In vitro Antifungal Activity of Fosetyl Al and Phosphorous Acid on *Phytophthora* Species.

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IN VITRO ANTIFUNGAL ACTIVITY OF FOSETYL Al AND PHOSPHOROUS ACID ON *PHYTOPHTHORA* SPECIES.

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SUMMARY - A direct mode of action of fosetyl Al and phosphorous acid is found *in vitro* on the natural Corn Meal Agar media, which has a low phosphate content which favors the antifungal activity of these two compounds. Ten species of *Phytophthora* can be classified in three groups : 1) high sensitivity, e.g. *P. cinnamomi, P. citrophthora, P. parasitica, P. palmivora* ; 2) medium sensitivity, e.g. *P. cactorum, P. cryptogea, P. vignae : 3)*low sensitivity, e.g. *P. capsici, P. infestans, P. megasperma.*

With fosetyl Al and phosphorous acid, the inhibition rate is not constant during the fungi growth, but appears to be progressively increasing. As a result EC_{50} and EC_{90} values are meaningless. According to the species studied, fosetyl Al is more or less inhibitory than H_3PO_3. *Phytophthora* species are not only different in regard to sensitivity to these compounds, but also in the antagonistic effect of phosphate ion. In some cases,phosphate (1 mM) nullified rapidly the effect of phosphorous acid (*P. citrophthora*) and for others only a slight reversion can be obtained (*P. parasitica*).

The importance of the direct mode of action *in vivo* is discussed.

ACTIVITE ANTIFONGIQUE *IN VITRO* DU PHOSETHYL Al ET DE L'ACIDE PHOSPHOREUX SUR LES ESPECES DE *PHYTOPHTHORA*.

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RESUME - Un mode d'action direct du phoséthyl Al et de l'acide phosphoreux a été trouvé *in vitro* sur le milieu «Corn meal agar». Celui-ci a une basse teneur en phosphate qui favorise l'activité antifongique de ces deux composés.

Dix espèces de *Phytophthora* peuvent être classées en trois groupes : 1) haute sensibilité : *P. cinnamomi, P. citrophthora, P. parasitica, P. palmivora* ; 2) moyenne sensibilité : *P. cactorum, P. cryptogea, P. vignae* ; 3) faible sensibilité : *P. capsici, P. infestans, P. megasperma.* Avec le phoséthyl Al et l'acide phosphorique, le taux d'inhibition n'est pas constant au cours de la croissance des champignons, mais paraît s'accroître progressivement.

Il en résulte que les valeurs de CE_{50} ou CE_{90} sont sans signification. Selon les espèces étudiées, le phoséthyl Al est plus ou moins inhibiteur que l'acide phosphoreux. Les espèces de *Phytophthora* ne diffèrent pas seulement pour leur sensibilité à ces composés, mais aussi pour l'effet antagoniste de l'ion phosphate. Dans certains cas le phosphate annule l'effet direct de l'acide phosphoreux (*P. citrophthora*) et dans d'autres une légère réversion est seulement constatée (*P. parasitica*).

L'importance du mode d'action direct *in vivo* est discutée.

Contrary to the other systemic fungicides used in the control of Oomycetes, fosetyl Al is clearly more active *in vivo*. Indeed mycelial growth is inhibited *in vitro* in high concentrations of fosetyl Al compared with anilides such as metalaxyl for which approximatly 1 ppm is sufficient to stop the *in vitro* and *in vivo* growth.

The efficiency of fosetyl Al in open field, could be explained partially by an indirect mode of action stimulating the plant's defence mechanisms (VO-THI-HAI et al., 1979 ; BOMPEIX et al., 1980, 1981, 1983 ; GUEST and BOMPEIX, 1984 ; GUEST, 1982, 1984 ; DURAND and SALLE, 1981 ; YUSUF, 1981).

These results have been discussed by FENN and COFFEY (1984) who found a high inhibitory effect of fosetyl Al and its active metabolite H_3PO_3, on Ribeiro's synthetic medium with a deficiency in phosphate (0.08 mM); this can be explained by phosphite-phosphate antagonism, already found *in vivo* (BOMPEIX et al., 1980).

