

Laboratory methods of evaluating tomato resistance to late blight (*Phytophthora infestans* (Mont.) de Bary)

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Abstract

The purpose of the presented investigations was to determine uniform laboratory methods of evaluating horizontal resistance of tomatoes to late blight. It was found that the best tests were infecting whole plants at the 5-leaf stage and infecting leaves removed from the middle part of the shoot. The best method of testing the resistance of fruit to infection was instilling a suspension of spores on the calyx with the sepals left in place.

INTRODUCTION

Late blight, caused by the fungus *Phytophthora infestans* (Mont.) de Bary, is the most serious disease of field-grown tomatoes in Poland. It occurs under our climatic conditions almost every year and, if stringent protective measures are not undertaken, causes heavy yield losses. The best method of fighting this disease would be the cultivation of resistant varieties. Since material sufficiently resistant to the particular races of this fungus has not been found yet anywhere in the world, it seems worthwhile to breed in the direction of obtaining varieties with horizontal resistance. Breeders who screen for horizontal resistance use a mixture of races of the pathogene as the inoculum. Parlevliet (1983) maintains that using single races for inoculation provides the best conditions of selecting for horizontal and vertical resistance, but only in the presence of genes for vertical resistance.

Systematic evaluation of the susceptibility of tomato varieties has not been conducted in Poland to date and the fragmentary results obtained in various research centers have often been contradictory. The main reason for differences in the classification of the same variety or line, aside from the high variability of the fungus, is the lack of a uniform system of evaluating resistance; this pertains both to this country and abroad.

Plants have been infected under laboratory conditions by spraying at various stages of growth, from cotyledons (Pshedetskaya and Kherepa-

nova, 1971), 2-3 leaf stage (Gallegly and Marvel, 1955), 5-leaf stage (Shirko, 1971), 10-12 leaves (Laterrot, 1975) even to and throughout blooming (Pshedetskaya and Kherepanova, 1971). Isolated leaves have also been infected using a pipette (Peirce, 1970; Brezhnev et al., 1978; Skvortsova, 1979, Dorozhkin et al., 1982) or a special apparatus (suspensor) which measures out the suspension in drops of a specified volume (Turkensteen, 1973). A pipette has also been used to infect leaf discs (Eggert, 1970a; Grümmer and Eggert, 1968; Günther et al., 1970; Dorozhkin et al. 1982). Only Khrobrykh (1957) infected isolated tomato shoots on a wider scale. Green fruit has been infected using numerous methods: by placing the mycelium under the skin (Shirko, 1963), instilling a conidiospore suspension on the calycine depression (Dorozhkin and Strel'skaya, 1966), injecting the suspension under the skin of the fruit (Laterrot, 1975) and by spraying the fruit evenly with a spore suspension (Eggert, 1970b; Brezhnev et al., 1978).

The objective of the investigations undertaken in 1983-1985 was to evaluate traditional and new methods of determining the horizontal resistance of tomatoes to late blight.

MATERIAL AND METHODS

The tomato varieties and lines used in these studies were chosen from those in our collection on the basis of previous observations of their field performance and literature data. The material was varied in respect to resistance and utilitarian traits. The set of varieties and breeding lines was not the same in each series of experiments because the results of the previous series had been taken into account. Only those tomatoes which were promising and the standards were tested several times. The standards were: West Virginia 700, West Virginia 106, Ottawa 30, *Lycopersicon hirsutum*, susceptibility standards: Rutgers and Moneymaker. The types of tests that were conducted and the numerical data on the studied material are presented in Table 1.

The seeds of tomatoes intended for artificial infection in June and July were planted on April 10 and May 1, while those that were to be used in August and

Table 1
List of objects of laboratory investigations

Kind of test	No. of exp.	No. of varieties and lines	No. of repetitions	No. of elements per repetition
Plants	10	51	2	10
Leaves	6	54	2-3	9-20
Leaf-discs	1	31	5	10
Stems	2	50	3	10
Fruits	5	47	2	10

September, on August 10 and September 1. The seeds were sown in fine high peat. The seedlings were transplanted into window boxes, depending on the size of the boxes, either 5 or 10-12 plants per box. Either 15 × 30 cm or 15 × 50 cm window boxes were used. The infection experiments were conducted in an incubation chamber. Observations on the natural infection of the plants under field conditions were also carried out.

PREPARATION OF THE INOCULUM FOR INFECTION

Depending on the time of year and current possibilities, the inoculum was prepared from two-week-old cultures on agar medium in test tubes, from one-week-old cultures on plant tissues in Petri dishes (Binties potato tubers), in containers on filter paper (leaves, tomato fruit) or from 10-day-old potted tomato seedlings. The sporangia were rinsed off with sterile distilled water. A Thom chamber was used to determine the spore concentration, which was then adjusted to $50 \times 10^3 / 1 \text{ cm}^3$. The suspension prepared in this way was incubated for 2 hours at a temperature of 10-12°C, after which it was transferred to room temperature for 20-30 min in order to free the zoospores. Depending on the experiment, the inoculum for infection was either an isolate of a specified race or a mixture of isolates.

