

Metalaxyl-resistant strains of *Phytophthora infestans* (Mont.) de Bary in Ireland

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Summary

Isolates of *Phytophthora infestans* obtained from different sources were tested for pathogenicity on cv. Kerr's Pink after different fungicide treatments. All isolates were pathogenic on untreated Kerr's Pink. Disease development after inoculation with all isolates was effectively controlled by a mancozeb spray. One isolate was effectively controlled by metalaxyl. The remaining two isolates were resistant to all levels of metalaxyl (50–700 $\mu\text{l/l}$). Similar results were obtained with cv. Home Guard. In vitro tests showed that metalaxyl-resistant strains grew faster on metalaxyl-containing medium than sensitive strains.

Introduction

The effectiveness of the acylalanine fungicide metalaxyl in controlling late blight caused by *Phytophthora infestans* (Mont.) de Bary was reported in 1977 (Urech et al., 1977). These results were confirmed by field trials in Ireland from 1976–78 (Frost & Dowley, 1979) where the fungicide was first used commercially in 1977. Its use increased rapidly so that by 1980 it was one of the major fungicides used to control late blight in Ireland. Using Bourke's System (Bourke, 1955), the meteorological conditions suitable for the spread of late blight during 1980 were 214% above average between 20 July and 18 August (Anon., 1980). These conditions resulted in the early onset of foliage blight and some crops sprayed with metalaxyl were observed to have high levels of blight compared to crops sprayed with other fungicides. Isolates of *P. infestans* were collected from different areas to determine if strains resistant to metalaxyl had developed.

Experimental

Three cultures of *P. infestans* were isolated from tubers obtained from different sources. Isolate 1 was obtained in 1978 from the Carlow area where metalaxyl was not widely used; isolate 2 was obtained in 1980 from a metalaxyl-sprayed crop in Co. Meath; isolate 3 was obtained in 1980 from a mancozeb-sprayed crop in Co. Kilkenny.

Another isolate, No 4, was obtained from Dublin University as race 4.11. The isolates were maintained on rye seed agar (Caten & Jinks, 1968) for several generations before inoculation.

In vivo experiments

Sprouted tubers of the blight-susceptible cultivar Kerr's Pink were planted in 15-cm pots in the greenhouse. When the plants had reached about 50 cm (12 extended leaves) they were variously sprayed to run-off with metalaxyl at 50, 100, 300 or 700 $\mu\text{l/l}$ or with mancozeb at 6000 $\mu\text{l/l}$ or with water alone (upper surface only). Twenty-four hours after spraying each plant was inoculated with a zoospore suspension of isolate 1, 2 or 3. Zoospores were induced to form by subjecting a suspension of 250 000 sporangia/ml to 5°C for 3 hours. After inoculation, each plant was covered with a plastic container to prevent drying-out and cross contamination between isolates. Plants were then incubated for 10 days in the greenhouse at 17–20°C with no supplementary lighting (12 h day length). Each treatment unit consisted of four plants and was replicated four times. Disease incidence was assessed after 7 and again after 10 days by the EPPO key (Martin et al., 1978). A similar experiment was carried out with cv. Home Guard and all four isolates of *P. infestans* but with a reduced number of spray treatments.

In vitro experiments

Plates of Rye Seed Agar (RSA) supplemented with different levels of metalaxyl were prepared. Standard discs (5 mm diam.) taken from the leading edge of each isolate were transferred to 4 RSA test plates per treatment. After 7 days the colony size was

Table 1. Foliage blight in cv. Kerr's Pink after different inoculation and fungicide treatments 7 and 10 days after inoculation.

Fungicide treatment ¹	Foliage blight ² (%)					
	isolate ³ 1		isolate 2		isolate 3	
	7 days	10 days	7 days	10 days	7 days	10 days
Unsprayed ⁴	25	68	75	75	50	75
Metalaxyl 50 $\mu\text{l/l}$	0	0	50	75	31	75
Metalaxyl 100 $\mu\text{l/l}$	0	0	69	75	44	75
Metalaxyl 300 $\mu\text{l/l}$	0	0	50	75	25	75
Metalaxyl 700 $\mu\text{l/l}$	0	0	56	75	25	75
Mancozeb	0	0.3	0.5	0.7	0	5

L.S.D. 5% level⁵ 11.39 (for 7 days) and 2.89 (for 10 days).

