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A NEW METHOD FOR EVALUATING TOMATO LEAF RESISTANCE TO *PHYTOPHTHORA INFESTANS* USING A SEEDLING TEST

ABSTRACT

A laboratory test for evaluating the tomato leaf resistance to *Phytophthora infestans* in the seedling stage has been developed. The test is suitable for evaluation of breeding lines and selec–tion within large populations. The following standard varieties and accessions representing a whole range of variability of known resistance were used: Moneymaker, New Yorker, West Virginia'63, West Virginia 700 and Ottawa 30. Various *P. infestans* isolates were used in the tests. Tomato seedlings grown in a liquid medium were cultured and tested in a growth chamber. The infection of individual seedlings was evaluated using 9–degree logistic key and data were statistically estimated. The results were reproducible in the same growth conditions. Ranking of infection degree was as follow: Moneymaker ≥ New Yorker > West Virginia'63 > West Virginia 700 = Ottawa 30 irrespectively of test conditions or the isolates applied. The laboratory ranking was consistent with field observations. The results of tests depended on concentration of liquid medium (lower infection at lower concentration), on the isolate used and its spore concentration. The method proposed meets all requirements necessary for breeding and research as a routine method for evaluation of the tomato leaf resistance to *P. infestans*.

Key words: late blight, *Lycopersicon esculentum*, method, *Phytophthora infestans*, resistance, seedling test, tomato

INTRODUCTION

The late blight caused by *P. infestans* is one of the most devastating diseases of field tomato in different climatic regions. Intensive investi–gations to solve this problem were started in a few countries in the 1940s and 1950s. Studies were carried out to find sources of resistance, investigate virulence of *P. infestans* isolates, as well as to determine the resistance inheritance. The selection of resistant lines was also be–gun. Mills (1940) and Ferguson *et al.* (1952) in Canada, Gallegly (1952), Walter and Conover (1952) in USA and Goodman (1957) in Ire–land started these investigations, followed by Grümmer et al. (1969) in

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Germany, Kubicka (1969) in Poland, Turkensteen (1973) in the Neth–erlands and Laterrot (1975) in France. First resistant varieties carry–ing the *Ph–1* gene were bred: New Hampshire Surecrop (Rich and Yeager 1957), Rockingham (Rich *et al.* 1962) and New Yorker (Robin–son *et al.* 1967). Then Gallegly (1964) bred West Virginia'63 , a variety partially resistant to the *P. infestans* isolates which infect the forms carrying *Ph–1* gene. Turkensteen (1973) stated that resistance of the line WV 700, which was a resistant progenitor of West Virginia'63 cultivar, was determined by *Ph–2* gene, and Laterrot (1975, 1994) bred several varieties with this gene.

Not only field experiments but also laboratory tests were run to study the resistance to this disease. Plants were usually grown in greenhouses, and tests were performed in greenhouses or in growth chambers. Some authors used 6 to 8–week–old plants for inoculation (Kubicka 1969, Turkensteen 1973, Laterrot 1975 and Nishio *et al.* 1985). In Turkensteen`s experiments (1973) the resistance of 8–week–old plants was higher than that of 6–week–old ones, and it did not change when plants grew older, therefore the author did not recommend screening the resistance of plants before they reach 8 weeks. Nevertheless, many authors used younger plants, namely Gallegly (1952, 1960): 3 – 7 weeks old, Conover and Walter (1953) at the stage of 2 – 4 leaves (about 3 weeks), Shirko and Kuzubova (1972) at the stage of 5 – 6 leaves (about 5 weeks) and Hartman and Huang (1995) 4 weeks old plant. On the other hand, Wilson and Gallegly (1960), who studied the effect of age (up to 7 weeks) and some other conditions on the expression of resistance, stated that plants were less infected as they grew older. The degree of infection was also influenced by nutrition and light, a lower medium concentration caused a lower degree of infection in some genotypes. Based on this experiment Gallegly (personal information, 1986) worked out a method of testing 2 to 3–week–old seedlings grown in poor soil (a mixture of soil and sand). Seedlings were cultured and tested in greenhouses in order to select breeding materials.

Evaluation of resistance to late blight by detached leaflets inocula–tion, a method widely used in potato investigations, is used much less often in tomato resistance studies. The resistance of various tomato forms was studied using the leaflet tests by Günter *et al.* (1970), Peirce (1970), Eggert (1972), Turkensteen (1973) and Nishio *et al.* (1985), while pathogenicity of *P. infestans* isolates was studied by Kubicka (1969). On the other hand, Horodecka (1989b) was of the opinion that leaf tests can be used as an additional method, but variation in plant response to the pathogen was better estimated when whole plants were inoculated, and she used such plants to estimate the pathogenic–ity of *P. infestans* isolates (Horodecka 1989a). Another method of re–sistance evaluation was applied by Grümmer *et al.* (1969). They observed a high correlation between field resistance and sporulation

tested on leaf discs, while Horodecka (1989b) recognized this method as not suitable.

No standardized method of investigating tomato–leaf resistance to late blight has been recommended therefore the experiments of different authors can hardly be compared. Gallegly's test on 2 to 3–week–old seedlings could play this role, as it is simple, fast, and suitable for studies on large populations of tomato. The authors of this paper repeated the test and confirmed Gallegly`s results (not published). Unfortunately, conditions in greenhouses are not stable and depend on climate and vegetation season. To obtain repeatable results it is necessary to grow and test plants under controlled conditions.