TESTING TECHNIQUES

Infection of whole plants

The plants intended for use in the laboratory tests were grown in a greenhouse. One to two days prior to the test they were transferred to an infection chamber and illuminated with sodium lamps (WLS) at 200 W/m². The plants were infected using a sprayer producing a fine mist which uniformly covered the leaves and stems. After inoculation the plants were tightly covered with foil and the walls and floor of the chamber kept constantly moist with water. Darkness was maintained for 20 hours, after which the plants were illuminated for three days for 10 hours per day, next for 12 hours per day. A temperature of 18-20°C and relative humidity of 90-100% were maintained.

Infection of isolated leaves

The leaves used in these tests were obtained from plants growing in a greenhouse. They were placed on moist filter paper bottom side up in containers with transparent lids. The inoculum was applied to the center of each leaf with a pipette. The leaves were turned over the next day and the

remainder of the inoculum was shaken off. Sporulation was assayed by soaking the leaves in identical volumes of water, mixing with a mixer and then counting the spore concentration using a Thom chamber.

Infection of leaf discs (disc test)

The discs (2 cm in diameter) were cut out of the central part of the leaf blade of the apical leaflets. They were arranged (10 discs per dish) bottom side up in Petri dishes lined with moist filter paper. The inoculum was applied with a pipette. The dishes were kept in a thermostat at 18-20°C. After the fungus fur appeared, each disc was separately placed in a test tube with 20 cm³ of water. The tubes were shaken using a WS-2 laboratory shaker for 30 min, after which the suspension was filtered through cheesecloth. The filtrate was mixed with a mixer for 1 min and the concentration of the spores determined.

Infection of isolated stems

The test was carried out using 8 cm long sections of the middle part of the main shoot of greenhouse-grown plants at the 5- or 10-leaf stage. The shoot sections were placed on moist filter paper in containers with transparent lids. The inoculum was applied using an electric sprayer.

Infection of green fruit

Good-sized, green tomato fruits were chosen randomly from field grown plants, 1 fruit from the first cluster of each plant. Their surfaces were disinfected with a 0.1% sublimate solution, rinsed with sterile water after which the fruits were placed on moist filter paper in closed containers. The following methods of infection were used:

1. Injection of the spore suspension under the skin using a medical syringe. The solution (2 cm³) was injected 1 cm away from the calycine depression;
2. Submersion in the inoculum of fruits with removed peduncles;
3. Placing 2 cm³ of inoculum (3 drops) on the trace left by the removed peduncle;
4. Placing 2 cm³ of inoculum on the peduncle with a pipette;
5. Spraying fruits with retained peduncles using a hand sprayer.

METHODS OF EVALUATING INFECTION

The evaluation was carried out when the organs (leaves, shoots, fruits) of the individual plants from the studied varieties or breeding lines showed similar reactions but the differences in degree of infection among the varieties or breeding lines were the greatest.

Whole plants

The first symptoms of disease were usually observed 3 days after inoculation. Evaluation of infection was carried out on days 7-10 by visually determining the degree of infection of each leaf or shoot according to a 6-point scale for leaves (Fig. 1) and a 7-point scale for stems (Fig. 2). The mean degree of infection of leaves and stems and percentage of diseased leaves were calculated on the basis of these observations.