¹ Fungizidbehandlung - Traitement fongicide; ² Krautfäule - Feuillage atteint de mildiou; ³ Isolat - Souche; ⁴ Unbehandelt - Témoin non traité; ⁵ Kleinste signifikante Differenz 5% Stufe - ppds au niveau de 5%

Tabelle 1. Krautfäule in cv. Kerr's Pink nach verschiedenen Inokulationen und Fungizidbehandlungen 7 und (10) Tage nach der Inokulation.

Tableau 1. Feuillage atteint de mildiou sur la variété Kerr's Pink après différentes inoculations et traitements fongicides 7 et (10) jours après inoculation.

measured and the rate of growth expressed as a percentage of growth on metalaxyl-free medium.

Results

Results with cv. Kerr's Pink showed that after 7 days incubation, isolate 1 was completely inhibited by all levels of metalaxyl (Table 1). Isolates 2 and 3 were pathogenic on plants treated with all levels of metalaxyl but were less virulent than on the control treatment, especially at the higher concentrations. Mancozeb significantly reduced disease development in all isolates and only isolate 2 showed any measurable level of infection. The overall results after 10 days incubation (Table 1) were similar but differences between unsprayed and metalaxyl-sprayed treatments were no longer apparent. The mancozeb treatment was highly effective in controlling disease although some blight did develop. Results with Home Guard (Table 2) were similar to those obtained with Kerr's Pink.

Table 2. Foliage blight in cv. Home Guard after differing inoculation and fungicide treatments (7 days after inoculation).

Fungicide treatment ¹	Foliage blight ² (%)			
	isolate ³ 1	isolate 2	isolate 3	isolate 4
Metalaxyl 700 µl/l	0	50	65	0
Unsprayed ⁴	75	75	75	75

¹ ⁴ Siehe Tabelle 1 - Voir tableau 1

Tabelle 2. Krautfäule in cv. Home Guard nach verschiedenen Inokulationen und Fungizid-behandlungen (7 Tage nach der Inokulation).

Tableau 2. Feuillage atteint de mildiou sur la variété Home Guard après différentes inoculations et traitements fongicides (7 jours après inoculation).

Table 3. Growth rate of *P. infestans* on RSA with different levels of metalaxyl as % of the control.

Isolate ¹	Colony growth (% of control) ²					
	0 µl/l	0.01 µl/l	0.1 µl/l	1 µl/l	10 µl/l	100 µl/l
1	100	34	0	0	0	0
2	100	60	50	46	50	45
3	100	95	89	80	84	84
4	100	58	30	22	21	14

¹ Isolat - Souche; ² Kolonie Wachstum in % der Kontrolle - Croissance des colonies en % de témoin.

Tabelle 3. Wachstumsrate von *Phytophthora infestans* auf Roggenagar (RSA) mit verschiedenen Gehalten von Metalaxyl, angegeben als % der Kontrolle.

Tableau 3. Taux de croissance de *Phytophthora infestans* sur RSA avec différentes concentrations de métalaxyl en pourcentage de témoin.

The addition of metalaxyl to the culture medium reduced growth rates in all isolates tested but isolates 1 and 4 were more affected than isolates 2 and 3. Isolate 1 was completely inhibited at levels of metalaxyl above 0.01 $\mu\text{l/l}$ (Table 3).

Discussion

Isolate 1 of *P. infestans* was capable of causing infection in unsprayed and mancozeb-sprayed Kerr's Pink but was completely inhibited by even the lowest rates of metalaxyl. Within the metalaxyl treatments, however, small necrotic specks could be observed indicating that while penetration had taken place the further development of the disease was prevented. This is consistent with the results of other researchers (Cohen et al., 1979; Staub & Young, 1980). Seven days after inoculation isolates 2 and 3 showed lower disease levels in the metalaxyl treatments compared to the unsprayed control, especially at the two higher levels of metalaxyl. This result is similar to that reported for a metalaxyl-tolerant strain of cucumber downy mildew (*Pseudoperonospora cubensis*). (Pappas, 1980). However, after 10 days incubation there were no significant differences between unsprayed and metalaxyl-sprayed treatments inoculated with isolates 2 and 3. A similar result was obtained with Home Guard where isolate 4 was also controlled by metalaxyl. The mancozeb application, however, gave good control of all isolates on Kerr's Pink. These results are substantiated by the *in vitro* studies where isolates 1 and 4 were strongly affected by the addition of metalaxyl while isolates 2 and 3 were only slightly affected. It is concluded from these experiments that strains of *P. infestans* (isolates 2 and 3) resistant to metalaxyl were present and may have been responsible for failures of this fungicide to control late blight in Ireland during 1980.

