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Inhibition by aluminum of mycelial growth and of sporangial production and germination in *Phytophthora infestans*

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Abstract

Mycelial growth on clarified V8 agar of the potato late blight pathogen *Phytophthora infestans* was inhibited when either aluminum chloride ($AlCl_3$, 6 H_2O) or aluminum sulfate ($Al_2(SO_4)_3$, 18 H_2O) was added to the culture medium at concentrations of 2.5–100 mg.l^{-1} Al^{3+}. Toxicity of Al^{3+} varied among the five *P. infestans* isolates tested, but toxicity of sulfate and chloride salts was similar for a given isolate. Overall sporangial production was affected in all five isolates by both Al^{3+} forms. Al^{3+} also decreased sporangial germination at concentrations equal to or greater than 10 mg.l^{-1} in two isolates. These data support the hypothesis of aluminum toxicity as a major factor in soil suppressiveness to *P. infestans*.

Introduction

The now widespread, simultaneous occurrence of both mating types of the potato late blight pathogen *Phytophthora infestans* (Mont.) de Bary has recently renewed the interest of plant pathologists for the soil stages of this destructive fungus. *P. infestans* is now known to be able to form oospores under field conditions in Europe [Drenth *et al.*, 1995]; these oospores remain viable for at least eight months in the soil, and can infect potato shoots [Pittis & Shattock, 1994; Drenth *et al.*, 1995]. Recent work showed that differences existed in the evolution of asexual inoculum of *P. infestans* between soils, and that a soil with low pH (3.8) and high exchangeable aluminum (Al^{3+}) content was suppressive to the pathogen [Andrivon, 1994a]. The suppressiveness of this soil was related to an increased fungistasis and lysis of *P. infestans* sporangia, with respect to conducive soils [Andrivon, 1994a]. Calcareous amendments, raising the pH up to 7.5, decreased but did not completely eliminate suppressiveness, suggesting that pH *per se* was not the primary cause of pathogen inhibition [Andrivon, 1994b].

Many acid soils are suppressive to fungal or bacterial plant pathogens, as was shown for *Fusarium coeruleum* [Tivoli *et al.*, 1990], *Phytophthora* spp. [Ko and Nishijima, 1985; Benson, 1993; Ann, 1994], *Thielaviopsis basicola* [Meyer and Shew, 1991], or *Streptomyces* spp. [Mizuno and Yoshida, 1993]. Aluminum toxicity has been associated with suppressiveness of several acid soils to *Verticillium albo-atrum* [Orellana *et al.*, 1975], *F. coeruleum* [Ridao *et al.*, 1990], *T. basicola* [Meyer and Shew, 1991], *Phytophthora parasitica* var. *nicotianae* [Benson, 1993], *Neurospora tetrasperma* [Ko and Hora, 1972], and *Streptomyces* spp. [Mizuno and Yoshida, 1993]. Concentration of exchangeable Al^{3+} in soil solution is typically higher at low soil pH, and Al^{3+} is inhibitory to spore germination, spore production, and mycelial growth in a number of fungi, including various *Phytophthora* species [Muchovej *et al.*, 1980; Schmitthenner and Canaday, 1983; Deluca and Shew, 1988; Weaver and Shew, 1992; Benson, 1993]. Al^{3+} was therefore suspected to be a factor in soil suppressiveness to *P. infestans* [Andrivon, 1994b]. However, no data on the effect of Al^{3+} on *P. infestans* are available in the literature. The aim of the experiments reported

in this paper was therefore to investigate the effect of Al^{3+} on mycelial growth, spore production and spore germination in the potato late blight fungus.

Material and methods

Fungal isolates. Five *P. infestans* isolates from the GRISP (Groupement Régional d'Intérêt Scientifique Phytosanitaire, Le Rheu, France) collection were used throughout the study. Three of the four A_1 isolates (6.88, 20.91, 108.91) originated from population surveys carried out in Brittany during 1988–1991, while the fourth (2.92), provided by Mrs M. Conus (INRA Avignon), was initially isolated from tomato in southern France in 1982. The A_2 isolate (A_2S), originally collected in Scotland, was provided by Dr B. Schöber-Butin in 1988. All isolates were maintained on clarified V8 agar and subcultured every 2–4 weeks.