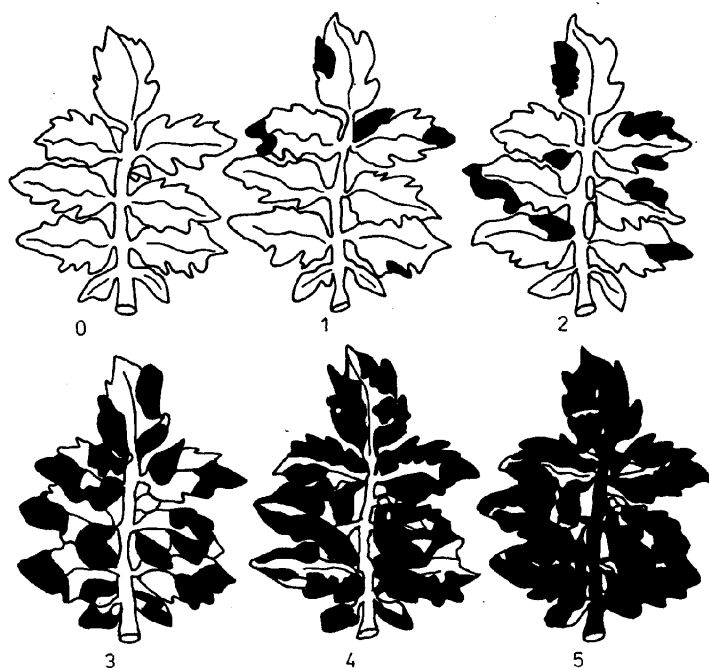


Fig. 1. Scale of infection of tomato leaves by *Phytophthora infestans*

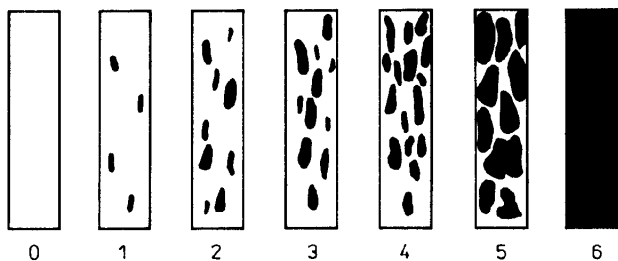


Fig. 2. Scale of infection of tomato stems by *Phytophthora infestans*

Isolated leaves

In series 1, 2 and 3 the isolated leaves were evaluated according to a 6-point scale (Fig. 1). The degree of infection of each leaflet was determined by this scale, after which the mean degree of infection of the whole leaf was calculated. In series 4, 5 and 6, a different concept of evaluation was adopted, namely that the basis of comparison of the size of the pathological spots is the area of rectangles inscribed on them. With this assumption, the object of measurement was the length of two perpendicular lines connecting the most distant points of the spot. These measurements were made using a ruler with an accuracy of ± 1 mm 3 and 6 days following inoculation.

The concentration of conidiospores washed off the diseased leaves 6 days after inoculation was also measured.

Discs in the disc test

The degree of infection of leaf discs was evaluated on the basis of the concentration of conidiospores 6 days after inoculation.

Fruits

The fruits were evaluated 4, 8 and 14 days after inoculation. After 4 days, the size of the spot was judged by eye and expressed as an approximate percentage of the fruit surface. After 8 and 14 days, a compass and ruler were used to measure the diameters of the spots and fur on them in their widest places. After 14 days, the fruits were cut open and the depth to which the pericarp was streaked with mycelium was measured.

The obtained measurements were expressed in relation to the transverse diameter of the fruit. The index P was calculated according to the following formula:

$$P = \frac{\text{diameter (of spot, fur or streaking)}}{\text{transverse diameter of fruit}} \cdot 100\%.$$

Statistical work-up of data

The following methods were used in the statistical analysis of the data:

- 2, 3 or 4 factor variance analysis in a random block system,
- linear regression and correlation analysis of pairs.

The significance of the differences was evaluated at $\alpha = 0.01$ for variance analyses and $\alpha = 0.05$ for correlation and regression analyses. The methods of testing plants were compared using the regression theory and verifying successive hypotheses by their precision and comparing the variance for the studied methods (Elandt, 1964).

RESULTS

THE SUITABILITY OF LABORATORY TESTS FOR DETERMINING THE HORIZONTAL RESISTANCE OF TOMATO LEAVES AND STEMS

Testing whole plants at 3 growth stages: 5, 10 and 13 leaves

It was found after conducting 10 series of infections that the 5-leaf stage was the best for infection. This conclusion was arrived at after verifying the hypotheses on the equivalence of the studied stages and comparing their variances (higher variance indicates that the studied varieties and lines are better differentiated in respect to infection). The results of verifying successive hypotheses of leaf infection are presented in Table 2. It was found that the conversion coefficient, B_0 , equals 0.52 for stems. This means that the infection of the stem at this stage of growth can be approximated by multiplying the result obtained at a different stage by 0.52. The results of infecting stems at the 5 and 10 leaf stages show significant correlation between their infection at these two stages, although the variance in the infection of stems in each experiment was smaller at the 10-leaf stage (Table 3).

The suitability of testing plants at the 5-leaf stage is supported by the reproducibility of the results obtained in different experiments. When the correlation coefficients among the resistance indicators in 16 of the same varieties and lines in successive series of infections were analysed, it was found that they equalled from 0.59 to 0.79 for the 5-leaf stage, while for the 10-leaf stage they were always less than the critical coefficient, 0.49.

In the assessment of laboratory methods of testing plant resistance, the correlation between the degree of infection of the plants under laboratory conditions and that of the same lines and varieties under natural infection conditions in the field is very important. The correlation between the natural infection of plants under field conditions and in laboratory tests on whole plants at the 5-leaf stage turned out to be significant (r equalled 0.45 to 0.55 at $R_{\text{tab.}} = 0.33-0.42$) (Table 4).

Leaf test

Six series of infections were carried out — 3 on leaves collected from the 8-leaf stage and blooming and 3 on leaves collected at the 5- and 10-leaf stages. It was found that the 8-leaf stage is more suitable for evaluation of resistance than the blooming stage, while the 5- and 10-leaf stages are equivalent.

The greater suitability of the 8-leaf stage was determined on the basis of variance analysis. The variance of the 8-leaf stage equalled from 0.95 to 2.05 in the successive experiments, while that of the blooming stage, 0.56 to 1.11 (Table 5). The differentiation of the degree of infection in the 8-leaf stage was greater in every experiment than in the blooming stage, which points to a greater difference in the reaction of younger plants to the pathogene than of

Table 2

Plants' usefulness in different phases of growth to the tomatoes tests of resistance to late blight

