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A New Approach to Measure Potato Susceptibility to *Phytophthora infestans*, a Causal Organism of the Late Blight

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SUMMARY
The proposed method makes it possible to evaluate the level of the foliage and tuber susceptibility of potato cultivars to *Phytophthora infestans* under field and laboratory conditions using a mathematical simulation approach.

KEY WORDS
Phytophthora infestans, late blight, potato resistance

INTRODUCTION

Potato cultivar resistance to *Phytophthora infestans*, a causal agent of the late blight, still plays a key role in the control of this disease. The use of resistant plants requires no actions from potato growers during the season; it does not harm the environment and is usually compatible with other disease management techniques; finally, sometimes such approach is sufficient to reduce the disease development to a tolerant level (Fry, 1982). That is why the testing of potato cultivar for the late blight resistance is an important part of the selection process in the breeding of new potato cultivars.

There are two known resistance types: vertical (absolute) and horizontal (partial). The first-type resistance is race-specific, since it is related to dominant genes (R genes), which present in wild *Solanum* species (mainly *S. demissum* and *S. stoloniferum*), used by breeders in crossings. R genes provide a hypersensitive reaction of infected tissues that resulted in the localization of the pathogen penetration point by necrotized tissues. The pathogen perishes, leaving only a small necrotic lesion on a leaf.

Numerous attempts to obtain a long-term resistance using the mentioned R genes were unsuccessful because of the development of virulent races, always existing in any *P. infestans* population. As a result, breeders started to use another type of resistance, named partial (horizontal) or field resistance (Turkensteen, 1993; Colon et. al., 1995). In contrast to the race-

specific resistance, this type of resistance just controls the development of the disease and does not suppress it completely. It is usually considered that this type of resistance is polygenic, since it is efficient against all *P. infestans* races and, therefore, has a more stable and prolonged effect, than the race-specific resistance. However, the possibility of genetic recombinations, appeared in "new" *P. infestans* populations due to a sexual process, provided the appearance of more aggressive pathogen strains that caused a gradual decrease of this type of resistance. As a result, the partial resistance of some potato cultivars to various late blight populations can significantly vary. For example, cv. Santé is considered to be moderately resistant to late blight in France, moderately susceptible in Netherlands, and susceptible in the Moscow region of Russia. Due to this fact, there should be a permanent control on the late blight infection level of the cultivated potato varieties.

The common method of estimation of the late blight resistance of potato in the field is based on the scoring of the foliage destruction usually performed at any certain stage of the plant development. The late blight resistance is also scored under laboratory conditions using artificially inoculated detached potato leaves by the measurement of the size of necroses or the level of sporulation. Results of such estimation are expressed in accordance with the 9-score scale (Colon *et al.*, 1995). We consider that the traditional late blight assessment methods can be improved; to do this, one should use the quantitative value of the LB-caused yield loss, calculated from the dynamics of dying-off of infected tops during the whole vegetation period, as the key evaluation factor.

This paper describes procedures required to realize the above-described idea.

FIELD TESTS FOR FOLIAR BLIGHT RESISTANCE

The field assessment of the partial resistance of tested potato cultivars to late blight is carried out on the natural or artificial background by measuring the level of a leaf infection each 10-12 days using a special scale (Table 1).

Table 1. *Scale for the assessment of the late blight infection of potato leaves (James, 1971)*

Level of infection, %	Description
0	- No any signs of infection.
0,1	- First single spore-bearing spots.
1,0	- Weak level of infection (5-10 lesions per a plant).
5,0	- About 50 lesions per a plant; 1 of 10 leaf lobes is infected.
25,0	- Almost all leaves are infected, but plants still keep a normal form. The field looks green.
50	- Each plant is infected; about 50% of the leaf area is dead. The field looks green with brown spots.
75	- The infection is spread over 75% of the leaf area. The field looks brown-and-green.
95	- Plants have only single leaves, but the stems are green.
100	- All leaves died, and stems are died or dry.

