EFFICACY OF TWO FUNGICIDES FOR THE MANAGEMENT OF PHYTOPHTHORA INFESTANS ON POTATO THROUGH DIFFERENT APPLICATIONS METHODS ADOPTED IN CONTROLLED CONDITIONS

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Abstract

In an attempt to develop an alternative strategy, it is interesting to assess the best method of fungicide application, the position of the leaflets treated and the effect of a reduced dose on the infection level. Two fungicides, Ridomil Gold® and Copper Nordox® were evaluated to control mildew infection caused by Phytophthora infestans on potato (cv. Bintje). Hyphal growth of P. infestans was reduced by using Ridomil Gold® at 50 mg/L a.i (33.96%) compared to Copper Nordox® (54.28%) and untreated control (100%). Both Ridomil Gold® and Copper Nordox® indicated that spray application method on the lower side revealed a reduction of infection level. The other methods such as dipping and placing drop using Copper Nordox® revealed a decrease of mildew necrosis areas. The infection levels inhibition reached 53.94% and 56.62%, respectively (control=100%). It could be suggested that the use of fungicides Metalaxyl at low dose preventively could be efficient to control potato late blight in vitro and might be used for controlling this disease of potato plants under field conditions.

Keywords: Phytophthora infestans, potato, dose, fungicide application, position treated leaflets.

I. INTRODUCTION

Potato late blight caused by the oomycete Phytophthora infestans (Mont.) de Bary (1876) is economically the most important and most destructive potato disease worldwide (Mizubuti et al. 2007) where the losses of potato produced due to late blight are more than five billion USD annually (Tsedaley, 2014). In the middle of the 19th century the pathogen was introduced into the Europe. In the 1845, P. infestans caused the Irish potato famine, when a million people starved and another million and a half emigrated out of Ireland (Smart and Fry, 2001). Late blight can occur anytime in the growing season and is especially damaging during cool and wet weather. This pathogen can affect all plant parts. Young lesions are small and appear as dark, water-soaked spots. These leaf spots will quickly enlarge under favorite conditions and a white mold will appear at the margins. Stems are similarly affected and complete defoliation (browning and shriveling of leaves and stems) can occur. Infected potato tubers have a dry, corky rot that may be brown or reddish. Tubers are symptomless at the initial stages of infection but often develop symptoms in storage. The fungus produces a foul odor in both crops where infection is severe (Florian et al. 2012; Abreha et al. 2015).

Effective management of this disease requires implementation of an integrated disease management approach. Although the most important measures are chemical controls, P. infestans could be controlled by fungicide treatment (contact, penetrating or systemic products) that enables destroy, weaken or suppress the pathogen applied throughout the crop cycle (Muchiri et al. 2009). Until 1970, the most commonly used fungicides including Bordeaux mixture and other copper products. Copper fungicides can be highly effective if it is applied preventively and with the complete coverage of the foliar surfaces, including the undersides of leaves. It has a contact action,
hindering the development of the fungus on the leaf surface (Hermeziu and Hermeziu, 2014). Subsequently, control is based on the use of systemic fungicides, especially Metalaxyl and Propamocarb. Metalaxyl belongs to phenylamides, a group of systemic fungicides with preventive and curative activity that is limited to Oomycetes (García et al. 2008; Beninal et al. 2009; Savazzini and Galletti, 2015). All of the phenylamide fungicides inhibit RNA polymerization within the synthesis of ribosomal RNA, resulting in the inhibition of mycelial growth, haustorium formation and sporulation (Gisi, 2002). Metalaxyl resistance originates from a very small proportion of resistant strains in the pathogen populations that are present before exposure to the fungicide treatment. Selection pressures result in an increased frequency of resistant strains (Rahman et al. 2008).

Wiik (2002) launches the hypothesis in field trial that reducing the number of treatments and doses of fungicides at the beginning of the crop, in combination of other fungicides with synergistic effects. We implemented our study basing on reduction of fungicide doses to investigate the underpinning process of doses changes and to assess the effect of different fungicides on Phytophthora control using as plant material the potato plant. The objectives set out to (i) evaluate the effect of reduced dose on the infection level of P. infestans in vitro; (ii) compare the different fungicide applications and the position of treated leaflets.