Strangely, they found a good inhibitory effect on Corn Meal Agar (CMA) which is a natural medium. This result is in contradiction with a number of papers written by other research groups (BOMPEIX, loc. cit. ; MORGAT

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et al., 1977 ; VEGH and LEBERRE, 1979 ; FARIH et al., 1981).

According to FENN and COFFEY the direct mode of action should be taken into consideration, but unfortunately they do not indicate the phosphate content of CMA and host-plants.

In the latter case, the phosphate concentration is approximately in the range of 5 to 50 mM (BIELESKI, 1973 ; BLIGNY et al., 1983 ; MIGINIAC-MASLOW et al., 1983), concentrations which are probably not able to give sufficient efficacy of fosetyl Al and H_3PO_3.

In the case of CMA, it could be possible that a low phosphate content exists. Consequently, we have measured the phosphate content in this medium, and then we have tried a wide range of *Phytophthora* species in order to compare the efficacy of fosetyl Al and H_3PO_3 *in vitro* and *in vivo*.

Finally the CMA has been supplemented with phosphate in order to verify the possibility of nullifying the phosphorous acid effect under those conditions.

MATERIALS AND METHODS

1. *Phytophthora* species.

Phytophthora species which have been used in this experiment are :

	obtained from :
P. cactorum	INRA, Versailles (Dr. LEROUX).
P. capsici af.	GERDAT (Drs LAVILLE and DE VALLAVIELLE,Montpellier) African isolate.
P. capsici f.	Rhône-Poulenc Agrochimie, Lyon.
P. cinnamomi f.	INRA,Versailles (Dr. VEGH).
P. cinnamomi ca	GERDAT (Californian isolate).
P. citrophthora	GERDAT (Corsica)
P. cryptogea	University P. and M. CURIE Paris (Dr. SAINDRENAN).
P. hevea	IRHO, Ivory Coast (Dr. RENARD)
P. infestans	INRA,Avignon (Dr. MOLLOT)
P. megasperma	INRA,Rennes (Dr. ROUXEL)
P. palmivora	GERDAT
P. parasitica	GERDAT *(P. nicotianae* var. *parasitica).*
P. vignae	CVS,Baarn (Netherlands).

Stock cultures were maintained on V_8-Ca CO_3 juice agar.

2. Chemicals.

Fosetyl Al (Aluminium tris-0-ethylphosphonate) is used in wettable powder (Aliette^R) and 80 % a.i. phosphorous acid solutions are partially neutralized at pH 6.5 with NaOH 1 N.

3. Measurement of fungitoxicity.

Toxicity toward linear mycelial growth is measured by adding fosetyl Al and phosphorous acid to the CMA buffered at pH 6.5 by MES.

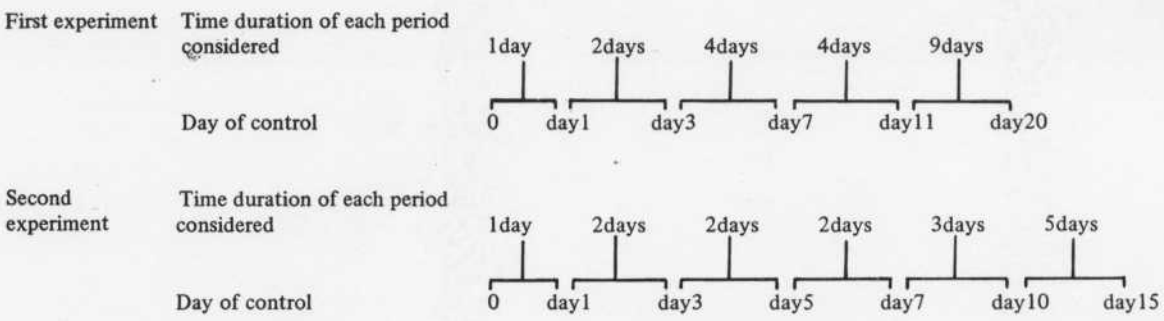
Petri dishes were 9 cm diameter, filled with 20 ml medium. Plates were inoculated with mycelium plugs (6 mm diameter) taken from the margin of 5-day-old cultures on V8-$CaCO_3$ agar, and dishes (six replicates) were incubated at 22°C in the dark.

