

# Incidence of Fosetyl Al and elicitors on the defence reactions of Citrus attacked by *Phytophthora* spp.

AKHTAR J. KHAN, Annie VERNENGHI and A. RAVISE\*

INCIDENCE OF FOSETYL Al AND ELICITORS ON THE DEFENCE REACTIONS OF CITRUS ATTACKED, BY *PHYTOPHTHORA* spp.

AKHTAR J. KHAN, Annie VERNENGHI and A. RAVISE

*Fruits*, Oct. 1986, vol. 41, n° 10 p. 587-595.

**SUMMARY** - Defence mechanisms against gummosis agents - *Phytophthora parasitica* and *P. citrophthora* - were compared in Valencia late susceptible and resistant Rough lemon and *Poncirus trifoliata*. After infection, were observed an increase of phenolics concentrations and a synthesis of new compounds. Valencia late defence reactions were stimulated by treatments with Fosetyl Al or by fungal elicitors; the compounds synthesized were similar to those found in tissues of resistant cultivars.

STIMULATION DES REACTIONS DE DEFENSE DE CITRUS A DES ATTAQUES DE *PHYTOPHTHORA* SPP PAR LE PHOSETYL D'ALUMINIUM ET DES ELICITEURS FONGIQUES.

AKHTAR J. KHAN, Annie VERNENGHI et A. RAVISE

*Fruits*, Oct. 1986, vol. 41, n° 10, p. 587-595.

**RESUME** - Les mécanismes de défense contre les infections à *Phytophthora* (gommose) sont comparés chez Valencia late sensible et chez Rough lemon ainsi que *Poncirus trifoliata* résistants au *Phytophthora parasitica* et au *P. citrophthora*. Ils correspondent à l'accumulation dans les tissus de composés phénoliques et à la synthèse de plusieurs produits toxiques pour les parasites. Ces réactions sont stimulées chez Valencia late par des traitements avec le Phosétyl Al ou par des éliciteurs fongiques ; dans ces conditions, les produits de défense accumulés sont semblables à ceux décelés dans les tissus des cultivars résistants.

## INTRODUCTION

The Citrus plants are attacked by several *Phytophthora* species causing trunk gummosis. The occurrence of disease is more common on the majority of Citrus varieties cultivated in tropical region and being limiting factor for their production (BOCCAS and LAVILLE, 1978). The fungicide Fosetyl Al possesses preventive and curative properties against the trunk gummosis of Citrus plants (LAVILLE and CHALANDON, 1982). Fosetyl Al has a little direct activity against mycelial growth of pythiaceae *in vitro* (VO-THI-HAI *et al.*, 1979) although it controls the disease caused by these pathogens in fields (BERTRAND *et al.*, 1977, FARIH *et al.*, 1981).

It was suggested that Fosetyl Al could be converted to a more toxic derivative for the pythiaceae in the plant tissues

(CLERGEAU and BEYRIES 1977, FARIH *et al.*, 1981). According to other authors, Fosetyl Al could inhibit the pathogens *in situ* by a gradual release of phosphorous acid and its remanence (TIMMER and CASTLE, 1985; DERCKS and BUCHENAUER, 1986). In fact, it was demonstrated *in vitro* a direct mode of action of Fosetyl Al and phosphorous acid causing a differential inhibition of *Phytophthora* spp. (BOMPEIX and SAINDRENAN, 1984). Nevertheless, the physiological reactions of the host like tomato against several *Phytophthora* spp (VO-THI-HAI *et al.*, 1979; BOMPEIX *et al.*, 1980; RAVISE *et al.*, 1981; TRIQUE *et al.*, 1981), grapevine against downy mildew (RAYNAL *et al.*, 1980), tobacco against *Phytophthora* spp. (GUEST, 1982) could suggest that the mechanism of Fosetyl Al is situated at the host-parasite interface (BOMPEIX *et al.*, 1981; GUEST and BOMPEIX, 1984). In fact, it is evident from several plant-fungus interaction studies that fungitoxic compounds of host origin-phytoalexins produced during infection - contribute to defence reaction (KUC and HAMMERSCHMIDT, 1978; BELL, 1981). In addition to fungal infection, the accumulation of phytoalexins could be triggered by molecules of microbial origin -

\* - AKHTAR J. KHAN - Department of Botany and Range Science - Brigham Young University - Provo Utah 84602 USA  
Annie VERNENGHI and A. RAVISE - Office de la Recherche scientifique et technique - Bondy - France.

ORSTOM Fonds Documentaire

N° : 23791 ex. 1

29 Cote : B

11 Date : 870731

elicitors - (ALBERSHEIM and VALENT, 1978; BAILEY, 1982) or by some fungicides (CARTWRIGHT *et al.*, 1977; VO-THI-HAI *et al.*, 1979).

The purpose of the present study is to investigate the following:

- comparison of defence reaction of a susceptible variety to that of resistant varieties;
- comparison of natural resistance mechanisms in genetically resistant varieties to that of a susceptible induced by Fosetyl Al;
- comparison of incidence of fungicide and elicitors of the defence reaction of Citrus according to their susceptibility to the pathogens.

## MATERIALS AND METHODS

### Plant materials.

The Citrus plants provided by INRA-IRFA-CIRAD station of Corse, Rough lemon, *Poncirus trifoliata* resistant and Valencia late susceptible to gummosis (de VALLA-VIEILLE and PERRIER, 1981) were grown in plastic pots kept in green house at 25°C.

