Induction of *Phytophthora citrophthora* mutants resistant to fosetyl-Al and phosphorous acid and study of their pathogenicity towards *Citrus*.

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INDUCTION OF *PHYTOPHTHORA CITROPHTHORA* MUTANTS RESISTANT TO FOSETYL-Al AND PHOSPHOROUS ACID AND STUDY OF THEIR PATHOGENICITY TOWARDS *CITRUS*

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SUMMARY - 34 phosphorous-acid-resistant mutants of *Phytophthora citrophthora* were selected on different media after chemical treatment with MNNG or UV light irradiation. These mutants were 3 to 7 times more resistant to phosphorous acid than the wild type strain. We isolated only 4 fosetyl-Al-resistant mutants, which were selected on CMA medium after UV irradiation ; their resistant levels to fosetyl-Al were weak (RL=2). Among 6 resistant mutants, none was pathogen on *Citrus* seedlings and 3 were pathogen on wounded *Citrus* leaves. When leaves were floated on a solution of fosetyl-Al these 3 mutants were stopped by 500 $\mu g.ml^{-1}$ of fosetyl-Al, a concentration which is lower than the EC50 of the wild type strain *in vitro*.

INDUCTION DE MUTANTS DE *PHYTOPHTHORA CITROPHTHORA* RESISTANTS AU PHOSETHYL-Al ET A L'ACIDE PHOSPHOREUX ET ETUDE DE LEUR POUVOIR PATHOGENE SUR *CITRUS*.

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RESUME - 34 mutants de *Phytophthora citrophthora* résistants à l'acide phosphoreux ont été sélectionnés sur différents milieux après des traitements mutagènes à la nitrosoguanidine ou aux rayons UV. Ces mutants se sont avérés être 3 à 7 fois plus résistants à l'acide phosphoreux que la souche sauvage. Seuls 4 mutants résistants au phoséthyl-Al ont pu être obtenus. Ils ont été sélectionnés sur du milieu CMA après irradiation de thalles aux UV et leur niveau de résistance au phoséthyl-Al est faible (NR=2). Parmi les 6 mutants résistants testés, aucun ne s'est avéré pathogène sur des plantules de *Citrus*. Toutefois, 3 d'entre eux sont pathogènes sur des feuilles isolées et blessées de *Citrus*. Cependant ils sont sensibles à une concentration de 500 $\mu g.ml^{-1}$ de phoséthyl-Al ; cette concentration étant inférieure à la Cl50 de la souche sauvage déterminée *in vitro*.

INTRODUCTION

Aluminium tris-O-ethyl phosphonate (fosetyl-Al), a systemic fungicide developed by Rhône-Poulenc, is efficient in the control of diseases caused by the fungi belonging to the class of Oomycetes and in particular against root rot of *Citrus* caused by *Phytophthora* spp. (*P. citrophthora, P. parasitica, P. citricola, P. palmivora*, ...) (LAVILLE and CHALANDON, 1982).

Fosetyl-Al is a weak inhibitor of mycelial growth *in vitro* (BOMPEIX and SAINDRENAN,1984), but is efficient at low concentrations *in vivo*. It seems that fosetyl-Al has an indirect mode of action which involves the stimulation of host defence mechanisms (GUEST, 1984 a ; KHAN *et al.*, 1986 ; SAINDRENAN *et al.*, 1988). Phosphite (the degradation product of fosetyl-Al in plant tissues) was also shown to act directly on the fungus in the coconut tree - *Phytophthora heveae* interaction (DARAKIS *et al.*, 1986). This complex mode of action is consistent with the observation that until now, no fosetyl-Al resistance in the field was mentionned.

The study of fungicide resistance can be undertaken in the laboratory by the search of resistant mutants in a mutagenized population. Chemical or physical mutagenesis, for instance, will cause genetic damages and therefore will lead to an increased number of induced mutations. Such an approach has been used before by BOWER and COFFEY (1985) to study resistance to fosetyl-Al in *Phytophthora capsici*. They selected several resistant mutants that retained pathogenicity to Pepper plants. Furthemore, there was a high correlation between resistance expressed *in vitro* and *in vivo*. However, the selection for resistance was done

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on phosphorous acid not on fosetyl-Al itself.