Radial mycelial growth was determined periodically by measuring diameters of each colony and subtracting the diameter plug.

Fosetyl Al concentrations are expressed in meq H_3PO_3 in order to facilitate the comparisons between fosetyl Al and H_3PO_3.

The concentration range used, varies from 0.42 mM (34.5 μg/ml) to 6.72 mM (552 μg/ml) in H3PO3 and from 0.84eq. mM (99 μg/ml) to 13.44eq. mM (1580 μg/ml) in fosetyl Al.

The inhibition rates are calculated during the following time periods :



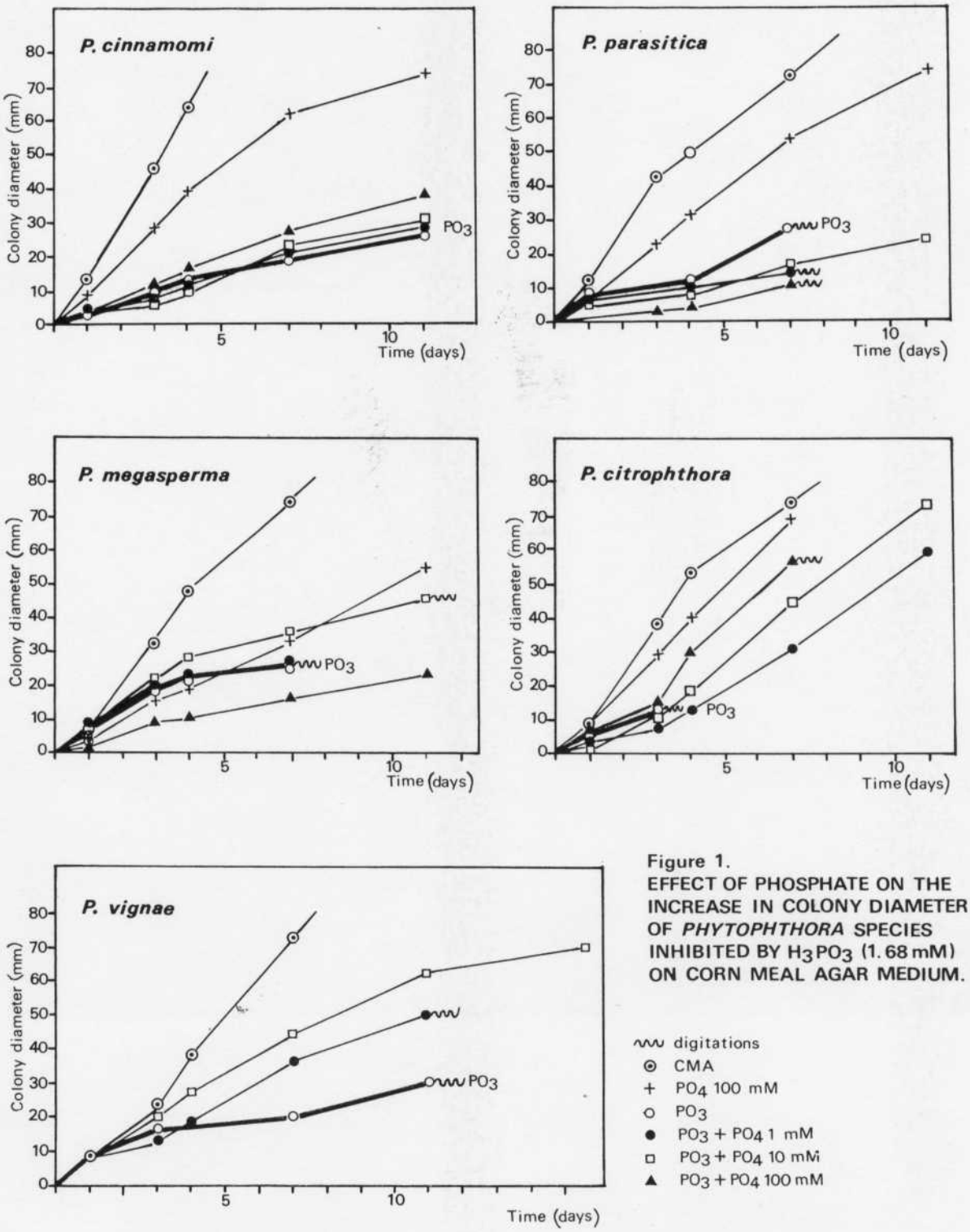


Figure 1.
EFFECT OF PHOSPHATE ON THE INCREASE IN COLONY DIAMETER OF *PHYTOPHTHORA* SPECIES INHIBITED BY H_3PO_3 (1.68 mM) ON CORN MEAL AGAR MEDIUM.

∿∿ digitations
⊙ CMA
\+ PO_4 100 mM
○ PO_3
● PO_3 + PO_4 1 mM
□ PO_3 + PO_4 10 mM
▲ PO_3 + PO_4 100 mM

4. Phosphate content of the CMA.

The ion phosphate content of the CMA has been determined by the HESS and DERR'S method (1975).

5. Effect of phosphate content.

To study the role of phosphate on the activity of phosphorous acid, CMA has been amended with 1, 10, and 100 mM in phosphate by adding a buffered solution of sodium phosphate (pH 6.5).

Results.

1. Content in phosphate of the CMA medium.

Without mineralisation (Pi available) we find 0.38 mM, a very low concentration that could improve the phosphite and fosetyl Al effect.

This content is much lower than in flowering plants (Cf. *supra*).

2. Inhibitory effect of fosetyl Al and H_3PO_3.

Contrary to the observations made on most fungicides we find that inhibition of the radial mycelial growth rate increases progressively during the fungi growth. It can be total (mycelium stopped in the middle of the Petri dishes) with concentrations as low as 0.42 mM (34.5 µg/ml) in phosphorous acid.