The aim of this study was to work out a laboratory test of the seedlings grown in an artificial medium under controlled conditions suitable for fast evaluation of resistance of breeding lines, as well as for selection of large tomato populations segregating for leaf resistance to *P. infestans*. Moreover, it was aimed to estimate the dependence of the test results on the conditions of testing and isolate applied.

The experiment was run in the Breeding Station of Horticultural Crops Ulrichów, Warsaw, Poland, 1989 – 1996. This paper was based on the results of selecting tests done for breeding purposes and some tests performed in order to find the best conditions for selection.

MATERIALS AND METHODS

Plant materials:

This study was run using the following tomato varieties and accessions (standards of resistance to *P. infestans*):

1. Moneymaker (Mon)	susceptible
2. New Yorker (NY)	*Ph–1* gene
3. West Virginia`63 (WV63)	*Ph–2* gene
4. Ottawa 30 (Ott 30)	*Ph–2* or more genes (discussion)
5. West Virginia 700 (WV700)	*Ph–2* or more genes (discussion)

Numbers 1–3 refer to the varieties of *Lycopersicon esculentum,* while lines WV700 (PI 204996 – Gallegly 1960) and Ott30 (PI 198674 – Kerr 1989, personal letter) are accessions of *L. esculentum* × *L. pimpinellifolium* (Clark et al. 1975). Seeds of WV63 and WV700 were received from Dr. R. Young, West Virginia Univ., Morgantown, USA. Other standards were obtained from Polish breeding collections.

P. infestans isolates:

The following isolates of *P. infestans* were used in the tests:

Designation/Year	Source	Country (Station)
1. H 2/83	potato	Holland
2. Ul 12/84	tomato NY	Poland (Ulrichów)
3. R 17/88	tomato	Poland
4. R 19/88	tomato	Poland

5. MP 269	potato	Poland
6. MP 270	potato	Poland
7. MP 272	potato	Poland
8. Ul 1/94	tomato	Poland (Ulrichów)
9. Ott 2/94	tomato Ott30	Poland (Ulrichów)
10. Mc 3/94	tomato	Poland (Mielec)
11. Ul 1/95	tomato	Poland (Ulrichów)
12. Sw 2/95	tomato	Poland (Świętosław)

Numbers 1 and 5–7 were received from Dr. H. Zarzycka, Młochów Research Center of Potato Research Institute, Poland, numbers 3 and 4 from Dr. E. Horodecka, Research Institute of Vegetable Crops, Dep. of Plant Breeding, Reguły, Poland. The authors of this paper collected other isolates from the leaves of susceptible tomatoes, except numbers 2 and 9, collected from resistant tomatoes.

Experimental procedures

The resistance tests were run by the modified method for frost resistance in cereals (Zagdańska and Rybka 1984). Culturing and testing of tomato seedlings were run in cupboards illuminated with fluorescent tubes, on the shelves mounted at various distances between them. Light intensity and temperature depended on this distance. The cupboards were placed in a growth chamber at a stable temperature of 12 ± 1°C. In 1989 – 1992 the photoperiod of 10 h day/14 h night was applied, and in 1993 – 1996 12/12 h.

The following variants of light intensity and temperatures were used:

Variant	Temperature [°C, day/night]		Light intensity [lux]	
	Before inoculation	After inoculation	Before inoculation	After inoculation
I	25/16	19/12	20000	5000
II	21/14	20/12	10000	10000

The seeds were germinated in Petri dishes at room temperature in diffused light until cotyledons were spread (about 1 week). Fig. 1 shows the scheme of seedling culturing and testing. Ten seedlings were placed on a piece of filter paper, covered with another piece, and then the filter paper with the seedlings was rolled (A). Thirty or forty seedlings of each standard were prepared for one test. Fifteen rolls were placed in a plastic box and a liquid medium was poured into the box (B). Additional medium was supplied when necessary. Boxes with the seedlings were placed in a growth chamber in lighted cupboards. After 2 – 3 weeks, when the seedlings had 1 – 2 leaves, they were inoculated by spraying. Boxes with the seedlings were placed in plastic containers with water to keep high humidity (C). Containers covered with a glass and with white paper were put into a cupboard under light for 24 h. Then the boxes with

seedlings were placed in trays with some water added (D) and put again in the cupboard under light.