In this paper, we investigated the fosetyl-Al and phosphorous acid resistance on the well defined interaction *Phytophthora citrophthora - Citrus*. We studied the possibility of isolating *P. citrophthora* resistant isolates after different techniques of mutagenesis and selection of resistant-mutants was done comparatively on fosetyl-Al and phosphorous acid. The fungal behaviour in the field was estimated by the scoring of pathogenicity and resistance of the mutants *in vivo*.

EXPERIMENTAL METHODS

Fungi.

The wild type strain Fp of *Phytophthora citrophthora* was obtained from an infection of *Citrus* plants in Corsica (France) and was kindly supplied by the laboratory of Phytopathology, CIRAD-IRFA (Montpellier, France). The fungi were maintained on pea agar medium (200 g of preserved garden pea for one liter of medium) at 26°C, in the dark.

Chemicals.

Fosetyl-Al (Aliette^{R}, 80% a.i., Rhône-Poulenc) and phosphorous acid [used as its disodium salt, Na_2HPO_3 $5\ H_2O$, Prolabo France], were buffered in 2-[N-morpholino] ethane sulfonic acid (MES) 40 mM, pH 6.5 (adjusted with 1.0 M NaOH). Fosetyl-Al was added to the medium after autoclaving (20 min at 120°C).

Mutagenesis.

The mutagenesis were carried out on mycelium or on zoospores from *P. citrophthora* using two mutagenic reagents.

- UV light (wavelenght of 254 nm) irradiation was performed using a quartz lamp with mercury vapour. Different doses (ergs mm^{-2}) were used.

- N-methyl-N'-nitro N-nitrosoguanidine (MNNG) was used at a final concentration of 30 $\mu g.ml^{-1}$ in water.

Mycelium mutagenesis.

Petri dishes containing 20 ml of pea agar medium were inoculated with a 4 mm diameter mycelial plug taken from the periphery of a 5 day-old culture and incubated in the dark at 26°C for 2 days. The mycelium was then irradiated with UV light (7500 or 12500 ergs mm^{-2}) and transferred on corn meal agar medium (CMA at 17 g ml^{-1}) or on Ribeiro's modified synthetic agar medium (RMSA) (FENN and COFFEY, 1984) supplemented with either fosetyl-Al or phosphorous acid for resistance detection (Table 1). The RMSA medium was used either at low (0.0084 mM) or high (0.84 mM) phosphate concentration.

Encysted spores mutagenesis.

10 ml of distilled water were added to each 10 day-old culture of *P. citrophthora* on pea agar medium and transferred to continuous light for 5 days in order to produce abundant sporangia. The plates were chilled at 4°C for 20 min and than incubated 30 min at room temperature until zoospores were released.

Zoospores were induced to encyst by adding 1 ml of undiluted sterilized pea broth to 10 ml of a zoospore suspension (10^5 ml^{-1}) in a plastic Petri dish (9 cm diameter). While encysting, the zoospores attached to the bottom of the dish and started to germinate within 1 h. The cysts were treated with UV light or MNNG 30 min after zoospore encystement. UV light irradiation was carried out at a dose of 600 ergs mm^{-2}. The MNNG solution was poured on the dishes, kept during 15 min, then removed and the cysts were washed with 5 ml pea broth for 15 min. After the mutagenic treatment, the cysts were overlayed with 15 ml CMA or RMSA medium containing a fongistatic concentration of either fosetyl-Al or phosphorous acid (see table 1). The mutagenized population in each Petri dish was 10^6 cysts.

The mutants selected for further characterization were those which had the best growth rate on the medium supplemented with fosetyl-Al or phosphorous acid.

Stability of resistance.

Single zoospores of the parental strain and of each of the

TABLE 1 - Fongistatic concentrations of inhibitor (fosetyl-Al[a] or phosphorous acid) used in mutagenesis experiments.

Selection medium	Fungal organites	Phosphorous acid ($\mu g.ml^{-1}$)	Fosetyl-Al ($\mu g.ml^{-1}$)
CMA	Mycelium Encysted zoospores	350 210	1000 800
Ribeiro's deficient in phosphate (0.0084 mM PO_4)	Mycelium	350	300

a : One mole of fosetyl-Al (molecular weight 354) is equivalent to 3 moles of phosphorous acid (molecular weight 82).

selected mutants were transferred 4 times and stored 6 months on a medium that lacked the inhibitor before testing again their *in vitro* resistance.