Zusammenfassung

Gegen Metalaxyl-resistente Stämme von Phytophthora infestans (Mont.) de Bary in Irland

Isolate von *Phytophthora infestans*, von verschiedenen Herkünften, wurden auf Resistenz gegen Metalaxyl geprüft. Eingetopfte Pflanzen (Sorte Kerr's Pink) wurden mit den gewünschten Konzentrationen von Metalaxyl (50–700 $\mu\text{l/l}$), Mancozeb (6000 $\mu\text{l/l}$) oder Wasser besprüht. 24 Stunden nach der Fungizidanwendung wurden alle Pflanzen mit einer Zoosporensuspension der Isolate 1, 2 und 3 von *Phytophthora infestans* inokuliert. 7 und 10 Tage nach der Inokulation wurde der Befall der Pflanzen bestimmt.

Isolat 1 wurde durch Metalaxyl wirkungsvoll bekämpft. Sieben Tage nach der Inokulation mit den Isolaten 2 und 3 zeigten die mit Metalaxyl behandelten Pflanzen einen geringeren Befall als die unbehandelten Kontrollen

(Tabelle 1). 10 Tage nach der Inokulation war jedoch kein signifikanter Unterschied zwischen den Kontrollen und den mit Metalaxyl behandelten Pflanzen (Tabelle 1). Die Behandlung mit Mancozeb hemmte effektiv den Befall nach Inokulation mit allen Isolaten. Gleiche Ergebnisse wurden mit der Sorte Home Guard erhalten (Tabelle 2). *In vitro* Prüfungen zeigten, dass Metalaxyl resistente Stämme schneller auf Metalaxyl haltigem Medium wuchsen als sensible (Tabelle 3).

Von diesen Versuchen ausgehend wird gefolgert, dass Metalaxyl-resistente Stämme von *Phytophthora infestans* vorhanden waren und sie für den Misserfolg dieses Fungizids, die Krautfäule in Irland 1980 zu bekämpfen, verantwortlich gewesen sein können.

Résumé

Races de Phytophthora infestans (Mont.) de Bary résistantes au métalaxyl en Irlande

Des souches de *Phytophthora infestans* provenant de différentes sources ont été examinées pour leur résistance au métalaxyl.

Des plantes en pots (var. Kerr's Pink) ont été pulvérisées à différentes concentrations de métalaxyl (50-700 µl/l), de mancozèbe (6000 µl/l) ou d'eau. 24 heures après l'application de fongicide, toutes les plantes ont été inoculées avec une suspension de spores provenant de souches 1, 2 et 3 de *Phytophthora infestans*. Les plantes ont été examinées, en ce qui concerne l'incidence de la maladie, 7 et 10 jours après inoculation.

La souche 1 était effectivement contrôlée par le métalaxyl. 7 jours après inoculation, les souches 2 et 3 présentaient sur les plantes traitées au métalaxyl un niveau de maladie plus bas que sur celles non traitées (tableau 1).

Cependant, dix jours après inoculation, il

n'y a avait aucune différence significative entre les plantes non traitées au métalaxyl lorsque l'inoculation était réalisée avec les souches 2 et 3 (tableau 1). Le développement de la maladie, après inoculation avec toutes les souches était effectivement contrôlé par le traitement au mancozèbe. Des résultats identiques ont été obtenus avec le cultivar Home Guard (tableau 2).

In vitro, les tests montraient que les races résistantes au métalaxyl se développaient plus vite que les races sensibles, dans des milieux contenant du métalaxyl (tableau 3).

Il ressort de ces expérimentations qu'il existe des races de *Phytophthora infestans* résistantes au métalaxyl et qu'elles peuvent être responsables des échecs enregistrés dans le contrôle du mildiou en Irlande en 1980.

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