Series of exp.	No. of obj.	Coefficient of regression for infection of leaves in phases	$R_{\text{tab.}}$	Regression equation	Verified hypothesis H_0 after throwing off H_0 : $b = 1$ about equivalent of phases	Coefficient of recounting B_0	Modified regression equation	Variance of phases		$r_{\text{cal.}}$ for infection of leaves in phases:			Chosen phase
								younger	older	y	x	z	
1	33	0.83*	0.24	$y = 0.96 + 0.53x$	$\beta \neq 1, \alpha \neq 0$	—	—	1.74	0.70	0.20	0.21	0.33*	y
2	33	0.72*	0.24	$x = 0.94 + 0.84z$	$\beta \neq 1, \alpha \neq 0$	—	—	0.70	0.95	—	0.21	0.33*	z
3	33	0.71*	0.24	$y = 1.56 + 0.52z$	$\beta \neq 1, \alpha \neq 0$	—	—	1.74	0.95	0.20	—	0.33*	y
4	26	0.84*	0.38	$y = -0.89 + 1.45x$	$\beta \neq 1, \alpha \neq 0$	0.84	$y = 0.84x$	1.79	0.59	0.71*	0.62*	—	y
5	26	0.69*	0.38	$x = 0.66 + 0.56z$	$\beta \neq 1, \alpha = 0$	1.23z	$x = 1.23z$	0.59	0.92	—	0.62*	0.50	z
6	47	0.47*	0.29	$y = 0.59 + 0.62x$	$\beta \neq 1, \alpha = 0$	0.76	$y = 0.76x$	0.69	0.40	0.75*	0.66*	—	y
7	51	0.36*	0.20	$y = 3.20 + 0.27x$	$\beta \neq 1, \alpha = 0$	—	—	0.83	0.46	0.75*	0.66*	—	y
8	18	0.59*	0.47	$y = 4.005 + 0.54x$	$\beta \neq 1, \alpha = 0$	—	—	1.87	1.04	0.76*	0.67*	—	y
9	17	0.69*	0.24	$y = 1.11 + 0.69x$	$\beta \neq 1, \alpha = 0$	—	—	1.41	1.43	0.20*	0.21*	—	y
10	6	0.57*	0.33	$y = 1.40 + 0.59x$	$\beta \neq 1, \alpha = 0$	—	—	1.49	1.54	0.46*	0.62*	—	x

y — phase of 5 leaves; x — phase of 10 leaves; z — phase of 13 leaves; α, β — parameters of regression equation.

Table 3

Correlations and variances of stems' infection in phase of 5 and 10 leaves

Series of exp.	Coefficient of correlation $r_{cal.}$	Coefficient of correlation $R_b.$	Variance of stems' infection in phases	
			5 leaves	10 leaves
1983	0.65*	0.38	0.80	0.35
1984	0.38*	0.33	14.37	8.81
1985	0.62*	0.47	17.33	11.66

Table 4

Statistical relationships between natural infection of leaves in the field and artificial infection in laboratory

Series of exp.	Phase of growth	Coefficient of correlation		Regression equation
		$r_{cal.}$	$R_{lab.}$	
1984/1	5 leaves	0.55*	0.42	$y = 1.64 + 0.05x$
1984/2	5 leaves	0.50*	0.42	$y = 2.30 + 0.05x$
1985	5 leaves	0.45*	0.33	$y = 1.61 + 0.04x$

y — infection of leaves in the field; x — infection of leaves in the laboratory.

older plants. This fact, along with the equivalence of the 5- and 10-leaf stages is confirmed by the verification of the hypotheses of the equivalence of stages (Table 5).

It was found upon analysing the infection of leaves taken from different layers that the variance of infection of the leaves from the middle layer was on average 37.45 while for leaves from the lower layer — 12.93 and upper layer, 13.61. This tendency was found in each experiment. This indicates that the degree of infection of the leaves from the middle layer best differentiates the studied plants.

The degree of infection of leaves was evaluated 3 and 6 days after infection. It was found on the basis of verification of hypothesis H_0 on the equivalence of infection coefficients that the degree of infection of leaves after 6 days is a better indicator, both in the case of the 5- and 10-leaf stage. The variance of the infection of leaves 3 days after infection was 0.03-0.18, whereas after 6 days, it equalled 6.34-37.46 (Table 6). Correlation coefficients were calculated on the basis of the results of a series of infections. They equalled from 0.33 to 0.49 (at $R_{lab.} = 0.47$) which points to the low reproducibility of the obtained results. No

Table 5

Usefulness of plants in different stages of growth to the leaf-testing of resistance to late blight

Series of exp.	No. of objects	Coefficient of correlation for leaves in 2 phases	Coefficient of correlation tab.	Regression equation	Verified Hypothesis H_0	Modified regression equation	Variance of phases		Chosen phase
							y	z	
1	14	0.56*	0.53	$y = 2.21 + 0.43x$	$\beta \neq 1 \quad \alpha \neq 0$	—	0.95	0.56	y
2	14	0.63*	0.53	$y = 0.29 + 0.85x$	$\beta \neq 1 \quad \alpha = 0$	$y = x$	2.05	1.11	y
3	28	0.54*	0.39	$y = 1.50 + 0.58x$	$\beta \neq 1 \quad \alpha \neq 0$	—	1.26	1.08	y
4	18	0.59*	0.47	$y = 0.90 + 0.84x$	$\beta = 1 \quad \alpha \neq 0$	$y = 0.90 + x$	0.70	0.80	y, x

Series 1-3: y — 8 leaves, x — florescence; Series 4: y — 5 leaves, x — 10 leaves; α, β — parameters of regression equation.