However, the time necessary to obtain the cessation of growth is frequently preceded by the formation of mycelial digitations and precise measurements become impossible. This phenomenon is represented on the growth curves by a sinusoïdal line (Figure 1).

3. Comparison of the effect of fosetyl Al and H_3PO_3.

Towards *P. capsici* and *P. megasperma* growth, fosetyl Al is more active than H_3PO_3 (Tables 1 and 5).

The contrary has been observed with *P. citrophthora*. Some other *Phytophthora* spp. such as *P. cinnamomi* are more inhibited by lower H_3PO_3 concentrations, but this result is reversed with higher concentrations (Tables 1 to 8). These contradictions can be explained by the effect of the aluminium ion which can be an inhibitor for *Phytophthora* growth.

4. Comparison of the effect of fosetyl Al and H_3PO_3, on ten Phytophthora *species.*

In preliminary experiments we have obtained some remarkable inhibitions on CMA but not on the following media : malt extract («cristomalt»), V8 juice agar, PDA.

Phytophthora species show wide differences as regards their sensitivity to fosetyl Al and H_3PO_3.

TABLE 1 - Percentage growth inhibition of *P. capsici* on CMA agar medium at five concentrations of fosetyl Al and phosphorous acid.

Isolates	mM	Percentage inhibition of radial growth		
		1d	2d	4d
P. capsici af.	0.84	0	22.4 a	-
Fosetyl Al	1.68	12.5 a	21.8 a	-
expressed in equivalents	3.36	34.3 b	50.8 b	70.3 a
mM H_3PO_3	6.72	88.0 c	96.8 c	100 b
	13.44	100 d	100 c	100 b
P. capsici af.	0.42	8.5 a	0 a	0 a
H_3PO_3	0.84	8.7 a	0 a	0 a
	1.68	8.6 a	0 a	47.7 b
	3.36	25.2 a	0 a	44.1 b
	6.72	43.5 b	14.6 b	34,4 b
P. capsici f.	0.42	30.0 a	3.2 a	0
H_3PO_3	0.84	40.1 a	6.9 a	0
	1.68	28.0 a	0 a	48.2 a
	3.36	39.0 a	3.4 a	42.4 a
	6.72	60.1 b	31.2 b	30,1 a

Values with the same letter (within a column) are not significantly different according to Duncan's Multiple Range Test (P = 0.05).

