

Occurrence of metalaxyl-resistant strains of *Phytophthora infestans* in Dutch potato fields

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In August 1980 Dutch potato growers from several areas in the country complained about the bad performance of the systemic fungicide metalaxyl in controlling potato late blight. It had been first noticed in fields treated with Ridomil 25 WP, a metalaxyl formulation. Later on in fields treated with Ridomil Speciaal, a formulated mixture of metalaxyl and mancozeb, problems also occurred. Both products had been used at recommended rates and spraying intervals. A survey revealed that in the fields concerned, the foliage was severely attacked. In some fields extensive formation of foci had taken place, in which tubers were also infected.

Since development of metalaxyl-resistance might be involved, samples of infected foliage and tubers were collected and isolates of the fungus *Phytophthora infestans* (Mont.) de Bary were made to establish their sensitivity to metalaxyl.

Isolations were made from infected tissue by placing it underneath 1 cm thick slices of potato tuber tissue (cv. Bintje or Eigenheimer) in 9-cm-Petri dishes and incubating at 15°C. After 5-7 days, the fungus usually sporulated on the surface of the slices. The isolates were weekly transferred onto fresh potato tuber tissue.

The sensitivity of the isolates to metalaxyl was determined by an *in vivo* test since it has been shown (Bruck et al., 1980; Bruin, 1980; Staub et al., 1979) that resistance *in vitro* does not always correlate with resistance *in vivo*. Discs (14 mm in diameter) were cut from potato leaves (cv. Eigenheimer) and placed upside down on fungicide solutions (10 ml) in 5-cm-Petri dishes (five discs per dish). Fungicide concentrations ranged from 100 µg to 0.01 µg a.i. ml⁻¹ in five 10-fold dilutions. Control discs floated on distilled water. The discs were inoculated with 10 µl of a sporangial suspension, containing 200-800 sporangia. The closed dishes were kept in a climate room at 15°C with 16 h light/day. After 7-9 days a disease severity index for each disc was assessed using the following scale: 0 = no disease; 1, 2, 3 and 4 = 0-25, 25-50, 50-75 and 75-100%, respectively, of the surface of the disc covered with sporangia. Results with several isolates from different locations are given in Table 1. Isolates with a high degree of resistance to metalaxyl were obtained from six of the seven fields where metalaxyl was used and failure of disease control was observed. Isolate 42, although isolated from a field where metalaxyl was used, proved to be as sensitive as

Table 1. Disease severity index of potato leaf discs floating on Ridomil 25 WP suspensions after inoculation with isolates of *Phytophthora infestans* from different sources.

Iso- late	Origin ¹	Location ²	Fungi- cide	Conc. Ridomil 25 WP (μ g a.i./ml)					
				0	0.01	0.1	1	10	100
3	Prominent (l)	Venray	man/fen	4.0	4.0	4.0	4.0	4.0	3.2
7	Prominent (l)	Venray	RidS	4.0	4.0	4.0	4.0	4.0	3.0
19	Bintje (l)	Wieringermeer	man/fen	3.4	3.6	0	0	0	0
24	Bintje (l)	Middelburg	man/fen	3.2	3.0	0	0	0	0
29	Bintje (l)	Weert	Rid	4.0	4.0	4.0	4.0	4.0	1.2
42	Astarte (l)	Valthermond	Rid	4.0	4.0	0	0	0	0
43	Prominent (l)	Borgercompagnie	RidS	4.0	4.0	4.0	4.0	4.0	0
52	Astarte (l)	Valthermond	Rid	4.0	4.0	4.0	4.0	4.0	0
72	Bintje (t)	Weert	Rid	4.0	4.0	4.0	4.0	4.0	3.4
84	Bintje (t)	Silvolde	Rid	4.0	4.0	4.0	4.0	4.0	4.0

¹ Cultivar and leaf (l) or tuber (t) from which the isolate was obtained.

² Each entry represents a different field.

³ Fungicide used to control potato late blight: Rid = Ridomil 25 WP, RidS = Ridomil Speciaal 58 WP (10% metalaxyl + 48% mancozeb) and man/fen = a maneb/fentin-acetate formulation.

Tabel 1. Aantastingsgraad van op Ridomil 25 WP suspensies drijvende aardappelbladponsjes na inoculatie met Phytophthora infestans isolaten van verschillende herkomsten.

the isolates 19 and 24, which both came from fields where a conventional fungicide was used and disease incidence was low. A second isolate from the same field as isolate 42 was also sensitive. The explanation for this phenomenon might be found in the particular location of this field on an experimental farm where unsprayed and heavily diseased plots were also present, which could have served as inoculum sources. Isolate 3, a metalaxyl-resistant one, came from a field where good control was achieved with a conventional fungicide, but which was close to another field sprayed with metalaxyl, where resistant strains were present. Isolate 3 might have originated from the latter field.

