1221-1225]. The present investigation has looked at the ethylene production rates of inoculated and uninoculated soybean (*Glycine max* [L.] Merr.) roots grown on 1 or 8 mM NO_3^- solution. Regardless inoculation, 8 mM fed roots produced significantly ($P<0.05$) more ethylene than those fed 1 mM, 1600 vs 1126 and 1763 vs 1503 pmol·(g DW)$^{-1}$·h^{-1} on average for uninoculated and inoculated roots respectively. Accordingly they also showed higher ethylene forming enzyme (EFE) activity than controls (38 vs 24 nmol·(g DW)$^{-1}$·h^{-1}, $P<0.05$). Mean nodule number recorded per plant 18 days after inoculation was 120 and 7 for 1 and 8 mM NO_3^- treated plants respectively. Silver (Ag^+) efficiently inhibits ethylene action in many plant tissues and supplied as silver thiosulfate promoted a 3-fold increase in nodule number per plant in these highly NO_3^- inhibited soybean plants. Further silver significantly stimulated (30 %, $P<0.05$) nodulation in soybean under conditions where limitation of nodulation would not be expected, i.e. 1 mM NO_3^- largely recommended in nodulation studies. The results suggest that endogenous ethylene might have a role in the control of nodulation in soybean. *Financial support from INIA, Grant AGR91-0549.*

19 21

Phytoalexin production is not related to necrose intensity in elicitin-treated tobacco

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When tobacco cells (*Nicotiana tabacum* var. *xanthi*) are treated with cryptogein, a proteinaceous elicitor from *Phytophthora cryptogea*, a strong extracellular alkalization and a transient AOS production are observed. These effects appear within a few minutes after treatment and are dose-dependent, the maximal responses being obtained for about 50-100 nM cryptogein. In experiments performed with *N. rustica* and *N. sylvestris* cells, we observe a similar extracellular alkalization whereas the AOS productiona is ten-fold lower. Moreover, cryptogein induces tobacco leaf necroses and *Nicotiana tabacum* var. *xanthi* appears to be more sensitive than *N. rustica* or *N. sylvestris* .

These results suggest that AOS production, observed in cell suspensions, could be responsible for the necrose induction observed in plants.

In these treatments we also analyse the phytoalexin accumulation. Contrary to AOS production, capsidiol accumulation is higher in *N. rustica* or *N. Sylvestris*, suggesting that AOS and phytoalexin productions are not directly related.

19 22

The effect of doubled ambient CO_2 concentration on winter wheat plants and on rhizosphere microflora

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Sterile seeds were inoculated by suspension of rhizosphere microflora and plants were grown in ventilated glass chambers with coarse silica sand as a root substrate under controlled conditions for 30 d. Microbial characteristics of root media (biomass, activity, CFU on various media) were regularly measured together with root exudation and with net photosynthetic rate of leaves and stems. As compared to control plants, root exudation of high-CO_2-grown plants was higher in the first days of experiment. After 20 d the decrease of root exudation which was preceded by the inhibition of net photosynthetic rate was observed in high-CO_2-grown plants but not in control plants. Microbial biomass and number of CFU in rhizosphere of high-CO_2-grown plants was higher than that of control plants up to 20 d and then was reduced in dependence on decrease in root exudation. Fluorescein diacetate hydrolysis characterizing microbial activity was higher in rhizosphere of high-CO_2-grown plants during the whole experiment.

19 23

What is the role of cerato-ulmin in Dutch elm disease ?

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The role of cerato-ulmin (C-U) in Dutch elm disease (D.E.D.), caused by *Ophiostoma ulmi sensu lato*, is still to be completely clarified. In a recent eccellent review on cerato-ulmin, Richards (1993, in: D.E.D. Research, cell. and mol. approaches, M.B. Sticklen & J. Sherald Eds.) pointed out the involvement of cerato-ulmin in D.E.D. because of seven major reasons. Our opinion is that some of these points could be revised according to the following results. It has been found that the synthesis of C-U is temperature-dependent, and thus some isolates of *O. ulmi* have been induced to produce abundant quantities of C-U (Tegli et al, 1994, Mycol. Res., in press). A specific C-U-probe of about 320 pb hybridizes with Hind III-digested total genomic DNA extracted from various isolates of *O. novo-ulmi*, *O. ulmi* and *O. piceae* (Cervone F., De Lorenzo G., Caprari C. Tegli S. and Scala A., 1994, unpublished data). C-U is a fungal hydrophobin on the basis of amino acid residues sequence (Stringer and Timberlake, 1993, The Plant Cell **5**, 145-146) and has been shown to be a component of cell walls of the mycelium of *O. novo-ulmi*, *O. ulmi* and *O. piceae* (Scala F., Del Sorbo G., Tegli S. and Scala A., 1994, unpublished data). C-U protects the D.E.D. fungi from the toxic effects of the elm phytoalexins (Wu et al., 1989, Eur. J. For. Path. **19**, 343-357), and spontaneous non C-U producing mutants of *O. novo-ulmi* are still pathogenic on *Ulmus carpinifolia* and *U. procera* (Tegli S., Scala A. and Brasier C.M., 1994, unpublished data).