TABLE 2 - Percentage growth inhibition of *P. cinnamomi* on CMA agar medium at five concentrations of fosetyl Al and phosphorous acid.

Isolates	mM	Percentage inhibition of radial growth			
		1d	2d	4d	4d
P. cinnamomi f.	0.84	16.0 a	41.0 a	57.8 a	-
Fosetyl Al	1.68	38.6 b	35.9 a	55.7 a	-
expressed in equivalents	3.36	38.5 b	69.6 b	74.4 b	100
mM H_3PO_3	6.72	100 c	100 c	100 c	100
	13.44	100 c	100 c	100 c	100
P. cinnamomi f.	0.42	38.9 a	49.5 a	32.9 a	43.7 a
	0.84	33.4 a	56.6 a	45.1 b	45.4 a
H_3PO_3	1.68	31.1 a	39.4 a	45.8 b	43.2 a
	3.36	22.2 a	43.9 a	44.5 b	47.5 a
	6.72	16.7 b	55.2 a	53.2 c	62.5 b
P. cinnamomi ca	0.42	0	2.0 a	10.4 a	21.0 a
	0.84	0	32.0 a	22.4 b	32.0 b
H_3PO_3	1.68	0	0.4 a	35.4 b	40.0 c
	3.36	0	6.8 a	32.2 b	47.0 d
	6.72	50.0	46.0 b	42.4 c	60.0 e

f = isolate from France ca = isolate from USA (California).
Values with the same letter (within a column) are not significantly different according to Duncan's Multiple Range Test (P = 0.05)

TABLE 3 - Percentage growth inhibition of *P. citrophthora* on CMA agar medium at five concentrations of fosetyl Al and phosphorous acid.

Isolates	mM	Percentage inhibition of radial growth				
		1d	2d	4d	4d	9d
Fosetyl Al	0.84	3.7 a	39.8 a	17.2 a	-	-
expressed in equivalents	1.68	27.4 b	25.2 a	22.1 a	-	-
mM H_3PO_3	3.36	52.6 c	81.2 b	86.9 b	100	-
	6.72	100 d	100 c	100 c	100	-
	13.44	100 d	100 c	100 c	100	-
	0.42	51.2 a	22.7 a	5.3 a	100	100
	0.84	55.3 a	23.8 a	8.6 a	100	100
H_3PO_3	1.68	63.4 a	47,9 b	23.1 b	100	100
	3.36	62.6 a	73.1 c	85.8 c	—	100
	6.72	100 b	100 d	86.6 c	—	100

Values with the same letter (within a column) are not significantly different according to Duncan's Multiple Range Test (P= 0.05).

They can be classified in three groups :

1) High sensitivity, e.g. *P. cinnamomi, P. citrophthora, P. parasitica, P. palmivora* for which 0.42 mM of H_3PO_3 or 0.84 mM of fosetyl Al lead to the total inhibition, but it needs at least 10-12 days to be observed.

2) Medium sensitivity : among them we find *P. cactorum, P. cryptogea, P. vignae.*

3) Low sensitivity such as *P. capsici, P. infestans* and *P. megasperma.*

5. Differences among isolates.

Different responses of the two isolates of *P. capsici*

TABLE 4 - Percentage growth inhibition of *P. infestans* on CMA agar medium at five concentrations of fosetyl Al and phosphorous acid.

Isolates	mM	Percentage inhibition of radial growth			
		3d	4d	4d	9d
Fosetyl Al expressed in equivalents mM H_3PO_3	0.84	0	0	0	0
	1.68	0	0	0	0
	3.36	0	0	25.8 a	100
	6.72	0	0	62.4 b	100
	13.44	0	100	100 c	100
H_3PO_3	0.42	0	0	0	0
	0.84	45.3 a	0	20.0 a	100
	1.68	9.5 a	16.2 a	18.4 a	100
	3.36	100 b	100 b	100 b	100
	6.72	100 b	100 b	100 b	100

Values with the same letter (within a column) are not significantly different according to Duncan's Multiple Range Test.
(P = 0.05).