Mycelial growth and spore production. Stock solutions containing 1000 $mg.l^{-1}$ Al^{3+} in sterile permutated water were prepared separately from either aluminum chloride ($AlCl_3$, 6 H_2O; Merck, Germany) or aluminum sulfate ($Al_2(SO_4)_3$, 18 H_2O; Bruchet Dano, France). Autoclaved clarified V8 agar was amended with either stock solution to reach final concentrations of 2.5, 5, 10, 25, 50, or 100 $mg.l^{-1}$ al^{3+}, and poured into plastic Petri dishes (90 mm diameter). Unamended medium was used as control.

Three dishes per isolate and aluminum form and concentration were seeded each with one mycelial plug (5 mm) cut with a cork borer from the margin of actively growing cultures of *P. infestans* on clarified V8 agar. Fungal growth was monitored on each plate after 4, 6, 8, 11, 13, 15, and 18 days incubation at 20 °C in the dark. After 18 days, each culture was flooded with 5 ml sterile water, sporangia were removed by gentle shaking and counted with an haemocytometer. Three counts per culture were made. Data are shown for one experiment with the complete set of Al^{3+} concentrations. Previous experiments with fewer doses gave similar results.

Germination of sporangia. Sporangial suspensions of isolates 6.88 and 108.91 were prepared by gentle shaking of 15-day-old cultures on clarified V8 agar after flooding with 5 ml sterile water, and their concentration was adjusted to 5.10^5 $sporangia.ml^{-1}$. Aliquotes of these suspensions were diluted in aqueous solutions of either aluminum chloride or aluminum sulfate, to reach final Al^{3+} concentrations of 5, 10, 25, or 100 $mg.l^{-1}$. Dilutions in sterile water were used as controls. After 24, 48, and 72 h incubation at room temperature (*c.* 20 °C), 50–150 sporangia per isolate and aluminum form and concentration were examined microscopically and assigned to one of the following categories: ungerminated, germinated directly through emission of a germ tube or of secondary sporangia, or germinated indirectly through release of zoospores. The frequency of sporangia in each class was then calculated. The experiment was performed twice.

Results

Mycelial growth. Aluminum sulfate or aluminum chloride added to the culture medium significantly reduced growth of the five *P. infestans* isolates tested, as indicated by the negative correlations observed between colony diameter of 18-day-old cultures and Al^{3+} concentration in the medium (Table 1). No significant differences were found between regression coefficients calculated for chloride and sulfate salts with isolates 20.91, 6.88, 108.91, and A_2S (pairwise t test, $P > 0.05$), indicating that both Al^{3+} forms affected growth similarly for a given isolate. The coefficient calculated with aluminum chloride and isolate 2.92 was excluded from comparisons, because of the poor fit of the regression ($P = 0.105$) to experimental data points. Comparisons of regression coefficients obtained with aluminum sulfate between isolates showed that Al^{3+} toxicity was greatest towards isolates 108.91 and 6.88, lower towards isolate A_2S, and least towards isolates 2.92 and 20.91 (Table 1).

Sporangial production. As for mycelial growth, Al^{3+} inhibited spore production in the five *P. infestans* isolates tested: negative correlations between the total number of spores produced by 18-day-old cultures of the fungus and Al^{3+} concentrations were significant ($P < 0.05$), except with aluminum chloride in isolate 2.92 and with aluminum sulfate in isolates A_2S and 20.91 (Table 2). However, variation in the number of sporangia produced per culture was much greater than variation in colony diameter for a given Al^{3+} concentration, resulting in lower correlation coefficients. The comparison of regression coefficients showed no difference between Al^{3+} forms in the two isolates 6.88 and 108.91; aluminum toxicity was greatest towards isolates 20.91, 6.88 and 108.91, and lowest for isolates A_2S and 2.92. Positive correlations between spore pro-