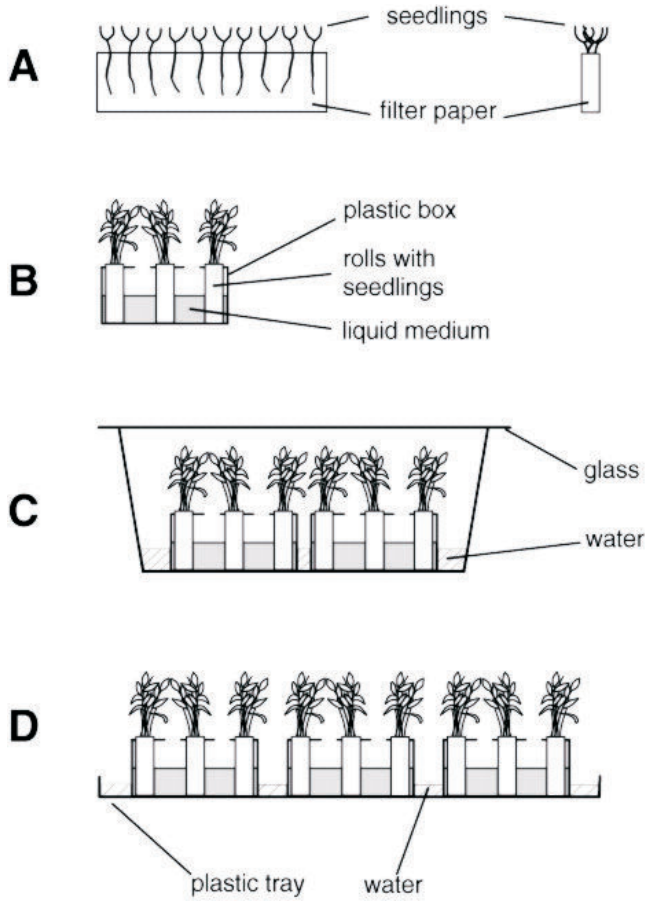

Fig. 1 The scheme of seedlings testing
A Preparation of tomato seedlings for testing (1 week after sowing)
B Seedlings growing before inoculation (2 – 3 weeks)
C Seedlings growing after inoculation (24 h)
D Disease incubation period (6 days).

The following liquid medium was applied for seedlings culture:

Macroelements	Concentration [g/l]
$Ca(NO_3)_2 \times 4H_2O$	0.820
KNO_3	0.147
KCl	0.071
KH_2PO_4	0.143
$MgSO_4$	0.143
NaFeEDTA	0.028

Microelements	[g/l] (1000 × concentration)
$CuSO_4 \times 5H_2O$	0.050
$ZnSO_4 \times 7H_2O$	0.100
H_3BO_3	0.550
$Al_2(SO_4)_3 \times 18H_2O$	0.050
$MnCl_2 \times 4H_2O$	0.350
$NiSO_4 \times 4H2O$	0.050
$Co\ (NO_3)_2 \times 4H_2O$	0.050
KJ	0.025
KBr	0.025
$Na_2MoO_4 \times 2H_2O$	0.050
LiCl (conc. solution)	100 µl

This medium diluted (1:4) was also used.

The isolates of *P. infestans* were maintained on leaves of susceptible tomatoes. New isolates were passaged on tomato leaves at least three times, and those that were used frequently were permanently maintained this way. Sporangia were washed off from the leaves with distilled water, then concentration of sporangia was counted in haemocytometer, and the suspension was adjusted to 50 spores/mm^3. The suspension was then incubated for 2 h at 10 – 12°C to release zoospores. After transfer to room temperature for 30 min the inoculum was used (diluted if necessary).

Test evaluation:

7 days after inoculation the necrotic area of leaves and cotyledons of individual seedlings was evaluated using a 9–degree logistic key (Van der Plank 1963, Pietkiewicz 1972, Försund 1987):

Degree	Necrotic area in %
1	99.6 –100.0
2	97.8 – 99.5
3	90.5 – 97.7
4	68.0 – 90.4
5	32.2 – 67.9
6	9.6 – 32.1
7	2.4 – 9.5
8	0.6 – 2.3
9	0.0 – 0.5

All tests were read by the same person for the sake of unified evaluation. The mean infection degree of seedling samples of each standard was calculated. These means were listed in tables, and numbered according to the test order. Tests that were done at the same time have the same number. Replications of tests are designated with additional numbers (e.g. 14/1, 14/2), variants of test conditions or isolates are designated with letters (e.g. 3a, 3b). Many tests had no replications performed at the same time, therefore in the analysis of variance, subsequent tests done exactly in the same conditions were treated as

replications. The data were analyzed by ANOVA and Tukey`s test for estimation of the differences between means in one–factorial or multi–factorial experimental design. The list of tests cited in this paper is as follows:

Table 1

List of tests cited in this paper

No. of test	Year	Medium conc. [%]	Isolates	Conc. of spores [mm^3]	No. of plants tested	No. of table
1, 2	1989	B	Ul 12/84	50	40	2
3	1989	D, B	Ul 12/84	50	40	2, 3
4, 5	1989	B	Ul 12/84	50	40	2
6, 7	1989	D, B	Ul 12/84	50	40	2, 3
8	1989	B	Ul 12/84	50	40	2
9	1989	D, B	Ul 12/84, R 17/88	50	40	3, 4
10 – 11	1989	B	H 2/83, R 19/88	50	40	4
12 – 17	1990	B	Ul 12/84	50	40	2
18, 19	1990	D	Ul 12/84	50	40	3
20	1991	B	Ul 12/84	50	30	2
21	1991	B	Ul 12/84	50	40	2
22 – 25	1991	B	Ul 12/84	50	30	2
26	1992	B	Ul 12/84	50	30	2, 3
27 – 29	1992	B	Ul 12/84	50	30	2
30	1992	D	Ul 12/84	50	30	3
31 – 33	1993	B	Ul 12/84	50	30	5
34, 35	1994	B	Ul 12/84, MP 269, MP 270, MP 272	12.5, 50	30	5
36	1994	D	Ul 12/84	50	30	7
37	1994	D	Ul 12/84	25, 50	30	7
38	1995	D, B	Ul 12/84	25	30	6, 7
39	1995	D	Ul 1/94	25	30	7
40	1995	D	Ul 1/94	25	30	7
41	1995	D	Ul 1/94, Ott 2/94	25	30	7
42	1995	D	Mc 3/94	25	30	7
43	1995	D, B	Ul 1/95	25	30	6, 7
44	1996	D	Ott 2/94	25, 50	30	7
45	1996	D, B	Ott 2/94, Sw 2/95	25	30	6, 7
46, 47	1996	D	Sw 2/95	25	30	7