TABLE 5 - Percentage growth inhibition of *P. megasperma* on CMA agar medium at five concentrations of fosetyl Al and phosphorous acid.

Isolates	mM	Percentage inhibition of radial growth			
		1d	2d	4d	4d
Fosetyl Al expressed in equivalents mM H_3PO_3	0.84	0	0	0	-
	1.68	0	5.2 a	13.6 a	-
	3.36	0	25.8 b	21.2 a	70.7 a
	6.72	0	60.6 c	75.4 b	100 b
	13.44	100	100 d	94.4 c	100 b
H_3PO_3	0.42	0	0	0	0
	0.84	0	0	0	0
	1.68	0	0	40 a	100
	3.36	0	0	44,4 a	100
	6.72	0	0	69.6 b	100

Values with the same letter (within a column) are not significantly different according to Duncan's Multiple Range Test
(P = 0.05).

are clear cut although slight. Up to 0.84 mM the inhibition is almost nil. The higher concentration of H_3PO_3 (6.72 mM) gives, in the two last periods of measurement, an inhibition of 34.4 % and 30.1 % respectively.

On the other hand, isolates of *P. cinnamomi* have a very different response for the low concentrations. The French isolate is more sensitive than the American one in the two experiments.

6. Antagonistic effect of the phosphate on the phosphorous acid activity.

The effect of phosphate varies considerably according to the species studied (Table 9).

A very weak effect has been obtained for two species (*P. parasitica, P. palmivora*). On the other hand, with *P. vignae, P. citrophthora, P. megasperma*, the antagonistic effect is particularly clear. Even with 1 mM, digitations are often suppressed and colonies become circular again (Figure 1).

These three species and *P. cinnamomi* and *P. capsici* can be considered very sensitive to the reverse effect of H_3PO_3 by phosphate.

It seems that each species is characterized by a specific interaction phosphite/phosphate.

For all species examined, check concentrations (1 mM

TABLE 6 - Percentage growth inhibition of *P. palmivora* on CMA agar medium at five concentrations of fosetyl Al and phosphorous acid.

Isolates	mM	Percentage inhibition of radial growth				
		1d	2d	4d	4d	9d
Fosetyl Al expressed in equivalents mM H_3PO_3	0.84	6.2 a	26.5 a	35.7 a	-	-
	1.68	57.5 b	41.2 b	42.1 a	69.0 a	100
	3.36	56.2 b	67.8 c	70.6 b	100 b	100
	6.72	75.0 c	84.9 d	100 c	100 b	100
	13.44	75.1 c	86.7 d	100 c	100 b	100
H_3PO_3	0.42	50.0 a	52.9 a	47.0 a	52.1 a	100
	0.84	48.2 a	29.5 a	85.1 b	58.0 a	100
	1.68	-	-	73.6 b	75.3 b	100
	3.36	-	-	80.9 b	81.6 b	100
	6.72	-	-	87.8 b	86.6 b	100

Values with the same letter (within a column) are not significantly different according to Duncan's Multiple Range Test (P = 0.05).

TABLE 7 - Percentage growth inhibition of *P. vignae* on CMA agar medium at five concentrations of fosetyl Al and phosphorous acid.

Isolates	mM	Percentage inhibition of radial growth				
		1d	2d	4d	4d	9d
Fosetyl Al expressed in equivalents mM H_3PO_3	0.84	17.4 a	3.0 a	17.0 a	20.0 a	-
	1.68	4.4 a	3.1 a	14.4 a	19.7 a	100
	3.36	4.5 a	0.5 a	18.5 a	14.0 a	100
	6.72	35.7 b	46.5 b	100	100	100
	13.44	21.7 b	94.1 b	100	100	100
			3 d			
H_3PO_3	0.42	-	0	0	11.7 a	6.3 a
	0.84	-	0	0	7.9 a	22.4 b
	1.68	-	0	45.0 a	46.6 b	66.9 c
	3.36	-	0	46.0 a	51.0 b	53.1 c
	6.72	-	0	54.0 a	51.0 b	82.0 d

Values with the same letter (within a column) are not significantly different according to Duncan's Multiple Range Test (P = 0.05).

and 10 mM) of phosphate show no difference with non amended CMA. 100 mM phosphate leads to a slight reduction of the mycelial growth rate.