Table 6

Usefulness of indicators of infection in leaf-test

Series of exp.	No. of objects	Phase of growth	$r_{\text{cal.}}$ for infection after 3 days (y) after 6 days (x)	$R_{\text{tab.}}$	Regression equation	Verified hypothesis H_0	Coefficient of recounting B_0	Modified of regression equation	Variance		Chosen indicator
									y	x	
1	18	5 leaves	0.62*	0.47	$y = 0.11 + 0.02x$	$\beta \neq 1 \quad \alpha = 0$	25.53	$y = 25.53x$	0.03	18.91	x
		10 leaves	0.56*	0.46	$y = -0.07 + 0.08x$	$\beta \neq 1 \quad \alpha = 0$	9.47	$y = 9.47x$	0.12	6.34	x
2	54	5 leaves	0.47*	0.27	$y = 0.15 + 0.02x$	$\beta \neq 1 \quad \alpha \neq 0$	—	—	0.06	37.46	x
		10 leaves	0.41*	0.27	$y = 0.06 + 0.05x$	$\beta \neq 1 \quad \alpha = 0$	7.71x	$y = 7.71x$	0.18	10.40	x

 α, β — parameters of regression equation.

significant correlations were found either between the degree of leaf infection of the tested varieties and breeding lines in the leaf test and their infection under natural field conditions.

The disc test

This test was found to be unsuitable because it is very work-consuming and gives completely unreproducible results.

Test on isolated stems

The results of 2 series of infections carried out on stems obtained from plants in the 5- and 10-leaf stages showed that the average degree of infection was always less, and its variability S^2 always greater in the stems from plants in the 5-leaf stage (Table 7). The greater suitability of testing stems from 5-leaf

Table 7

Statistical characterization of mean degree of infection of tomatoes stems

Series of exp.	Phase of plant's growth	Mean degree of infection	SD	S^2	$V\%$
1	5 leaves	3.67	1.63	2.64	53.36
	10 leaves	5.00	1.15	1.32	28.36
2	5 leaves	3.04	1.64	2.68	58.89
	10 leaves	4.02	1.24	1.53	30.76

SD — standard deviation; S^2 — variance; V — coefficient of variation.

stage plants is also indicated by its correlation with the infection of stems in the test on whole plants ($r = 0.66$ at $R_{\text{tab.}} = 0.46$). This correlation was insignificant for the stems from plants at the 10-leaf stage. In contrast to the variance analysis indicating that the younger stage is better for infecting stems, verification of the hypothesis ($H_0: \beta = 1, \alpha = 0, y = x$; y — 5-leaf stage; x — 10-leaf stage) points to the equivalence of both studied growth stages. Upon comparison of the assessment of the infected stems after 4 days and 14 days, it was found that the degree of infection after 14 days was a better indicator. Although the correlation between both assessment dates is significant for both growth stages (r for the 5-leaf stage was 0.75, for the 10-leaf stage 0.68 at $R_{\text{tab.}} = 0.27$), comparison of the differentiation of the studied varieties and lines was found to be better 14 days after infection (Table 8).

Table 8
Variances of infection of stems after 4 and 14 days since inoculation

Phase of plant's growth	Evaluation after days	Variance of infection
5 leaves	4	1.15
	14	2.68
10 leaves	4	1.23
	14	1.53

THE SUITABILITY OF LABORATORY TESTS FOR DETERMINING THE RESISTANCE OF FRUIT

The tomato fruit plays a very important role in the resistance of the plant to potato blight, but it is very difficult to infect it under artificial conditions.

From among the 6 tested ways of infecting fruit, the best was found to be instilling a suspension of spores on the calycine depression with its sepals left remaining.

From the 2 methods initially compared (injection and submersion), the former was eliminated since the latter method of submersion in the inoculum gave much higher degrees of fruit infection. The infection index, *P* equalled 22.28-24.15% for submersion and 12.59-19.20% for injection. The differentiation of the infection of the studied tomatoes was also greater. In addition, it is a much easier method which better imitates natural infection and is less work-consuming than the injection method. Figure 3 presents the infection of fruits of 2 of the studied varieties and breeding line, Roma, Moneymaker, West Virginia 700, using 5 methods of inoculating with the same mixture of isolates:

- 1 — submersion of fruit in a suspension of spores,
- 2 — instilling a spore suspension into the calycine depression after the peduncle was cut off,
- 3 — instilling a spore suspension into the calycine depression with the sepals remaining,
- 4 — spraying fruit with peduncles remaining,
- 5 — spraying fruit with peduncles removed.

As was expected, the variety Moneymaker which is the standard of high susceptibility, was infected the most, regardless of the method of inoculation. The highest rate of infection was obtained using method 2, the lowest, method 4. West Virginia 700, the standard of resistance, reacted similarly. The variety Roma, which is considered to be a moderately resistant variety, exhibited a lower degree of infection by all methods except no. 5. Under field conditions, the fruit of this variety is slightly less resistant than of West Virginia 700.

