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POPULATIONS OF *PHYTOPHTHORA INFESTANS* IN THE SLOVAK REPUBLIC IN 1996–2003

ABSTRACT

The effective management of protection against *Phytophthora infestans* must be established on the perfect knowledge of the pathogen. From a practical point of view this means identification of races and mating types as well as the pathotypes resistant to systemic compounds of fungicides. The main goal of our project was to characterize populations of late blight, especially in the most important area of seed potato production. Recognition of races and mating types and evaluation of resistance to metalaxyl of the isolates in potato–growing area were performed in laboratory tests. Isolates of late blight for laboratory tests were collected in commercial and research potato crops in the regions of Poprad and Kežmarok. The first occurrence of metalaxyl–resistant strains was noticed in 1999, and the greatest incidence was recorded in 2000. In the following years a significant decrease was observed. This was due, among others, to omitting fenylamide fungicides in crop protection in our region. The occurrence of isolates virulent to potato with *R2*, *R5*, *R9* and *R11* genes was noted neither in 1996 nor in 1997, and tested isolates had maximum six virulence factors. Since 1999, the occurrence of isolates with seven, eight or even ten virulence factors has been recorded. Presence of A2 mating type was not confirmed in our area until 2002. In 2003, more A2 than A1 isolates were found. It is likely that problems concerning the ability of oospores to survive in soil as well as sexual reproduction of the pathogen may appear also in our potato–growing area.

Key words: late blight, mating type, metalaxyl, *Phytophthora infestans*, race

INTRODUCTION

Late blight is the main phytopathological problem in growing the potato all over the world. Fungus–like *Phytophthora infestans* has run over considerable genetic changes in the last few years. Plant protection needs to be highly reliable. Meanwhile, the occurrence of resistance to the effective compounds of systemic fungicides has recently been reported in many articles. The new populations show broad–spectrum virulence that is considered to be responsible for the collapse of late blight resistant clones (including resistant cv. Alva in Slovakia in 1999). The changes in population of late blight are related to earlier start of attack, stem lesions, higher pathogenicity and aggressiveness, a higher

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frequency of primary infections, resistance to phenylamides, occurrence of oospores and stronger tuber infections.

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MATERIAL AND METHODS

Isolates of late blight for laboratory tests were collected in the years 1996–2003 in commercial and research potato crops in the regions of Poprad and Kežmarok – the seed potato production area. Each year, 40–50 isolates were tested. The detailed results of tests can be found in Forišeková (1997, 2002).

Isolation from naturally infected material

The procedure described by Dowley and O'Sullivan (1985) was applied. Infected tissues were surface sterilised in 0.5% NaOCl for 3 min, blotted on a sterile paper towel, rinsed with sterile distilled water and plated on rye agar. The isolation plates were incubated in darkness for 48–72 h at 15°C. Spores and mycelia from the infected tissue pieces were examined with the aid of a dissecting microscope, then transferred to fresh rye media amended with antibiotics (100 ppm of penicillin G, pimaricin and polymyxin each) and incubated in darkness at 18°C. Agar plugs of 4–5 mm diameter containing each fungal isolate were transferred to unamended rye agar to initiate pure culture.

Culture maintenance

P. infestans isolates were maintained in darkness at 20–21°C on rye agar medium. Mycelial mass transfers to fresh media were made at 30–day intervals with plugs cut from the outer zone of active hyphal growth.

In vitro response to metalaxyl

In vitro response to metalaxyl of each isolate was assessed by comparing radial mycelial growth in metalaxyl amended rye agar media to growth in metalaxyl free controls. The method described by Deahl *et al.* (1993) was used. Metalaxyl was added to agar prior to autoclaving to final yield concentrations of 0.10 ppm and 100 ppm. For each test, inocula consisted of agar plugs of 5 mm diameter cut from the outer zones of active growth from cultures aged 10–20 days. The plugs were placed on fresh media with or without metalaxyl amendment, followed by incubation at 21°C in darkness. Measurements were made after 5–7 days for faster growing isolates and after 7–10 days for slower growing ones. All *P. infestans* isolates were designated as metalaxyl–resistant (MR), in–

termediate (MI) or sensitive (MS), based on the rate of growth (MR >60%, MI 10–60%, MS <10% growth).

