Effect of *Phytophthora infestans* on potato yield in dependence on variety characteristics and fungicide control

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ABSTRACT

The epidemiology of potato late blight (*Phytophthora infestans*) was observed over the three-years with 110 potato varieties from various maturity groups treated or not treated with fungicides. The determined severity values were transformed into AUDPC coefficients characterizing rate of disease spreading across the crop in connection with varietal characteristics, fungicide application and year effects. The analysis of the pathogen population for the distribution of races virulent to usually used resistance genes indicated non-efficiency of these genes; resistance could only be increased by breeding for polygenically determined horizontal resistance. There were important yearly variations observed for the epidemiology of *P. infestans*. A correlation was detected between increasing AUDPC value and yield reduction in both fungicide treated and non-treated variants. AUDPC value of the treated variant is to a certain extent positively correlated with the value of the non-treated variant. However, particular interaction between potato genotype and applied fungicide program was shown, which could positively or negatively affect disease course in dependence on the variety.

Keywords: potato late blight; potato varieties; fungicide protection; area under the disease progress curve

Potato late blight caused by the oomycete *Phytophthora infestans* is economically the most important and most destructive potato disease worldwide. The disease causes annual losses of several billion dollars and it is a global threat for potato growers (Cooke and Lees 2004). The pathogen apparently originates from Central Mexico (Zimnoch-Guzowska et al. 2003). In the middle of the 19th century the pathogen was introduced into the US and Europe, where it destroyed a great of part the potato crop and is widely known as the cause of the Irish potato famine in 1845 (Smart and Fry 2001).

Phytophthora infestans is a hemibiotrophic pathogen attacking living parts of plants from the family *Solanaceae*. The pathogen is economically significant on potato and tomato. The pathogen causes lesions with necrotic cells in the middle, surrounded by a ring of gradually necrotizing tissue. Once infected, plants initially appear healthy, before necrotic lesions develop. Under favourable weather conditions, the pathogen can destroy potato foliage in 10 to 15 days and potential yield can be reduced by 50 to 70% (Tymčenko and Jefronová 1987).

In developed countries, potato late blight control is mainly based on intensive application of fungicides (Song et al. 2003). However, late blight epidemiology is also impaired by natural resistance of varieties provided by introgression of resistance genes. The genetic background of a variety together with environmental conditions that are not conducive for the pathogen results in field resistance. Expression of so-called 'age resistance'

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is also important, when the pathogen only infects ontogenetically older parts of the plants. Natural resistance plays an important role in plant protection and optimization of fungicide protection. Therefore, breeding for resistance is a critical part of integrated late blight control in potatoes.

Current resistance of commercially used varieties of *Solanum tuberosum* ssp. *tuberosum* L. to potato late blight can be vertical or horizontal in character (Bradshaw and Mackay 1994). Specific (vertical) resistance is the resistance to a certain pathogen race. It is oligogenic resistance and confers a relatively high level of resistance and is less environment dependent (years, growing localities); however, it is overcome by the emergence of new virulent races. The hexaploid botanical species *S. demissum* Lindl. is used as a source of specific (vertical) resistance for crossing with *S. tuberosum* ssp. *tuberosum* (Ordoñez et al. 1997). A total of eleven *R* genes (*R*1–*R*11) were classified based on mutual compatibility and incompatibility of specific races and differential clones (Malcolmson and Black 1966). These *R* genes are inherited on the basis of Mendelian laws of inheritance as single dominant factors (Abu-El Samen et al. 2003). Pathogen races are then indicated by a number, which corresponds to an *R* gene for host resistance that can overcome that particular race (Erwin and Ribeiro 2005).

Non-specific (horizontal) resistance is the polygenic resistance, controlled by a large number of minor genes and is when the host can resist a large number of pathogen races. It is specified by relatively lower level of resistance, more environment dependent (year and locality, i.e. especially temperature and air and soil moisture), and it is not overcome by emerging of new pathogen races. The development of horizontal resistance is the major aim of potato breeding programs for resistance to *P. infestans* (Tian et al. 2006).