The suitability of the methods employed was evaluated on the basis of

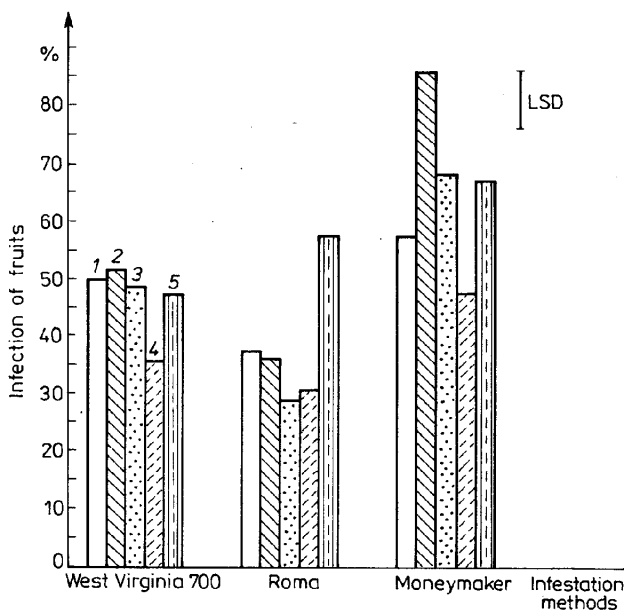


Fig. 3. Infection of the fruits of 3 studied lines of varieties infected by 5 methods

regression and correlation analysis. Methods 1, 3 and 4, 5, whose correlation coefficients P for infection – spot after 4 days and spot after 14 days, were significant (Table 9) were chosen. For methods 1, 3 and 4, 5, and then 1, 5 and 3, 5, variance was also compared, selecting the method for which the differences in the infection of the fruit were greater between varieties than within a variety. When the infection variance within a variety was compared it was found that

Table 9
Correlation infections of fruits in methods 1-5

Compared methods	Infection after 4 days		Infection after 14 days	
	$r_{cal.}$	Regression equation	$r_{cal.}$	Regression equation
1, 2	-0.06	$y = 27.73 - 0.05x$	0.58*	$y = 45.97 + 0.48x$
1, 3	0.51*	$y = 5.31 + 0.37x$	0.38*	$y = 36.61 + 0.42x$
1, 4	0.24	$y = 2.07 + 0.04x$	0.32	$y = 44.05 + 0.37x$
1, 5	0.24	$y = 20.64 + 0.26x$	0.50*	$y = 41.64 + 0.54x$
2, 3	0.66*	$y = -11.06 + 0.77x$	0.34	$y = 30.95 + 0.44x$
2, 4	0.52*	$y = -0.05 + 0.12x$	0.26	$y = 51.41 + 0.35x$
2, 5	0.67*	$y = 1.23 + 0.95x$	0.11	$y = 69.12 + 0.14x$
3, 4	0.20	$y = 2.26 + 0.04x$	0.45*	$y = 39.25 + 0.47x$
3, 5	0.19	$y = 22.74 + 0.29x$	0.36*	$y = 56.41 + 0.35x$
4, 5	0.62*	$y = 14.86 + 3.95x$	0.42*	$y = 52.10 + 0.39x$

for methods 4 and 5, and 1 and 3, the differences between them, with the exception of Roma, are statistically insignificant, which allows these three methods to be considered equally precise. Methods 1 and 3 are equally useful for evaluating the resistance of fruit to late blight and both are better than methods 4 and 5 (Table 10).

The best method for use in practical breeding seems to be no. 3 since it is the least work-consuming, requires a small amount of inoculum and is relatively precise, since the amount per fruit is measured out.

When the value of the various fruit infection indicators P was determined, it was assumed that resistance to infection is expressed by the size of the spot 4 days after inoculation, resistance to spreading of the fungus — by the size of the spot and depth of streaking after 14 days and resistance to sporulation — by the size of the fur of sporulating mycelium 14 days after inoculation.

After conducting 5 series of infections of fruit in 3 series, no correlation was found between the particular infection indexes e.g. fruit without external symptoms was often heavily streaked with mycelium inside. However, in 2 series (in 1984 and 1985), such correlations did, albeit weak, occur. In 1984, a correlation was found between the infection after 4 days and the mycelium fur ($r = 0.38$), and the size of the spot after 14 days ($r = 0.34$), and between the size of the spot after 14 days and streaking ($r = 0.41$ at $R_{\text{tab.}} = 0.29$). In general, when the first index rose, the others did too. In the 1985 series, significant correlations were found between the size of the spot after 14 days and the depth of streaking of the pericarp ($r = 0.92$ at $R_{\text{tab.}} = 0.49$).

In looking for better differentiation in infection of tomatoes, inoculation with races 0 and 1 was done by method no. 3. It was found that it is more favourable to use an inoculum with a greater pathogenicity. Race 1 gave better differentiation of the studied varieties and breeding lines and gave better reproducibility in the test (Table 11).

Comparison of the infection of fruit of the same varieties and breeding lines obtained under artificial, laboratory conditions (method 3) and their infection under natural field conditions, showed that the artificial infection of fruit with highly pathogenic isolates gives representative results for field conditions.