Races

Races were determined by inoculation of detached leaflets of *R*–gene differential series from CIP, Peru, grown in a glasshouse. One 20 µl droplet of inoculum was placed on the abaxial surface of each leaf and the dishes were then covered and incubated at 18°C for 7 days with a 16 h day length. Positive reaction consisted of complete necrosis and sporulation on leaf surface.

Mating type

Mating type determination according to Fry and Smart (1999) was done *in vitro*. A small agar plug with mycelium of the sample was placed in the centre of a plate. On one plate an agar plug with mycelium of known A1 strain was placed on either side of the sample. The procedure was repeated on other plate, this time using a known A2 strain. Obser–vations on the presence of oospores were conducted for 5–10 days. If oo–spores were seen on the plate with known A1 but not on the plate with known A2, the sample was considered to be A2. If opposite situation was observed, the sample was considered to be A1.

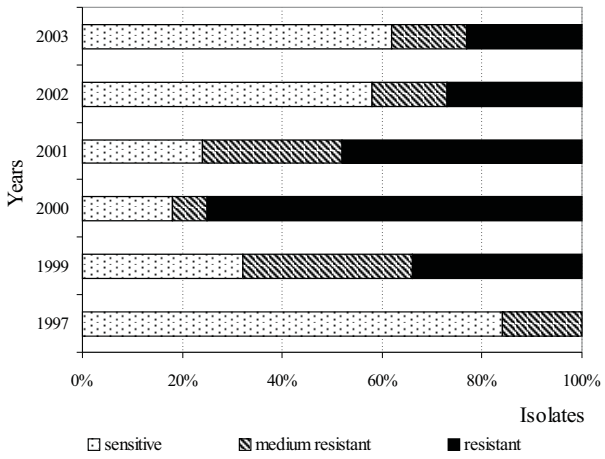

Fig. 1. Resistance to metalaxyl of *Phytophthora infestans* isolates collected the Slovak Republic in 1997–2003

RESULTS AND DISCUSSION

The appearance of resistant isolates was confirmed for predominantly metalaxyl–sensitive isolates (Fig. 1). The levels of resistance to metalaxyl among isolates obtained from blighted plant tissues were similar to those reported in other studies. In Slovakia only sensitive iso–

lates were found till 1996. The first appearance of metalaxyl–resistant strains was confirmed in 1999 (34% resistant isolates), the greatest incidence was recorded in 2000 (75% of isolates). In the following years a significant decrease (2001 – 48%, 2002 – 27%, 2003 – 23%) was observed. This was due, among others, to omitting fenylamide fungicides in crop protection in our region.

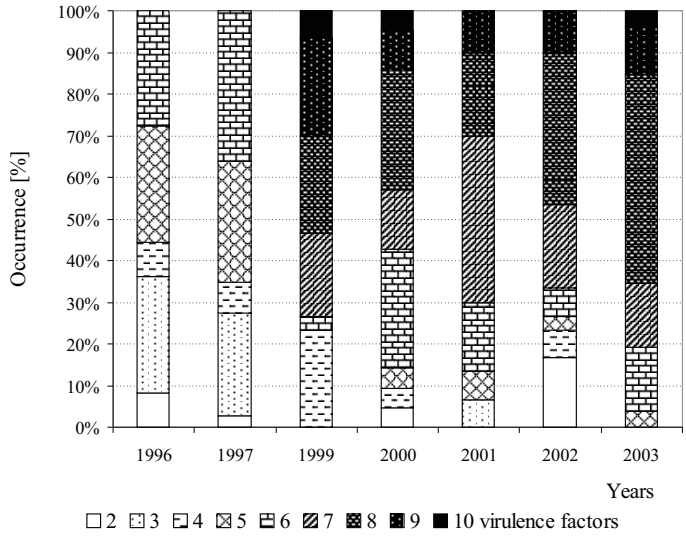

Fig. 2. The occurrence of *Phytophthora infestans* isolates with various numbers of virulence factors in Slovak population in 1993–2003