B – basic, D – diluted

RESULTS

Infection of resistance standards

In all of tests presented in this paper, varieties and accessions were ranged in the same way. In most tests applied plants of Moneymaker var. were infected totally (Table 2 – 7). In the tests run in 1989 – 1992 New Yorker var. was partially resistant (Table 2 – 4), and significant differences appeared between Mon and NY. The NY means depended on the kind of

test and varied from 3.0 to 8.7, while the total means calculated from all tests varied from 4.8 to 7.5, depending on the conditions of growth and testing. In 1993 – 1996 NY was strongly infected and there were very few tests in which Mon and NY responses to the pathogen were but slightly different (Table 5 – 7). The reaction of NY did not depend on isolate used. The variety West Virginia`63 was partially resistant, and it was less in–fected than NY. The differences between NY and WV63 were significant (Table 5 – 7) in all tests except those presented in Table 2. The accessions Ottawa 30 and West Virginia 700 were highly resistant (Table 2 – 7). They were the least infected, or were not infected at all, and the infection of these

Table 2

Results of tests on tomato seedlings grown in medium of basic concentration, inocu–lated with Ul 12/84 *P. infestans* isolate at concentration of 50 spores/mm^3 in 1989 –1992

No of test	Year	Mean degree of infection					Remarks
		Mon	NY	WV63	Ott30	WV700	
1	1989	1.0	4.3		8.4	7.5	
2		1.0	4.1		7.8	7.2	
3a		1.0	3.3		8.9	8.9	1)
4		1.0	4.6		8.4	8.4	
5		1.0	5.5		8.7	8.9	
6a		1.0	6.9		9.0	8.9	1)
7a		1.0	6.1		9.0	9.0	1)
8		1.1	4.8		8.8	9.0	
12	1990	1.0	3.8		8.0	7.8	
13		1.0	4.3		7.8	8.2	
14/1		1.0	5.0		7.6	6.6	
14/2		1.0	5.0		7.3	6.2	
15		1.0	5.2		7.5	7.3	
16		1.0	3.0		6.0	5.7	
20/1	1991	1.0	4.9		7.4	7.5	
20/2		1.0	5.5		7.8	7.6	
21		1.0	5.4		8.3	8.6	
22		1.0	4.2		7.2	7.8	
23		1.0	3.2		7.3	7.2	
24		1.0	4.5		7.8	7.9	
25		1.0	5.2		7.6	7.2	
26a	1992	1.0	5.3		6.9	7.5	1)
27		1.0	5.6		7.3	6.7	
$\bar{x}$	89–92	1.0	4.8		7.9	7.7	LSD 0.05=0.58
$\bar{x}$	for 1)	1.0	5.4		8.5	8.6	
17	1990	1.0	4,5	5.7	8.8	8.6	2)
28	1992	1.0	6.2	6.6	9.0	8.9	2)
29		1.0	6.2	6.0	8.6	6.8	2)
$\bar{x}$	for 2)	1.0	5.6	6.1	8.8	8.1	LSD 0.05 =1.90

[1)] Results of tests No.3a, 6a, 7a and 26a to be compared with the same tests in Table 3 (for ANOVA see Table 8). [2)] Results of tests No 17, 28 and 29 including WV63.

Table 3

Results of tests on tomato seedlings grown in diluted (1:4) medium, inoculated with Ul 12/84 *P. infestans* isolate at concentration of 50 spores/mm^3 in 1989 – 1992

No of test	Year	Mean degree of infection				Remarks
		Mon	NY	Ott30	WV700	
3b	1989	1.2	6.8	9.0	9.0	1)
6b		2.2	8.7	9.0	8.9	1)
7b		1.5	8.1	9.0	9.0	1)
9a		1.0	6.1	8.9	9.0	
18	1990	1.0	7.5	8.7	8.7	
19		1.7	7.9	8.8	8.6	
26b	1992	1.8	7.3			1)
30		1.0	7.3			
	89–92	1.4	7.5	8.9	8.9	
	for 1)	1.7	7.7	9.0	9.0	

1) Results of tests No. 3a, 6a, 7a and 26a to be compared with the same tests in Table 2 (for ANOVA see Table 8)

Table 4.

Results of tests on tomato seedlings grown in medium of basic concentration, inoculated with three *P. infestans* isolates at concentration of 50 spores/mm^3 in 1989

No of test	Isolate	Mean degree of infection			
		Mon	NY	Ott30	WV700
9b	R 17/88	1.0	6.7	9.0	9.0
10a	H 2/83	1.0	5.4	9.0	8.8
10b	R 19/88	1.3	4.5	9.0	9.0
11a	H 2/83	1.0	7.0	9.0	9.0
11b	R 19/88	2.3	5.6	9.0	9.0

lines was significantly lower than of WV63. Evaluation of the infection of Ott30 and WV700 in individual tests showed some differences, but the means of many tests show no difference between these accessions.

