

STIMULATION OF DEFENCE REACTIONS IN CITRUS BY FOSETYL AL  
AND FUNGAL ELICITORS AGAINST PHYTOPHTHORA spp. .AKTHAR J. KHAN and A. RAVISE  
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## SUMMARY

Defence mechanisms of a Citrus cultivar susceptible to gummosis are stimulated by treatments with fosetyl-Al or by fungal elicitors. The compounds synthesized are similar to those found in tissues of resistant cultivars.

## INTRODUCTION

The fungicide fosetyl-Al can effectively control the susceptibility of Citrus to gummosis caused by *Phytophthora* spp. (Laville and Chalandon 1982). Several studies have shown that fosetyl-Al has little direct effect on the parasite *in vitro* (Vo Thi Hai *et al.* 1979) although it induces the resistance *in vivo* after infection (Bompeix *et al.* 1981, Guest 1984, Vernenghi 1985). In the case of Citrus gummosis we suppose that fosetyl-Al acts by influencing host defence mechanisms as in tomato or dichlorocyclopropane in rice (Cartwright *et al.* 1977). The aim of the present study was to investigate following :

- comparison of natural defence reaction in tolerant Citrus with that of a susceptible Citrus after treatment;
- comparison of incidence of the fungicide and of elicitors on defence reaction of susceptible Citrus;

## MATERIALS AND METHODS

Tolerant cultivars -*Poncirus trifoliata* and Rough Lemon- and susceptible -Valencia Late- were raised in the glass house for 5-7 months. They were inoculated with *P. citricola*, *P. citrophthora*, *P. parasitica* strains of determined pathogenicity provided by the "Institut de Recherches sur les Fruits et Agrumes". Stem inoculations were done with mycelial discs. The inoculated plants of Valencia Late were treated weekly with fosetyl-Al (200 µg/plant) or with elicitors -glucosamine (40 µg/plant), arachidonic acid (50 µg/plant) or both at the same concentrations. Amino-oxyacetic acid (AOA) a competitive inhibitor of phenylalanine ammonia lyase was applied to a set of plants treated with fosetyl-Al.

Plants were harvested after 4 weeks of incubation. After methanolic extraction, phenolics were estimated by Folin-Ciocalteu reagent. The accumulation of defence compounds was analysed by thin layer chromatography (TLC) and high performance liquid chromatography (HPLC) using different solvents. *In vitro*, the toxicity of the extracts was assessed by inhibition of *Cladosporium cladosporioides* growth on TLC. Identification of inhibitors was carried out by Mass spectrometry, Nuclear Magnetic Resonance and Infra-Red spectrometry in the Laboratoire des Mediateurs Chimiques (I.N.R.A.-C.N.R.S.).

## RESULTS

Total phenolics increased significantly after inoculation, mainly in Rough Lemon and in *Poncirus Trifoliata*; in tissues of Valencia Late synthesis was considerably enhanced by the treatments with fosetyl-Al and elicitors (Table I).

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Table I :Concentration of phenolics in Valencia Late after inoculation and various treatments (concentrations expressed as equivalent of chlorogenic acid  $\mu\text{g/g}$  fresh weight).

Treatment	Inoculated	Fosetyl-Al	Fosetyl-Al + ADA	Glucosamine	Arachidonic acid	Glucosamine + Arachidonic acid
Stem	642	780	650	946	1270	1078
Root	846	1045	760	980	1155	1076

TLC analysis revealed accumulation of compounds in tolerant hosts which were also detected in Valencia Late after treatments Table 2).

Table 2 : Comparison of TLC analysis of Valencia Late extracts in 2 eluants after spraying with p-nitroaniline (PN) or antimony chloride (SB) reagents.

Table 2a : (Hx : Ae 2 : 1)

Rf	0,17	0,33	0,40	0,46	0,50	0,53	0,60	0,63	0,70	0,83	0,90
PN	++	+	0	-	-	+	-	0	-	+	-
Sb	+	+++	0	+++	0	++	+	+	+	0	+

Table 2b : (Clf :Ae : Me 50 : 40 : 5)

Rf	0,10	0,40	0,60	0,69	0,77	0,83	0,86	0,91	0,94
PN	0	0	++	++	+++	+++	++	++	+
Sb	0	0	++	++	+++	+++	++	++	+

+ = Increase in synthesis ; 0 = No increase ; - = Not detected ; + = New synthesis.

According to these results, several biosynthetic pathways were triggered. A sesquiterpen ( $R_f$  0.63 in Hx/Ae) and a coumarin -xanthyletin- ( $R_f$  0.77 in Clf:Ae:Me) were detected besides anthoxylin (Hartmann and Nienhaus 1974). Other inhibitors were mainly phenolic compounds, benzoic and cinnamic acid derivatives. It is evident from present results that fosetyl-Al and elicitors triggered in Valencia Late tissues the synthesis of compounds which were constitutive in Rough Lemon inoculated by *P. citrophthora*. We observed only quantitative differences between extracts of inoculated plants treated with fosetyl-Al or with elicitors. The application of AOA significantly suppressed the synthesis of phenolics in plants treated by fosetyl-Al, mainly the compounds eluted between 40% and 80% MeOH on silica C 18 column (Figure 1).