Table 1. Regression and correlation coefficients in linear regressions of colony diameter of 18-day-old cultures of five *Phytophthora infestans* isolates and Al^{3+} concentration in the culture medium

Isolate	Al^{3+} form [1]	Regression coefficient [2]						Correlation coefficient	P> F
2.92	C	− 1.450	[3]					0.36	0.105
	S	− 0.143	a					0.50	0.020
20.91	C	− 0.332		b				0.82	< 0.001
	S	− 0.194	a	b				0.48	0.025
A_2S	C	− 0.466			c	d		0.94	< 0.001
	S	− 0.468			c	d		0.94	< 0.001
6.88	C	− 0.681				d	e	0.90	< 0.001
	S	− 0.638			c	d	e	0.85	< 0.001
108.91	C	− 0.532			c	d	e	0.92	< 0.001
	S	− 0.638				d	e	0.95	< 0.001

[1] C: aluminum chloride; S: aluminum sulfate. Six concentrations of Al^{3+} (2.5, 5, 10, 25, 50 and 100 $mg.l^{-1}$) and three replicate plates per isolate/aluminum form and concentration were used.
[2] Coefficients followed by the same letter are not significantly different (pairwise t test, $P > 0.05$)
[3] Coefficient excluded from comparisons because of the poor fit of regression to experimental data

Table 2. Regression and correlation coefficients in linear regressions of total number of sporangia produced by 18-day-old cultures of five *Phytophthora infestans* isolates and Al^{3+} concentration in the culture medium

Isolate	Al^{3+} form [1]	Regression coefficient [2]						Correlation coefficient	P > F
2.92	C	− 0.070	[3]					0.35	0.113
	S	− 0.094	a					0.48	0.027
20.91	C	− 1.138					d	0.72	0.001
	S	− 0.546	[3]					0.30	0.192
A_2S	C	− 0.145	a	b				0.48	0.027
	S	− 0.122	[3]					0.38	0.084
6.88	C	− 0.403			c			0.71	0.001
	S	− 0.626			c	d		0.82	< 0.001
108.91	C	− 0.485	a	b	c	d		0.49	0.022
	S	− 0.502		b	c	d		0.52	0.016

[1] C: aluminum chloride; S: aluminum sulfate. Six concentrations of Al^{3+} (2.5, 5, 10, 25, 50 and 100 $mg.l^{-1}$) and three replicate plates per isolate/aluminum form and concentration were used.
[2] Coefficients followed by the same letter are not significantly different (pairwise t test, $P > 0.05$)
[3] Coefficient excluded from comparisons because of the poor fit of regression to experimental data

duction and colony diameter were found in all isolates except 108.91, for which the correlation was not significant at $P = 0.05$ (data not shown).

Sporangial germination. Overall germination was markedly higher in isolate 6.88 than in isolate 108.91 (Fig. 1). Aluminum sulfate and aluminum chloride at concentrations equal to or greater than 10 $mg.l^{-1}$ Al^{3+} decreased germination of both isolates, but no clear differences between aluminum forms could be observed. However, at 5 $mg.l^{-1}$, the chloride salt was more inhibitory than the sulfate salt (Fig. 1). Overall germination (Fig. 1) and direct germination (Fig. 2) patterns were very similar, due to the very low poportion of indirect germination. For both isolates, no significant differences between Al^{3+} forms were found in the proportion of germ tubes and secondary sporangia among germinated sporangia, although the emission of secondary sporangia tended to be higher in the sulfate solution than in the chloride solution at 5 $mg.l^{-1}$ Al^{3+} (data not shown).