There were only slight differences between replications of the tests carried out at the same time in the same conditions and with the same isolate (Table 2, tests 14/90 and 20/91, Table 7 tests 39/95). On the other hand, distinct differences were observed between some different tests. There were tests, in which all standards were infected more than in some other tests of weak infection (compare test 16/89 with 6/89 and 7/89, Table 2). In a few cases evaluation of one variety (particularly NY) differed from those found in most tests (see test in 3/89, Table 2 and compare tests 39 and 40/ 95 with 41a/95, Table 7). Nevertheless, the ranking was generally the same, with the exception of the test 29/92 (Table 2).

Isolate effect

In Table 4 the results of infection in 1989 with three isolates of *P. infestans* are presented. Ranking of the standards was the same as with

the isolate Ul 12/84 which was used simultaneously (Table 3). The infection of NY was lower than that of Mon, while the lines Ott30 and WV700 were not infected at all. At the same time, other eleven isolates

Table 5.

Results of tests on tomato seedlings grown in medium of basic concentration, inoculated with four *P. infestans* isolates at concentrations of 12.5 and 50 spores/mm^3 in 1993 and 1994

No of test	Year	Isolate	Conc. of spores in mm^3	Mean degree of infection					Remarks
				Mon	NY	WV63	Ott30	WV700	
31	1993	Ul 12/84	50	1.0	1.0	5.6	6.8	7.6	1)
32				1.0	1.0	4.8	7.8	8.5	1)
33				1.0	1.2		9.0	8.7	1)
34a	1994	Ul 12/84	50	1.0	1.0	6.3	8.6	8.5	1), 2)
35a		MP 269		1.0	1.1	7.0	8.8	8.6	2)
35b		MP 270		1.0	1.0	1.7	7.5	7.0	2)
35c		MP 272		1.0	1.0	3.8	8.4	7.7	2)
34b	1994	Ul 12/84	12.5	1.0	2.0	8.7.	9.0	9.0	3)
35d		MP 269		2.2	4.4	8.6	9.0	9.0	3)
35e		MP 270		1.0	2.3	6.4	8.7	8.9	3)
35f		MP 272		1.9	4.1	7.6	9.0	8.9	3)
$\bar{x}$	for 1)	Ul 12/84	50	1.0	1.1	5.7	8.1	8.3	$LSD_{0.05}$ = 1.25
$\bar{x}$	for 2)		50	1.0	1.0	4.7	8.3	7.9	$LSD_{0.05}$ = 2.54
$\bar{x}$	for 3)		12.5	1.5	3.2	7.8	8.9	9.0	$LSD_{0.05}$ = 1.71

1) Results of tests No. 31, 32, 33 and 34a on seedlings inoculated with *P. infestans* isolate Ul 12/84 at concentration of 50 spores/mm^3
2) Results of tests No. 34a, 35a, 35b, and 35c on seedlings inoculated with 4 isolates at concentration of 50 spores/mm^3
3) Results of tests No. 34b, 35d, 35e, and 35f of seedlings inoculated with 4 isolates at concentration of 12.5 spores/mm^3

Table 6

Results of tests on tomato seedlings grown in medium of basic concentration, inoculated with four *P. infestans* isolates at concentration of 25 spores/mm^3 in two replications in 1995 and 1996

No. of test	Year	Isolate	Mean degree of infection				
			Mon	NY	WV63	Ott30	WV700
38a	1995	Ul 12/84	1.0	1.0	4.2	8.5	8.5
43a		Ul 1/95	1.0	1.3	3.0	7.0	8.3
45a	1996	Ott 2/94	1.0	1.0	5.0	5.8	5.9
45b		Sw 2/95	1.0	1.1	6.9	8.6	8.4
$\bar{x}$			1.0	1.1	4.8	7.5	7.8

Results to be compared with the same tests in Table 7 (for ANOVA see Table 9)

collected from tomato and potato plants were used. They were less aggressive than those presented in Table 4, but did not change the ranking order.

In Tables 5 – 7 the results of tests conducted in 1993 –1996 with nine isolates of *P. infestans* are presented including Ul 12/84 which was used

previously. In the tests in which four isolates at spore concentrations of 50 and 12.5/mm^3 were used, MP 270 was found to be the most aggressive (Table 5, test 35b/94). Also Ul 12/84 was very aggressive in some tests, and strongly infected WV63 (at spore concentration of 50/mm^3, Table 7, tests 36 and 37/94). In most other cases, the differences of results obtained by inoculation with various isolates were not larger than the differences between tests run with the same isolate. There were no significant differences in ANOVA among isolates Ul 12/84, Ul 1/94, Ott 2/94 and Sw 2/95 used in tests 37b, 38b, 40, 41a,b, 44b, 45d and 46 (each isolate used in two tests) when a concentration of 25 spores/mm^3 was applied, while standards differed highly significantly (Table 7). On the other hand, ANOVA of the tests 38, 43 and 45 conducted with the isolates Ul 12/84, Ul 1/95, Ott 2/94 and Sw 2/95 run at two medium concentrations in two replications (Tables 6 and 7) showed highly significant differences between the effects of isolates and highly significant interaction standard × isolate (Table 9). The isolates Ul 1/95 and Ott 2/94 were significantly

Table 7

Results of tests on tomato seedlings grown in diluted (1:4) medium, inoculated with six *P. infestans* isolates at concentrations of 25 and 50 spores/mm^3 in 1994–1996

