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Seed treatment of peas with fosetyl-Al against Aphanomyces euteiches

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Abstract

In 1983, seed dressing of peas with fosetyl-Al (Aliette) compared favourably with all other treatments on a field heavily contaminated with foot and root rot pathogens. Experiments carried out in 1983 and 1984 could not establish the reason for this phenomenon. Artificial inoculation with the most frequently isolated pathogens (*Fusarium oxysporum*, *Phoma medicaginis* var. *pinodella*, *Pythium ultimum* and *Fusarium solani* f.sp. *pisi*) of sterilized soil before planting treated seeds did not reproduce the field observations, but glasshouse experiments using soil from the experimental field did. Glasshouse experiments in 1989, after *Aphanomyces euteiches* had been identified as a frequently occurring pea pathogen in the Netherlands, confirmed the favourable effect of fosetyl-Al as a seed treatment when *A. euteiches* was in the pathogen flora.

Additional keywords: Pisum sativum, Aliette, foot rot, root rot.

1 Introduction

Foot and root rot – in the following shortened to root rot - is one of the most important constraints in pea production. The disease complex is widespread in the traditional pea-growing areas of the Netherlands. When EC policy made pea-growing financially more attractive by 1980, the acreage for dry pea production increased. Consequently, cases of heavy root rot occurred.

The problem of root rot is worldwide. By seed dressing with mixtures of fungicides, the effects of both seed and soil contamination can be attenuated (Trawally et al., 1984; Salter and Smith, 1986; Tu, 1988). These treatments offer protection especially during seedling establishment. The use of modern fungicides with activity against *Oomycetes* has increased the effect of seed dressing through an extended spectrum of activity.

Complete pea crop failure in 1981 on a field at Dinteloord, in the South-West of the Netherlands, on heavy clay, led to field trials on this field in order to assess the effect of seed dressings on root rot. The superiority of fosetyl-Al in suppressing plant death, remained unexplained at that time.

Since Oyarzun and Van Loon (1989) established the importance of *Aphanomyces euteiches* as a cause of root rot and seedling death in the Netherlands, the results of the older field trial could be tentatively explained as a fungicide effect of fosetyl-Al on *A. euteiches*. To examine this hypothesis, additional experiments with seed dressing and artificial contamination of sterilized soil with *A. euteiches* versus natural soil were undertaken.

2 Materials and methods

2.1 Field experiment in 1983

A field experiment was carried out in a field at Dinteloord (South-West Netherlands) on heavy clay soil, where complete pea crop failure occurred in 1981. Part of the field was cropped to wheat in 1982, part to pea again. Peas were grown on this field in 1983. The rotations are indicated as p-w-p and p-p-p respectively. Treated seeds of the cultivar Finale were sown in five replicates on the p-w-p plots and in three on the p-p-p plots. Seed treatment consisted of:

A. Apron 70 SD (35% metalaxyl + 35% captan), 2 g kg^{-1} seed;
B. Captan (83% a.i.), 2 g kg^{-1} seed;
C. Carbendazim (50% a.i.), 2 g kg^{-1} seed;
D. Carbendazim (50% a.i.), 4 g kg^{-1} seed;
E. Apron 35 SD (35% metalaxyl) + carbendazim (50% a.i.), (2+2) g kg^{-1} seed;
F. Aliette (80% fosetyl-Al) + captan (83% a.i.), (2+2) g kg^{-1} seed;
G. Aliette (80% fosetyl-Al) + carbendazim (50% a.i.), (2+2) g kg^{-1} seed.

Because of prolonged rain, plots (2.15 × 12 m) were sown late, on May 26 in ten rows at 20 cm distance. The germination count on June 15 showed 52 to 59 plants per m^2. From June 29, root rot severity and numbers of dead plants were regularly assessed. Root rot was scored according to Basu et al. (1976), where a disease index (DI) 0 represents healthy, white roots, and a completely rotten root results in DI 4. DI 5 was added to signify premature death of the plant. At each sampling five plants were scored and their average DI noted. Percentage dead plants was scored by counting at random over 6 m rowlength. On July 11 samples were taken for isolation of root pathogens. The most heavily attacked plots were harvested on August 10, when they were already perfectly dry, the others on August 23. Yield data, standardized at 14% humidity, were only taken from the central parts of the plots which had not been used for sampling in the course of the experiment.