### Fungal cultures.

The pathogenic strains of *Phytophthora citrophthora* (Smith and Smith) Leon; *P. parasitica* Dastur and *P. citricola* Swada were provided by IRFA. Cultures were maintained on pea agar synthetic medium at 27°C.

### Chemicals.

The systemic fungicide Fosetyl Al [Aluminium tris (0-ethyl)-phosphonate] provided by Rhone-Poulenc Phytosanitaire was used for stimulation of defence reaction. For comparison, two fungal elicitors were applied: glucosamine - an oligomer of chitosane, elicitor of *Fusarium* sp. - and arachidonic acid - one of the elicitors of *Phytophthora infestans* - provided by Sigma chemicals. Their activity was compared to that of a competitive inhibitor of phenylalanine ammonia lyase, aminoxy-acetic acid (AoA) (Sigma chemicals).

### Inoculation and treatments.

Inoculations were made with 7 days old mycelium discs of *P. citrophthora*, *P. parasitica* and *P. citricola* on the stems of 5 - 6 months old Valencia late, Rough lemon and *Poncirus trifoliata* plants, respectively. The inoculated

Valencia late plants were treated weakly with Fosetyl Al (200 µg/plant) or with elicitors, glucosamine (40 µg/plant) or arachidonic acid (50 µg/plant). The inhibitor AoA was applied to a set of plants inoculated and treated with Fosetyl Al in comparison with treated reference and treated after inoculation. Similarly a set of Rough lemon plants were treated with Fosetyl Al or glucosamine in the same concentration used for Valencia late. Plants were harvested after 4 weeks of incubation at 27°C.

### Extraction of fungistatic compounds.

After harvesting, a determined amount of shoots and roots were chopped in small pieces and dilacerated with a tissue grinder in 90 % methanol. The extraction was performed in dark at room temperature during 24 hours. Then the samples were filtered and residues re-extracted with the same solvent. The filtrates were pooled and dried in a rotatory Büchi evaporator at 40°C under vacuum. The dry residue was dissolved in methanol (1 ml corresponding to 5 g of fresh weight). These extracts were used for total phenolics estimation, chromatographic studies, partial purification of fungal inhibitors and *in vitro* toxicity tests for *Phytophthora citrophthora*.

### Dosage of phenolics.

The total phenolic compounds were estimated by Folin-Ciocalteu method using chlorogenic acid as reference at 725 nm with a Beckman 25 spectrometer.

### Chromatographic analysis.

#### • Thin layer chromatography (TLC).

The accumulation of fungitoxic compounds was analysed by silica gel TLC in several solvent systems: hexane-ethylacetate (2:1); hexane-ethylacetate-methanol (60:40:3); chloroform-ethylacetate-methanol (50:40:5); chloroform-methanol (95:5); chloroform and dichloromethane. The fluorescence of the compounds was observed under ultraviolet (U.V.) at 254 and 336 nm. Compounds were visualized also by spray reagents: diazotised p-nitroaniline for phenolics and antimony chloride saturated in chloroform at 120° (aliphatic and aromatic compounds).

#### • High performance liquid chromatography (HPLC).

The results obtained with TLC were confirmed by HPLC Touzart et Matignon Chromatem apparatus on a C-18 silica column (7 µ - 3/8' x 30 cm) with U.V. detection at 280 nm using a gradient of methanol from 5% to 99% in an 5 o/oo acetic solution as eluant.

#### • Partial purification of fungitoxic compounds.

These inhibitors were purified by successive treatments, first by preparative chromatography on thick layers of silica gel Gf (254), using the same eluants than for analytic TLC. Further purifications were achieved on atmospheric columns of silica or of Sephadex LH-20 (Fluka chemicals) eluted sequentially by chloroform, isopropanol and methanol. The toxic fractions were treated by isocratic HPLC with double detection - U.V. and refractometer - either on silica column ( $5 \mu - 3/8' \times 30 \text{ cm}$ ) or on CN-silica column ( $5 \mu - 3/8' \times 30 \text{ cm}$ ) eluted with Hexane and Ethylacetate in increasing proportions.

#### Structure determination.

The structure of several inhibitors was determined by mass spectrometry (MS) with a G C-M S Nermag R10-10C coupled with a computer and by Nuclear Magnetic Resonance (MNR) with a Bruker apparatus 200 MHz in  $\text{CDCl}_3$ . The infra red spectrum was recorded on a Perkin Elmer 397 in K Br (KHAN *et al.*, 1985).

#### Toxicity test.

The toxicity tests of the methanolic extracts (50 mg - 200 mg f.w./ml) and purified inhibitors (25  $\mu\text{g}$  - 200  $\mu\text{g}/\text{ml}$ ) were carried out for the inhibition of *Cladosporium cladosporioides* on TLC plates and of *P. citrophthora* in concavity slides (TAQUET *et al.*, 1985, VERNENGLI, 1985; KHAN *et al.*, 1985). The synergy of *in vitro* inhibition of *P. citrophthora* by the association of a coumarin with other constitutive phenolics mainly benzoic and cinnamic acids derivatives was also investigated.