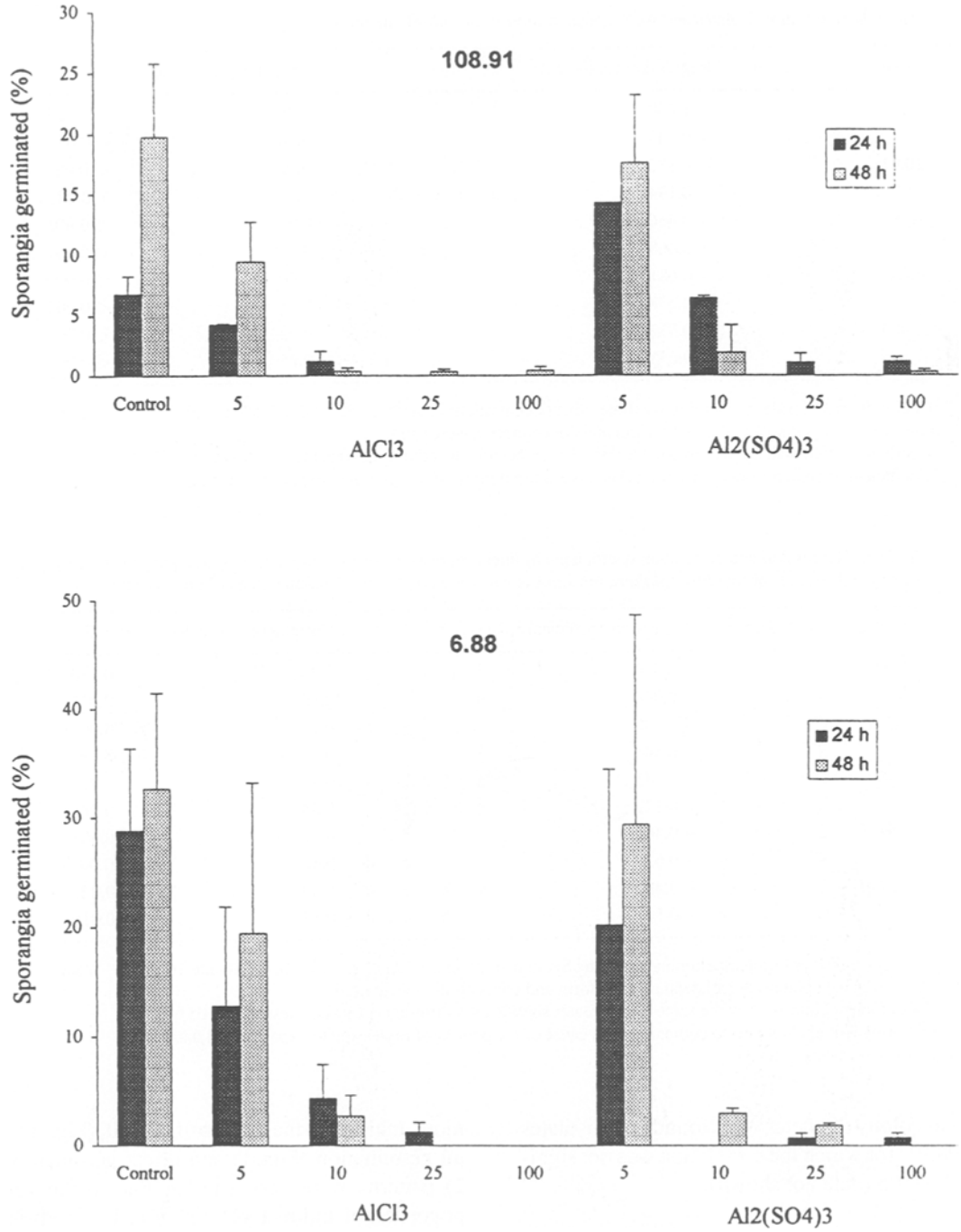

Fig. 1. Proportion of sporangia of isolates 108.91 and 6.88 of *Phytophthora infestans* germinated (directly or indirectly) after 24 and 48 h incubation in aqueous solutions of aluminum chloride ($AlCl_3$) or aluminum sulfate [$Al_2(SO_4)_3$] containing 5, 10, 25 or 100 mg.l^{-1} Al^{3+}.