2.2 Glasshouse experiments

2.2.1 Experiments carried out in 1983 and 1984. Soil from the experimental field was taken to the laboratory, placed in trays and then treated seeds of pea, cv. Finale, were sown. Part of the soil was steam-sterilized and inoculated with the pathogens which were most frequently found on roots of diseased plants from the 1983 field experiment (see 2.1), viz: *Fusarium oxysporum*, *F. solani*, *Phoma medicaginis* var. *pinodella* and *Pythium ultimum*. They were grown for two to three weeks in a cornmeal/soil medium, which was added at 2% (w/w) alone or in mixtures.

Seed treatment consisted of different combinations of fungicides:

1. Apron 35 SD (35% metalaxyl), 2 g kg^{-1};
2. Apron 70 SD (35% metalaxyl + 35% captan), 2 g kg^{-1};
3. Aliette (80% fosetyl-Al), 2 g kg^{-1};
4. Aliette (80% fosetyl-Al) + captan (83% a.i.), (2+2) g kg^{-1};
5. Carbendazim (50% a.i.), 2 g kg^{-1};
6. Apron 35 SD (35% metalaxyl) + carbendazim (50% a.i.), (2+2) g kg^{-1};
7. Aliette (80% fosetyl-Al) + carbendazim (50% a.i.), (2+2) g kg^{-1};

8. Captan (83% a.i.), 2 g kg^{-1};
9. Apron 70 SD (35% metalaxyl + 35% captan) + carbendazim (50% a.i.), (2+2) g kg^{-1};
10. Aliette (80% fosetyl-Al) + captan (83% a.i.) + carbendazim (50% a.i.), (2+2+2) g kg^{-1};
11. TMTD (50% thiram), 2 g kg^{-1};
12. Control.

For each treatment, six to eight rows containing eight seeds each were sown, randomly distributed over the trays. Root rot was assessed (see 2.1) on various dates, from 26 to 44 days after sowing, four or six rows per date. The experiments were repeated three times. In two of the experiments the fresh weight of the above-ground parts of the plants was measured.

2.2.2 Experiment done in 1989. A clay loam soil with proven infestation by *A. euteiches* was taken from a continuous pea cropping experimental field in Lelystad (Research Station for Arable Farming and Field Production of Vegetables, PAGV). Part of the soil was steam sterilized. To half of this soil a sand and a zoospore culture of *A. euteiches* was added. 1.6 l of a maize-meal/sand culture of the pathogen was mixed with 60 l of soil before sowing. Fifteen days after planting, a 20 ml zoospore suspension was added to the pots of this treatment. The zoospore suspension was obtained by growing *A. euteiches* for five days at 26 °C in a liquid maltose/peptone medium, then replacing the medium by distilled water, while continuing the aeration. After 20 h the zoospore suspension had 250 000 spores per ml. It was diluted to 15 000 spores per ml. The soils are coded G1 (steamed soil); G2 (steamed soil + *A. euteiches*); G3 (naturally infested soil). The soil was air dried to such a degree that crumbling to small aggregates (< 0.8 mm) was possible. Pots (11 cm diameter) were filled with a bottom layer of 100 g sand, followed by the test soil up to 9 cm height. Fourteen seeds of cultivars Marzia and Finale were sown per pot. Subsequently, the soil was covered with 15 g (about 1 cm) of vermiculite, and brought to about 25% water content (pF = 2). After germination only 10 plants per pot were maintained. Seed treatment consisted of a sticker plus:

I. control;
II. Apron (35% metalaxyl), 2 g kg^{-1};
III. Aliette (80% fosetyl-Al), 4 g kg^{-1};
IV. Wakil (40% oxadixyl + 16% cymoxanil), 1.25 g kg^{-1};
V. Apron (35% metalaxyl) + captan (83% a.i.) + bavistin (50% carbendazim), (2+2+2) g kg^{-1};
VI. Aliette (80% fosetyl-Al) + captan (83% a.i.) + bavistin (50% carbendazim), (4+2+2) g kg^{-1};
VII. Wakil (40% oxadixyl + 16% cymoxanil) + captan (83% a.i.) + bavistin (50% carbendazim), (1.25+2+2) g kg^{-1};
VIII. Captan (83% a.i.) + bavistin (50% carbendazim), (2+2) g kg^{-1}.