## RESULTS

#### Symptoms development.

The susceptible variety, Valencia late, exhibited at the site of inoculation typical diffuse and brownish green, depressed and soaked necrosis with clear margin. The rootlets and secondary roots are also attacked, their cortex being brown to dark, easily dissociated and removed. In the case of genetically resistant varieties a dry necrosis was observed at the site of inoculation with dark extension of

5 - 10 mm upward and downward beneath the bark. Similar symptoms appeared on stems of Valencia late treated with Fosetyl Al or elicitors. These treatments did not modify significantly the symptoms on resistant Rough lemon.

#### Estimation of total phenolics.

The concentrations of phenolic compounds were significantly increased by inoculation of *Phytophthora* spp. In tissues of roots and shoots of Valencia late (Table 1) the accumulation was triggered by treatments with Fosetyl Al or with elicitors. Conversely, application of AoA depressed drastically the synthesis of phenolics in plants of Valencia late inoculated and treated with Fosetyl Al. In resistant varieties (Table 2) the accumulation of phenolics was more important than in susceptible and modulated by Fosetyl Al in the case of Rough lemon.

#### Chromatographic analysis.

Chromatographic studies by TLC and by HPLC revealed that the defence reaction of Rough lemon and *Poncirus trifoliata* was correlated with a stimulation of several biosynthetic pathways. Similar compounds appeared in tissues of susceptible Valencia late after treatment with Fosetyl Al or elicitors.

#### • TLC analysis.

The development of plates in different solvent systems revealed the synthesis *de novo* of several compounds, the concentrations of other being increased. Using Hexane-Ethylacetate (2:1) as eluant (Table 3), 4 new products were detected: 1 phenolic (Rf 0,83) and 3 non aromatic (Rf 0,63 - 0,70 - 0,90). According to the GC-MS data, the product eluted at Rf 0,63 could be a sesquiterpen of eudesman group; further structural studies are needed for complete determination. In the system chloroform-Ethylacetate-Methanol (50:40:5) was revealed the accumulation of 6 phenolics (Table 3 and fig. 1) among these Xanthoxylin (Rf 0,94) described by HARTMANN and NIENHAUS (1984), Xanthyletin (Rf 0,77) a new coumarin and its isomer KHAN *et al.*, 1985).

TABLE 1 - Comparison of phenolic concentrations in tissues of stems or roots of Valencia Late plants healthy or after inoculation and treatments. Results are expressed as equivalent of chlorogenic acid  $\mu\text{g}/\text{g}$  f. w.

Treatment	Healthy	Inoculated	Inoculated + Fosetyl Al	Inoculated Fosetyl Al + AoA	Inoculated + Glucosamine	Inoculated + Arachidonic acid	Inoculated + Glusamine + Arachidonic acid
Stem	350	542	780	650	946	1 270	1 070
Root	560	846	1 045	760	980	1 155	1 080

TABLE 2 - Comparison of phenolic concentrations in tissues of stems or roots of plants of *Poncirus trifoliata* and Rough Lemon. Treatments concern only Rough Lemon. Results are expressed an equivalent of chlorogenic acid  $\mu\text{g/g f. w.}$

	Treatment	Healthy	Healthy + Fosetyl AI	Inoculated	Inoculated + Fosetyl AI	Inoculated + Glucosamine
Rough Lemon	Stem	948	950	1 070	1 259	1 375
	Root	1 422	1 406	1 490	1 550	1 636
<i>Poncirus trifoliata</i>	Stem	699	-	1 016	-	-
	Root	720	-	980	-	-