Discussion

The present study was undertaken to test the aluminum toxicity to the potato late blight pathogen, as no report on this topic existed in the literature. Both aluminum chloride and aluminum sulfate were found to inhibit mycelial growth, sporangial production, and sporangial germination in *P. infestans*, at concentrations

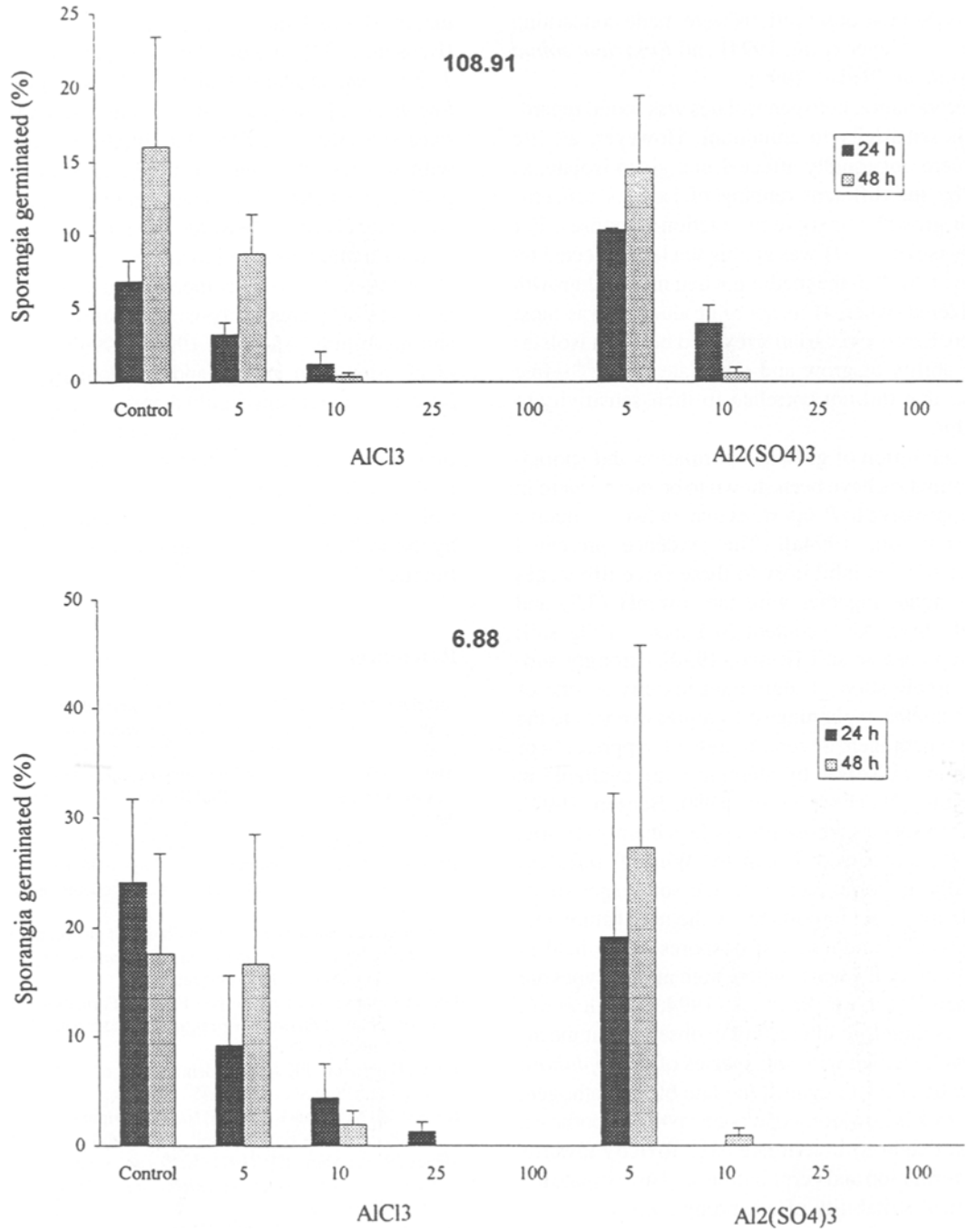

Fig. 2. Proportion of sporangia of isolates 108.91 and 6.88 of *Phytophthora infestans* germinated directly after 24 and 48 h incubation in aqueous solutions of aluminum chloride ($AlCl_3$) or aluminum sulfate [$Al_2(SO_4)_3$] containing 5, 10, 25 or 100 $mg.l^{-1}$ Al^{3+}.