MATERIAL AND METHODS

Establishment and evaluation of field varietal trials. Field trials were established between 2007 and 2009 at the research station of the Potato Research Institute in Valečov (Czech Republic). The station is situated 460 m a.s.l., the soil is medium to heavy with a loamy to sand-loamy topsoil. Cultural parameters did not vary over the three years of the trials. Certified category 1 seed potatoes was used; a representative of the varieties grown in the Czech Republic and Europe was evaluated. Briefly, 110 potato (*Solanum tuberosum* L.) varieties were used in all trials. Twenty-five tubers of each variety were planted into a row in a random trial design. The varieties included various maturity groups, resistance, and/or susceptibilities to late blight, various cooking types and food and industrial uses. The distribution of the varieties were based on maturity group: 28 were very early varieties (VE), 31 were early varieties (E), 35 were medium early varieties (ME) and 16 were medium late (ML) to late varieties (L).

The treatments included fungicide or no fungicide applications against late blight. Fungicide treatments were initiated as preventive measures after crop canopy closure, six applications were performed at 10-day intervals during the vegetative period using electric knapsack sprayers (Vermorel 2000 Berthoud, Villefanche, France). Each fungicide treatment was applied at a volume of 600 L of water per hectare. In 2007 and 2008 the following fungicide program was used: 1. and 2. Ridomil Gold MZ Pepite (mancozeb 0.96 kg/ha, metalaxyl-M 0.06 kg/ha); 3. and 4. Casoar (chlorothalonil 0.75 kg/ha, propamocarb-hydrochloride 0.75 kg/ha); 5. and 6. Altima 500 SC (fluazinam 0.2 kg/ha). In 2009 the program was: 1. and 2. Ridomil Gold MZ Pepite (mancozeb 0.96 kg/ha, metalaxyl-M 0.06 kg/ha); 3. and 4. Revus (mandipropamid 0.15 kg/ha); 5. and 6. Ranman (cyazofamid 0.08 kg/ha) + Ranman Aktivator (heptamethyltrisiloxan modified by polyalkylfenoxide 0.126 kg/ha).

The trial crops were only subjected to natural infection pressure of the pathogen; no spreaders were used in the crop. The evaluation for foliar damage was initiated after reaching critical value of late blight negative prognosis in a given year. The value was determined based on meteorological measurements according to Ullrich and Schrödter (1966). Disease progress expressed as percentage of late blighted foliage was evaluated at weekly intervals. The results for individual varieties in given year were converted to AUDPC (Area Under the Disease Progress Curve) values based on the formula according to Viljanen-Rollinson and Jeger (2001):

$$\text{AUDPC} = {}^{a}\Sigma_{i=1}\,\{[Y_i + Y_{(i+1)}]/2\} \times [t_{(i+1)} - t_i]$$

Where: t is a number of days since measurement initiation and Y is percentage of late blighted foliage. In addition, the results were evaluated in program Statistica CZ (Statsoft Version 9.0).

Study of pathogen population. The pathogen population adjanced to the field trial was analysed for the distribution of races virulent to genes derived from *Solanum demissum* in or-

der to acquire a better view on possible impacts of pathogen population structure in relation to natural resistance of trial varieties. For analysis of race distribution of *P. infestans* isolates, tetraploid differential clones of *S. tuberosum* ssp. *tuberosum* L. set 'Black Differentials' were used. The set contained 9 genotypes carrying one resistance gene each (*R*1, *R*2, *R*3, *R*6, *R*7, *R*8, *R*9, *R*10, *R*11), one genotype was containing four genes simultaneously (*R*1234) and one control genotype without any resistance genes (rr). The set was added with *S. bulbocastanum* clones (PI243510) considered for carriers of *Rpi-blb 1* gene (Van der Vossen et al. 2003). Collection of *P. infestans* isolates and *in vitro* culture is described by Mazáková (2008). For phenotypic assessment of resistance in these clones infection test was used related to inoculated leaf disks cultured for 3 days in a moist chamber. A spore suspension containing approximately 2 × 10^4 sporangia/mL of solution was used.