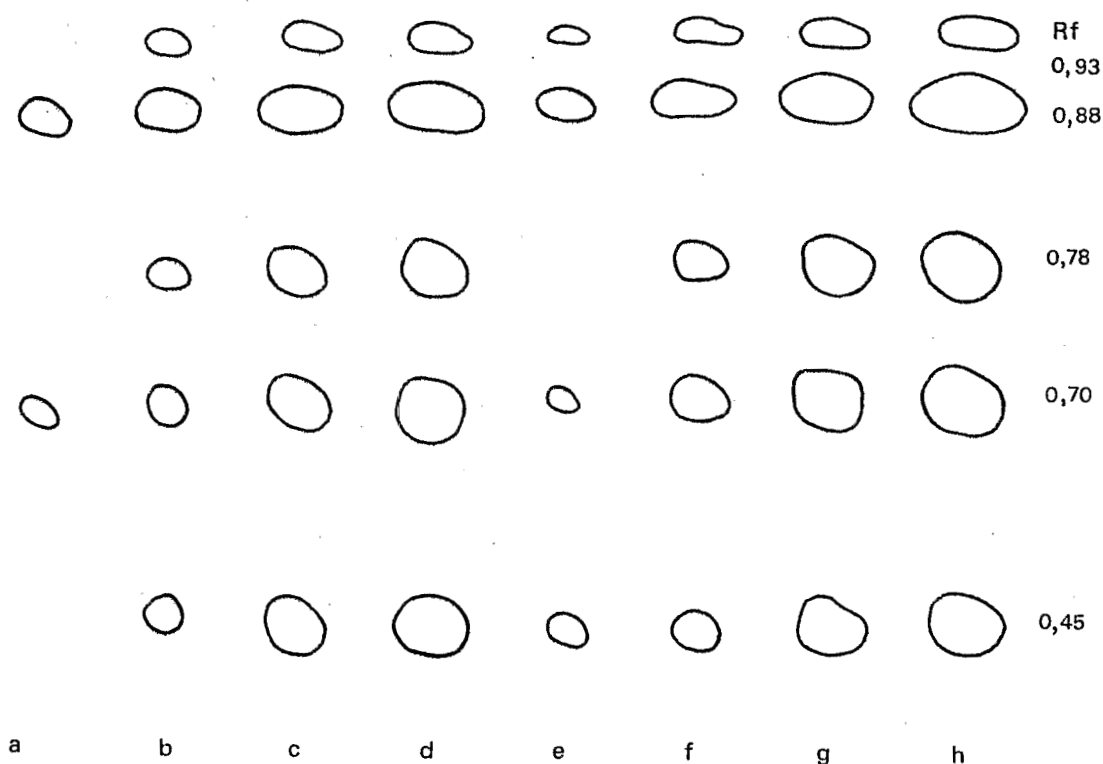


FIGURE 1 - Diagramm of TLC analysis in the system chloroform-Ethylacetate-Methanol (50 : 40 : 5) of methanolic extracts of stem tissues corresponding to 50 mg of fresh weight :

- of Valencia Late (a - d),

- of Rough Lemon (e - h).

healthy = a and e, inoculated = b and f, inoculated and treated with Fosetyl AI = c and g, or with glucosamine = d and h. Spray reagent : p - nitro-aniline, the size of spots is proportional to concentration

Rf = 0,45 and 0,88 unidentified phenols

Rf = 0,70 presumed tuberosin, a precursor of Xanthyletin

Rf = 0,78 Xanthyletin

Rf = 0,93 Xanthoxyllin. These compounds inhibit *in vitro* the growth of *Phytophthora citrophthora*.

TABLE 3 - Comparison of TLC analysis of Valencia Late extracts with Hexane-Ethylacetate (2 : 1) as eluant. The compounds are visualized after spraying p. nitro-aniline (PN) for phenolics or antimony chloride at saturation in chloroform (15 min at 120°C) for other compounds.

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	+

- = not detected in the reference ; 0 = no increase in synthesis ; + = increase in synthesis ;  
 + = new synthesis after inoculation and treatments.

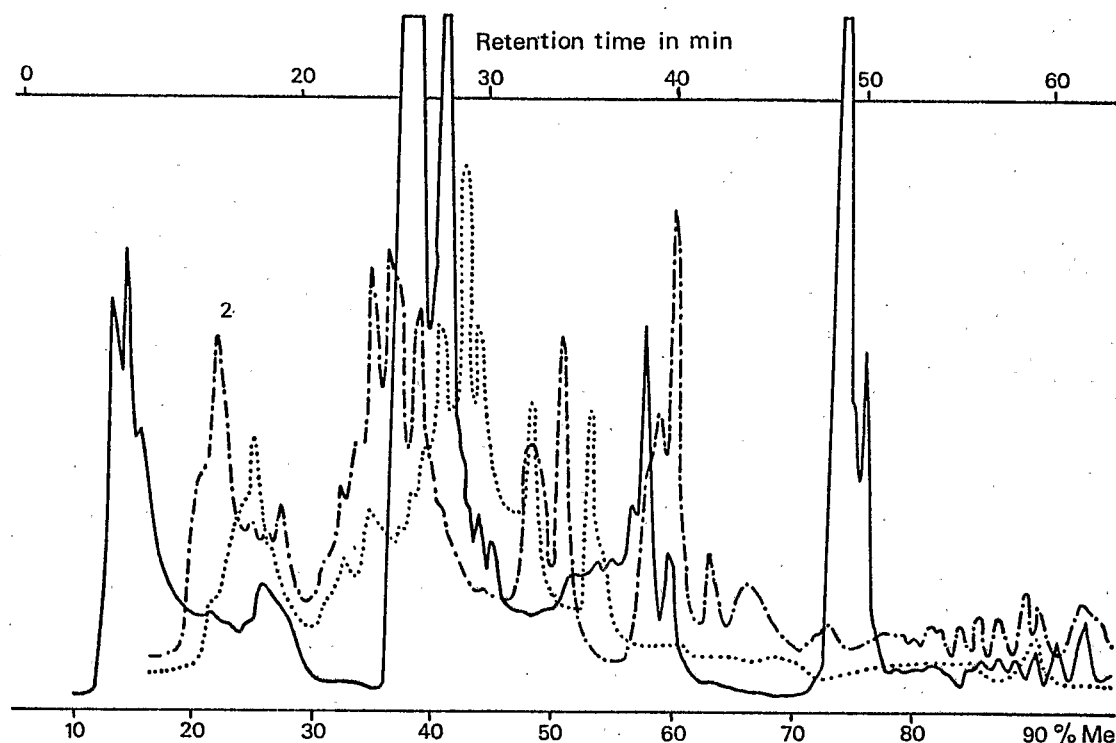


FIGURE 2 - HPLC traces of analysis on a C-18 silica column with a gradient of 10 to 99 % methanol in a 5 o/oo acetic solution of methanolic extracts of Valencia Late stem tissues :

..... = inoculated by *P. citrophthora*  
 - - - - = inoculated and treated with Fosetyl Al,  
 ————— = inoculated and treated with glucosamine  
 (U.V. detection at 250 nm, flow rate 1,5 ml/min).