Basing on this assessment data one can determine the area under the disease progress curve (AUDPC), in the course of the vegetation season, the corresponding yield losses caused by the early destruction of leaves (%), and the late blight resistance level (in scores).

The program of such calculation (Fig. 1) is placed at the website of the All-Russian Research Institute of Phytopathology (Rogozhin and Filippov, 2011; http://vniif.ru/index.php?option=com_content&view=article&id=40&Itemid=30&lang=ru).

This program is based on the known van der Plank hypothesis (1968), which assumes a direct ratio between the AUDPC on the potato foliage and yield losses. According to our long-term field studies (Gurevich, Filippov, and Tverskoy, 1977), this dependency can be expressed by the following equation:

$$\omega = \frac{AUDPC}{q} \cdot 100,$$

where ω is a yield loss (%) caused by an early leaf decay, q is the number of days between the bud formation phase and the decay of non-infected leaves. The average q value for the early, intermediate, and mid-late potato cultivars is 46, 52, and 84 days, respectively. If the foliage is killed by the frost or desiccant, or the harvesting is carried out before the natural dying-off of the foliage, then q is considered to be the number of days passed between the bud formation phase and the moment of the foliage death (Rogozhin and Filippov, 2012).

The calculated yield losses are then converted to the scores characterizing the level of the late blight resistance in accordance with the 9-score scale, where 9 scores represent the highest resistance level.

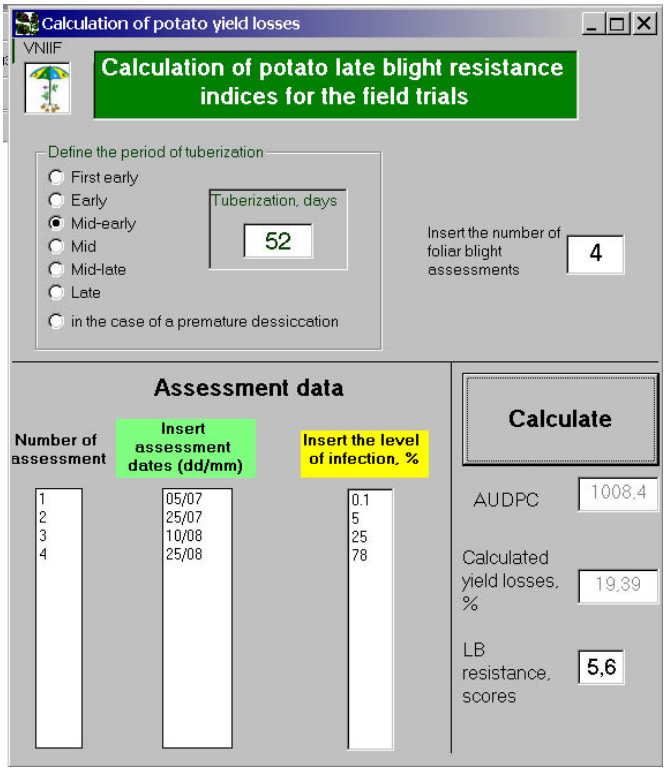


Figure 1. *Working window of the program for the calculation of potato yield losses, caused by the late blight and the resulting early destruction of leaves*

DETACHED LEAF TESTS FOR FOLIAGE BLIGHT RESISTANCE

The quantitative manifestation of the partial LB resistance within the same potato cultivar depends on the infection load, the level of aggressiveness of *P. infestans* strains, and weather conditions. Therefore, an objective assessment can be performed by the arrangement of field trials in regions, which are usually favorable for the late blight development (such as the Sakhalin island and Central Mexico), or under standard laboratory conditions with the use of special tests and the mathematical simulator of the epidemic development (Filippov et.al., 2004).

