GROWTH CURVES OF *PHOMA TRACHEIPHILA* (PETRI) KANC. ET GIK. ON LIQUID MEDIA AND TOXICITY OF CULTURE FLUIDS

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Phoma tracheiphila (Petri) Kanc. et Gik. is the causal agent of mal secco, a severe tracheomycotic disease of citrus in the Mediterranean area.

Since the early studies on mal secco it was suggested that metabolites secreted by the fungus are involved in the pathogenesis of the disease (Petri, 1930). A phytotoxic non specific glycopeptide, «malseccin», was isolated in Israel from culture filtrates and infected tissues and it was shown to induce some symptoms of the disease (Nachmias *et al.*, 1977; Nachmias *et al.*, 1979).

Many experimental evidences, however, indicate that different phytotoxic compounds might contribute to the symptoms expression of mal secco (Scrivani, 1954; Pennisi e Graniti, 1987). Cell wall-degrading enzymes produced by *P. tracheiphila* were also suggested as virulence factors (Graniti, 1969).

The aim of the present study was to investigate the interactions between pectic extracellular enzymes and other metabolites in determining the phytotoxicity of cultural liquids of *P. tracheiphila*.

The production of pectic enzymes was induced by growing the fungus in a substrate containing a specific carbon source. The time-course of mycelial growth, polygalacturonase activity and phytotoxicity was determined in the culture filtrates of two strains of the fungus grown on basal salt medium (Czapek Dox) supplemented with different concentrations of citrus pectin. The parameters examined were affected by the inoculum density and pectin concentration at which the cultures were started.

Both phytotoxicity and polygalacturonase activity peaked during the exponential growth of the cultures. Secondary peaks of phytotoxicity were detected in the stationary phase. The results suggest that different phytotoxic metabolites are produced at different times during the growth kinetic.

ON THE PHYTOTOXIN FROM CULTURE FILTRATES OF *PHYTOPHTHORA NICOTIANAE*

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The root and crown rot of tomato (*Lycopersicon esculentum*) caused by *Phytophthora nicotianae* (*P. parasitica*), is a serious disease of some tomato-growing areas of Italy.

The severity of the damages to the cultivation and the difficulty of the chemical and agronomic control of the pathogen, led to purify the phytotoxin produced in the culture filtrates, to use the active compound in a programme of genetic improvement and to study its role in the pathogenesis.

The fungus was grown on a synthetic medium containing sucrose, *L*-asparagine, yeast extract, mineral salts and vitamins. The shaken cultures were incubated for 12 days at 26° C.

The culture filtrates, which were tested for toxicity on tomato cuttings, did not show the occurrence of any lipophilic phytotoxin, but of an hydrophilic and polymeric compound, stable at 70° C for 30'. The culture filtrates were submitted to precipitation with cold acetone; the resulting phytotoxic supernatant was dialyzed against bidistilled water, using a tube with a cut off of 3500 dalton. The resulting non permeated activity was purified by gel filtration, affording a very active fraction.

The chemical and electrophoretic analysis strongly suggested for such fraction a nature of an acidic glycoprotein with an apparent molecular weight of 13000-14000 dalton.

The toxin showed strong phytotoxicity still at 0,05 mg/ml. Work is in progress to further define the chemical and biological characterization of the active compound.

EFFECT OF FUSARIC ACID ON THE VACUOLAR MEMBRANE

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The effect of fusaric acid, a phytotoxin of *Fusarium* spp., on the tonoplast was investigated.

In the classical test of betacyanin release from red beet root disks, the toxin fed at millimolar concentrations induced a marked release of betacyanins, suggesting that the tonoplast functionality in the intact cell was somehow affected (Radice and Pesci, this same issue).

A possible direct effect on the tonoplast was thus tested both at morphological and biochemical levels. Fusaric acid (up to 15 mM) did not affect the intactness of *Acer pseudoplatanus* isolated vacuoles, observed at the optical microscope. In microsomal preparations from cultures cells of both *Acer pseudoplatanus* and *Arabidopsis thaliana*, fusaric acid at concentrations from 0.25 mM to 3 mM inhibited (–10 to –35%) the nitrate-sensitive (tonoplast) ATPase, whereas it was ineffective on another tonoplast enzyme activity, the K^+-stimulated pyrophosphatase.

The structural analogues picolinic and nicotinic acids at the same concentrations showed a similar behaviour (inhibition of nitrate-sensitive ATPase and no effect on K^+-stimulated PPase).

These results indicate that fusaric acid can directly affect some tonoplast function without inducing a general alteration of the state of the membrane.