

Efficacy of fungicidal protection of newly developing potato leaves against *Phytophthora infestans*

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Abstract

Three experiments aiming to explore the efficacy of fungicides to protect newly developing potato leaves were carried out between 2000 and 2002. Contact, translaminar and systemic fungicides were applied in field trials at Lelystad in the centre of the Netherlands. Efficacy of the fungicides was established using a bioassay in which detached new grown leaflets (cv Bintje) were inoculated with *Phytophthora infestans*. The interval between the last fungicide application and picking the leaves to be used in the bioassay varied between 4 and 11 d. Disease incidence was assessed after incubation for approximately a week.

Protection of the newly developed leaves depended on the intrinsic property of the fungicides, time since the last fungicide application and growth rate of potato leaves in the field since the last fungicide application. Shorter time intervals between spraying and inoculation resulted in better (re)distribution of (contact) fungicides on newly developed leaves resulting in better protection. Also, there was less dilution of the fungicide due to less leaf expansion during a 4 d compared with 7 d spray interval. A similar effect occurs when crop growth rate decreases.

Ridomil Gold MZ, with the systemic active ingredient metalaxyl and Ranman (cyazofamid) resulted in the best protection of newly developed leaves at the beginning of the growing season when crop growth rate was high. Contact fungicides containing cyazofamid or to a lesser extent, mancozeb can protect newly developing leaves, due to good redistribution of the compounds.

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1. Introduction

Potato crops are commonly sprayed preventively with fungicides to protect them against the late blight pathogen, *Phytophthora infestans*. Spray intervals required to maintain protection depend on the intrinsic fungicidal properties of the products used, the weather conditions, disease pressure and growth rate of the potato crop, and may result in application of 10–18 sprays per growing season in the Netherlands (Schepers, 2002). Increased aggressiveness of the new blight population in Europe (Day and Shattock, 1997; Flier and Turkensteen, 1999) has probably led to shorter infection cycles and more rapid epidemic development (Flier et al., 2002), resulting in more intensive

fungicide application. The narrower window of opportunity for application of fungicides to control late blight has resulted in the increased importance of new growth protection.

Systemic fungicides are readily translocated acropetally (Davidse et al., 1991), whereas local systemics are not (Edgington et al., 1980). For example, in potato, cymoxanil was not acropetally systemically translocated, but translocated translaminarily (Cohen and Grinberger, 1987). Dimethomorph is considered locally systemic, and translaminar translocation takes place, although little transport occurs out of treated leaves (Schwinn and Margot, 1991), indicating little real systemicity. Protectant fungicides such as maneb, chlorothalonil and captan remain on the surface of the plant (Edgington et al., 1980). To date only fungicides with a systemic mode of action are assumed to protect new growth effectively. Bruhn and Fry (1982)

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showed redistribution of contact fungicides from upper leaf layers to lower leaf layers by rainfall. Thus contact fungicides can be redistributed to other leaf layers. There are indications which suggest that non-systemic fungicides may protect newly developing leaves as well depending on growth rate of the crop and rainfall to facilitate redistribution (Spits and Schepers, 2001; Spits and Schepers, 2002). The objective of this research was to determine the efficacy of preventive protection of newly developing leaves following application of contact, translaminar and systemic fungicides.

2. Materials and Methods

2.1. Field applications

Seed tubers (cv. Bintje) were planted 35 cm apart on ridges in a sandy loam soil at Lelystad. Crop management followed good agricultural practice. Experiments were set up in 2000, 2001 and 2002. Fungicide sprays were carried out every 7 d in experiments in 2000, 2001 and 2002 (Tables 1 and 2). Additionally, a treatment was included in which sprays were carried out every 4 d with each of the fungicides in 2001. In the 2002 experiment the build up of efficacy of fungicides and the influence of postponing the first spray on the level of protection of leaves in different leaf layers was tested. For this purpose two spray strategies were included (Table 1). Fungicide treatments including the untreated control were randomised in four complete

blocks in 2000 and 2001. A split – plot design was applied in 2002. Spraying strategies were allotted to the split strata (main plots). Fungicide treatments were randomly allotted to plots within the strata (sub-plots). Plot length was 8 m in each experiment. Plot width varied and was 3.75 m in 2000, 4.5 m in 2001 and 5.25 m in 2002.