Identification of late blight races started at our institute in 1996. Tested isolates had maximum six virulence factors (Fig. 2). More complex isolates occurred in 1999 when new races were detected in our region, and the attack of late blight was very strong. That year, the resistance of cv. Alva was broken for the first time. Since 1999 the occurrence of isolates with seven, eight or even ten virulence factors has been noted (apart from virulence factor 9). In the years 2001 and 2002 isolates with maximum nine virulence factors were detected (besides virulence factors 5 and 9). In 2003, again only the isolate virulent to potato with *R9* gene was not detected in our area.

Presence of the A2 mating type was not confirmed in our area until 2002. In 2002 the A2 mating type was identified in 15.5% of tested isolates, in 2003 the proportion of A2 isolates (62.5%) was greater than that of A1 isolates (Fig. 3).

It is likely that problems concerning both the survival of oospores in soil and sexual reproduction of the pathogen may appear also in our potato–growing area. The precondition for effective protection of potato crop from late blight are correctly scheduled treatments in the period preceding the first appearance of the pathogen, with the use of fungi–

cides against which there is no resistance within the late blight popula–tion. It is also important to breed potatoes for resistance without *R*–genes to avoid breaking of resistance.

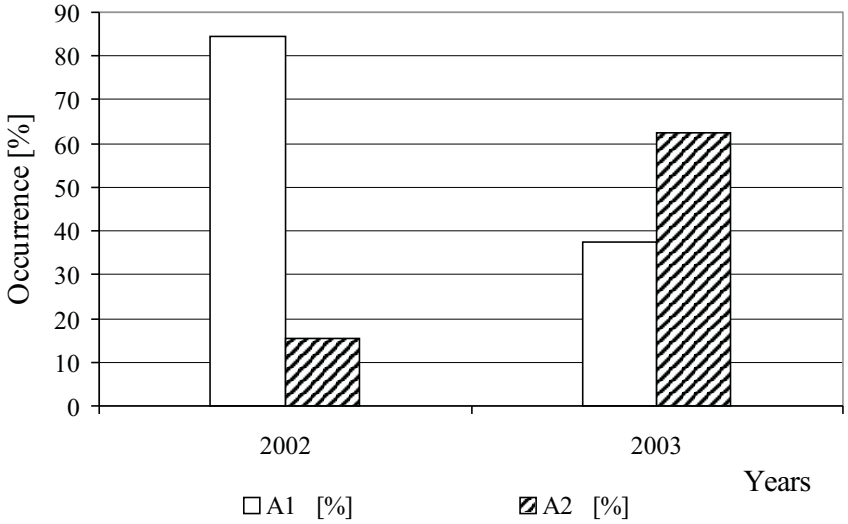

Fig. 3. Mating type of *Phytophthora infestans* isolates collected in the Slovak Republic in 2002 and 2003

REFERENCES

Deahl K.L., Inglis D.A., DeMuth S.P. 1993. Testing for resistance to metalaxyl in *Phytoph–thora infestans* isolates from Northwestern Washington. Am. Potato J. 70: 779–795.

Dowley L.J., O'Sullivan E. 1985. Monitoring metalaxyl resistance in populations of *Phytoph–thora infestans*. Potato Res. 28: 531–534.

Forišeková K. 1997. Ochrana proti plesni zemiakovej (Protection against late blight). Final report VŠÚZ Veľká Lomnica, 38 p.

Forišeková K. 2002. Prognóza, signalizácia a monitoring výskytu rás v ochrane proti plesni zemiakovej (Prognosis, signalling and monitoring of races in protection against late blight). Final report VŠÚZ Veľká Lomnica, 53 p.

Fry W.E., Smart Ch.D. 1999. The return of *Phytophthora infestans*, a pathogen that just won't quit. Potato Res. 42: 279–282.