Experimental design was of a split-plot type, with cultivars allocated to main plots and five replicates of the eight treatments to 40 subplots. Temperature in the glasshouse was 22/17 °C (day/night); light was supplemented for 12 h using mercury lamps. Disease index (DI) on a scale 0 - 4, fresh weight of plants and dry matter were determined at three weeks after sowing, six days after adding the zoospore suspension in G2.

A fresh soil sample from the field at Dinteloord was tested for the presence of *A. euteiches*.

3 Results

3.1 Field experiment in 1983

The first dead plants were observed on June 22, and as early as five weeks after planting (June 29) many plants had died in most of the treatments. Root rot DI was generally high. This trend was confirmed on July 13 and July 20. Due to the increasing crop mass, plant counts were impossible at later dates. Only the treatments with fosetyl-Al (F and G) maintained a full crop stand until the end of the vegetation period (see Figure 1). They also had a remarkably lower DI at the first sampling, but slightly converged with the other treatments later (Table 1).

No difference in root rot development could be found between the rotations p-w-p and p-p-p.

Yield was an unreliable parameter due to heavy bird damage (data not shown).

3.2 Glasshouse experiments

3.2.1 Experiments in 1983 and 1984. Table 2 shows DIs of roots and fresh weight of plants at different sampling dates. In both experiments, lowest DI and highest fresh

Fig. 1. Field experiment done in 1983. Large difference in plant stand when seed treatments contained fosetyl-Al (background) or not (front).

Table 1. The effect of seed treatment with various fungicides on root rot of peas grown under field conditions (1983).

Treatment	Sampling date					
	June 29		July 13		July 20	
	DI	% dead plants	DI	% dead plants	DI	% dead plants
p-p-p						
A	2.6	38	3.9	51	4.0	55
B	4.0	49	4.0	58	3.9	73
C	3.6	31	3.9	43	4.2	53
D	3.4	12	4.0	31	4.1	28
E	2.8	17	3.7	32	3.2	33
F	1.8	7	2.8	9	3.4	11
G	1.7	2	3.0	1	3.3	2
p-w-p						
A	3.4	30	3.8	52	4.1	54
B	3.2	34	3.9	63	4.2	57
C	2.8	17	3.8	30	3.8	35
D	3.0	12	3.3	28	3.9	29
E	2.8	11	3.6	26	4.0	29
F	2.1	1	3.1	3	3.6	6
G	2.1	1	3.2	1	3.0	1
SED	0.38	3.8	0.24	4.0	0.21	5.4
signific.	***	***	**	***	***	***

For treatment codes: see text (2.1).
DI = disease index of the roots (scale 0-5; 0 = healthy)
% dead plants = percentage of dead plants of total dead + living.
p-p-p and p-w-p represent rotations (p = peas; w = wheat)
SED for comparing treatments within each of the rotations p-p-p or p-w-p. Significance: * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$.

weight were always found for treatments containing fosetyl-Al, either alone or in mixture. Figure 2 shows the strong negative correlation that exists between fresh weight and disease index. The effect of root rot on fresh weight was much stronger at 44 than at 28 or 26 days after sowing, but this was not true for the correlation coefficients, which were -0.8170 ($P < 0.01$), -0.8816 ($P < 0.001$) and -0.9633 ($P < 0.001$) respectively. The experiments using soil inoculation with pea root pathogens individually or in mixture gave no consistent seed treatment effect. In any case fosetyl-Al did not outperform the other fungicides (results not shown).

3.2.2 Experiment done in 1989. A highly significant effect of soils and seed treatment was found (Table 3). DI was higher on naturally infested soil than on sterilized soil inoculated with *A. euteiches*. On sterilized soil the DI was negligible. Treatments

Table 2. Effect of seed treatment with different fungicides on root rot (DI) and fresh weight of pea seedlings grown in the glasshouse in field soil heavily infested with root rot pathogens.

Treatment	Experiment 1				Experiment 2	
	fresh weight		disease index (DI)		fresh w.	DI
	28 days	44 days	28 days	44 days	26 days	26 days
1	6.16	6.64	4.03	4.43	3.67	3.85
2	7.52	10.16	3.75	4.08	4.22	3.25
3	9.36	13.12	2.97	3.80	5.17	3.28
4	9.68	12.88	2.70	3.53	5.90	3.03
5	6.32	10.88	4.40	4.29	3.12	4.03
6	5.84	10.00	4.53	4.38	4.47	3.40
7	8.72	11.04	3.15	4.08	6.32	2.72
8	7.92	6.24	3.10	4.44	3.72	4.03
9	6.24	9.60	4.30	4.34	3.67	3.70
10	8.56	11.36	2.92	3.93	7.08	2.32
11	7.04	8.96	3.42	4.18	3.27	4.10
12	8.56	9.60	3.85	4.38	4.30	3.65
SED	0.88	1.76	0.50	0.21	0.79	0.44
Signific.	***	*	**	**	***	**

(* = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$)
For treatment codes: see text (2.2.1); fresh weight in g per plant; DI 0-5 (0 = healthy; 5 = dead).