In 1984, the correlation between the field infection expressed as the percentage of diseased fruit in the total yield, and the laboratory infection P expressed as the size of the spot 14 days after inoculation was rather high ($r = 0.67$ at $R_{\text{tab.}} = 0.29$). For the remaining artificial infection indexes, the correlations were also statistically significant, but much smaller ($r = 0.42-0.57$). Similarly as in 1984, in the 1985 season the varieties and lines whose fruit was less diseased under laboratory conditions, especially by race 1 (the P index ranged from 10 to 35%) were also less infected under natural field conditions (the percentage of diseased fruit in the total yield ranged from 0 to 8.3%). The varieties and lines whose fruit was more heavily infected in the laboratory tests (the P index ranged from 35 to 100%) were also heavily infected in the field and had from 8.5 to 33.1% diseased fruit in the total yield (Table 12, Phot. 1).

Table 10
Evaluation of methods of fruits' inoculation

Correlated methods	Infection after 4 days P				Infection after 14 days P				Correlation of infection after 4 and 14 days in succeeding methods $R_{\text{tab.}}$ 0.36		Variances of fruits' infection in succeeding methods								Chosen method
	H_0	B_0	Modified regression equation	H_0	B_0	Modified regression equation	H_0	B_0			Among varieties	West Virginia 700	Roma	Moneymaker					
1, 3	$\beta \neq 1$	$\alpha = 0$	0.51	$y = 0.51x$	$\beta = 1$	$\alpha = 0$	—	$y = x$	0.40*	0.58*	39.26	41.53	41.81	42.87	27.48	37.05	44.60	36.92	1, 3
4, 5	$\beta \neq 1$	$\alpha = 0$	6.46	$y = 6.46x$	$\beta = 1$	$\alpha = 0$	—	$y = x$	0.11	0.30	45.37	42.44	44.50	43.20	43.14	40.40	47.80	42.52	(4) 5
1, 5	—	—	—	—	$\beta \neq 1$	$\alpha = 0$	—	—	0.40*	0.30	39.26	42.44	41.81	43.20	27.48	40.40	44.60	42.52	1
3, 5	—	—	—	—	$\beta \neq 1$	$\alpha \neq 0$	—	—	0.58*	0.30	41.53	41.44	42.84	43.20	37.05	40.40	36.92	42.52	3

p — The hypotheses were not verified for 1, 5 and 3, 5 (infection after 4 days) because the correlation was insignificant; B_0 — coefficient of recounting for 2 investigated methods; α, β — parameters of regression equation; (4) — method equivalent to 5 but a bit worse.

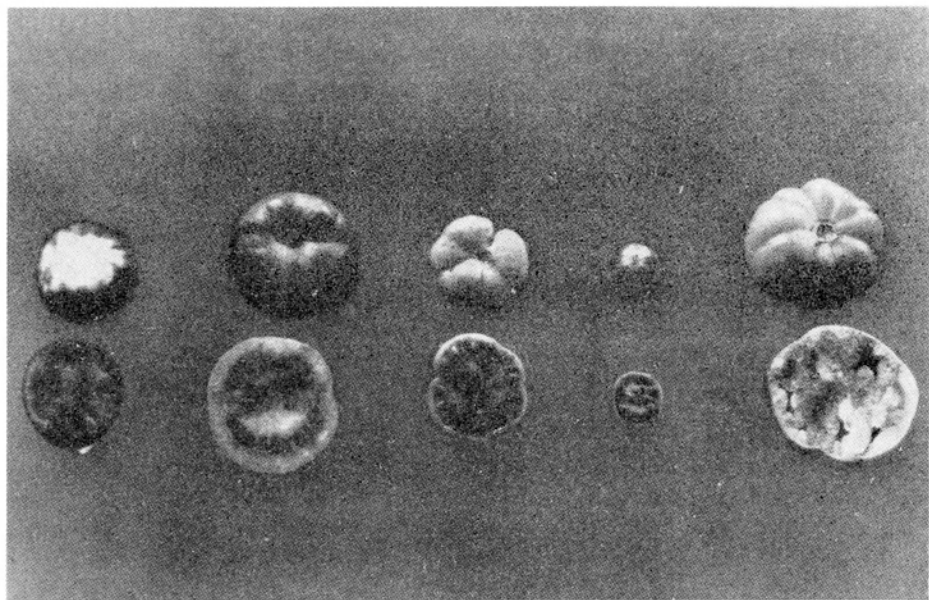
Table 11
Coefficients of correlation (r) for test's repetitions
 $r_{\text{tab.}} = 0.47$

Symptom of fruit infection%	Race 0	Race 1
Spot after 4 days	0.39	0.61*
Spot after 14 days	0.32	0.71*
Fruit's streaked with mycelium after 14 days	0.05	0.91*

Table 12
Infection of fruits in natural and artificial conditions in 1985

Variety Line Wild species	Artificial infection		Natural infection-field
	spot after 14 days		percent of disease fruits in total yield
	race 0	race 1	
Caline	12.50	66.00	8.55
Goniec 13	4.51	65.00	13.09
Kirys	3.10	100.00	18.10
Moneymaker	1.60	94.00	33.10
New Yorker	1.00	78.00	12.33
Pieraline	1.50	91.00	16.57
Robot	20.55	35.00	8.33
Roma	1.85	15.37	1.65
Rutgers	11.05	65.00	16.99
Szkarłatna Kula	1.60	100.00	10.36
No. 155/84	1.36	18.33	5.37
No. 62/74	0.72	24.99	7.20
No. 246/72	3.33	21.55	7.42
PI 224675	0.00	21.00	0.05
PI 341988	10.00	34.25	5.22
Genewa T-5	1.66	33.10	5.33
Ottawa 30	0.00	31.00	1.10
West Virginia 700	0.20	16.50	0.00
<i>Lyc. hirsutum</i>	1.00	10.00	0.00
<i>Lyc. cerasiforme</i>	11.70	95.00	13.11
\bar{x}	4.46	50.75	9.19
SD	5.61	32.79	8.06
V%	125.78	64.61	87.70

\bar{x} — mean; SD — standard deviation; V — coefficient of variation.