Measurement of fungitoxicity.

The effect of fosetyl-Al and phosphorous acid on the radial growth of *Phytophthora citrophthora* was determined on several media containing a range of fosetyl-Al (10 to 3500 $\mu g.ml^{-1}$) or phosphorous acid (6 to 3000 $\mu g.ml^{-1}$) concentrations.

The inoculum was a 4 mm diameter mycelial plug taken at the margin of a 5 day-old pea medium culture. Radial linear growth was measured after 7 days of incubation in the dark at 26°C, and EC50 and EC90 values were calculated from linear regression analysis of the percent growth inhibition *versus* log of the fungicide concentration. Experiments were repeated at least 3 times.

Pathogenesis of fungicide-resistant mutants.

Pathogenicity tests were carried out on *Citrus poncirus xc. sinensis* cv. jambhiri lush (Rough lemon) plants, which are susceptible to *P. citrophthora.*

Inoculation on Citrus seedlings.

Two months old seedlings were inoculated by soaking the roots in either a suspension of zoospores (10^6 ml^{-1}) or a mycelial mixture (a 15 day-old liquid culture in pea medium stirred and diluted 10 times in distilled water).

After inoculation, the plants were potted again and incubated in a greenhouse at 26°C. Uninoculated control plants were dipped into water. The infected or dead plants were scored 10 days after inoculation.

Inoculation on Citrus leaves.

Leaves cut from 6 month-old plants were inoculated with a 4 mm diameter mycelial plug (taken from the periphery of a 5 day-old culture grown on pea agar medium), after wounding on the main vein. After inoculation, the leaves were put in Petri dishes filled up with MES buffer at pH 6.5. The lesions were measured 3 days after inoculation.

In vivo **behaviour of resistant mutants.**

Leaves taken from 6 month-old plants were inoculated as described before and floated on a solution of fosetyl-Al at 500 $\mu g.ml^{-1}$ or on MES buffer as control. The lesions were measured 3 days after incubation in the light at 26°C.

RESULTS

Selection of resistant mutants.

A total of 34 phosphorous acid- resistant mutants were selected on agar medium supplemented with phosphorous acid; 24 mutants were isolated on CMA medium and 10 on RMSA medium deficient in phosphate (Table 2). Both mutagenic reagents, UV light and MNNG, produced approximately the same number of resistant mutants.

Only 4 mutants were selected as fosetyl-Al resistant. They were isolated on fosetyl-Al supplemented CMA medium from UV light irradiated mycelium. We could not isolate any fosetyl-Al resistant mutant on RMSA medium deficient or not in phosphate, in spite of the large number of mutagenized zoospores (3.10^7) or thalli (1640).

The selected resistant mutants were tested for their resistance stability. Their resistance to fosetyl-Al and phosphorous acid were the same after 4 transfers and 6 months storage in the absence of inhibitor (data not shown).

In vitro **EC90 values.**

The EC90 values obtained with the phosphorous acid-resistant mutants showed that 21 strains were also resistant to fosetyl-Al while 13 strains were as sensitive as the wild type strain to fosetyl-Al.

The EC90 values determined with the 21 mutants resistants to phosphorous acid and cross-resistant to fosetyl-Al ranged from 724 to 1480 $\mu g.ml^{-1}$ for phosphorous acid and from 1072 to 3800 $\mu g.ml^{-1}$ for fosetyl-Al (Table 3).

TABLE 2 - Summary of results of mutagenesis experiments showing the number of phosphorous acid-resistant mutants screened on an agar medium supplemented with phosphorous acid.