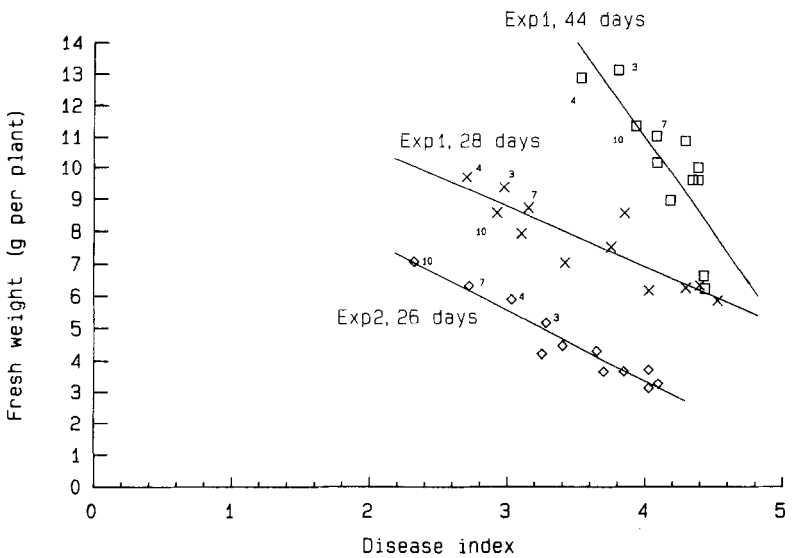

Fig. 2. Negative correlation between DI of the roots and fresh weight of pea plants assessed at 28 and 44 days after sowing in experiment 1 and at 26 days in experiment 2. The numbers refer to the seed treatments (see 2.2.1). Note lowest DI and highest weight for treatments containing fosetyl-Al (nos. 3, 4, 7, 10).

Table 3. Effect of seed treatments on root rot (DI) and fresh weight of pea plants sown in soils with different pathogen microflora.

Treatment	Soil type					
	G1 (sterilized)		G2 (sterilized + *A. euteiches*)		G3 (continuous peas)	
	DI	weight	DI	weight	DI	weight
I	0.69	10.95	3.21	4.65	3.41	3.40
II	0.91	9.04	3.23	4.54	3.44	3.54
III	0.57	10.57	1.37	6.95	3.01	4.46
IV	0.71	10.08	3.25	4.94	3.38	4.02
V	0.61	11.13	3.15	4.80	3.41	3.60
VI	0.56	12.77	1.59	6.90	2.80	4.81
VII	0.73	10.52	3.24	4.53	3.39	3.64
VIII	0.64	11.30	3.13	4.85	3.37	3.81
SED	0.13	0.43	0.13	0.43	0.13	0.43
signif.	***	***	***	***	***	***

For treatment codes: see text (2.2.2).
DI 0 – 4 (0 = healthy; 4 = dead)
weight: fresh weight in g per plant.
All data are averages of two cultivars, assessed three weeks after sowing.

Table 4. Effect of seed treatments on root rot (DI) and fresh weight of pea plants of two different varieties.

Treatment	Finale		Marzia	
	DI	weight	DI	weight
I	2.46	7.72	2.41	4.95
II	2.57	6.48	2.49	4.93
III	1.43	9.37	1.86	5.29
IV	2.41	7.87	2.48	4.83
V	2.37	7.52	2.41	5.50
VI	1.39	10.27	1.91	6.05
VII	2.39	7.39	2.51	5.07
VIII	2.35	8.26	2.41	5.05
SED	0.11	0.35	0.11	0.35
signif.	***	***	***	***

For treatment codes: see text (2.2.2).
DI 0 – 4 (0 = healthy; 4 = dead).
weight: fresh weight in g per plant.
All data are averages of three soil treatments, assessed three weeks after sowing.