DISCUSSION

Contrary to anilides which lead to a constant rate of inhibition during the fungi growth, fosetyl Al and H_3PO_3 involve an increasingly progressive inhibition.

According to the points of measurements on the culture media, the inhibition rate can be low or high.

Consequently it becomes very difficult to accurately calculate EC_{50} or EC_{90} values by only measuring two points of the fungi growth.

For example, *P. palmivora* EC_{50} with fosetyl Al is situated at approximately 1.68 mM (Table 6).

However, the same concentration leads to EC_{100} after the eleventh day of culture. The total inhibition can be obtained with only 0.42 mM in H_3PO_3, but this concentration gives an inhibition rate of 50 % for a long time.

The same reasoning can be applied to the EC_{90}. Only the precise comparison of the inhibition rates is significant.

As a result of these fundamental observations a more

TABLE 8 - Percentage growth inhibition of several species of *Phytophthora* at 1.68 mM H_3PO_3, with short sequences.

Isolates	Percentage inhibition of radial growth					
	1d	2d	2d	2d	3d	5d
P. cactorum	24,2 a	42,9 a	36,0 a	29,2 a	62,2 b (1)	100 (1)
P. capsici (f)	7,9 a	19,5 a	5,1 a	-	-	-
P. capsici (af)	6,3 a	6,2 a	4,8 a	-	-	-
P. cinnamomi(f)	32,8 a	74,6 b	73,7 b	80,8 c	82,2 c	100 (2)
P. cinnamomi (ca)	20,9 a	58,0 b	61,2 b	61,0 b	60,1 b	100 (2)
P. cryptogea	24,2 a	23,3 a	18,2 a	29,0 a	51,5 b	100 (1)
P. megasperma	3,1 a	27,0 b	33,8 b	54,3 c	60,5 d	100 (2)
P. palmivora	47,6 a	48,7 a	44,3 a	62,1 b	68,0 b	100 (2)
P. parasitica	50,0 a	66,0 a	55,9 a	75,6 b	72,0 b	100 (2)

Values with the same letter (line) are not significantly different (P= 0.05).
(1) side effect ; (2) digitations f : French isolate af : African isolate ca : Californian isolate

TABLE 9 - Antagonistic effect of phosphates on the direct mode of action of phosphorous acid used at 1.68 mM (138μg/ml) in the CMA supplemented with $Na_2H\ PO_4$-NaH_2PO_4 (buffered solution pH 6.5).

Isolates	Sodium Phosphate		
	1 mM	10 mM	100 mM
P. cactorum	+	+	0
P. capsici	+++	+++	+++
P. cinnamomi	+	+	++
P. citrophthora	+++	+++	+++
P. cryptogea	+	+	+
P. heveae	+	+	+
P. infestans	+	+	0
P. megasperma	+	++	+++
P. palmivora	0	0	0
P. parasitica	0	0	0
P. vignae	+	++	-

+++ Efficacy of phosphorous acid almost nullified
++ Efficacy partially nullified
+ Efficacy slowly nullified
0 No or weak effect
- Not tested

useful classification can be suggested in : 1) high sensitive species (e.g. *P. palmivora*) ; 2) medium sensitive species (e.g. *P. cactorum*) ; 3) low sensitive species (e.g. *P. capsici*).

Some of our results could allow us to explain the efficacy of fosetyl Al by a direct mode of action *in vivo*.

1. With post-harvest treatments against fruit rot due to *Phytophthora* spp., only the surface of the fruit is involved. The direct effect is probably the only one responsible for the efficacy of the fosetyl Al on apples (BOMPEIX et al., 1979) on oranges (GAULLIARD and PELOSSIER, 1983) and when the trunk paint technique is used (LAVILLE and CHALANDON, 1982).