Selection medium	Mutagenic reagent	Fungal organites	Number of resistant mutants
CMA	UV light	Thalli (28)	6
		Encysted zoospores (2.10^7)	8
	MNNG	Encysted zoospores (10^7)	10
RMSA deficient in phosphate (0.0084 mM PO_4)	UV light	Thalli (196)	10

TABLE 3 - *In vitro* resistance to fosetyl-Al and phosphorous acid of ten phosphorous acid-resistant mutants of *P. citrophthora* compared to the parental strain Fp

Isolate [a]	Phosphorous acid		Fosetyl-Al	
	EC90[b]	RL[c]	EC90[b]	RL[c]
Fp	215		795	
UVT4	1480	6.8	2951	3.7
UVT1	1413	6.5	2138	2.6
NTG8	1350	6.2	2512	3.1
UVT3	1260	5.8	3800	4.7
NTG11	1202	5.5	1950	2.4
UVT5	1122	5.2	1413	1.7
NTG1	1042	4.8	1698	2.1
UVC1	933	4.3	1072	1.3
UVC8	811	3.7	1202	1.5
UVC7	724	3.3	1148	1.4

a : Fp is the sensitive parental isolate; isolates with NTG designation are phosphorous acid-resistant mutants screened after a MNNG treatment of Fp encysted zoospores. Isolates with UVC or UVT designation are phosphorous acid resistant mutants screened after an UV light treatment of Fp encysted zoospores or mycelium respectively.

b : EC90 values ($\mu g.ml^{-1}$) for radial growth inhibition due to fosetyl-Al or phosphorous acid on CMA medium are obtained from regression lines plotting percentage inhibition on a probit scale versus log concentration.

c : RL value is the resistance level of the resistant mutants.
RL is calculated as the ratio between the EC90 value of the resistant mutant and the EC90 value of the parental strain.

The calculated resistance levels of mutants ranged between 3.3 and 6.8 for phosphorous acid and between 1.3 and 4.7 for fosetyl-Al. Therefore, the phosphorous acid-resistant mutants showed a lower resistance level for fosetyl-Al than for phosphorous acid.

The EC90 values were also determined for the 4 fosetyl-Al-resistant mutants; the results showed that these mutants were also resistant to phosphorous acid. Their growth on CMA medium gave EC90 values ranged from 1148 to 1202 $\mu g.ml^{-1}$ for phosphorous acid and from 2188 to 3388 $\mu g.ml^{-1}$ for fosetyl-Al (Table 4). These mutants were approximately six times more resistant to phosphorous acid than the parental strain, and twice more resistant to fosetyl-Al.

Substrat dependance of the *in vitro* resistance.

The EC90 values of fosetyl-Al or phosphorous acid for the resistant mutants were much lower on RMSA medium at low phosphate concentration than on CMA or pea agar medium which contained 1.24 mM and 0.6 mM PO_4 respectively (Table 5).

Different phosphate concentrations were compared for fungal inhibition by fosetyl-Al on RMSA medium. For each fungal mutant, the resistance to fosetyl-Al increases with the amount of phosphate in the medium (Table 6).

***In vivo* behaviour of the resistant mutants.**

Inoculation on Citrus seedlings.

The wild type strain Fp was pathogen on Citrus seedlings whatever the mode of inoculation, zoospore suspension or mycelial mixture. Plants were dead ten days after inoculation.

Roots of Citrus seedlings were inoculated with 3 phosphorous acid-resistant mutants (NTG1, UVT3 and UVC1) and 4 fosetyl-Al-resistant mutants (A1, A2, A3 and A4). No symptoms were visible on the leaves, even after one month post-inoculation. However, when seedlings were depoted, we observed an infection of the roots with all the mutants. Because of their low pathogenicity, these mutants cannot be tested for their fosetyl-Al resistance on Citrus seedlings.

Inoculation on Citrus leaves.

Citrus leaves were inoculated with the wild type strain Fp and 6 resistant mutants : 4 phosphorous acid-resistant mutants (UVT1, UVT3, UVT4 and NTG8) and 2 fosetyl-Al-resistant mutants (A1 and A4). Only the 3 resistant mutants (UVT1, UVT3 and NTG8 were as pathogen as the wild type strain. Their mycelium invaded the whole leaf 5 days after inoculation. The other resistant mutants tested were not pathogen.

TABLE 4 - *In vitro* resistance to fosetyl-Al and phosphorous acid of 4 fosetyl-Al-resistant mutants of *P. citrophthora* compared to the parental strain Fp.