The laboratory assessment method, developed in our institute, is based on the joint use of the artificial inoculation of detached potato leaves and the mathematical model, simulating the late blight development under standard favorable meteorological conditions and at the given primary infection level. This model, based on the measurement of the inoculation efficiency, size of necroses, and sporulation productivity, reproduces the dynamics of the foliage destruction during a vegetation season and calculates the correspondence of this dynamics to the yield losses caused by the late blight of potato (Gurevich, Filippov, and Tverskoy, 1979). The method makes it possible to assess the cultivar resistance to the most aggressive *P. infestans* strains, including exotic ones under isolated laboratory conditions.

The tests are carried out on detached leaves, collected from the studied potato cultivars and inoculated with the studied *P. infestans* isolates, and, in parallel, on detached leaves of the standard potato cultivar, inoculated by the standard *P. infestans* strain.

Plants of the tested cultivars and the standard cultivar (30 plants of each cultivar) are grown under field conditions. During the phase of development of 7-9 leaves, a mid-level leaf is detached from each plant for the testing. Then leaves are transferred into laboratory premises and inoculated with the selected pathogen strains. Each tested "cultivar-isolate" pair is compared with the standard pair. The comparison of data, obtained in the course of experiments for each "cultivar-isolate" pair (number and diameter of necrotic lesions and the sporulation productivity) makes it possible to conclude about any differences in the aggressiveness of isolates from different regions and, therefore, about the level of resistance of tested cultivars.

In the proposed method we use the cv. Santé as a standard cultivar and the N161 *P. infestans* strain as a standard. The field yield loss of the above-mentioned cultivar, infected with the chosen strain, makes 30% under the weather conditions favorable for the disease development.

Using the tests, one can measure the basic parameters of the infection cycle on each tested cultivar as compared to the standard cultivar.

1. Inoculation efficiency measurement

The test is carried out using 10 leaves for each cultivar. The leaves are inoculated by spraying with a suspension of zoosporangia (30000 spores/m^2); the volume of the suspension is 5 ml per a cuvette. After the inoculation, leaves are incubated in a wet chamber for 3 days at 18^{o}C; then the area of leaves was determined using a photoplanimeter, and the number of necrotic lesions per 1 cm^2 was calculated.

2. Measurement of necrotic lesions

Potato leaves are inoculated with a suspension of zoosporangia (1-2 drops per a leaf) using a microdispenser. The concentration of zoospores is the same as for the previous operation (stage I). Inoculated leaves are incubated in a wet chamber for 18 h at 20^{o}C. Then the drops of suspension are removed by a filter paper and the leaves are placed into a wet chamber again for 3 days. On the 4th day the diameter of necrotic lesions is measured (Fig. 2).

Figure 2. *Example of infected potato leaves used to assess the size of necrotic lesions and the sporulation productivity*

3. Measurement of the sporulation productivity

For this measurement one can use the leaves from the previous test.

The intensity of the spore formation is assessed using two methods. The more exact way is to calculate the number of conidia per one lesion using a Goryaev's count number. To do this, one should put 10 leaf lobes with necrotic lesions into a glass beaker and add 15 ml of distilled water (1.5 ml per a lesion). After shaking, the leaves should be removed, and the remaining water volume should be measured. Then the number of conidia per lesion should be calculated using the Goryaev's chamber.

All after-measurement calculations are performed separately for potato cultivars of three maturing groups. The program developed on the basis of the above-mentioned measurements, calculates the AUDPC value, yield losses, and the level of the late blight resistance of the tested cultivar under fixed conditions favorable for the disease development (Fig. 3). The program can be found on the above-mentioned website of our institute.

