RESISTANCE TO METALAXYL OF PRYTOPHTHORA INFESTANS ISOLATES OCCURRING IN POLAND IN 2006–2010

S. Sobkowiak, J. Śliwka, M. Chmielarz, R. Lebecka and E. Zimnoch-Guzowska

Abstract

In total 517 Phytophthora infestans isolates were assessed for resistance to metalaxyl. They were collected from 15 regions of Poland in 2006–2010. The most of isolates were assessed in provinces: podkarpackie (130 isolates: 107 sensitive, 19 intermediate and 4 resistant), mazowieckie (116 isolates: 96 sensitive, 12 intermediate and 8 resistant), wielkopolskie (77 isolates: 39 sensitive, 13 intermediate and 25 resistant) and malopolskie (53 isolates: 39 sensitive, 6 intermediate and 8 resistant). The majority of evaluated isolates was sensitive to metalaxyl (72.3%). The frequency of isolates resistant to metalaxyl was the greatest in 2006 (33%) and 2007 (20%) and the lowest in 2008 (6.4%) and 2010 (3.3%). The intermediate isolates ranged from 9.6% in 2006 to 16.7% in 2007. The occurrence of resistant to metalaxyl P. infestans isolates indicates that this chemical may not always be effective against late blight.

Key words: metalaxyl, Phytophthora infestans, resistance of isolates

Introduction

Metalaxyl, a fungicide belonging to the group of phenylamides, was used to fight late blight on potato and tomato for the first time to in the seventies of the twentieth century. Resistance to metalaxyl may be a phenotype marker during the...
studies of *Phytophthora infestans* isolates (Fabritius et al. 1997), along with mating type and virulence on the Black’s differentials. Previous studies indicate a fluctuation of the number of resistant *P. infestans* isolates, depending on the application of metalaxyl on the field (Sobkowiak and Lebecka 2006). Many authors indicated variability in *P. infestans* populations for resistance to phenylamides (Goodwin et al. 1996, Miller et al. 1997, Cohen 2002).

*Phytophthora infestans* isolates, that cause of potato late blight, differ in their resistance to phenylamide (Goodwin et al. 1996). Evaluation of resistance to metalaxyl of *P. infestans* isolates collected in Poland in 1995–2005, gathered in the collection of Plant Breeding and Acclimatization Institute – National Research Institute, Research Division at Młochów was presented at the conference in Kołobrzeg in 2006 (Sobkowiak and Lebecka 2006). In this study fluctuation of resistance of isolates depending on the application of phynloamide in the field was identified. According to Goodwin et al. (1996) and Kapsa (2001), the cause of emergence of the resistant pathogen in unprotected fields is the migration from the protected fields.

This paper presents the resistance to metalaxyl of *P. infestans* isolates collected from 15 Polish provinces in 2006–2010.

**Materials and methods**

In 2006–2010 517 isolates from 15 Polish provinces (Table 1) were collected and evaluated for resistance to metalaxyl according to the method described by Bakonyi et al. (2002). Potato leaves with symptoms of late blight were collected from experimental plots (137 isolates), production fields (305) and garden plots (75). Rye agar medium with metalaxyl at concentrations of 5 and 100 mg/l was prepared in Petri dishes with a diameter of 85 mm. Control were Petri dishes with the rye agar medium without metalaxyl. In the middle of dishes with the artificial medium, 9-mm discs cut out from fresh overgrown mycelium were placed. The cultures were then incubated in darkness at a constant 16°C temperature. Measurements of the diameter of colony growth of tested isolates were performed when the appropriate response to metalaxyl of the standard isolates were revealed. In each year of the study, the following standard isolates were used: two sensitive isolates MP511 74001 (The Netherlands) and MP512 28/05 (United Kingdom), two intermediate isolates MP538 01/03/2002 and MP539 04/13/2002 (Hungary) and resistant isolate US-8 (United States of America). The colony growth was expressed as a percent, and was calculated on the basis of measurements of pathogen colony diameter on each metalaxyl medium in relation to colony growth in control dishes (Phot. 1). The evaluated isolates were assigned to each category of resistance to metalaxyl according to the following scale:

1) isolates sensitive: the relative growth of colonies < 40% on medium containing 5 and 100 mg/l of metalaxyl,
Resistance to metalaxyl of *Phytophthora infestans* isolates occurring in Poland...