2. Convergences exist between results of *in vitro* tests and the efficacy in the open field. High sensitive species are generally well controlled by fosetyl Al treatments (e.g. Citrus gummosis). On the other hand *P. megasperma* and

P. capsici are poorly or not controlled by the same treatment.

In addition, the effect of the phosphates is not always obvious on high sensitive species, which leads us to think that the Pi content of plant tissues could not really be an obstacle to the direct mode of action of fosetyl Al, at least for some species.

From this point of view, *P. capsici* could add a low sensitivity to phosphite and an easy reversion of its effect by phosphate. We checked this phenomenon *in vivo* (BOMPEIX et al., 1980).

The same is probably true for *P. megasperma*.

3. LAVILLE et al.'s results (1979, 1982) always show a delayed effect of fosetyl Al compared to metalaxyl for instance. Fosetyl Al effect seems to be a very progressive poisoning of the *Phytophthora* species. Consequently the delayed efficacy in Citrus orchards can be explained.

Other observations seem in contradiction with an important direct mode of action, and promote an indirect mode of action or at least a more complex one.

1. The H_3PO_3 content of protected organs against *Phytophthora* are not very well known. It is generally believed to be in the range 5 to 20 μg/g f.w. Which is particularly insufficient for almost all the species tested if we consider the effects observed in open field.

2. Contradictions exist between the variable efficacy of the fosetyl Al on one species of *Phytophthora* (e.g. *P. palmivora*) with different host-plants.

This can be interpreted as either a lack of knowledge of the H_3PO_3 content *in vivo* for an unprotected plant, or an absence of stimulation of the plant defence mechanisms.

Some species such as *P. cactorum* and *P. cryptogea* are very well controlled on various host-plants, although *in vitro*, their sensitivity is in the medium range. (BOMPEIX et al., 1984 ; BOLAY et al., 1984 ; MONTGOMERIE and KENNEDY, 1979).

3. The antagonistic effect of phosphate seems important on species which are well controlled in open field.

This is the case of *P. citrophthora*. It is difficult to explain how a content of H_3PO_3 as low as 5 to 20 μg/g f.w. can be active in the presence of phosphate in *Citrus*, which is sufficient enough to nullify its effect. However, this parasite, as *P. parasitica*,not very sensitive to the reversal effect of phosphate,is well controlled by fosetyl Al treatments.

4. Inhibitors of the biosynthesis of plant defence metabolites (AOA, AOPP, glyphosate) nullify the efficacy of fosetyl Al but not that of metalaxyl. This fact reinforces the idea that plant defence mechanism plays a major role (BOMPEIX et al., 1981).

5. The CMA medium is very poor in nutriments and its biochemical content is completely different from the host-plant, not only in phosphate content, but for other nutriments as well.

In these conditions what value can we attribute to the *in vitro* assay ? GUEST (1984) has shown effectively that the nature of the medium culture greatly influences the results of inhibition. Now we can suggest that the mode of action proposed by GUEST and BOMPEIX (1984) could be valid. The primary site of action of fosetyl Al (H_3PO_3) would be situated at the level of phosphite-phosphate interaction-or competition-probably inside the cytoplasm of the fungus, so that a self poisoning process takes place. This phenomenon would be inadequate to destroy the parasite *in vivo* but would involve the plant defence mechanisms in a secondary phase.

According to the species and host-plant, the direct mode of action, and or, the indirect mode of action would play the major role.

As far as the occurence of resistant strains is concerned, the interpretation of this complex mode of action does not modify our conception, and it appears highly unprobable to find any resistant strain. COHEN and SAMOUCHA 1984) think they have obtained cross-resistance between fosetyl Al and metalaxyl but CLERGEAU et al. (1984), BOMPEIX et al. (1984), do not confirm this possibility.

Several new results have to be taken in consideration :
1) the progressive inhibition obtained with these compounds
2) the extreme diversity of sensitivity of the *Phytophthora* species to fosetyl Al and H_3PO_3.
3) the extreme diversity to the reversal effect of phosphate.

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