Isolate[a]	Phosphorous acid		Fosetyl-Al	
	EC90[b]	RL[c]	EC90[b]	RL[c]
Fp	206		1445	
A1	1202	5.8	3388	2.3
A2	1148	5.6	2630	2
A3	1175	5.7	2188	1.5
A4	1175	5.7	2692	2

a : Fp is the sensitive parental isolate ; isolates with A designation are fosetyl-Al resistant mutants selected after UV light irradiation of Fp mycelium.

b : EC90 values ($\mu g.ml^{-1}$) for radial growth inhibition due to fosetyl-Al or phosphorous-acid on CMA medium are obtained from regression lines plotting percentage inhibition on a probit scale versus log concentration.

c : RL value is the resistance level of the resistant mutants.

TABLE 5 - *In vitro* resistance to fosetyl-Al and phosphorous acid of the parental strain and two mutants compared on different agar media.

Isolate[a]	RMSA medium (0.0084 mM PO_4)		CMA (1.24 mM PO_4)		Pea agar (0.6 mM PO_4)	
	EC90[b]	RL[c]	EC90[b]	RL[c]	EC90[b]	RL[c]
Fosetyl-Al						
Fp	74		1445		759	
A1	488	6.5	3388	2.3	1175	1.5
A3	269	4	2188	1.5	1148	1.5
Phosphorous acid						
Fp	19		206		372	
A1	1479	78	1202	5.8	3090	8.3
A3	1698	90	1175	5.7	3500	9.5

a : Fp is the sensitive parental strain ; A1 and A3 are two fosetyl-Al-resistant mutants.

b : EC90 values ($\mu g.ml^{-1}$) for radial growth inhibition due to fosetyl-Al or phosphorous acid are obtained from regression lines plotting percentage inhibition on a probit scale versus log concentration.

c : RL value is the resistance level of the resistant mutants.

TABLE 6 - Percent inhibition [a] of linear radial growth of *P. citrophthora* isolate by fosetyl-Al [b] on RMSA medium containing different concentrations of phosphate.

Isolate[c]	Concentrations of phosphate (mM PO_4)		
	0.0084	0.084	0.84
Fp	98.8	88.5	46.8
A1	25.8	23.5	6.4
A3	63.0	33.0	7.7

a : Percent inhibition was determined after a 7-day culture

b : Concentration of fosetyl-Al is 100 $\mu g.ml^{-1}$.

c : Fp is the parental strain ; A1 and A3 are two fosetyl-Al-resistant mutants.

When Citrus leaves were floated on a solution containing 500 $\mu g.ml^{-1}$ of fosetyl-Al the resistant mutants UVT1, UVT3 and NTG8 like the wild type strain did not develop lesions.

DISCUSSION

The exposure of *Phytophthora citrophthora* to either the chemical mutagen MNNG or UV light irradiation resulted in the possibility of screening a few mutants resistant to either fosetyl-Al or phosphorous acid. It seems easier to obtain *in vitro* phosphorous acid resistant mutants than fosetyl-Al ones. However, our results are now insufficient to calculate the frequency of resistant mutant. All the mutants selected either on fosetyl-Al or phosphorous acid, showed resistance levels which do not exceed 5 for fosetyl-Al and 7 for phosphorous acid (in the presence of high concentrations of phosphate). These resistance levels are weak compared to those obtained on different species of *Phytophthora* with metalaxyl (DAVIDSE, 1981 ; JOSEPH and COFFEY, 1984 ; ABDELLAOUI-MAANE *et al.*, 1988) where resistant mutants selected *in vitro* after chemical mutagenesis, were 50 to 1000 times more resistant to metalaxyl than the wild type strain. BOWER and COFFEY (1985) selected high level resistant mutants of *Phytophthora capsici* with fosetyl-Al *in vitro*. But in this case, two facts must be pointed out : the EC90 values of the resistant mutants were determined with extrapolation from a regression analysis and the mutants were selected on a culture medium deficient in phosphate. As shown in table 5, the resistance levels of mutants are very different depending on the growth substrate and especially the phosphate content. We found that the EC90 values are 10 times higher on RMSA than on CMA medium. GUEST (1984 b) have reported that factors which optimize conditions for fungal growth reduce the activity of fosetyl-Al and the variety of nutrients and environmental factors seems to act unspecifically rather than by relieving metabolic blockages. In regard to these results, we have to be careful for the translation between the *in vitro* behaviour and what happens *in planta*.