2) isolates intermediate: the relative growth of colonies ≥ 40% on medium containing 5 mg/l of metalaxyl and relative growth of colonies < 40% on medium containing 100 mg/l of metalaxyl,

3) resistant isolates: the relative growth of colonies ≥ 40% on medium with metalaxyl in both concentrations.

**Results and discussion**

The majority of evaluated *P. infestans* isolates, collected in 2006–2010 was sensitive to metalaxyl (72.3%; Table 2). The highest percentage of most isolates sensitive to metalaxyl (80%) was found in Poland in 2008 and 2010, and the lowest in 2006 (57.4%). In previous studies, the majority of the isolates tested in 1995–2005 was also sensitive to metalaxyl (85.3% of 204 evaluated; Sobkowiak and Lebecka 2006). Much less of isolates sensitive to metalaxyl (from 27.6 to 53.9% of 602 tested) recorded Kapsa (2001) in Poland in 1995–1999. A similar frequency of sensitive isolates to metalaxyl was reported in the European Union (EU) in the European Database „Eucabligh” in 2006–2009 – from 23 to 49% among the total of 1682 evaluated isolates (www.eucabligh.org). All of 257 *P. infestans* isolates evalu-
ated in the neighbouring Czech Republic in 2005–2006 were classified as sensitive to metalaxyl (www.eucablight.org).

Results of this study showed that the occurrence of isolates resistant to metalaxyl in Poland was the largest in 2006 (33%) and 2007 (20%) and the lowest in 2008 (6.4%) and 2010 (3.3%). In a study conducted in Poland in 1995–2005 there were 23 isolates resistant to metalaxyl that equaled to 11.2% (Sobkowiak and Lebecka 2006). In Poland, the higher percentage of isolates resistant to metalaxyl (from 27.6 to 53.9) in fields protected against P. infestans was recorded in 1995–1999 (Kapsa 2001). Higher frequency of resistant isolates was also found in the EU. In 2006–2009 they accounted from 32 to 75% (www.eucablight.org).
In all the study years, the share of the intermediate isolates was similar and also the least frequent among resistance groups: ranging from 9.6% in 2006 to 16.7% in 2007. Sobkowiak and Lebecka (2006) showed that in 1995–2005 frequency of the intermediate isolates in Poland was lower (3.5%) in comparison with the results presented in this paper. In other studies, the range of occurrence of isolates characterized by an intermediate phenotype in 1995–1999 in Poland was wide and ranged from 4.8% to 32.4% (Kapsa 2001). In 2006–2009, in the EU countries the presence of intermediate isolates ranged from 1% to 21% (www.eucablight.org).

In France, 811 isolates of *P. infestans* were examined in 2006–2009. Among them those resistant to metalaxyl balanced from 40% to 78%, intermediate from 1% to 31%, and sensitive from 19% to 29% (www.eucablight.org). In the studies, resistant and intermediate isolates were occurring less frequently. In earlier studies, carried out in 1995–2005 (Sobkowiak and Lebecka 2006) frequency of metalaxyl resistant and sensitive isolates were at a similar level compared with the results of this study, while intermediate isolates occurred rarely.

Genetic basis of the resistance to metalaxyl remains not fully defined. Probably this resistance is controlled by a dominant gene (Lee et al. 1999) or incompletely dominant gene (Fabritius et al. 1997). This resistance can be also determined by multiple loci (Cooke and Lees 2004). According to Kapsa (2001), resistant forms of pathogen to metalaxyl may be formed as a result of intensive chemical protection.

During five years of research, the majority of *P. infestans* isolates evaluated for resistance to metalaxyl has been tested in provinces: podkarpackie (130 isolates: 107 were susceptible, 19 intermediate and only 4 resistant), mazowieckie (116 isolates: 96 susceptible, 12 intermediate and 8 resistant), wielkopolskie (77 isolates: 39 susceptible, 13 intermediate and 25 resistant) and malopolskie (53 isolates: 39 susceptible, 6 intermediate and 8 resistant; Table 1).