The first fungicide application was carried out when plants were 20 cm high. The various fungicides used in the experiments are listed in Table 2. Fungicides were applied in a suspension of 250 l ha⁻¹ at a pressure of 250 kPa, using a tractor pulled trial-site sprayer (Sosef) with Teejet XR110.04 nozzles.

2.2. Bioassay

Top leaves were picked 4 or 7 d after the last fungicide spray and just before the next scheduled fungicide application. Twenty leaflets from four randomly selected individual growing points (leaves) were taken from each plot. Four leaves of each plot were picked and placed in plastic containers with the leaf stems placed in wetted Oasis. The four containers of each treatment coincided with the replicates. Containers were wrapped in polythene bags to maintain a high relative humidity after inoculation and placed according to the field lay-out in the climate chamber. The adaxial site of the distal leaflet and four lateral leaflets closest to the top leaflet of each leaf were inoculated with *P. infestans*. Metalaxyl-sensitive isolate IPO98014, race 1.2.3.4.7.11, of *P. infestans* was used in all experiments. IPO98014 was originally isolated in 1998 in the north-east of the Netherlands. The pathogen was stored in liquid nitrogen until use. The pathogen was maintained on slices of potato tubers (cv. Bintje) under high relative humidity at 15 °C in the dark in 2000. Potato leaves (cv. Bintje) were used to maintain the pathogen in 2001 and 2002. Sporangial inoculum was obtained by rinsing 1-week-old infested tuber slices or potato leaves with tap water. The crude suspension was sieved through cheese cloth and sporangia were collected on a 15 µm sieve and resuspended. Sporangial density was established microscopically using a haemocytometer and adjusted to 10⁴ (2000) or 5 × 10³ (2001 and 2002) sporangia ml⁻¹. The inoculum was stored for 1.5 h at 7 °C to facilitate zoospore release. A single 40 µl droplet was placed in the middle of a leaflet. Leaflets were incubated at 15 °C, with an 8 h light period and under a high relative humidity (98%) in a climate chamber for 5–7 d (Evenhuis et al., 1996; Flier and Turkensteen, 1999).

2.3. Crop growth and disease assessment

Growth rate of plants between spraying and inoculation was determined by observation of growing points that were sprayed with red paint just before each fungicide application. An estimate was made of the number of new emerged leaves at the time of picking, since the last fungicide application. All new leaf layers above the leaves sprayed

Table 1
Diagram of the trial procedure at Lelystad

Day	2000 7 d interval	2001 7 d interval	2001 4–5 d interval	2002 A2	2002 A3
0	F	F	F		F
4			T1 + F		F
7	T1	T1 + F			
8	F		T2 + F		
11				F	T1 + F
13			T3 + F		
14		T2 + F			
15	T2				
16	F			F	F
18			T4 + F		
21		T3		T2 + F	T2 + F
22		F	T5		
23	T3		F		
24				F	F
27			T6		
28		T4		T3	T3
32				T4	T4
35				T5	T5

Experiments started at day 0 on 19-6-2000, 20-6-2001, 21-6-2001 and 3-6-2002 with the first fungicide treatment (F). At regular intervals newly expanded leaves were picked and inoculated with *P. infestans* in a bioassay (T). A strategy (A2) in which the first spray application was postponed was compared to a strategy (A3) which started at day 0 in 2002. Disease assessments were made 5–7 d after inoculation.

Table 2
Treatments to test the preventive efficacy of fungicides on new growth in field experiments