All the compounds accumulated in tissues, after partial purification by TLC and HPLC, were tested for toxicity to *Cladosporium cladosporioides*; a strong inhibition was obtained with the sesquiterpene, the coumarin, the acetophenone and phenolics eluted between 40%-70% MeOH on silica C18 column. In this case,

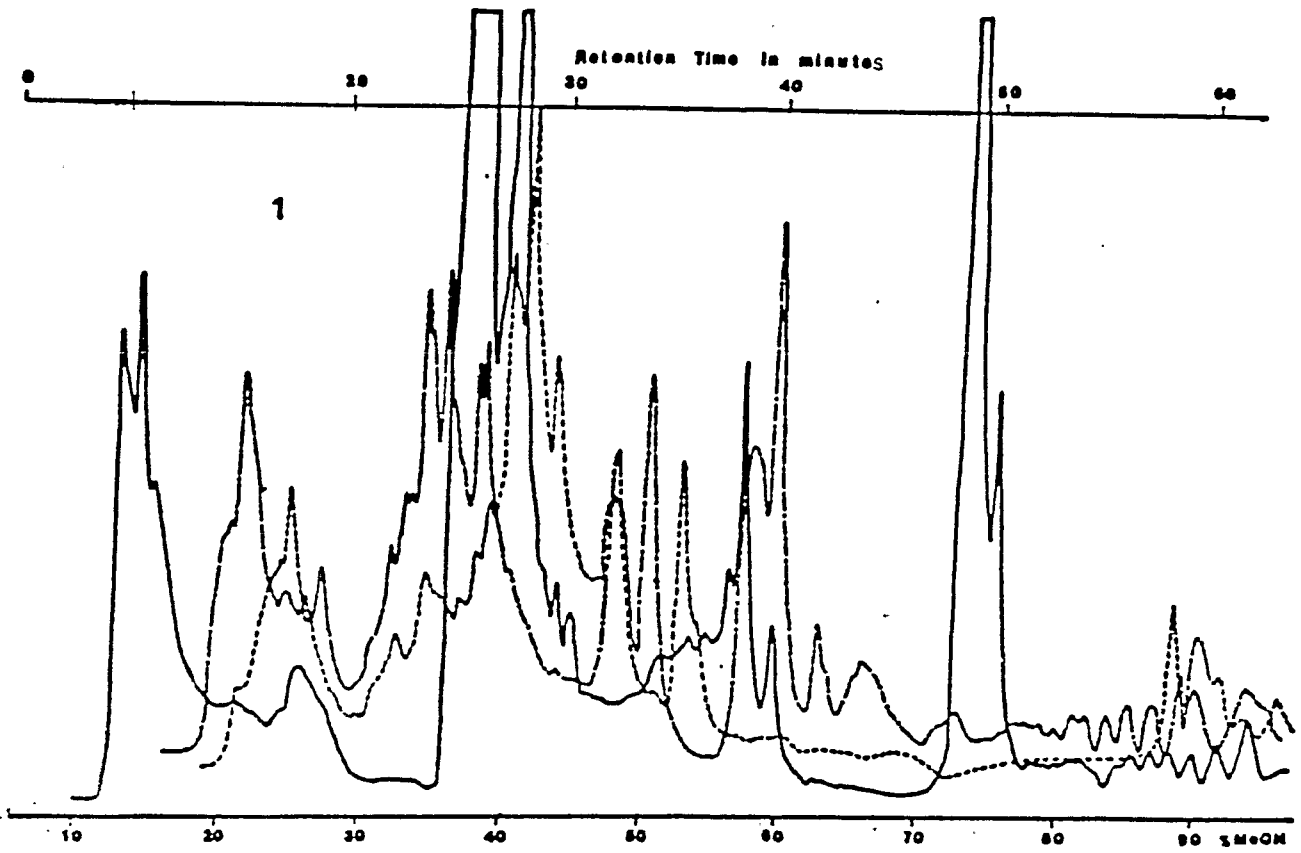


Fig. I : Comparison of HPLC analysis of the shoot extracts of Valencia Late inoculated with *P. citrophthora* (.....), inoculated and treated with fosetyl-Al (---) or with glucosamine (—).

we observed strong correlation between the extracts from Rough lemon and from Valencia Late treated with fosetyl-Al or elicitors.

#### DISCUSSION AND PROSPECTS

The present findings indicate that treatment with fosetyl-Al induces defence mechanisms like elicitors. Chromatographic analysis shows considerable similarity between the natural reaction of a tolerant variety and that of susceptible Valencia late after treatment. It is known for host-pathogen interactions of capsicum and tobacco (Guest) or tomato (Vernenghi) that fosetyl-Al induces defence mechanisms. In the case of Citrus, we suppose that independently of its direct activity (Bompeix and Saindrenan 1984), the fungicide triggers the host reaction in the same way that the elicitors.

Studies using Fosetyl-Al could be a tool to determine the mechanisms of resistance of Citrus to Phytophthora spp.. Possible applications could concern improving disease control in practice and also the screening of hybrid root stocks for their ability to give defence reactions against Phytophthora spp..

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#### ABBREVIATIONS

Ae = ethyl acetate; Clf = chloroform; Hx = hexane; MeOH = methanol

Titre : Stimulation des réactions des citrus aux Phytophthora par le phosétyl d'Al et par des éliciteurs ioniques.

Résumé : le phosétyl d'Al, comme des éliciteurs, stimule les réactions de défense contre la gommose; les substances inhibitrices accumulées sont analogues à celles élaborées par des cultivars naturellement résistants aux Phytophthora spp..