#### ● HPLC analysis.

The results obtained by gradient chromatography on a silica C-18 column of methanolic extracts of roots and shoots of resistant and susceptible varieties improved the

observations of TLC. Using a gradient of 5% to 99% of methanol in an acetic acid 5 o/oo solution, the main modifications of the traces after inoculation were detected in 4 zones: 10-20%, 35-45%, 50-60%, 70-80% (fig. 1 and 2). Only a part of these compounds were identified and localized on the gradient:

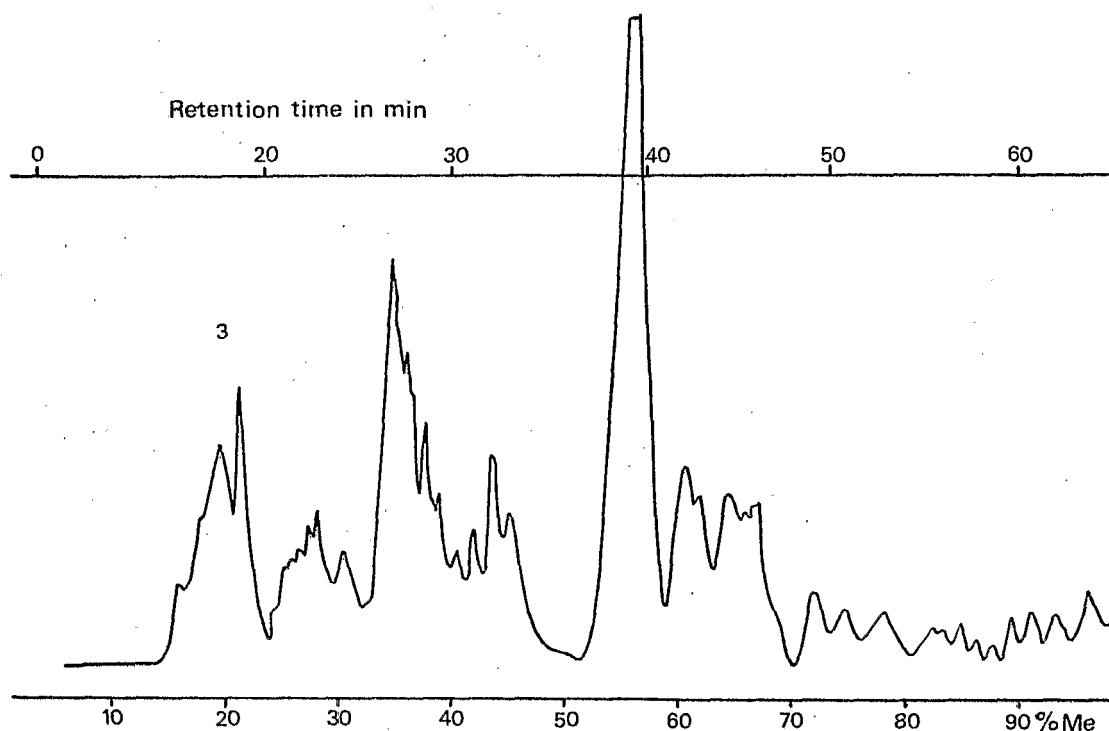


FIGURE 3 - HPLC traces of analysis on a C-18 silica column with a gradient of 10 to 99 % methanol in a 5 o/oo acetic solution of methanolic extracts of stem of Rough Lemon inoculated by *P. citrophthora*.

TABLE 4 - Comparison of TLC analysis of Valencia Late extracts with Chloroform-Ethylacetate-Methanol (50 : 40 : 5) as eluant. Experimental see table 3.

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

- 3,4 - dihydroxy-benzoic acid (20-30%),
- 3,4 - dimethoxy-benzaldehyde (35-50%),
- ferulic acid and derivatives (50-60%),
- caffeic acid and derivatives (60-80%),
- Xanthyletin and isomer (60-75%),
- Xanthoxylin (75-80%), these data must be confirmed,
- a sesquiterpen (85-90%).

The purifications were performed by successive thick layer chromatography, LH-20 gel columns and preparative HPLC (see materials and methods).

#### Modulation of defence reaction.

Figure 1 shows diagrammatically the migration on TLC, in

chloroform-Ethylacetate-Methanol (50:40:5) as eluant, of major inhibitory compounds synthesized after inoculation in shoots of susceptible Valencia late and resistant Rough lemon. In both cases the treatments with Fosetyl Al increased the synthesis and/or the accumulation of these inhibitors. Similar results were observed after treatments with glucosamine and arachidonic acid.

In the same way, HPLC analysis on a C-18 silica column indicated an accumulation of several products eluted between 20-30%, 40-65%, 70-80% of methanol in the gradient (fig. 2 and 3). In comparison with that of inoculated Valencia late, tissues extracts of plants treated with fungicide or with elicitors contained more phenolics - mainly benzoic and cinnamic acids derivatives and coumarins - In

our experiments, we observed important similarities between HPLC traces after treatments and that of resistant Rough lemon. Fosetyl Al and Glucosamine enhanced the accumulation in Rough lemon tissues of the same compounds than in Valencia late. The stimulation of benzoic acid derivatives and coumarins synthesis seemed more efficient with glucosamine treatment (fig. 2). The application of AoA significantly suppressed the synthesis of phenolics in plant infected or infected and treated with Fosetyl Al mainly the compounds eluted between 40% and 80% of Methanol on silica C-18 column.