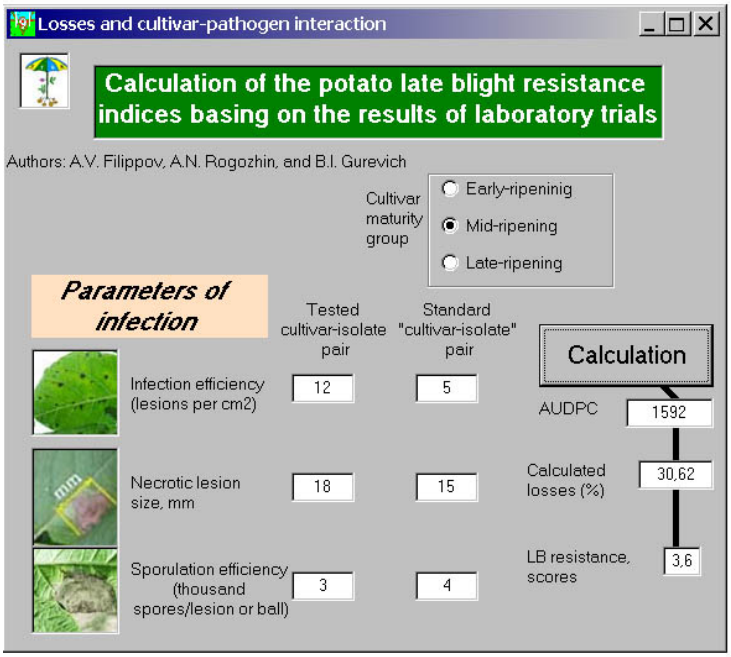


Figure 3. *Working window of the program calculating the level of the leaf blight resistance of potato cultivars under conditions, favorable for the disease development, on the basis of the measurement of the main parameters of the infection cycle on detached leaves*

TUBER SLICE TESTS FOR TUBER BLIGHT RESISTANCE

To assess the late blight resistance of potato tubers under laboratory conditions, we propose to use a Lapwood method (Lapwood, 1965, 1967) with some modifications.

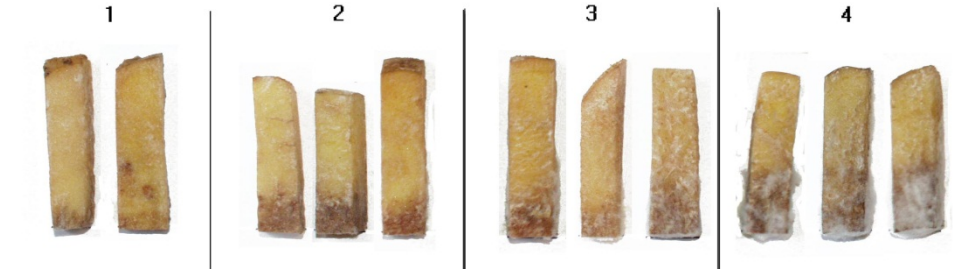
Potato tubers are sliced into pieces (7×5×40 mm) in the twenty-fold repeatability. One end of each piece is submerged for 3-5 seconds into a zoosporangial suspension poured into Petri dishes (2-3-mm layer). After a 6-day incubation, the length of the infected zone is measured by a ruler (mm), and the mycelial covering intensity is determined using a 4-score scale (Fig. 4).

Tuber slices of the cv. Santé, inoculated with the N161 strain, are used as a standard. According to the expert assessments, the level of the tuber resistance of the cv. Santé to the N161 strain is equal to 5.5 scores of the 9-score scale, where 9 scores correspond to the maximal resistance level. The cv. Santé and the strain 161 can be replaced by any other "standard" cultivar-isolate pair with the known result of their interaction, expressed in scores. From the practical point of view, it is desirable that the tuber resistance level of the selected "standard" cultivar towards the selected *P. infestans* isolate would be within the range of 4-7 scores.

Based on the measurements of the size of necrotic lesions and the level of the mycelial covering of tuber slices, the cultivar resistance index is calculated using the equation (1):

$$x = \frac{\sum(a \times b)}{n}, \qquad (1)$$

where *x* is the resistance index, *a* is the average size of the lesion of the tested cultivar as compared with the standard one, *b* is the average mycelial covering intensity as compared with the standard (equal to 1), and *n* is the number of slices.