Although, 3 resistant mutants tested were pathogen on Citrus leaves, they were not pathogen on Citrus seedlings. Root inoculation was made without wounding, thus the mutants seems to have lost their ability to penetrate plant tissues. Moreover, these 3 mutants were sensitive to 500 $\mu g.ml^{-1}$ of fosetyl-Al as well as the wild type strain, after leave inoculation. This concentration is lower than the *in vitro* EC50 value of fosetyl-Al for the wild type strain Fp (EC50= 625 $\mu g.ml^{-1}$). So, fosetyl-Al seems more efficient *in vivo* than *in vitro*, confirming the results of other workers. But, the correlation between the *in vivo* and *in vitro* EC50 or EC90 values could be possible if phosphite content of leaf tissues was determined. An accumulation of phosphite at the inoculation site could be the cause of the higher efficiency of fosetyl-Al *in vivo*, although such a phenomenon was not found in the cowpea-*Phytophthora cryptogea* interaction (SAINDRENAN *et al.*, 1986). Without these informations, any conclusion is pure illusive. However, the reduction of pathogenicity observed with the resistant mutants selected *in vitro* explains why no resistant strain appeared in the field until now.

Cross resistance between fosetyl-Al and phosphorous acid observed for most of mutants, support the view that the two compounds have the same mode of action. Phosphorous acid is more efficient than fosetyl-Al on natural strains of *P. citrophthora*. However, the fosetyl-Al resistant mutants showed a higher level of sensitivity to fosetyl-Al than to phosphorous acid when the two compounds are applied at the same concentration ($meqPO_3$). Moreover, all the cross-resistant mutants to fosetyl-Al and phosphorous acid were more resistant to phosphorous acid than to fosetyl-Al when compared to the wild type strain. These results might suggest that the 2 compounds are different at the uptake level or because of the toxicity of the aluminium cation. Phosphite uptake by fungal cells has been determined by BARCHIETTO *et al.* in 1988 but no data is available about fosetyl-Al uptake. Thus, determination of the absorption of phosphite by the *in vitro* resistant mutants could give more information on the differential mode of action of fosetyl-Al and phosphorous acid.

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REFERENCES

ABDELLAOUI-MAANE (S.), SENG (J.M.), SAINDRENAN (P.) and BOMPEIX (G.). 1988.
Fosetyl-Al is effective against mutants of *Phytophthora capsici* resistant to metalaxyl.
Cryptogamie, 9 (1), 47-56.

BARCHIETTO (T.), SAINDRENAN (P.) and BOMPEIX (G.). 1988.
Uptake and utilization of phosphite by *Phytophthora citrophthora* and *Nectria haematococca* in relation to its selective toxicity.
Pest. Sci., 22, 159-167.

BOMPEIX (G.) and SAINDRENAN (P.). 1984.
In vitro antifungal activity of fosetyl-Al and phosphorous acid on *Phytophthora* species.
Fruits, 39 (12), 777-786.

BOWER (L.A.) and COFFEY (M.D.). 1985.
Development of laboratory tolerance to phosphorous acid, fosetyl-Al and metalaxyl in *Phytophthora capsici*.
Canadian J. of Plant Pathol., 7, 1-6.

DARAKIS (G.), BLIZOUA-BI (P.), BARCHIETTO (T.), RENARD (J.L.), SAINDRENAN (P.) and BOMPEIX (G.). 1985.
Distribution du phoséthyl-Al dans les tissus de cocotier après traitement : hypothèses sur son mode d'action sur le *Phytophthora heveae*.
in Fungicides for crop protection, B.C.P.C. Monograph, 31 (2), 273-276.

DAVIDSE (L.C.). 1981.
Resistance to acylalanine fungicides in *Phytophthora megasperma* f. sp. *medicaginis*.
Neth. J. Pl. Path., 87, 11-24.

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.). 1984 a.
Modification of defence responses in tobacco and capsicum following treatment with Fosetyl-Al [Aluminium tris (0-ethyl phosphonate)].
Physiological Plant Pathology, 25, 125-134.

GUEST (D.I.). 1984 b.
The influence of cultural factors on the direct antifungal activities of Fosetyl-Al, Promocarb, Metalaxyl, SN 75196 and Dowco 444.
Phytopath. Z., 111, 155-164.