II. MATERIAL AND METHODS

A. P. infestans MP-324 isolate response to fungicides

Fungal material

P. infestans isolate MP-324 (mating type A1) obtained from infected stems of potato plant in Poland were used in this investigation. Before initiating, P. infestans isolate were transformed onto PDA culture media plates and grown at 25°C in the dark for 10 to 15 days. The sporangia were harvested using 10 mL sterile water and a cotton swab. The suspensions were placed at 4-10ºC. After 2-3 hours, the zoospores were released. The suspensions were filtered again with filter, in which the bore diameter was 12 µm to remove sporangia wall. The zoospore suspensions were adjusted to 4.10^4 zoospores/ml with a hemacytometer (Bruck et al. 1981).

Vegetal material

Potato variety tested is Bintje provided by Pr. José Ignacio Ruiz de Galarreta Gomez (laboratory Neiker Tecnalia, Bilbao Spain). This variety presented as mini-tubers of potatoes (2nd generation) (Table 1), that was planted in greenhouse early April in pots with 42 pots (three mini tubers for each) and irrigated three times per week and no chemical treatment was performed. Detached 60 days-old leaflets are used to evaluate the mildew infection level after washed and surface-sterilized in 1% sodium hypochlorite solution for 2 min (Ryckmans, 2011).

<table>
<thead>
<tr>
<th>Table 1. Characteristics of the potato cv. Bintje.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genetic origin</td>
</tr>
<tr>
<td>Sensibility to late blight</td>
</tr>
<tr>
<td>Breeder</td>
</tr>
<tr>
<td>Maturity</td>
</tr>
</tbody>
</table>

B. Treatment and incubation

Two commercial formulations of fungicides representing 3 chemical groups were in order to assess a three concentration of 10, 50 and 100 mg of active ingredient per liter (a.i.l^1) and sterile water used as control (Table 2) (Cooke et al. 2006).

<table>
<thead>
<tr>
<th>Chemical Group</th>
<th>Fungicide</th>
<th>Trade name</th>
<th>Manufacturer</th>
<th>Formulation</th>
<th>Recommended dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenylamides</td>
<td>Metalaxyl-M 4%</td>
<td>Ridomil Gold</td>
<td>Syngenta</td>
<td>40 g/kg or 640 g/kg WG</td>
<td>2.5 kg/ha</td>
</tr>
<tr>
<td>and Carbamates</td>
<td>and 64% Mancozeb</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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In this study, three applications methods of fungicides were adopted (dipping, placing of fungicide drop and spraying). The experiment was carried out in a completely randomized design, with three repetitions. Each replication consisted of 27 potato leaflets.

**Dipping treatment**

Leaflets potatoes were dip treated for 15 min in individual fungicide in buckets (Figure 1a) (Yang and Clausen, 2007).

**Placing of fungicide drop treatment**

On the lower side of the detached leaflets, drop fungicide of Ridomil Gold® was inoculated at different concentration with sterile micropipette at volume of 40 µl. The same experiment is established for Copper Nordox® (Figure 1c) (Goufo et al. 2008).

**Spraying treatment**

The upper side of potato leaflets were sprayed to runoff with the desired concentration of fungicide treatments and then allowed to dry for 1-2 hrs, similarly, on the lower side of leaflets. Spray distance of 130 mm and spray angle of 90° were chosen (Figure 1b) (Bruck et al. 1981; Cohen et al. 1993). Treated potato leaflets were placed on wet filter paper in Petri dishes, and were inoculated with *P. infestans* 24 hrs later. The treated leaflets were incubated at temperature of 18°C and a photoperiod of 18 hrs.

![Figure 1. Applications methods of fungicides: a. dipping, b. spraying, c. placing of drop.](image)

**Inoculation**

After 24 hr of treatment, 40 µl of the sporangial suspension (4.10⁴ zoospores/ml) was deposited on the lower side of the detached leaflets, and incubated at 18°C at a photoperiod of 18 hr (6 hr dark). The concentration value of suspension was determined by a Malassez slide (Barquero et al. 2005).

**C. Infection level evaluation**

Ten days later, the infection level was expressed in terms of percentage of mycelial growth inhibition and calculated according to the following formula: \( N = \left( \frac{T}{C} \right) \times 100 \); \( N \): level of infection. \( T \): surface of leaf with mycelium of *P. infestans*. \( C \): total leaf area.