No. of test	Year	Isolate	Conc. of spores in mm^3	Mean degree of infection					Remarks
				Mon	NY	WV63	Ott30	WV700	
36	1994	Ul 12/84	50	1.0	1.0	2.1	6.4	7.1	
37a		Ul 12/84	50	1.0	1.0	1,0	6.8	6.1	
37b		Ul 12/84	25	1.0	1.0	6,5	9.0	9.0	2)
38b	1995	Ul 12/84	25	1.0	1.0	7.7	8.9	9.0	1), 2)
39/1		Ul 1/ 94		1.0	1.0	7.1		8.7	
39/2		Ul 1/94		1.0	1.0	7.2.		8.9	
39/3		Ul 1/94		1.0	1.0	7.1		8.7	
39/4		Ul 1/94		1.0	1.0	7.6		9.0	
40		Ul 1/94		1.0	1.0	7.0	8.7	8.7	2)
41a		Ul 1/94		1.0	3.9	8.3	9.0	9.0	2)
41b		Ott 2/94		1.0	1.0	7.4	9.0	9.0	2)
42		Mc 3/94		1.0	1.0	7.3	9.0	8.9	
43b		Ul 1/95		1.0	1.5	5.1	8.8	8.7	1)
44a	1996	Ott 2/94	50	1.0	1.0	6.9	8.9	8.9	
44b		Ott 2/94	25	1.0	1.0	7.9	9.0	9.0	2)
45c		Ott 2/94	25	1.0	1.0	6.9	7.8	7.8	1)
45d		Sw 2/95	25	1.0	1.4	8.2	8.8	8.8	1), 2)
46		Sw 2/95	25	1.0	1.3	8.2	9.0	9.0	2)
47		Sw 2/95	25	1.0	1.0	6.8	8.7	8.7	
$\bar{x}$	for 1)		25	1.0	1.2	7.0	8.6	8.6	
$\bar{x}$	for 2)		25	1.0	1.5	7.7	8.9	8.9	$LSD_{0.05}$ = 0.82

1) Results of tests No. 38b, 43b, 45c and 45d to be compared with Table 6 (for ANOVA see Table 9).
2) Results of tests No. 37b, 38b, 40, 41a, 41b, 44b, 45d and 46 used in ANOVA to compare 4 isolates

more aggressive than Ul 12/84 and Sw 2/95. Although these results were

obtained in various tests, the isolates Ott 2/94 and Sw 2/95 were applied in the same test, No. 45/96.

Effect of testing conditions

Table 2 shows the results of tests of tomato seedlings grown in medium of basic concentration under conditions of light and temperature according to variant I, and Table 3 presents the results obtained with the seedlings grown in diluted (1:4) medium in conditions of variant II. The results of tests run at the same time were higher in Tables 3 and 7 than in Tables 2 and 6 respectively, and this difference was significant

Table 8

ANOVA of results marked with [1] in Tables 2 and 3

Source	Degrees of freedom	Mean square	F value
Standards (A)	3	95.53	135.80**
Medium conc. (B)	1	5.82	8.27**
AB	3	1.51	2.14
Error	24	0.70	
Total	31		

Standards	Mon	NY	Ott30	WV700	
$\bar{x}$	1.3	6.7	8.6	8.7	$LSD_{0.05} = 1.16$
Medium	basic	diluted			
$\bar{x}$	5.9	6.8			$LSD_{0.05} = 0.61$

Table 9

ANOVA of results of Table 6 and Table 7 marked with [1]

Source	Degrees of freedom	Mean square	F value
Replications (variants)	1	0.15	0.55
Standards (A)	4	201.95	762.79**
Medium conc. (B)	1	14.45	54.58**
AB	4	3.02	11.41**
Isolate (C)	3	4.90	18.50**
AC	12	2.46	9.29**
BC	3	0.43	1.62
ABC	12	0.46	1.72
Error	39	0.27	
Total	79		

Standards	Mon	NY	WV63	Ott30	WV700	
$\bar{x}$	1.0	1.2	5.9	8.0	8.2	$LSD_{0.05} = 1.04$
Medium		basic		diluted		
$\bar{x}$		4.4		5.3		$LSD_{0.05} = 0.46$
Isolates	Ott 2/94	Ul 1/95	Ul 12/84	Sw 2/95		
$\bar{x}$	4.3	4.6	5.1	5.4		$LSD_{0.05} = 0.44$

(Tables 8 and 9). This holds also when the means of all tests from those tables were compared. It means that the seedlings grown in diluted medium were less infected.

The majority of tests on tomato grown in both medium concentrations in 1993 – 1996 were run according to variant I . Exceptionally, tests 38/95, 43/95 and 45/96 grown in two medium types were conducted in two variants of light and temperature (I and II) at the same time. The ANOVA showed a highly significant effect of medium, but there was no significant influence of variants on the infection degree (Table 9). Following this analysis, variants were treated as replications, and in Tables 6 and 7 the means of two variants are presented.

In a few tests run in 1994 and 1996 the effect of spore concentration of some isolates was observed. Lowering concentration from 50 to 12.5 spores/mm^3 weakened the infection rate to the extent depending on the isolate used (Table 5, tests 34 and 35/94). The susceptible variety Mon was less infected when concentration of 12.5/mm^3 of two out of four isolates was applied, NY was less infected by all isolates and MP 270 caused strong effect on the degree of WV63 infection. In tests 37/94 and 44/96 (Table 7) two isolates at concentrations of 50 and 25 spores/mm^3 were applied. Mon and NY were totally infected, regardless of concentration. The highest effect of a change in concentration was observed with WV63 when Ul 12/84 was applied. This variety was totally infected with the inoculum of 50 spores/mm^3, but showed considerable resistance to this isolate at the concentration of 25/mm^3

DISCUSSION

At the beginning of the work on the method of evaluation of tomato leaf resistance to *P. infestans* by seedling tests, the following requirements were taken into consideration.