ranging from 2.5–100 $mg.l^{-1}$ Al^{3+}, extending reports concerning other *Phytophthora* species [Muchovej *et al.*, 1980; Schmitthenner and Canaday, 1983; Deluca and Shew, 1988; Weaver and Shew, 1992; Benson, 1993]. The comparable activity of both aluminum salts on the different life stages of the pathogen tends to indi-

cate that the anion does not affect Al^{3+} toxicity in *P. infestans*. Similar observations were made concerning *T. basicola* [Meyer *et al.*, 1994] and *Fusarium solani* var. *coeruleum* [Ridao, 1990].

Some variation between isolates was found regarding their sensitivity to aluminum. However, all life stages were not equally affected in a given isolate, as shown by the different ranking of isolates concerning their growth and spore production responses. For instance, isolate 20.91 was among the least affected by addition of Al^{3+} in the medium when mycelial growth is considered, whereas its spore production was most inhibited. Extensive variation existed between isolates in their ability to grow and sporulate on Al^{3+}–free medium, that did not correlate to their sensitivity to aluminum.

The inhibition of growth, sporulation and sporangial germination have been shown to be more severe in a soil suppressive to *P. infestans* than in two conducive soils [Andrivon, 1994a]. The evidence presented here that Al^{3+} is inhibitory to these three life stages of the fungus, together with the low pH (3.8) and extremely high Al^{3+} content (6.5 meq / 100g soil) of the suppressive soil [Ridao, 1990], strongly support the implication of aluminum toxicity as one of the mechanisms explaining soil suppressiveness to the late blight pathogen. Several reports of suppression of *Phytophthora* diseases by aluminum are available in the literature [Muchovej *et al.*, 1980; Benson, 1993], but concern species whose life cycle is mainly telluric, such as *P. parasitica* or *P. capsici*. While *P. infestans* is basically an aerial pathogen, the soil stages of its life cycle may gain importance in the near future, due to the possible production of oospores in the field in many regions of the world where both mating types are established [Pittis and Shattock, 1994; Drenth *et al.*, 1995]. It is therefore interesting to observe that mechanisms well studied with soil species of *Phytophthora* may also be efficient against the late blight pathogen. Further work is now needed to confirm this hypothesis, and in particular to investigate Al^{3+} toxicity towards oospore formation and germination, and investigate the agronomical suitability of amending soils with Al^{3+} as a means of disease control. The extremely high exchangeable aluminum content of the suppressive soil studied is phytotoxic to many plant species, and is not to be attained in agricultural soils. However, significant inhibition of the several asexual life stages of *P. infestans* was observed *in vitro* at Al^{3+} concentrations of 10–25 $mg.ml^{-1}$ (ie, 1.1–2.8 meq /100 g). Reports of suppression of other *Phytophthora* diseases with concentrations six to twelve times lower in culture media (0.5 to 1 meq exchangeable Al^{3+} / 100 g soil [Benson, 1993]) and of other plant pathogens, such as *T. basicola*, at about 1 meq / 100g soil [Meyer and Shew, 1991], suggest that aluminum amendments at rates sufficient to control late blight and compatible with soil fertility might be possible. They would be particularly helpful if efficient against oospores, as no convenient control measures are currently available to deal with this source of inoculum.

Another important component of soil suppressiveness was an increased lysis of *P. infestans* sporangia and mycelium [Andrivon 1994a, 1994b]. The impact of aluminum on growth and antagonistic activity of lytic microorganisms would therefore be worth assessing, both *in vitro* and in the soil. These investigations are needed for a better understanding of the soil ecology of *P. infestans*, prerequisite to the setup of control strategies aiming at the major threat represented by the soilborne sources of inoculum of the late blight fungus.

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