**D. Experimental design and data analysis**

The trials were conducted according a completely randomized design with 4 treatments repeated 3 times. Therefore, the statistical analysis was performed using GEN-STAT. 12. The comparison of means was determined according the LSD test (Least Significant Difference test = Newman-Keuls’s test) to \( P-value = 0.05 \).

**III. RESULTS AND DISCUSSION**

*P. infestans* MP-324 isolate response to fungicides
Evaluation of the appropriate dose

Highly significant differences (P-value < 0.001) were observed among different doses compared to the control. Both Ridomil Gold® and Copper Nordox® inhibited in vitro sporangia germination at 50 and 100 mg/L a.i. At these concentrations, Ridomil Gold® consistently suppressed spore germination more effectively than Copper Nordox® with values of 33.96% and 31.37%, respectively (Table 3).

Table 3. Infestation level by P. infestans isolate using different doses of fungicides (Ridomil®Gold and Copper®Nordox).

<table>
<thead>
<tr>
<th>Doses (mg.L⁻¹)</th>
<th>Ridomil®Gold level of infection (%)</th>
<th>Copper Nordox level of infection (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>100 a⁵</td>
<td>100 a</td>
</tr>
<tr>
<td>10</td>
<td>76.82 b</td>
<td>82.21 b</td>
</tr>
<tr>
<td>50</td>
<td>33.96 c</td>
<td>54.28 c</td>
</tr>
<tr>
<td>100</td>
<td>31.37 c</td>
<td>55.08 c</td>
</tr>
</tbody>
</table>

L.S.D. (5%) = 18.32  L.S.D. (5%) = 24.95

³ Infection level means of 27 leaflets (3 replicates).
⁵ Column followed by the same letter are not significantly different according LSD test at 5%.

The exclusive usage of copper compounds cannot ensure adequate protection levels while good effectiveness was obtained with several active ingredients (chlorothalonil, cyazofamid, dimethomorph, fenamidone, fluazinam, iprovalicarb, metalaxyl-M, QoI, zoxamide) employed in different protection schedules (Berardi et al. 2006; Dongiovanni et al. 2006). The mixtures of propamocarb HCL + mancozeb and fenamidone + mancozeb were effective in controlling late blight compared with the application of mancozeb only or the untreated control. However, none of the fungicide mixtures was more effective than metalaxyl. The relatively enhanced disease control obtained with fungicide mixtures under moderate to severe late blight conditions suggests that fungicide mixtures could be a promising approach for late blight management in established epidemics compared with the use of a protectant fungicide (Muchiri et al. 2009). Metalaxyl-treated plants sustained less late blight damage compared with the plants exposed to fungicide mixtures. It was reported that metalaxyl is effective for controlling diseases incited by oomycetes as it is absorbed by the leaves and roots under various environmental conditions (Easton and Nagle, 1985). This may be the case in our experimental setting where controlled conditions exist, but metalaxyl is still effective on late blight. On other late blight research reports, which showed that mancozeb applied as a protectant was effective in reducing the impact of late blight (Namanda et al. 2004).

Our results are in agreement with other research for the dose of Ridomil Gold® MZ 68 WG. In fact, García et al. (2008) has reported that the fungicide dose to discriminate between sensitive and resistant strains could be at 50 and 100 mg L⁻¹ a.i. The work performed by Spits et al. (2007) showed that in the second half of the season tuber blight must be taken into consideration, which limits possibilities to reduce the dose rates for several but not all varieties.

Efficacy of Ridomil Gold® on the infection level of P. infestans

No significant differences in late blight severity among leaflets potato treated with Metalaxyl and Mancozeb were observed for the different fungicide application methods (P-value > 0.05). Otherwise, no significant effect of the level of disease infection between the interaction of dose and application method.

Application of fungicide using spray application method on the lower side revealed a significant (P-value < 0.05) reduction of infection level (42.22%), whereas the interaction “dose and position of the leaflets” generated no significant effect of infection level (P-value > 0.05) (Table 4).

Table 4. Infestation level by P. infestans isolate at different positions of treated potato leaflets (upper and lower side).