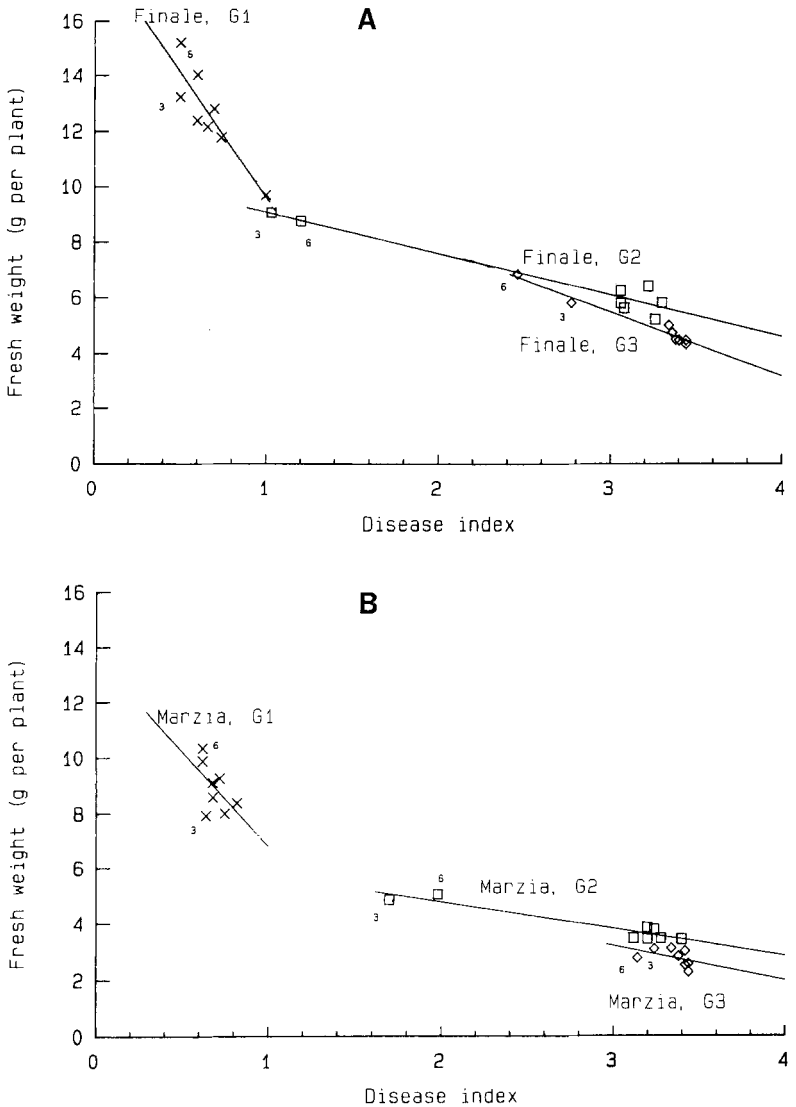

Fig. 3. The effect of root rot (DI) on the fresh weight of plants for three different soil treatments. A: 'Finale'; B: 'Marzia'. Points represent the eight seed treatments (see 2.2.2). Treatments III (3) and VI (6), containing fosetyl-Al are indicated. G1: steam-sterilized soil; G2: steam -sterilized soil inoculated with *A. euteiches*; G3: field soil heavily contaminated with pea root pathogens.

III and VI, which were the only ones containing fosetyl-Al, showed much lower DIs and higher weights than the other treatments, especially with inoculated soil G2 (results were virtually equal for fresh and dry weight; only the former are given here). The DI for 'Marzia' and 'Finale' did not generally differ much. However, the favourable effect on weight by seed treatments III and VI was much stronger for 'Finale' than for 'Marzia' (Table 4).

A strong correlation existed between DI and fresh (or dry) weight for 'Finale' ($r = -0.9282$; $P < 0.001$), and a less pronounced one for 'Marzia' ($r = -0.7063$; $P = 0.05$). Considering the correlations per cultivar and per soil treatment, all of them are strong and highly significant for 'Finale', whereas for 'Marzia' the only significant one is for treatment G2 ($r = -0.9504$; $P < 0.001$) (Fig 3).