In addition to the isolates mentioned in Table 1 a number of other isolates have been tested. When several isolates were obtained from the same field they proved to be either all resistant or all sensitive except for one case where one sensitive and three resistant isolates were obtained from a field on an experimental farm. Here again an unsprayed plot was nearby, from which the sensitive isolate might have originated.

Since the weather conditions in the summer of 1980 were very favourable for late blight development, all resistant isolates may have originated from one single parent strain in which resistance developed. To test this hypothesis a race determination was carried out for some of the isolates, using a set of differentials carrying different R-genes. Resistance to metalaxyl was found in two different races which strongly suggests that the resistant isolates originated from different sources (Table 2). It is

Table 2. Race determination of *Phytophthora infestans* isolates collected in 1980.

Isolate	Origin ¹	Location ²	Metalaxyl sensitivity ³	Race ⁴
19	Bintje	Wieringermeer	S	1.3.4.7.10.11
24	Bintje	Middelburg	S	4.10.(11)
29	Bintje	Weert	R	4.7.(8)
34	Bintje	Weert	R	4.7.(8)
42	Astarte	Valthermond	S	1.3.4.7.10.(11)
49	Prominent	Borgercompagnie	S	1.3.4.7.10.(11)
50	Prominent	Borgercompagnie	R	1.3.4.7.10.11

¹ Cultivar from which the isolate was obtained.

² Each entry represents a different field.

³ S = metalaxyl-sensitive; R = metalaxyl-resistant.

⁴ () = reaction on the differential concerned, is weak.

Table 2. Fysiodeterminatie van in 1980 verkregen *Phytophthora infestans* isolaten.

striking that two of these isolates belonged to race 4.7.(8), which has never been found in the Netherlands before.

To see whether metalaxyl-resistant isolates exhibited cross resistance to other fungicides with activity against *P. infestans* the test was also carried out with furalaxyl and milfuram, which are both structurally related to metalaxyl and with cymoxanil, fosetyl-Al and propamocarb. The metalaxyl-resistant isolates examined were also resistant to furalaxyl and milfuram. No difference in sensitivity to cymoxanil, fosetyl-Al and propamocarb was observed between the metalaxyl-sensitive and the resistant strains. The lowest completely inhibiting concentration was 10 µg a.i. ml⁻¹ with the latter three compounds for all isolates examined.

We conclude from these data that the failure of metalaxyl to control potato late blight is caused by the occurrence of metalaxyl-resistant strains in the fields concerned. After registration in 1979, metalaxyl was used that same year in about 5% of the potato fields and in 1980 in about 50% of the fields, the majority of which were sprayed with Ridomil 25 WP, so the development of resistance has been extremely rapid. Several reasons can be mentioned for this:

- a) the ease with which mutants resistant to metalaxyl arise in a population as has been demonstrated in the laboratory for other fungi (Bruin, 1980; Davidse, 1981);
- b) the wheater conditions in 1980 which were very favourable for the development of a potato late blight epidemic, and
- c) the almost exclusive use of metalaxyl in 50% of the acreage so that resistant mutants once they arose could multiply rapidly.

The outbreak of a potato late blight epidemic in 1980 in the Netherlands caused by a metalaxyl-resistant population of *P. infestans* resulted in considerable financial losses for many potato growers, since even a low percentage of infected tubers renders a crop almost unmarketable and the costs of removing infected tubers by hand are extremely high.

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Samenvatting

*Vóórkomen van metalaxyl-resistente stammen van *Phytophthora infestans* in aard-appelpercelen in Nederland*

In de zomer van 1980 gaf in een aantal gevallen het systemische fungicide metalaxyl een zeer onvoldoende werking te zien tegen de aardappelziekte. *Phytophthora infestans*-isolaten die uit de desbetreffende percelen werden verkregen vertoonden in een drijftoets een zeer geringe gevoeligheid voor het fungicide in vergelijking met isolaten uit percelen waar gebruik werd gemaakt van maneb/fentin-acetaat combinaties en waarin nagenoeg geen aantasting voorkwam.

De oorzaak van de onvoldoende werking van metalaxyl moet worden gezocht in het ontstaan van metalaxyl-resistente stammen in de populatie, die als gevolg van de voor de aardappelziekte geschikte weersomstandigheden en de veelvuldige toepassing van alleen metalaxyl zich ongebreideld konden vermenigvuldigen.

Metalaxyl-resistentie kwam voor in verschillende fysio's. Dit wijst erop dat de resistentie op meerdere plaatsen is ontstaan. Opmerkelijk was dat één van de resistente fysio's nooit eerder in Nederland was geïsoleerd.

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