RESULTS AND DISCUSSION

Analysis of *Phytophthora infestans* race range. The analysis of *Phytophthora infestans* isolates indicated that pathogen population at the experimental locality of Valečov (Czech Republic) included all races virulent to *S. demissum* genes detectable by used differential set over the duration of the trial. Therefore, we can conclude that the effect of pathogen was stable within the trial and resistance and/or susceptibility of evaluated varieties in individual years is a consequence of interaction between variety genotype and external environment effect (incl. protective measure in treated variant). Virulence to the *Rpi-blb 1* gene was not detected. Considering breeding of varieties it is apparent that increasing of resistance in potato varieties could be only done based on introgression of known and so far not widely used major genes for resistance to potato germplasm (e.g. genes *Rpi-ber* or *Rpi-blb*) and strengthening of non-specific – horizontal resistance (Visker et al. 2003, Tian et al. 2006, Park et al. 2009). Found structure of *P. infestans* population indicates that further increase of potato resistance in breeding programs will not be possible only on the basis of known *S. demissum* major genes in future. Results unambigously confirm a continual need to search new resources expressing polygenically determined horizontal late blight resistance and their use in potato breeding programmes.

Evaluation of epidemiological impact of *P. infestans* on potato yield. For statistical assessment of 110 potato varieties for response to presence of *Phytophthora infestans* pathogen, analysis of variance of main effects was used. Yield belongs to agronomic most important parameters in potato growing, which is markedly influenced by a series of factors. For this reason an effect of variety, fungicide treatment, year and maturity group on potato yields was studied. The analysis indicated that variety, fungicide treatment and year are the main factors contributing to yield variability ($^{***}P < 0.001$). It was found that maturity has a neglectable effect on yield variability compared to previous factors. The probable reason is the fact that maturity is only a consequence resulting from genetic background of a variety, and therefore it is integral part of intervarietal variability. This fact is also proven by results of evaluation of individual varieties, where, for example, not every medium early variety indicated higher yield than very early variety. The effect of fungicide treatment and year was evaluated by multi-factor ANOVA

Table 1. Multi-factor ANOVA, where potato yield (t/ha) is variable and fungicide treatment × year is sort criterion

Number	Fungicide protection	Year	1	2	3	4	5	6
			46.403	39.482	54.225	44.735	28.506	31.552
			P-value					
1	yes	2007						
2	yes	2008	**0.000130**					
3	yes	2009	**0.000026**	**0.000020**				
4	no	2007	0.890794	**0.009054**	**0.000020**			
5	no	2008	**0.000020**	**0.000020**	**0.000020**	**0.000020**		
6	no	2009	**0.000020**	**0.000024**	**0.000020**	**0.000020**	0.361204	

bold – statistically significant difference

Table 2. Multi-factor ANOVA, where AUDPC is variable and fungicide treatment × year is sort criterion

Number	Fungicide protection	Year	1	2	3	4	5	6
			2341.4	1926.4	809.65	2882.7	3606.3	2689.7
			P-value					
1	yes	2007						
2	yes	2008	**0.002200**					
3	yes	2009	**0.000020**	**0.000020**				
4	no	2007	**0.000031**	**0.000020**	**0.000020**			
5	no	2008	**0.000020**	**0.000020**	**0.000020**	**0.000020**		
6	no	2009	**0.019020**	**0.000020**	**0.000020**	0.494272	**0.000020**	

bold – statistically significant difference

(Table 1). The results indicate that no statistically significant difference between yields of treated and non-treated variant was detected in 2007. It shows that infection pressure of the pathogen was mild in 2007 and the effect of fungicide treatment was not expressed in yield. Mean yields of non-treated variants during 2008 and 2009 also do not statistically differ and comparable infection pressure of the pathogen and its effect on yield could be concluded. However, level of yield in response to fungicide treatment is different in these two years. This fact can be probably explained by a change of chemical products in the fungicide sequence, and/or other more closely undefined factors.