Treatment	Active ingredient (s)	Mobility	Dose rate active ingredient (l or kg ha ⁻¹)	Dose rate product (l or kg ha ⁻¹)	Field expts
Untreated	—	—	—	—	2000–2002
Curzate M ^a	Cymoxanil (4.5%) Mancozeb (68%)	local-systemic contact	0.11 1.70	2.5	2000–2002
Acrobat	Dimethomorph (7.5%) Mancozeb (67%)	local-systemic contact	0.15 1.34	2.0	2000–2001
Tattoo C	Propamocarb-hydrochloride(375 g l ⁻¹) Chlorothalonil (375 g l ⁻¹)	Systemic Contact	1.01 1.01	2.7	2000
Tattoo C	Propamocarb-hydrochloride(375 g l ⁻¹) Chlorothalonil (375 g l ⁻¹)	Systemic Contact	0.56 0.56	1.5	2001–2002
Ridomil	Metalaxyl-M (4%)	Systemic	0.10	2.5	2000–2001
Gold MZ	Mancozeb (64%)	Contact	1.60		
Shirlan	Fluazinam (500 g l ⁻¹)	Contact	0.20	0.4	2000–2002
Dithane DG	Mancozeb (75%)	Contact	3.00	4.0	2001–2002
Aviso DF	Metiram (57%)	Contact	1.43	2.5	2002
Ranman ^b	Cymoxanil (4.8%) Cyazofamid (400 g l ⁻¹)	local-systemic Contact	0.12 0.08	0.2	2001–2002

^aThe dose rate at the first application in field experiments was 2.0 kg ha⁻¹ and second application 2.25 kg ha⁻¹.

^bRanman was sprayed in combination with the dilutant ADDIKF in a dose rate of 0.25 and 0.15 l ha⁻¹ in 2001 and 2002, respectively.

with red paint were considered to have emerged since the previous application of the fungicides. New growth was defined as growth and development of leaves present at the time of the last fungicide application as well as newly formed leaflets or leaves. The protection of the new grown leaves by the applied fungicides was assessed in a bioassay. The percentage of leaflets with lesions was assessed 5–7 d after inoculation.

2.4. Data analysis

Data were analysed using Genstat 6.0 (Payne et al., 2002). Least significant differences were calculated at a significance level of $P = 0.05$. To be able to compare within experiments between different sampling dates, time was treated as a factor.

3. Results

3.1. Spray interval 7 d 2000

After the first fungicide application none of the fungicide treatments resulted in a significant reduction of the number of lesions on the newly formed leaves as compared to the untreated control (Table 3). Disease incidence observed in the bioassay decreased as fungicide applications increased. Application of Ridomil Gold MZ (4% metalaxyl + 64% mancozeb; Syngenta), Tattoo C (37.5% propamocarb + 37.5% chlorothalonil; Bayer) and Acrobat (7.5% dimethomorph + 67% mancozeb; BASF) resulted in a significant reduction of the number of leaflets with lesions as compared to the untreated control after the second fungicide application (Table 3). After the third fungicide

application all fungicides resulted in a significant reduction of the number of leaflets with lesions as compared to the untreated control. Lesions on new leaves ranged from 62% when Shirlan (50% fluazinam; Syngenta) was used to 6% when Ridomil Gold MZ was applied. Ridomil Gold MZ resulted in significantly fewer lesions than all other fungicides after three applications.

3.2. Spray interval 7 d 2001

Disease incidence established in a bioassay on detached leaves was 72% (Table 3) after the first application of Ridomil Gold MZ, which was significantly lower than the untreated control (100%). After two applications leaflets from plots treated with Ridomil Gold MZ Dithane DG (75% mancozeb; Dow) and Ranman (40% cyazofamid; Belchim) showed significantly fewer lesions than the untreated control. Three fungicide applications, of all fungicides, except Tattoo C, resulted in significantly fewer lesions as compared to the untreated control. Ridomil Gold MZ resulted in significantly fewer lesions on leaves than all other fungicides, except Ranman. After four applications all fungicides almost completely controlled the disease and there were significantly fewer lesions compared to the untreated control.

In general, the disease incidence on newly formed leaf layers decreased with increasing number of fungicide applications from 89% after one application to 3% after four applications. The differences in disease incidence after inoculation, assessed after the first and the second application were only significant for Dithane DG and Ranman. When fungicides were applied three times, all fungicide treatments, except Acrobat and Tattoo C,

Table 3
Percentage of leaflets with lesions of *P. infestans* following several applications with various fungicides