Phot. 1. Streaking by *Phytophthora infestans* mycelium of the pericarp of tomato fruits 14 days after inoculation

DISCUSSION AND CONCLUSIONS

The results obtained in this study have shown that when evaluating the resistance of tomato plants, whole plants in the 5-leaf stage should be tested first, after which the leaf test should be carried out for more precise information on the nature of the resistance. Infecting whole plants makes it possible to evaluate the infection of both leaves and stems and most resembles natural conditions, making it possible to observe the plant's immune reactions. One of the advantages of testing whole plants is the possibility of leaving selected individuals for reproduction. The remaining tests on isolated leaves and stems do not generally give such possibilities.

Although the leaf test gives results which correlate less with field results than the test on whole plants, it is more useful for evaluating such aspects of horizontal resistance as resistance to infection, streaking or sporulation of the pathogene.

Many authors have evaluated the resistance of the tomato on plants at various stages of growth. Gallegly (1960) found that resistance which is coded by many genes will not be expressed in very young plants, especially in the cotyledon stage, and that doubts about its development or regression with age can only be checked from the 15-leaf stage and up. This was later confirmed by numerous experiments.

Shirko (1971) considered the 5-leaf stage as best for evaluation, Laterrot (1975), the 10-12-leaf stage and Pshedetskaya and Kherepanova (1971) blooming as best. Gallegly (1960) proposed that infection be carried out at 6-weeks, which usually corresponds to the 10-leaf stage. This author has shown that the best results are achieved by infecting both whole plants as well as leaves isolated at the 5-leaf stage. Testing at such a young age is advantageous for biological reasons when resistance screening since the plants tolerate the conditions in the infection chamber well. Organizationally this is also preferable since the plants are in the greenhouse for just short lengths of time, do not take up much space and can be easily moved. In addition, smaller plants are easier to inoculate by spraying evenly, and less inoculum is used.

The disc test, recommended by Grümmer and Eggert (1968), Günther et al., (1970), turned out to be the least suitable. This test did not give good reproducibility of results or correlation with the results of other tests, and was also very work-consuming.

Evaluation of various laboratory tests for determining the resistance of fruit showed that the best method is instilling a suspension of conidiospores on the calycine depression with its sepals left in place. Other methods, such as placing mycelium under the skin of fruit (Shirko, 1963), spraying (Eggert, 1970; Brezhnev et al., 1978), injecting inoculum under the skin (Laterrot, 1975) were shown to be much inferior.

When different methods of infecting were used, it was noticed that fruit of the same varieties and breeding lines developed different degrees of infection. The degree of infection was always greater when the fruits were submerged in the inoculum than when it was injected under the skin, this in spite of the necessity of penetrating the barrier of the covering layer. The fungus spores probably have worse conditions in the juice of the fruit than in water, and for that reason, infection by injection progresses more slowly. It is also possible that the mycelium penetrates the sepals much more quickly than the fruit skin.

The conducted observations have also demonstrated that the infection of the fruit must be evaluated using several indicators, such as infection 4 days after inoculation, spot and fur size, penetration of pericarp by mycelium, because they represent various elements of resistance which are not always correlated with each other. Usually, when one indicator rose, so did the others, with the exception of mycelium fur which increased slightly with spot size, but decreased with increased infection after 4 days and streaking. This suggests that resistance to sporulation is a different type of resistance and is unrelated to infection after 4 days and streaking. The slight increase in fur size with increasing spot size is probably related to only the size of the infected area on which the conidiostalks arise. Sporulation seems to be the most deceptive resistance indicator, since it is the most dependent on external conditions.

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Metody oceny odporności pomidorów na zarazę ziemniaka (*Phytophthora infestans* (Mont.) de Bary) w warunkach laboratoryjnych

Streszczenie

Celem badań było ustalenie jednolitej laboratoryjnej metodyki określenia odporności pomidorów na zarazę ziemniaka. Oceniano test na całych roślinach, w fazie 5, 10, 13 liści właściwych, testy liściowe i krążkowe. Badano odporność zarówno roślin, jak i owoców.

Stwierdzono, że najlepszym testem jest zakażenie całych roślin w fazie 5 liści właściwych. Test liściowy przeprowadzony na liściach środkowego piętra rośliny jest przydatny do oceny elementów odporności w materiale wyselekcjonowanym po teście na całych roślinach.

Do określenia odporności owoców najlepszą metodą spośród 6 badanych okazało się wkraplanie inokulum w zagłębienie kielichowe zielonych owoców z pozostawionymi działkami kielicha. Test taki pozwala na ocenę kilku wskaźników porażenia, takich jak: wielkość plamy, nalotu i przerastania perykarpu owocu grzybnią.