In the potato production fields fungicides were used while on experimental plots these chemicals were not applied. However, no information was obtained on the use of fungicides on garden plots. In experiments conducted in 2006–2010

### Table 2

<table>
<thead>
<tr>
<th>Year of evaluation</th>
<th>Isolates</th>
<th>resistant</th>
<th>intermediate</th>
<th>sensitive</th>
<th>total number</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>number</td>
<td>%</td>
<td>number</td>
<td>%</td>
<td>number</td>
</tr>
<tr>
<td>2006</td>
<td>31</td>
<td>33.0</td>
<td>9</td>
<td>9.6</td>
<td>54</td>
</tr>
<tr>
<td>2007</td>
<td>6</td>
<td>20.0</td>
<td>5</td>
<td>16.7</td>
<td>19</td>
</tr>
<tr>
<td>2008</td>
<td>5</td>
<td>6.4</td>
<td>9</td>
<td>11.5</td>
<td>64</td>
</tr>
<tr>
<td>2009</td>
<td>28</td>
<td>12.4</td>
<td>34</td>
<td>15.1</td>
<td>163</td>
</tr>
<tr>
<td>2010</td>
<td>3</td>
<td>3.3</td>
<td>13</td>
<td>14.4</td>
<td>74</td>
</tr>
<tr>
<td>Total</td>
<td>73</td>
<td>14.1</td>
<td>70</td>
<td>13.5</td>
<td>374</td>
</tr>
</tbody>
</table>
there was no association between the use of fungicides and occurrence of resistant forms of P. infestans to metalaxyl. However, Cooke and Lees (2004) reported the influence of fungicides on the occurrence of isolates resistant to metalaxyl. These results are consistent with the results obtained in 1995–2005 by Sobkowiak and Lebecka (2006) where the higher frequency of metalaxyl resistant isolates came from fields protected against potato late blight. Relatively, the largest number of resistant P. infestans isolates occurred in western Poland in provinces lubuskie (57%), wielkopolskie (32%) and kujawsko-pomorskie (30%) (Table 1).

In the present study, however, there was no association between the presence of metalaxyl resistant P. infestans isolates in the field. Resistant to metalaxyl isolates on the unprotected plantations may be the result of migration of P. infestans (Goodwin et al. 1996, Deahl et al. 2002).

In this experiment, the standard P. infestans isolates (two sensitive: MP511 and MP512, two intermediate: MP538 and MP539 and resistant US-8) in all tests reacted according to the expected category of resistance to metalaxyl.

**Conclusions**

1. In 2006–2010 in Poland the majority of tested P. infestans isolates were sensitive to metalaxyl.
2. The occurrence of P. infestans isolates resistant to metalaxyl indicates that fungicides from the group of phenylamides may not be fully effective in control of late blight.

**Streszczenie**

ODPORNOŚĆ NA METALAKSYL IZOLATÓW PHYTOPHTHORA INFESTANS WYSTĘPUJĄCYCH NA TERENIE POLSKI W LATACH 2006–2010

W latach 2006–2010 oceniono 517 izolatów Phytophthora infestans pod względem ich odporności na metalaksyn. Zebrano je z 15 województw Polski. Najwięcej izolatów oceniono w województwach: podkarpackim – 130 izolatów (107 wrażliwych, 19 pośrednich i 4 odporne), mazowieckim – 116 izolatów (96 wrażliwych, 12 pośrednich i 8 odpornych), wielkopolskim – 77 izolatów (39 wrażliwych, 13 pośrednich i 25 odpornych) i małopolskim – 53 izolaty (39 wrażliwych, 6 pośrednich i 8 odpornych). Większość ocenianych izolatów była wrażliwa na metalaksyn (72,3%). W prezentowanych badaniach frekwencja izolatów odpornych na metalaksyn była największa w 2006 (33%) i 2007 roku (20%), a najmniejsza – w 2008 (6,4%) i 2010 roku (3,3%). Frekwencja izolatów pośrednich zawierała się w przedziale od 9,6% w 2006 roku do 16,7% w 2007 roku. Występowanie odpornych na metalaksyn izolatów P. infestans wskazuje, że ta substancja aktywna nie zawsze jest skuteczna w zwalczaniu zarazy ziemniaka.
Resistance to metalaxyl of Phytophthora infestans isolates occurring in Poland... 35

Literature


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