Experiment	Lesions on new grown leaflets (%)												
	2000			2001 long interval				2001 short interval					
Interval ^a (d)	7	7	7	7	7	7	6	4	4	5	5	4	4
Application (#)	1	2	3	1	2	3	4	1	2	3	4	5	6
Growth rate (# new leaf layers)	2	1.5	1.0	1.5	1.5	1	<1	1	1	1	1	0.5	<0.5
Untreated	100.0	99.0	100.0	100.0	98.8	100.0	45.0	96.3	85.0	100.0	98.8	85.0	40.0
Curzate M	100.0	84.4	60.4	93.8	78.8	58.8	3.8	43.8	41.3	46.3	90.0	0.0	0.0
Acrobat	100.0	71.9	40.6	87.5	77.5	71.3	2.5	50.0	32.5	41.3	86.3	0.0	0.0
Tattoo C ^b	100.0	53.1	28.1	97.5	95.0	78.8	5.0	57.5	46.3	50.0	51.3	0.0	0.0
Ridomil Gold MZ	93.7	44.8	6.3	72.5	73.8	16.3	0.0	11.3	10.0	25.0	13.8	0.0	0.0
Shirlan	100.0	91.7	61.5	97.5	88.8	73.8	7.5	60.0	30.0	61.3	86.3	0.0	0.0
Dithane DG	—	—	—	87.5	53.8	41.3	0.0	47.5	23.8	26.3	75.0	0.0	0.0
Ranman	—	—	—	85.0	22.5	21.5	0.0	22.5	3.8	7.5	25.0	0.0	0.0
LSD ($P = 0.05$)	4.5	20.4	26.4	8.8	21.9	29.4	18.3	19.1	23.2	24.5	32.0	—	—
LSD ($P = 0.05$)	19.5			21.8				22.0					

The preventive efficacy of fungicides on new growth was tested at Lelystad in 2000 and 2001.

^aInterval between spray treatment and sampling.

^bDose rate of Tattoo C was 2.7 and 1.5 kg ha⁻¹ in 2000 and 2001, respectively.

resulted in significantly fewer lesions than after one application. Significantly fewer lesions were observed after four than after one application of all fungicides.

3.3. Spray interval 4 d in 2001

Disease incidence on detached leaves ranged from 4% to 61% compared to the untreated control (Table 3). Ranman and Ridomil Gold MZ treatment resulted in the fewest lesions. After four applications, Ranman (25%), Ridomil Gold MZ (14%), Tattoo C and Dithane DG leaves had significantly fewer lesions compared to the untreated control (99%). After five or six applications, all fungicides totally protected the growing point. A cumulative effect of several fungicide applications was not clearly demonstrated. After the second fungicide application, a decrease in number of lesions was observed compared to one application. This decrease was significant for Shirlan and Dithane DG. More lesions were observed after fungicides were applied three times compared to two applications and were higher after four compared to three applications of the same fungicides. There were significantly more lesions on leaves where Acrobat, Shirlan and Dithane were applied four times compared to once.

3.4. Spray strategies in 2002

Ranman gave the best preventive protection after two applications (T1, A3; Table 4), while application of Shirlan resulted in more lesions as compared to the other fungicides used. The level of protection of a developing growing point was not improved after four applications

(A3) compared to two applications (A2) at T2. There were significant differences between the applied strategies on the disease incidence of *P. infestans* on the new grown potato leaves, depending on the fungicide used. However, no major effects of the strategies on late blight control were observed. Of the fungicides tested only Dithane had significantly fewer lesions after six applications compared to four. Efficacy of the other fungicides did not improve as number of sprays increased.

Four applications (T2, A3) of Ranman resulted in the fewest lesions. The number of lesions on Ranman-treated leaves was significantly less than with Shirlan or Aviso DF (57% metiram + 4.8% cymoxanil; BASF). The percentage of leaflets with lesions was similar or more following six (T3, A3; 11–85%) compared to four applications (T3, A2; 12–69%) of the same fungicide. Ranman sprays resulted in significantly fewer lesions than all other fungicides, except Curzate M (4.5% cymoxanil + 68% mancozeb; Dupont). There were more lesions after six applications compared to four (T3) when Shirlan, Dithane DG, Tattoo C and Aviso DF were used. As at T2, Dithane DG resulted in fewer lesions than the other contact fungicide (Shirlan). Aviso DF sprays resulted in more lesions than the other translaminar fungicide Curzate M (both containing cymoxanil).