#### Toxicity tests.

Toxicity tests of the methanolic extracts were performed by the inhibition of mycelial growth of *P. citrophthora* *in vitro*. Extracts of Rough lemon, were found more toxic than *Poncirus trifoliata* or Valencia late and the toxicity was increased according to the concentration of extracts (Table 5 and 6). Fosetyl Al or elicitor treatment in Valencia late increased the toxicity with a lethal dose of 200 mg f.w./ml (Table 5). A strong inhibition of fungus mycelium was observed in extracts of inoculated and treated Rough lemon, and the inhibition was found more intense in glucosamine treated (lethal dose = 100 mg f.w./ml) extract as compared to Fosetyl Al treated (lethal dose = 150 mg f.w./ml) extract (Table 6).

A strong inhibition of *Cladosporium cladosporioides* sporulation on TLC plates (spots of 10-20 mm in diameter)

was observed with 200 µg of purified Xanthyletin. *In vitro*, the inhibition of *P. citrophthora* began with 25 µg/ml of coumarin and the growth was completely inhibited with 75 µg/ml during at least one week of incubation. As published recently, the association *in vitro* of coumarin with several phenolics increased their toxicity by synergy (KHAN *et al.*, 1985).

#### DISCUSSION - CONCLUSION

The results indicate that Fosetyl Al or elicitors trigger several biosynthetic pathways for defence reaction in Citrus plants against gummosis caused by *Phytophthora* spp. The modulation of defence mechanisms in treated susceptible Valencia late seemed similar to that of genetically resistant varieties.

The appearance of brown stripes in wood of resistant varieties at inoculation site coincided with the cessation of fungus growth. Similar response was exhibited by susceptible Valencia late after treatment corresponding probably to the stimulation of defence mechanisms. Fosetyl Al or elicitors increased the total phenol concentration in susceptible Valencia late like in resistant varieties for defence response.

According to several authors (VO-THI-HAI *et al.*, 1979; BOMPEIX *et al.*, 1980 and 1981; RAYNAL *et al.*, 1980) treatments with Fosetyl Al trigger the synthesis and accu-

TABLE 5 - Comparison of the toxicity of Valencia Late extracts of tissues healthy, inoculated or inoculated and treated with Fosetyl Al or glucosamine, at different concentration *in vitro* for *Phytophthora citrophthora*. Results are expressed as mycelial growth in µm on concavity slides after 48 hours of incubation at 28°C.

Tissue extract ≠ mg f.w./ml	Healthy	Inoculated	Inoculated + Fosetyl Al	Inoculated + Glucosamine
50	624	605	425	380
100	400	370	253	182
150	290	260	123	97
200	230	185	-	-

- : no growth.

TABLE 6 - Comparison of the toxicity of Rough Lemon extracts of tissues healthy, healthy and treated with Fosetyl Al, inoculated and treated with Fosetyl Al or glucosamine. Experimental see table 5.

Tissue extract ≠ mg f.w./ml	Healthy	Healthy + Fosetyl Al	Inoculated + Fosetyl Al	Inoculated + Glucosamine
50	515	430	297	230
100	350	240	190	-
150	218	207	-	-
200	196	-	-	-

mulation of phenolics in tissues of infected leaves of tomato and grapevine.

In our experiments, Chromatographic analysis indicated a similarity between the natural defence reaction of resistant Rough lemon or *Poncirus trifoliata* and susceptible Valencia late after treatment with Fosetyl Al or elicitors. Stimulation of defence reaction in treated Valencia late is similar to other interactions of *Capsicum* or tobacco with *Phytophthora nicotianae* (GUEST, 1984) or tomato - *P. parasitica* (VERNENGI, 1985). Compared to untreated plants, Fosetyl Al or elicitors treated plants accumulated the coumarins and other phenolics at greater rate. According to HPLC traces, glucosamine seemed a best inducer of defence response in Citrus than Fosetyl Al.

Similarly, an enhanced phytoalexin accumulation was observed in rice plants treated with systemic fungicide, dichlorocyclopropane (CARTWRIGHT *et al.*, 1980). Like Fosetyl Al, cyclopropane derivatives possess little direct activity against rice blast fungus *in vitro*.

Enhanced, phytoalexin accumulation has been also reported in response to inoculation with *Phytophthora megasperma* in soybean hypocotyls treated with metalaxyl a systemic fungicide, at lower concentration than lethal dose *in vitro* (STOSSEL *et al.*, 1980, WARD, 1984). Nevertheless, Fosetyl Al like phosphorous acid inhibit *in vitro* the growth of some species of *Phytophthora*, the more sensitive at concentrations ranging from 0,42 to 13,44 millimoles/ml being *P. cinnamomi*, *P. citrophthora*, *P. parasitica* and *P. palmivora* (BOMPEIX and SAINDRÉ-NAN, 1984). On Ribeiro's medium with a deficiency in phosphate, FENN and COFFEY (1984) found a high inhibitory effect of Fosetyl Al. These results can be explained by phosphite-phosphate antagonism *in vivo* (BOMPEIX *et al.*, 1980) like *in vitro* (BOMPEIX and SAINDRÉ-NAN *loc. cit.*). Actually, effectiveness of Fosetyl Al and metalaxyl against *Phytophthora parasitica* in orchards of sweet orange is not correlated with biochemical analysis (TIMMER and CASTLE, 1985). At high concentration - 4,000 and 5,000 ppm - Fosetyl Al proved to be highly active against *Phytophthora fragariae* on strawberry and *Bremia lactucae* of lettuce (DERCKX and BUCHE-

NAUER, 1986). According to these authors, a *de novo* synthesis of phenolics in the hosts tissues seemed a consequence of fungicidal efficiency on parasites and of partial alteration of hosts tissues. In that studies, the concentrations of fungicides used for treatments were not comparable to ours and the authors did not investigate the toxicity *in vitro* of the total extracts of tissues or of purified phenolics accumulated in the hosts.