1	2	3	4
Mycelial covering is very weak or absent **(1 score)**	Weak, but clearly visible mycelial covering **(2 scores)**	Moderate mycelial covering **(3 scores)**	Abundant mycelial covering over the whole infected zone **(4 scores)**

Figure 4. *Scale for the visual assessment of the mycelial covering intensity*

The calculated indices are then converted into scores using a special chart (Fig. 5). It is also possible to use a special program located at the ARRIP site (Fig. 6).
The methods presented in this paper are offered for the use as a procedure for the state registration of new potato cultivars in Russian Federation.

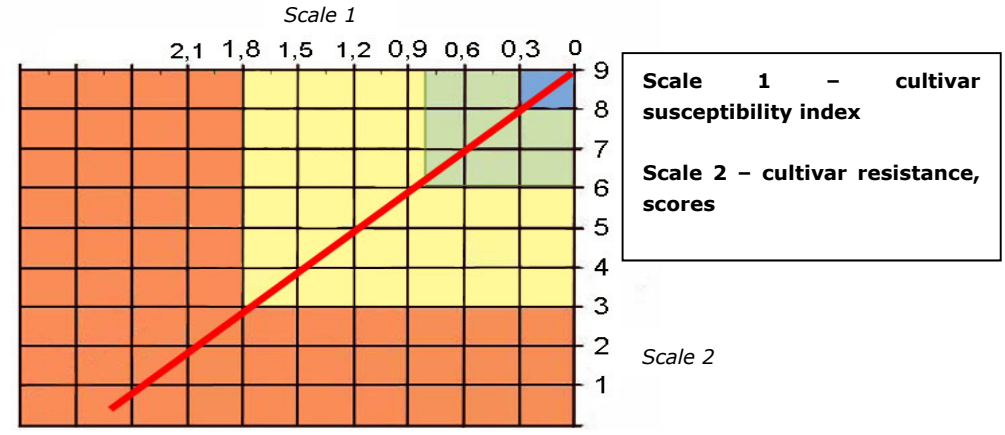


Figure 5. *Chart for the assessment of the late blight resistance of potato tubers*

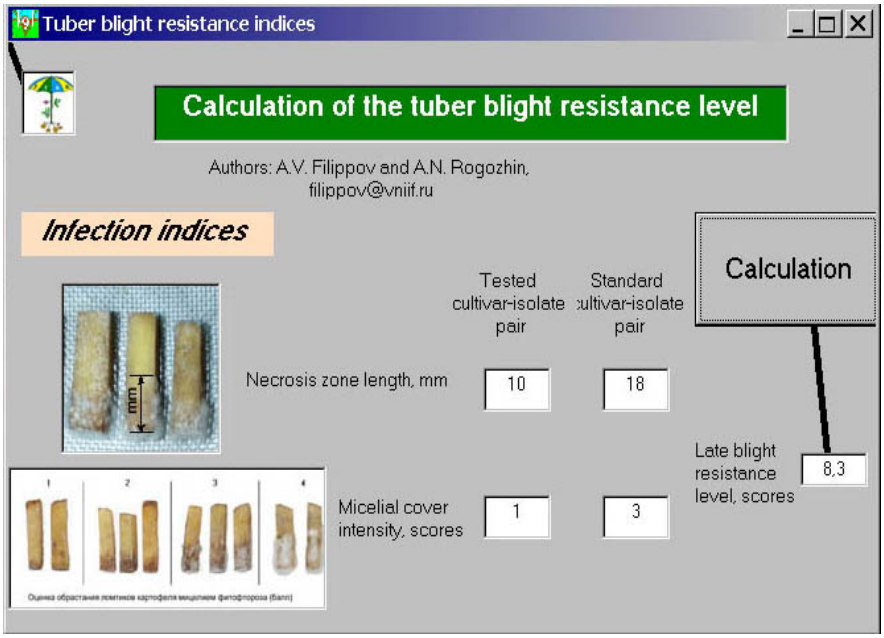


Figure 6. *Working window of the program calculating the level of the tuber blight resistance of potato cultivars*

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