4 Discussion

Fosetyl-Al is known to be a specific fungicide against Oomycetes. As such it is recommended for seed treatment of peas against *Peronospora pisi* and *Pythium* spp. In the Netherlands, it was also recommended because it was thought to have some effect against root rot (Anon., 1989), but the actual role was unknown. The discovery of the frequent occurrence of *A. euteiches* in the Netherlands, combined with the well-known lethal effect of this pathogen towards young pea plants (J.M. Kraft, pers. comm.), threw some new light on the 1983 field experiment and the corresponding glasshouse experiments. The experiment done in 1989 was specifically designed to verify whether the unexplained effect of fosetyl-Al in former experiments could indeed be ascribed to its effect on *A. euteiches*. The experiment was conclusive, since:

a. The effect of treatments III and VI was strongest in G2, which only contained *A. euteiches* as a pathogen;
b. In naturally infested soil (G3), of all treatments only III and VI were at all effective. In the presence of many other pathogens, which had built up in this soil over 10 consecutive years of pea growing, their effect was still significant, though much less pronounced than in G2.
c. These results agree with Oyarzun's (internal report PAGV, 1988) discovery of the predominant role of *A. euteiches* in determining root rot.
d. Figures 2 and 3 show striking similarities, making the equivalence of the experiments even more convincing.
e. *A. euteiches* proved to be largely present in soil samples from the experimental field at Dinteloord.

It is clear from the experiment that other fungicides with specificity to *Oomycetes* such as metalaxyl and cymoxanil have no effect on *A. euteiches*. This is not surprising, since metalaxyl is even used in a selective medium for *A. euteiches* (Pfender et al., 1984; cf. Tu, 1988).

In highly contaminated soil with a history of several pea crops, the pathogen microflora will always consist of a mixture of pathogens. Both *P. pisi* and *A. euteiches* have very persistent resting spores. Seed treatment should therefore always contain fungicide active against these fungi, notwithstanding irregular annual variation in the severity of attack. Fosetyl-Al combines effectiveness against *P. pisi* and *A. euteiches*, two of the most damaging pathogens, with environmental safety.

We are not aware of any previous report concerning the specific activity of fosetyl-Al towards *Aphanomyces*, a genus in which several pathogenic species are described. Tsvetkova and Guseva (1980) reduced development of *A. euteiches* by seed treatment with hydroxyisoxazole (Tachigaren). Allen et al. (1987) failed to cure *Aphanomyces* root rot of bean with a soil drench of fosetyl-Al or metalaxyl, whereas fenaminosulf was effective. Remarkably, seed treatment was less effective in their experiment, whereas in general it should prove more effective. According to Salter and Smith (1986), for

example, there is a seed treatment effect on root rot that lasts for three months. Other authors claim no long-lasting effect (e.g. Kraft, 1982; Kraft and Papavizas, 1983), but only one which prevents pre-emergence and seedling damping-off. Often, metalaxyl is claimed to be effective (Trawally, 1984) or even more effective than fosetyl-Al (Salter and Smith, 1986). The present experiments indicate that *A. euteiches* must be absent from the soil in such cases.

Fosetyl-Al is known to have a very low *in vitro* activity against *Oomycetes*. At least part of its activity is indirect (Bompeix, 1982; Guest, 1984a, b; Afek and Sztejnberg, 1989; Smillie et al., 1990), stimulating the defence reactions of the plant or preventing their suppression (Dunstan and Grant, 1990). This mechanism might explain why development of resistance among the pathogens has not caused problems.

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Samenvatting

Zaaizaadbehandeling van erwten met fosetyl-Al tegen Aphanomyces euteiches

In 1983 bleek op een zwaar met voetrotpathogenen besmet veld zaaizaadbehandeling van erwten met fosetyl-Al (Aliette) gunstig af te steken bij alle andere behandelingen. De oorzaak kon toen niet worden gevonden. Kunstmatige besmetting van gesteriliseerde grond vóór het planten van behandelde zaden met de meest geïsoleerde pathogenen (*Fusarium oxysporum*, *Phoma medicaginis* var. *pinodella*, *Pythium ultimum* en *Fusarium solani* f.sp. *pisi*) gaf resultaten die niet overeenstemden met die van het proefveld, maar kasproeven met grond van het proefveld deden dat wel. Nadat was aangetoond dat het erwtepathogeen *Aphanomyces euteiches* in Nederland veel voorkomt, bevestigden nieuwe kasproeven in 1989 het gunstige effect van fosetyl-Al als zaaizaadbehandeling wanneer *A. euteiches* deel uitmaakt van de pathogene bodemflora.

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