1. Evaluation of the seedlings infected with *P. infestans* should be consistent with the observations of leaf infection in the field and ranking should be the same as in the Gallegly`s seedling test.

2. A standard variety susceptible to *P. infestans* should be totally infected under conditions of the test.

3. Results of tests should be reproducible.

4. Evaluation of large tomato populations, selection of the most resistant seedlings and their further growth after replanting to soil should be possible .

Standard varieties and lines chosen for all experiments represent a whole range of variability of known tomato leaf resistance to *P. infestans*, namely: Moneymaker – the susceptible variety, New Yorker – carrying the *Ph–1* gene, resistant to T_0 race, West Virginia'63 – with *Ph–2* gene, the variety partially resistant to the race T_1, West Virginia 700 and Ottawa 30 – accessions expressing the highest level of the leaf resistance (Gallegly 1960 and 1964, Robinson *et al.* 1967, Turkensteen 1973 and Laterrot 1975).

Infection of different genotypes and their response to isolates

Gallegly`s test run on 2 to 3–week–old seedlings grown in green–houses in poor soil allowed to rank varieties and lines in the following order of resistance: susceptible varieties>WVa 106>WVa 36>WV63> WV700 (Gallegly, personal information). The resistance of WVa 36 and WVa 106 was determined by *Ph–1* gene (Gallegly 1960). Our field ob–servations made during many years in several places in Poland with the resistant standards and breeding lines can be summarized as follows: First late blight symptoms were always observed on susceptible lines, usually the same each year. Practically no infection of NY was observed during a slow development of epidemics or the infection was later and weaker than that of susceptible varieties. On the other hand, in case of severe epidemics, there was no difference between NY and susceptible varieties in the level of infection. The cultivars West Virginia`63, Pieraline and all breeding lines having *Ph–2* were always infected later than NY and the epidemics increased slower. The weakest and latest infection was always observed on WV700 and Ott30. During summers of 1997 and 1998, when the epidemics was particularly strong, these ac–cessions were less infected than Pieraline. At the end of September 1998, when the Pieraline plants were already killed, the accessions WV700 and Ott30 were green, although markedly infected.

Gallegly and Marvel (1955) first found that WV700 carries at least two dominant genes determining leaf resistance. According to Gallegly (1960) this resistance is due to expression of one dominant gene and poligenes . In contrast, Turkensteen (1973), Laterrot (1975 and 1994) and Moreau *et al.* (1998) assumed that leaf resistance of this accession was governed by one dominant gene *Ph–2* causing leaf and stem resis–tance. Moreover, Laterrot (1994) proved lately that WV63 and varieties which he bred using Gallegly`s lines carry *Ph–2*. Turkensteen (1973) and Laterrot (1975) did not compare WV700 and WV63 in laboratory tests. Although in their field trials WV63 was infected more than WV 700 and Ott30, this was explained by differences in growth types. All our tests as well as field observations showed lower degree of resistance of WV63 than that of WV700 and Ott30. A similar difference was observed in the field conditions by Shirko and Kozubova (1972), Nishio *et al*. (1985) and Markovič (pers. inf., 2000) and also by Günter *et al.* (1970) using a leaflet test. The difference in the resistance level between WV700 and WV63 was not always displayed in the same way. Our ob–servations, as well as of other authors indicate difference between ex–periments, becoming larger during strong epidemics or in tests with aggressive isolates. The only explanation for a weaker infection of WV700 than of WV63 is participation of other gene (or genes) controlling leaf resistance in WV700, beside *Ph–2*.

Various isolates were collected and the response to them was studied to find proper ones for evaluation and selection of breeding material. In the tests conducted on leaflets (unpublished data), the authors tried to

find an isolate infecting the *Ph–2* gene lines more than those of *Ph–1*. Neither these efforts were successful nor the tests performed on seed–lings showed any difference in virulence. In our tests WV63 was signifi–cantly less infected than NY (except for few cases) and significantly more than WV700. The difference between these two forms in the infec–tion level was particularly evident when a higher (50 spores/mm^3) con–centration of the aggressive isolates was applied (Tables 6 and 7). Response to infection of used standards, in majority of tests ranked as follow: Mon≥ NY>WV63>WV700 = Ott30, independently of the isolate applied. It corresponds to the results of other authors` experiments in the lab or field trials in which varieties and lines carrying different blight–resistance genes were included. The only different and surpris–ing results were obtained by Nishio *et al.* (1985): The West Virginia`63 (*Ph–2*) and Nova (*Ph–1*) cultivars proved to be susceptible in their field and lab experiments, while the accessions WV700 (*Ph–2* plus other genes) and WVa 36 (*Ph–1*) belonged to the most resistant group.

