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THE GENERATION OF RESISTANCE TO METALAXYL IN *PHYTOPHTHORA INFESTANS* (MONT.) DE BARY

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INTRODUCTION

Potato late blight, caused by *Phytophthora infestans* (Mont.) de Bary, is the economically most important potato disease in Serbia. The new genotypes of P*. infestans* are being detected in the countries from which a large quantity of potato seed has been imported to Serbia for its own potato production, in particular from Holland, Germany and Hungary. Control of *P. infestans* in Serbia has relied on intensive use of fungicides often without any appropriate programs. Discovery and development of the first systemic fungicides from phenylamides class was a significant improvement and one of the most important contributions to agrochemical industry. However, the intensive use of metalaxyl led to the rapid selection of metalaxyl-resistant strains of *P. infestans* in Europe within one year of its introduction. The objective of this study was to test the sensitivity of *P. infestans* isolates collected in Serbia over the period 2005-2007, to the metalaxyl and generate resistance in *P. infestans* to metalaxyl using mycelial adaptation.

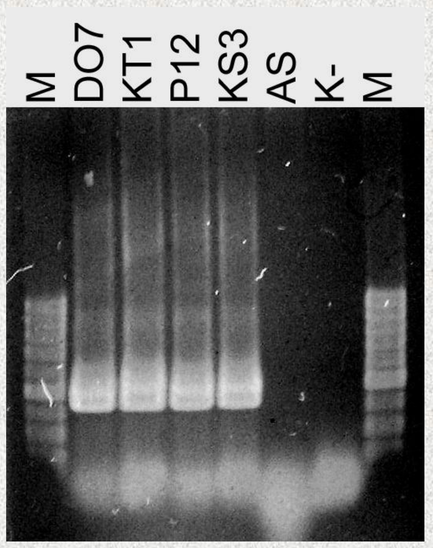
MATERIALS AND METHODS

Samples collection and isolation

Isolates were collected from the major potato growing regions in Serbia during 2005 and 2007 growing season (Table 1). *P. infestans* was isolated from the infected potato leaves collected from eight different locations according to the methods described by Mukalazi et al. (2001) and Zhu et al. (2008). identity of isolates of *P. infestans* was confirmed by polymerase chain reaction (PCR) using species-specific primers (Tooley, 1998) and their morphological traits according to Erwin and Ribeiro (2005). The isolates were kept on potato dextrose agar (PDA) at 5^{o}C in the Culture Collection of the Institute of Pesticides and Environmental Protection, Belgrade.

Table 1. *Phytophthra infestans* isolates and their origin

Code of isolate	Location	Year of isolation
DO7	Dobanovci	2005
GU6	Guča	2005
KS3	Kosjerić	2005
KS2	Kosjerić	2005
KT1	Kotraža	2006
KT2	Kotraža	2006
KV1	Kraljevo	2006
KV5	Kraljevo	2006
P11	Prijepolje	2007
P12	Prijepolje	2007
PR1	Prilike	2007
VK1	V. Kamenica	2007



Amplicon visualization 456 bp size. M: 100 bp size marker with fragments in bp from top to bottom: 1000, 900, 800, 700, 600, 500, 400, 300, 200 and 100; DO7 (Dobanovci) KT1 (Kotraža), P12 (Prijepolje) KS3 (Kosjerić), A - *Alternaria solani*, K - negative control (water)

Resistance generation: mycelial selection, calculation of EC_{50} and resistance factor (RF)

Colonized agar plugs, 4 mm in diameter, were transferred from the margin of an actively growing culture of each isolate, mycelium-side down, onto modified rye B agar amended with 10.0 mg/l metalaxyl and incubated at 21°C in the dark with three replicate plates per concentration. The metalaxyl concentration used for selection was previously found to be highly inhibitory, yet sublethal for all isolates of *P. infestans* examined. Final colony diameter was measured after 11 days. Mycelial fragments of the tested isolates were transferred five times onto rye B agar amended with 10.0 mg/l of metalaxyl. After that, the selected isolates were tansferred 10 times onto rye B agar without fungicide and EC_{50} values were calculated (Stein, 2002).

The EC_{50} (fungicide concentration which inhibits mycelial growth by 50%) was determined for each isolate and data on fungicide concentration and relative inhibition were analysed using probit analysis, according to Finney (1971).

The resistance factor (RF) was expressed as the ratio of the EC_{50} and the lowest EC_{50} of the isolates tested (Gouot, 1994). The level of resistance factor (RF) was expressed according to following scale (Gouot, 1994):

- **RF < 3 – sensitive isolates;**
- **RF = 3 – 20 > – moderate resistant isolates;**
- **RF = 100 > – high resistant isolates.**

RESULTS

Sensitivity of *P. infestans* isolates to metalaxyl are shown in Table 2. Among the 12 *P. infestans* isolates, the KS3 isolate showed the highest basic sensitivity (EC_{50} = 0.3 mg/l), whereas the P11 isolate had the lowest values (EC_{50} = 3.9 mg/l). The EC_{50} values of the remaining 10 isolates were between 0.4 and 2.7 mg/l (Table 2). All isolates tested exhibited RF < 20. Resistance factors of metalaxyl for 50% isolates were below 3, and for 41.7% ranging from 3 to 13.

Table 2. Calculated EC_{50} values and resistance factors for isolates of *Phytophthora infestans* and the same isolates repeatedly cultured on metalaxyl-amended medium

Isolate code	Basic sensitivity (BS)		Subcultured sensitivity (SS)			
			Five subcultures		Ten subcultures	
	EC_{50} (mg/l)	RF	EC_{50} (mg/l)	RF	EC_{50} (mg/l)	RF
DO7	1.7	5.7	31.07	12.9	23.5	11.2
GU6	1.6	5.3	20.06	8.5	15.6	7.4
KS3	0.3	1.0	2.4	1.0	2.1	1.0
KS2	0.8	2.7	9.2	3.8	7.3	3.5
KT1	0.9	3.0	14.4	6.0	12.6	4.1
KT2	0.8	2.7	12.3	5.1	9.5	4.5
KV1	0.7	2.3	13.6	5.6	11.5	5.5
KV5	0.4	1.3	10.9	4.5	8.9	4.2
P11	3.9	13.0	95.6	39.8	82.5	39.3
P12	2.7	9.0	81.9	34.1	71.4	34.0
PR1	2.2	7.3	80.3	33.4	69.3	33.0
VK1	1.1	1.0	29.5	12.9	26.6	12.7

EC_{50} - Fungicide concentration which inhibits mycelial growth by 50%; RF – The resistance factor was expressed as the ratio of the EC_{50} and the lowest EC_{50} for the isolates tested;

The EC_{50} values and resistance factor for all isolates on medium amended with metalaxyl at 10.0 mg/l increased with subculture number for all isolates (Table 2). With most isolates, the largest increase occurred between the initial and fifth subcultures, whereas changes at subsequent subcultures generally remained the same. Isolates P11, P12, PR1 and DO7 had highest EC_{50} values for (SS) than for (BS). For all isolates the calculated resistance factor (RF) was numerically larger for the (SS) than for the (BS).