<table>
<thead>
<tr>
<th>Positions of the treated leaflets</th>
<th>level of infection (%)⁶</th>
</tr>
</thead>
<tbody>
<tr>
<td>Upper side</td>
<td>79.91 a⁵</td>
</tr>
<tr>
<td>Lower side</td>
<td>42.22 b</td>
</tr>
</tbody>
</table>

L.S.D. (5 % level) = 13.95

⁶ Infection level means of 27 leaflets (3 replicates)
Whatever the methods of application (dipping, placing of drop and spraying) Ridomil Gold® was penetrated the leaflets and was mobilized into potato. Consequently, the fungicide banned the synthesis of some or more specific stages of metabolism (nucleic acids, lipids and amino acids) of zoospores (Pérez and Forbes, 2008). Thus, this fungicide which is double-acting is able to create a physical barrier preventing the germination and penetration of the inoculums.

**Efficacy of Copper Nordox® on the infection level of *P. infestans***

The results presented in table 5 indicated that methods of fungicides applications had a significant difference (*P*-value < 0.05). While, there was no significant difference for interaction “dose * method application of fungicides”. Thus, the lowest infestation level was generated with application method by dipping and placing of drop, in comparison with spraying of 85.86%.

*Table 5. Infestation level by *P. infestans* isolate on treated potato leaflets with various application methods.*

<table>
<thead>
<tr>
<th>Application Methods</th>
<th>level of infection (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spraying</td>
<td>85.86 a&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Placing of drop</td>
<td>56.62 b</td>
</tr>
<tr>
<td>Dipping</td>
<td>53.94 b</td>
</tr>
<tr>
<td>LSD (5%) = 21.61</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> Infection level means of 27 leaflets (3 replicates).  
<sup>b</sup> Column followed by the same letter are not significantly different according LSD test at 5%.

According to the results of infection level, spraying method on the lower side of leaflets had a good effect compared with treatment of the upper side. The highest infestation level was 98.94%, followed by 47.96%. In the case of position leaflets treated with Copper Nordox®, highly significant effect (*P*-value < 0.001) was detected (Table 6).

*Table 6. Infection levels of MP-324 isolate on potato leaflets at different positions (upper and lower side).*

<table>
<thead>
<tr>
<th>Position of treated leaflets</th>
<th>level of infection (%)&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Upper side</td>
<td>98.94 a&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Lower side</td>
<td>47.96 b</td>
</tr>
<tr>
<td>LSD (5%) = 9.2</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> Infection level means of 27 leaflets (3 replicates).  
<sup>b</sup> Column followed by the same letter are not significantly different according LSD test at 5%.

Spraying on the lower side of leaflets potatoes with dose 50 or 100 mg/L has reduced the infection of *P. infestans* with level values of 19.51% and 19.12% respectively. While, spraying method on the upper side exhibited the most symptom expression with 100% of infection level (Figure 2).
Visker et al. (2003) reported that leaf position of potato proves to be most significant of all considered factors and to have the largest effect on the linear lesion growth rate of *P. infestans*. Copper is very important to maintain a good layer where the treatment will take place. Subsequently, Nordox Cuivre® (copper oxide 75%) has created a physical barrier (Fernández et al. 1999). This fact could be responsible of denaturation of the enzymes of the respiratory chain by affecting the zoospores structure of *P. infestans* by acting on the different stages of germination and penetration (Schwinn and Margot, 1991). Mixtures of systemic and protectant fungicides were equally effective for the control of late blight in various experiments (Samoucha and Gisi, 1987). Mixtures have also been shown to be very effective when applied prior to infection (Cianchetti et al. 2000).

**IV. CONCLUSION**

This study aimed to ascertain the effectiveness of fungicides in the control of *Phytophthora infestans* and combined with application method and position of the leaflets treated. The degree of potato downy mildew attack in controlled conditions was obviously influenced by concentration of active ingredient. Present work showed the efficiency of tested fungicides applied with lower dose (50 mg/L a.i.1\(^{-1}\)). Thus, it appears that the tested active ingredients could be recommended at its lowest dose to control the disease. This information might be valuable to implement spraying programs considering the optimum number of applications and fungicides selection in order to achieve both satisfactory control of the disease and reduced risk of resistance.

**BIBLIOGRAPHY**


Phytophthora infestans