In our studies we used a weakly pathogenic strain of *P. citrophthora* and the plants were grown on a sandy clay substrate, moist but well drained. In that conditions, the plants of Rough lemon behaved like those of resistant *Poncirus trifoliata* and we observed an important similarity of defence reactions against *P. citrophthora*. In fact, Rough lemon regarded as genetically sensitive to *P. citrophthora* can behave like resistant or tolerant in fields against weakly pathogenic strains by means of a fast cicatrization of infected tissues and a good regeneration of rootlets. According to LAVILLE (personal communication) similar reactions in fields are observed in the case of lime Rangpur and *Citrus volkameriana*.

On the basis of a stimulation of fungitoxic compounds synthesis in plants of Valencia late and Rough lemon treated with Fosetyl Al, we can suppose that the fungicide could contribute to the enhancement of Citrus defence mechanisms like fungal elicitors. This hypothesis seems supported by the inhibition of secondary metabolites synthesis by AoA in Citrus or by glyphosate in soybeans (WARD, *loc. cit.*). As suggested in a recent review (BOMPEIX, RAVISE and SAINDRÉ-NAN, 1985), further investigations will deal with the interactions between Fosetyl-Al or elicitors and the host parasite interface.

#### ACKNOWLEDGEMENTS

Authors are thankful to Mr PELLETIER (ORSTOM) for his technical assistance in greenhouse and to Mr KUNESCH, EINHORN, MALOSSE and Mrs RAMIANDRASOA and CHUILON (Laboratoire des Médiateurs chimiques) for elucidation of inhibitors structures. Financial assistance by french government to A.J. KHAN is gratefully acknowledged.

#### BIBLIOGRAPHY

- ALBERSHEIM (P.) and VALENT (B.S.). 1978.  
Host-pathogen interactions in plants.  
Plants when exposed to oligosaccharides of fungal origin, defend themselves by accumulating antibiotics.  
*J. Cell Biol.*, 78, 627-643.
- BAILEY (S.A.). 1982.  
Mechanisms of phytoalexins accumulation.  
in *Phytoalexins*, J.A. BAILEY and J.W. MANSFIELD,  
Eds., John WILEY and SONS, New York, 289-318.
- BELL (A.A.). 1981.  
Biochemical mechanisms of disease resistance.  
*Ann. Rev. Plant Physiol.*, 32, 21-81.
- BERTRAND (A.), DUCRET (J.), DEBOURGE (J.C.) et HORRIERE (D.). 1977.  
Etude des propriétés d'une nouvelle famille de fongicides : les mono-éthylphosphites métalliques. Caractéristiques physico-chimiques et propriétés biologiques.  
*Phytriatri. Phytopharm.*, 26, 3-18.
- BOCCAS (B.) et LAVILLE (E.). 1978.  
Les maladies à *Phytophthora* des agrumes.  
Ed. SETCO-IRFA, Paris, 162 p.
- BOMPEIX (G.), RAVISE (A.), RAYNAL (G.), FETTOUCHE (F.) et DURAND (M.C.). 1980.  
Modalités de l'obtention des nécroses bloquantes sur feuilles