Estimation of AUDPC (Area under the Disease Progress Curve) value for each trial variant in given year is the objective evaluation of disease course. Yield dependence on AUDPC value was detected using of simple linear regression ($r = -0.56$), confirming the presupposition that the more hardly variety resists the attack of the pathogen, the more easily the pathogen destroys a larger part of yield. The disease course expressed as AUDPC value was found to contribute to a change in yield by 31%. It means that a series of other factors will contribute to change in yield levels. Genetic yielding potential of a variety is undoubtedly one of them. Another aspect could be a general expectation that with an increase of variety yield and quality potential the value of variety resistance decreases.

AUDPC values were further evaluated by analysis of variance of main effects in regard to the effect of variety, fungicide treatment, year and maturity group. The most important factor affecting variability of disease course appears to be variety ($^{**}P < 0.01$); this is confirmed by the results of Namanda et al. (2004) and Kirk et al. (2001). Statistically significant differences were detected between individual years and variants of treatment ($^{**}P < 0.01$). In accordance with results of Christ (1990) crops without fungicide protection showed higher AUDPC values compared to treated crops. Multi-factor ANOVA for combination of treatment effects and year did not detect any significant differences between non-treated variants only in 2007 and 2009 (Table 2). It means that infection had a similar course in those years. A difference was determined in onset of infection, which was 14 days earlier in 2009 (Figures 1 and 2) and this was mainly reflected in yield at the end (Table 1). Maturity contributes to variability of AUDPC values only minimally; it is in accordance with the results of yield variability evaluation.

The relationship between AUDPC values in non-treated and fungicide treated potato varieties was evaluated by regression and correlation analysis. Positive correlation was detected between both

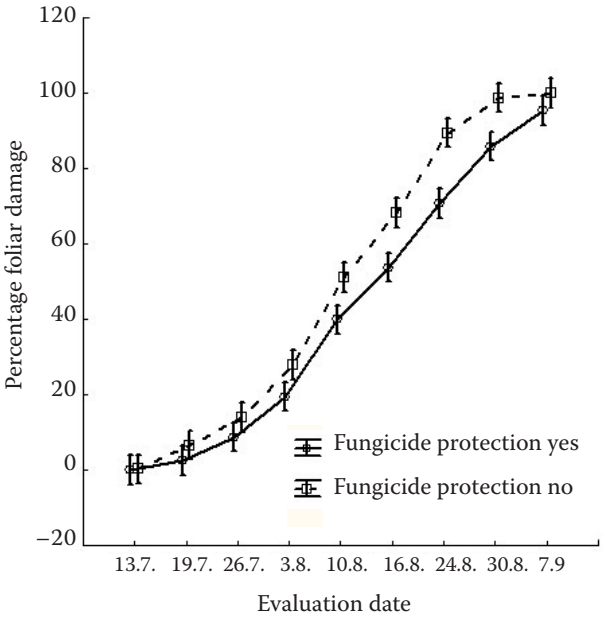

Figure 1. The average course of late blight in fungicide treated and non-treated potato varieties in 2007