Disease incidence was 14% and 37% at 8 and 11 d (T4 & T5), respectively after the last application with Ranman. Disease incidence was significantly lower when Ranman was applied compared to all other fungicides used. Differences in protection efficiency between other fungicides were small. Dithane DG resulted in significantly fewer lesions than Tattoo C, sprayed according to strategy

Table 4
Percentage of leaflets with lesions of *P. infestans* after several applications with various fungicides

Experiment	Lesions on new grown leaflets (%)									
	Strategy A2					Strategy A3				
Treatment	T2	T3	T4	T5	T1	T2	T3	T4	T5	
Time Interval (d) ^a	5	5	8	11	7	5	5	8	11	
Application (#)	2	4	4	4	2	4	6	6	6	
Growth rate (# new leaf layers)	<1	1	>1	>1	>1	<1	1	>1	>1	
Untreated	81.6	98.8	92.0	86.3	68.0	81.6	98.8	92.0	86.3	
Shirlan	62.5	66.3	68.4	68.6	60.2	60.6	71.3	50.7	65.6	
Curzate M	3.9	33.8	50.9	63.0	82.9	19.3	32.5	45.7	73.1	
Dithane DG	23.6	27.4	42.9	59.7	65.3	23.2	50.1	63.2	76.8	
Ranman	2.3	12.4	20.4	14.5	8.5	0.0	11.3	14.1	36.6	
Tattoo C	11.8	36.6	70.8	69.6	53.8	32.3	45.0	48.6	87.9	
Aviso DF	29.6	68.8	63.4	54.6	74.2	38.8	85.0	66.9	76.8	
LSD ($P = 0.05$)	20.4	30.1	17.5	21.8	19.2	23.7	30.1	22.9	23.2	
LSD ($P = 0.05$)	23.9				24.1					

The preventive efficacy of fungicides on new growth was tested at Lelystad in 2002. Strategy A3 involved spraying from the start. The first application was postponed in strategy A2 and coincided with the third application of strategy A3.

^aInterval between spray treatment and sampling.

A2, but not when strategy A3 was applied. The number of lesions in strategy A3 (six applications) tended to be higher as compared to strategy A2 (four applications) at 5 and 11 d, but not at 8 d, depending on the fungicide used.

5. Discussion

New growth consists of the formation of completely new leaves and also of leaf expansion since the last spray application of existing leaves, and is determined by crop growth rate.

5.1. Efficacy of fungicides at low and intermediate growth rates

No completely new leaves are developed within a spray interval at low to intermediate growth rates (<1.5 new leaf layer per week). Protection can be attributed to redistribution of contact fungicides on the expanding leaves as well as redistribution of (locally)-systemic fungicides in the leaflets. Leaf expansion itself might result in fungicide rates on the leaf dropping below the minimal concentration necessary to control late blight.

Protectants like Ranman, Shirlan and Curzate M (with a short lasting preventive efficacy of cymoxanil) also showed a reduction in number of lesions after multiple applications. A possible explanation for this observation may be the redistribution of the contact fungicides cyazofamid, fluazinam and mancozeb from lower leaf layers to the developing growing point by splash of rain droplets or vapour activity (Schepers and Meier, 2003). Bruhn and Fry (1982) showed redistribution of protectant fungicides by rain water from upper canopy layers to lower leaf layers.

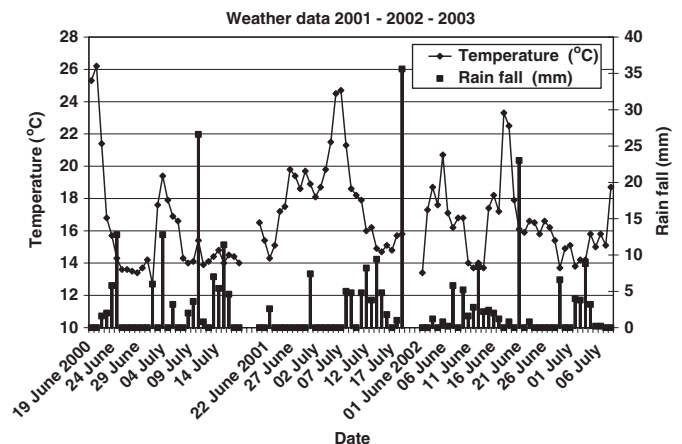


Fig. 1. Temperature and rainfall during the experiments carried out in Lelystad, The Netherlands.