- détachées de tomate par l'action du tris-0-éthylphosphonate d'aluminium, hypothèse sur son mode d'action *in vivo*.  
*Ann. Phytopathol.*, 12, 337-351.
- BOMPEIX (G.), FETTOUCHE (F.) et SAINDRENAN (P.). 1981.  
Mode d'action du Phoséthyl Al.  
*Phytopath. Phytopharm.*, 30, 257-272.
- BOMPEIX (G.) and SAINDRENAN (P.). 1984.  
*In vitro* antifungal activity of Fosetyl Al and phosphorous acid on *Phytophthora* species.  
*Fruits*, 39, 777-786.
- BOMPEIX (G.), RAVISE (A.) et SAINDRENAN (P.). 1985.  
Comportement des fongicides au niveau des relations hôtes-parasite.  
*Conf. au Coll. Franco-brit., Fongicides et protection des plantes, 100 ans de progrès ; BCPC monograph n° 31*, 107-118.
- CARTWRIGHT (D.), LANGCAKE (J.), PRYCE (D.) and LEWORTY (D.P.). 1977.  
Chemical activation of host defence mechanisms as a basis of crop protection.  
*Nature*, 267, 511-513.
- CARTWRIGHT (D.), LANGCAKE (J.) and RIDE (J.P.). 1980.  
Phytoalexin production in rice and its enhancement by a dichlorocyclopropane fungicide.  
*Physiological Plant Pathology*, 17, 259-267.
- CLERJEAU (M.) et BEYRIES (A.). 1977.  
Etude comparée de l'action préventive et du pouvoir systémique de quelques fongicides nouveaux (Phosphite, prothiocarbe).  
*Phytopath. Phytopharm.*, 26, 73-83.
- DERCKS (W.) and BUCHENAUER (H.). 1986.  
Untersuchungen zum Einfluss von Aluminiumfosetyl auf den Pflanzlichen Phenolstoffwechsel in den Pathogen - Wirt - Beziehungen *Phytophthora fragariae* Erdbeere und *Bremia lactucae* - Salat.  
*J. Phytopathology*, 115, 37-55.
- FARIH (A.), TSAO (P.H.) and MENGE (J.A.). 1981.  
Fungitoxic activity of Efosite aluminium on growth, sporulation and germination of *Phytophthora parasitica* and *P. citrophthora*.  
*Phytopathology*, 71, 934-936.
- FENN (M.E.) and COFFEY (M.D.). 1984.  
Studies on the *in vitro* and *in vivo* antifungal activity of Fosetyl Al and phosphorous acid.  
*Phytopathology*, 74, 606-611.
- GUEST (D.I.). 1982.  
Chemically induced host resistance. The mode of action of some systemic, anti-oomycete disease control chemicals.  
*Ph. D. Thesis, University of Melbourne*.
- GUEST (D.I.). 1984.  
Modification of defence responses in tobacco and capsicum following treatment with Fosetyl Al.  
*Physiological Plant Pathology*, 25, 125-134.
- GUEST (D.I.) and BOMPEIX (G.). 1984.  
Fosetyl Al as tool in understanding the resistant response in plants.  
*Phytophthora New-Letter*, 12, 62-69.
- HARTMANN (G.) and NIENHAUS (F.). 1974.  
The isolation of xanthoxylin from the bark of *Phytophthora* and *Hendersonula* infected Citrus lemon and its fungitoxic effect.  
*Phytopath. Z.*, 81, 97-113.
- KHAN (A.J.), KUNESH (G.), CHUILON (S.) and RAVISE (A.). 1985.  
Structure and biological activity of xanthyletin a new phytoalexin of Citrus.  
*Fruits*, 40, 807-811.
- KUC (J.) and HAMMERSCHMIDT (R.). 1978.  
Acquired resistance to bacterial and fungal infection.  
*Ann. Appl. Biol.*, 89, 313-317.
- LAVILLE (E.) et CHALANDON (A.). 1982.  
Synthèse des résultats obtenus avec le Phoséthyl Al dans la lutte contre les maladies à *Phytophthora* des agrumes.  
*Fruits*, 37, 11-17.
- RAVISE (A.), BOMPEIX (G.) et FETTOUCHE (F.). 1981  
Stimulation des réactions de défense de la tomate aux *Phytophthora* spp. par le tris-0-éthylphosphonate d'aluminium.  
*Comm. Coll. International sur la Protection des Cultures tropicales, Lyon 8 juillet*.
- RAYNAL (G.), RAVISE (A.) et BOMPEIX (G.). 1980.  
Action du tris-0-éthylphosphonate d'aluminium sur la pathogénie de *Plasmopara viticola* et sur la stimulation des réactions de défense de la vigne.  
*Ann. Phytopathol.*, 12, 163-175.
- STOSSEL (P.) WARD (E.W.B.), LAZAROVITS (G.), BARRIE (S.D.) and HUWIN (C.H.). 1980.  
Glycoollin production associated with control of *Phytophthora* rot of soybeans by the systemic fungicide Metalaxyl.  
*Phytopathology*, 70, 738-740.
- TAQUET (B.), RAVISE (A.), RENARD (J.L.) et KUNESH (G.). 1985.  
Modulation des réactions de défense du palmier à huile contre le *Fusarium oxysporum* f. sp. *Elaeidis* (Schecht) Toovey. Applications : prémunition et stimulation chimique.  
*Phytopath. Z.*, 112, 298-314.
- TIMMER (L.W.) and CASTLE (W.S.). 1985.  
Effectiveness of metalaxyl and Fosetyl Al against *Phytophthora parasitica* on sweet orange.  
*Plant disease*, 69, 741-743.
- TRIQUE (B.), RAVISE (A.) et BOMPEIX (G.). 1981.  
Modulation des infections à *Phytophthora* spp. provoquées chez la tomate.  
*Comm. 20e Coll. Sté Fse Phytopathologie, Brest 7 mai 1981*, résumé dans *Agronomie*, 1, 823.
- DE VAILLAVIELLE (C.) et PERRIER (X.). 1981.  
Etude sur la pathogénie de *Phytophthora citrophthora* (S.M. et S.M.) Leonian, sur la gamme de porte-greffe d'agrumes par deux méthodes d'analyse multidimensionnelle.  
*Agronomie*, 1, 527-534.
- VERNENGI (A.). 1985.  
Réactions de défense du *Lycopersicon esculentum* MILL. à des infections cryptogamiques : mise en évidence de phytoalexines et de leurs propriétés inhibitrices.  
*Thèse de spécialité, Université de Paris VI*.
- VO-THI-HAI, BOMPEIX (G.) et RAVISE (A.). 1979.  
Rôle du tris-0-éthylphosphate d'aluminium dans la stimulation des réactions de défense des tissus contre le *Phytophthora capsici*.  
*C.R. Acad. Sci., Sér. D.*, 288, 1171-1174.
- WARD (E.W.B.). 1984.  
Suppression of metalaxyl activity by glyphosate : evidence that host defence mechanisms contribute to metalaxyl inhibition of *Phytophthora megasperma* f. sp. *glycinea* in soybeans.  
*Physiological Plant Pathology*, 25, 381-386.