19 24

Specificity and efficiency in nodulation of actinorhizal plants by *Frankia*

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Actinorhizal plants are capable of beeing infected by and forming nodules containing the actinomycete *Frankia*. Species of *Casuarinaceae* are native to the southern hemisphere, while some species of *Elaeagnaceae* can be found in temperate areas. In contrast to *Rhizobium*, *Frankia* is actively fixing nitrogen both in a free-living and in a symbiotic state.

Nodulation specificity of four *Casuarina* species and one *Elaeagnus* species was examined for five sources of *Frankia*. Efficiency of nodules, i.e. capability of nitrogen fixation, was evaluated by use of the acetylene reduction assay. Also, PCR amplification of DNA from nodules was used in order to verify that the added *Frankia* was actually forming that particular nodule.
Nodulation will be discussed in terms of specificity and efficiency.

19 25

The role of membranes in intercellular interrelations in phytopathosystems at obligate parasitism

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One of the more intriguing aspects of obligate phytoparasitism is the question of mechanisms of haustorial/host metabolism through the extrahaustorial membrane(EM), the invaginated by growing haustoria host plasmalemma. Development of haustoria is associated with reorganisation of host endomembranes and the EM differs from the host plasmalemma in a number of respects.

Ultrastructural study of phytopathosystems at rust and powdery mildew infection of cereals indicates the possibility of exo and endocytosis as the way of intercellular metabolism.It is possible that the prevalent zone of exocytosis of host metabolites is the epical zone of the EM and the endocytosis of fungal metabolites prevails in the distal zone of haustorial body.The results indicate different morphology of specific dictyosome subcompartment,the trans-Golgi reticulum and different dense and ultrastructure of secretory vesicles in phytopathosystems with various direction of fungous metabolism. These observations raise important questions regarding the functional significance of common and specific mechanisms of haustorial /host metabolism in different pathosystems at obligate parasitism.

19 26

Pathogenesis-related proteins in barley induced by powdery mildew infection.

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Various proteins are expressed in plants as response to biotic and abiotic stresses. One class of these proteins induced by the pathogenesis are the so-called pathogenesis related proteins (PR proteins). The physiological function of PR proteins is mostly unknown. Extracellular PR proteins in intercellular spaces of barley leaves were detected by PAGE as early as in the beginning of powdery mildew pathogenesis (24 - 72 h). In this early phase of pathogenesis an increased "leakage" (exocytose) of some proteins into leaf intercellular spaces was stated, regardless of the host-parasite genotype compatibility or incompatibility. The pattern of PAGE-separated proteins was influenced by host-parasite compatibility. PR proteins were more expressed at incompatibility than at compatibility.

19 27

Protein excretion from *Frankia* strains HFPCcl3 and R43

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Extracellular proteins have been carefully studied in some bacteria. However the information available for the symbiotic nitrogen-bacteria *Frankia* is limited to the characterization of extracellular proteins in *Frankia* isolate BR. In *Frankia* strains HFPCcl3 and R43 the amount of protein excreted to medium BAP reached the maximum after the end of the exponential phase and was much higher in strain R43, with 26.9 μg(prot.) ml^{-1}(medium) than in strain HFPCcl3, with 5.4 μg(prot.) ml^{-1}(medium). The maximum of protein secretion is followed by a drastic decrease where after the cultures develop into a stationary phase. The extruded proteins were purified by dialysis followed by acetone precipitation. After SDS-PAGE analysis the polypeptide patterns showed a high number of secreted proteins and also a different pattern between the strains R43, HFPCcl3 and with the extruded proteins obtained from the mixed culture of the two strains, showing a different metabolic behaviour compared to the situation when the two strains were growing separately. The high number and amount of extracellular proteins found in *Frankia* strains HFPCcl3 and R43 reveal that the protein secretion is an important metabolic process that should be well characterized to understand the role of these proteins in *Frankia* metabolism and/or infection.