JOSEPH (M.C.) and COFFEY (M.D.). 1984.
Development of laboratory resistance to metalaxyl in *Phytophthora citricola*.
Phytopathology, 74, 1411-1414.

KHAN (A.), VERNENGHI (A.) and RAVISE (A.). 1986.
Incidence of fosetyl-Al and elicitors on the defence reactions of Citrus attacked by *Phytophthora* spp.
Fruits, 41 (10), 587-595.

LAVILLE (E.) and CHALANDON (A.). 1982.
Synthèse des résultats obtenus avec le phoséthyl-Al dans la lutte contre les maladies à *Phytophthora* des agrumes.
Fruits, 37 (1), 11-17.

SAINDRENAN (P.) and BOMPEIX (G.). 1986.
Rôle des phytoalexines dans la réponse de *Vigna unguiculata* traité par le phoséthyl-Al à l'infection par *Phytophthora cryptogea*.
C.R. Acad. Sc. Paris, série III, 10, 411-414.

SAINDRENAN (P.), BARCHIETTO (T.), AVELINO (J.) and BOMPEIX (G.). 1988.
Effects of phosphite on phytoalexin accumulation in leaves of cowpea infected with *Phytophthora cryptogea*.
Physiological and Molecular Plant Pathology, 32, 425-435.

INDUZIERUNG VON *PHYTOPHTHORA CITROPHTHORA* MUTANTEN, DIE GEGEN PHOSETYL-Al UND PHOSPHORISCHE SÄURE RESISTENT SIND, SOWIE STUDIUM IHRES PATHOGENEN POTENTIALS GEGENÜBER ZITRUSFRÜCHTEN.

Claire NEEMA, G. BOMPEIX und X. MOURICHON.

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KURZFASSUNG - 34 gegenüber der phosphorigen Säure resistente Mutanten von *Phytophthora citrophthora* wurden nach mutagenen Behandlungen mit Nitrosoguanidin und Ultraviolettstrahlen auf verschiedenen Substraten gezüchtet. Es hat sich erwiesen, dass die Resistenz der gewonnenen Mutanten gegenüber der phosphorigen Säure um 3 bis 7 mal stärker ausfällt als jene des wildwachsenden Stamms. Mit Blick auf die Resistenz gegenüber Phosetyl-Al konnten lediglich 4 Mutanten ausfindig gemacht werden, die auf einem CMA-Substrat nach UV-Bestrahlung der Bestockungstriebe gezüchtet worden sind.; ihre Resistenz gegen Phosetyl-Al ist schwach (NR=2). Von den 6 getesteten, resistenten Mutanten hat sich in Bezug auf *Citrus*-Keimpflanzen keiner als pathogen erwiesen. Drei davon sind es jedoch auf isolierten und beschädigten *Citrus*-Blättern. Sie reagieren empfindlich auf eine Phosetyl-Al-Konzentration von 500 $\mu g \cdot ml^{-1}$, welcher Konzentrationswert unter der *in vitro* bestimmten C150 des wildwachsenden Stamms liegt.

INDUCCION DE MUTANTES DE *PHYTOPHTHORA CITROPHTHORA* RESISTENTES AL PHOSETYL-Al Y AL ACIDO FOSFOROSO Y ESTUDIO DE SU PODER PATOGENO SOBRE *CITRUS*.

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RESUMEN - 34 mutantes de *Phytophthora citrophthora* resistentes al ácido fósforoso se han seleccionado sobre diferentes medios después de los tratamientos mutagenos con la nitrosoguanidina o con los rayos UV. Estos mutantes se han revelado 3 a 7 veces más resistentes al ácido fósforoso que la población salvaje. Solo has podido obtenerse 4 mutantes resistentes al fosetyl-Al. Se han seleccionado sobre medio CMA después de irradiación de talos con los UV y su nivel de resistencia al fosetyl-Al es escaso (NR=2). Entre los 6 mutantes resistentes sometidos a test, ninguno se ha revelado patógeno sobre plantulas de *Citrus*. Sin embargo, 3 de entre ellos son patógenos sobre hojas nisladas y heridas de *Citrus*. Con todo, son sensibles a una concentración de 500 $\mu g \cdot ml^{-1}$ de fosetyl-Al ; siendo inferior esta concentración a la C150 de la población salvaje determinada *in vitro*.