With splash dispersal, it is feasible that low dose rates of fungicides may disperse to upper leaf strata. Precipitation was found on many days during our experiments (Fig. 1), indicating splash dispersal of fungicides could have happened. Mancozeb (Dithane DG) sticks less to the leaf surface compared to fluazinam (Shirlan). A less sticky formulation of the active compound may facilitate redistribution and therefore increase the efficacy of the fungicide to protect the growing point. But it might also decrease protection of treated foliage due to fungicide runoff. Alternatively redistribution might occur through vaporization. A strong gas phase activity is shown by metalaxyl, which aids the distribution within the canopy (Schwinn and Margot, 1991), which might also be the case with Shirlan with a similar vapour activity to cymoxanil or

Ranman. Although weather might play a role, protection of new growth by Ranman and Dithane was evident in different experiments and over a wide range of weather conditions.

5.2. Efficacy of fungicides at high growth rates

At high growth rates (≥ 1.5 new leaf layers) only truly systemic fungicides, and Ranman were able to reduce the number of lesions on the developing growing point. The redistribution ability of locally systemic fungicides cannot compensate sufficiently for fast leaf expansion and leaf formation. A fairly good redistribution combined with limited translaminar movement coupled with a high intrinsic efficacy of cyazofamid may explain the good protection of new growth by Ranman. Systemic fungicides, such as Ridomil Gold MZ, can redistribute inside the plant to such an extent that at least a partial protection of the completely new formed leaves is achieved. This is in concordance with Cooke and Little (1992) who observed control of late blight on new growth only when oxadixyl (systemic active ingredient) was sprayed in a mixture, but not with mancozeb (contact) or cymoxanil (translaminar) plus mancozeb. In a field situation complete protection of new growth by Ridomil Gold MZ was observed with spray intervals of 14 d (Eberle and Urech, 1978). In these experiments Ridomil Gold MZ was less efficient, probably due to a much higher inoculum pressure in the bioassay or differences in pathogenicity of the *P. infestans* isolate used.

5.3. Practical implications to late blight control

During the period between spraying the fungicides and inoculation, the uptake of the fungicides was normal. Therefore, at inoculation the presence or absence of fungicides in the leaves used in the bioassay is comparable to what could be expected in the field situation.

Vaporisation of fungicides in closed containers may control unprotected leaflets and thus over-estimate the efficacy of the fungicide on the preventive protection of new growth.

Although vapour pressure of Shirlan is higher than that of Ranman and comparable to metalaxyl-M, the preventive protection of new growth by Shirlan was lower than by Ranman and Ridomil (Tomlin, 2002; Mitani et al., 1998; Anonymous, 2002). These facts indicate that vaporisation of the fungicides does not have a pronounced effect in the control of late blight in our bioassay.

Because a high (artificially applied) disease pressure was used in the experiments, results cannot be translated directly into practical spraying strategies, because the effect of the fungicides might have been under-estimated in our bioassay. However, the relative efficacy of fungicides can be established satisfactorily in a bioassay. An experiment carried out in 1995 showed that the efficacy of fungicides in whole plant assays was largely comparable to efficacy in a bioassay on detached leaves (Evenhuis et al.,

1998). An experiment was carried out comparing the efficacy of fungicides on newly developing leaves both under field conditions and in a bio-assay in 2004. A strong correlation was found between protection levels in the bio-assay and under field conditions (unpublished data). Therefore results from our study could be used at the beginning of the season when new leaves are formed within spray intervals. Farmers should choose a fungicide which can protect new growth sufficiently, taking into account the crop growth rate. Applying fungicides that contain active ingredients with a curative efficacy to growth tips can also protect newly formed leaf layers (Johnson et al., 2000). In this study only the preventive protection of new growth by fungicides was investigated. Fungicides which move systemically through the plant or have good redistribution properties, combined with a high intrinsic efficacy are well suited to protect new growth.

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Disclaimer

Mention of trade names is for the purpose of specific information on the fungicides used and does not imply recommendation or endorsement by Applied Plant Research, Wageningen University Research Centre.

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