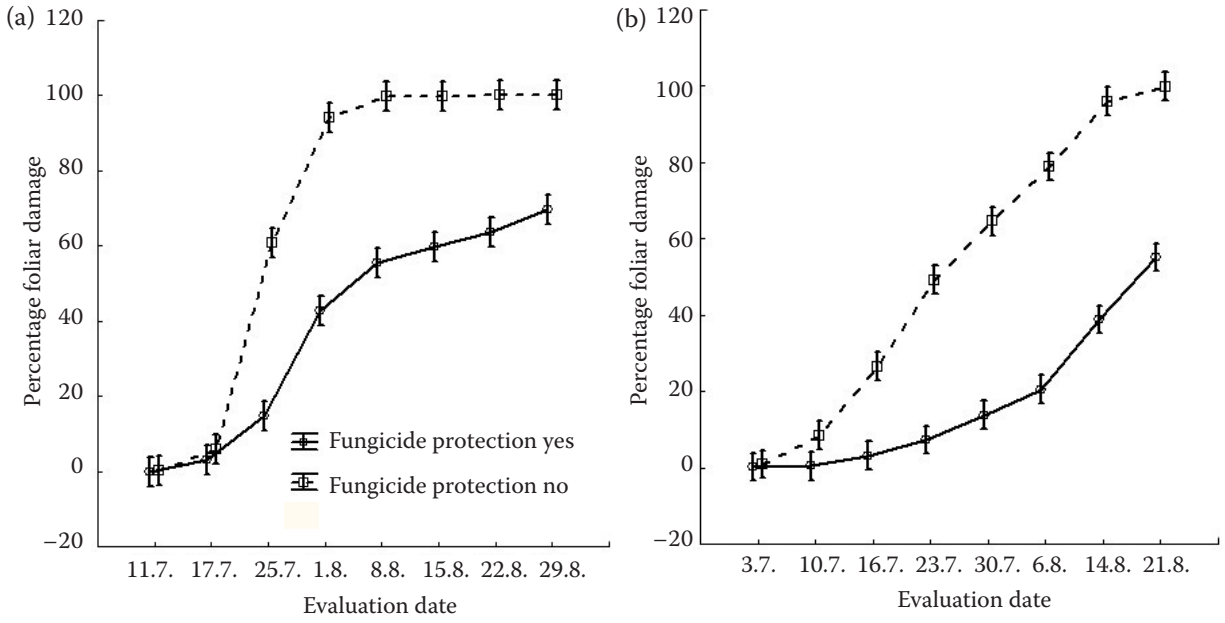

Figure 2. The average course of late blight in fungicide treated and non-treated potato varieties in (a) 2008 and (b) 2009

variants (r = 0.64). AUDPC value for treated variant is connected with values for non-treated variant only in 41%. It means that response of individual varieties to fungicide treatment is very variable. It was rather proved that in general selected sequence of fungicide treatments markedly limits development of the disease, but it could not be at large determined with how many %. This fact cannot be generalized for use of selected sequence in following years or a certain variety. Hypothetically, interaction between fungicides and genetic background of individual varieties could be another factor influencing correlation. Totally, four varieties (Tomensa, Collette, Red Anna, Westamyl) did not show significant differences in mean yields between treated and non-treated variant. High field resistance to late blight could be considered for them, when fungicide treatment did not influence yield under given conditions. For seven varieties markedly lower mean yields were found in the variant with fungicide treatment. They were the varieties Monaco, Rebel, Antoinet, Komtesa, Leoni, Sinora and Nomade. The probable reason could be phytotoxic action of any applied fungicide. Singh et al. (2003) detected phytotoxicity of several optical isomers of metalaxyl. Although no visual symptoms were recorded, such as yellowing or growth retardation, phytotoxic action of fungicides could be expressed in inhibition of plant metabolism, and/or reduction in intensity of assimilates accumulation in storage organs. It indicates that genotype × fungicide treatment interaction does not always have to have a positive impact on yield. However, for 90% of varieties a positive effect of fungicide treatment was determined on yields. The fact is interesting that mean AUDPC values were lower in all 110 varieties in treated variant. This confirms the ability of fungicide treatments to reduce the detrimental impact of the pathogen. In consequence in practice, it could mean that criteria for selection of optimal variety for ecological growing are quite different from criteria for integrated farming. In ecological growing especially the level of field resistance will decide resulting from genetically controlled resistance, whereas in integrated system response of genotype to a given fungicide treatment will also decide, which could express as induction of resistance or phytotoxicity or on contrary simulation of development of yield-forming components.

The results further indicate that AUDPC value absolutely represents disease course, regardless of at what stage of vegetation infection started. A certain disadvantage could be the fact that AUDPC cannot directly describe impact on total yield and this was proven by correlation analysis. For these reasons course of late blight epidemiology cannot be reliably done in context with expected yield and conversely.

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