Problems of pathogenicity, aggressiveness and virulence

The isolates of *P. infestans* which were used many times a year, were permanently maintained on tomato leaves not to lose pathogenicity, which could be lost on an agar medium (Wilson and Gallegly 1955, Turkensteen 1973, Laterrot 1975, Zarzycka 1996). The results obtained by Horodecka (1989a), who evaluated pathogenicity of isolates cultured on artificial media, was probably this case. In her work, 13 out of 32 iso–lates collected from tomatoes, were poorly pathogenic to the susceptible tomato–leaves. On the other hand, Mills (1940) and Turkensteen (1973) observed an increase of pathogenicity of the isolates collected from po–tato after several passages on tomato leaves. Graham *et al.* (1961) also noticed a change of virulence as a result of passaging on tomato and po–tato resistant accessions. Similar observation was made during this study (data not shown) when the potato isolates cultured on potato slices were slightly aggressive to tomato, but became aggressive to NY and WV63 after a few weeks of culturing on tomato leaves. Also the iso–late Ul 12/84 grew and sporulated faster after a few years of permanent maintenance on the detached tomato leaves. Tomato leaves seem to be the best substrate to prepare inoculum for tomato resistance tests.

Very few authors used natural substrates to prepare an inoculum for tomato resistance studies. Tomato leaves were used by Turkensteen (1973), Nishio *et al.* (1985) as well as Hartman and Huang (1995), to–mato fruit by Conover and Walter (1953) while Mills (1940) used potato slices. The inoculum prepared to investigate pathogenicity of *P. infestans* isolates was usually cultured on agar media (Gallegly 1952, Kubicka 1969, Gunter *et al.* 1970, Laterrot 1975, Horodecka 1989a). Wilson and Gallegly (1960) also propagated isolates on an agar medium to investigate the effect of potato isolates on tomato and *vice versa*, and observed large differences in pathogenicity of these isolates. In contrast

to their findings, Kubicka (1969) did not find any marked differences among isolates collected from the field–infected potato leaves, directly used for inoculum preparation. All seven isolates studied were pathogenic to tomato.

There is no evidence that the investigated *P. infestans* isolates belong to different tomato races. Various tomato–standard responses to isolates were caused by different aggressiveness rather than different virulence of isolates. Variability of the infection degree due to inoculum concentration and the conditions of seedlings growth and testing confirm this opinion. Infection of NY was particularly affected by conditions. The tomato forms with *Ph–1* like those with *Ph–2* gene respond intensively to changing conditions, therefore their reaction was typical of partial resistance. A typical hypersensitive response to the race T_0 of resistant tomato varieties possessing *Ph–1* gene was so far observed by Gallegly and Marvel (1955), Gallegly (1960) and Matthawson (1977). Although necrotic reaction was also detected by Günter *et al.* (1970), but in their studies sporulating spots were sometimes caused by T_0 race.

Infection level affected by conditions of the test

According to Wilson and Gallegly (1960) a lower medium concentration resulted in lower tomato susceptibility to late blight. Similarly in our studies with a few isolates the seedlings cultured in the diluted (1:4) medium were less infected than those cultured in the medium of basic concentration.

Basing on the present knowledge it is very difficult to explain the reaction of NY in the tests. The seedlings of this variety were partially resistant in all tests performed in 1989 – 1992, however they were as susceptible as Mon in all tests in 1993 – 1996 regardless of the isolate applied. At first, the authors tried to explain this change by an alteration of pathogenicity of the Ul 12/84 isolate, later by some modification in testing procedures. Finally, having analyzed all the tests, we have concluded that this change could not be explained by changes in virulence, therefore it must be ascribed to the alteration of conditions. Wilson and Gallegly (1960) observed the influence of light intensity, a day length, and temperature on expression of resistance. However, in our investigations two variants (I and II) of temperature and light intensity had no significant effect on test results and did not cause alteration of NY reaction. Then photoperiod was taken into consideration as in 1993 the day length was prolonged. Preliminary tests on the day length effect on the infection level did not give any consistent results which would explain the NY reaction. Possibly, the expression of resistance determined by *Ph–1* gene strongly depends on conditions, so their careful monitoring in performed tests is necessary.

Use of the method

The evaluation method of tomato–leaf resistance using seedling test meets all the above mentioned requirements and have many advan–tages. It makes possible to test a large number of seedlings in a short time (4 – 5 weeks from sowing to evaluation). Two cameras of a total area of 1 m^2 are enough for testing 1000 seedlings every second week. The whole technical work can be done by one trained person. Results of tests are repeatable providing the conditions are the same. Large popu–lations can be characterized as well as the most resistant seedlings can be selected. This method allowed the authors to select several breeding lines as resistant as WV700 (Michalska and Pazio 1997) and to breed the variety Awizo F_1 (Gajc–Wolska and Michalska 2000) which com–bines leaf resistance determined by *Ph–2* gene with fruit resistance (Bednara *et al.* 1996). Besides, this method can be used for studying vir–ulence and aggressiveness of isolates, effects of isolate maintenance as well as influence of abiotic factors on resistance expression etc. For breeding purposes some modifications of the method are also possible. The medium concentration, isolate used and its spores concentration can be altered according to the resistance level of selected materials. Well–known standard varieties and lines allow to compare results of different tests and to show a real resistance level of selected lines. Using the same method and the same resistance standards, results of different authors in various places can be comparable.

Some problems may occur because all dried seedlings, no matter what is the reason of the decay, must be qualified as susceptible ones. Other pathogens may disturb test performance. Therefore the testing condi–tions as well as all procedures should be precisely determined and care–fully followed to keep the results repeatable.

The seedlings infection degree was evaluated using the logistic key which was recommended by van der Plank (1963) and Försund (1987) as suitable for estimation of epidemics progressively developing in the field. Pietkiewicz (1972) proposed to adopt this key for evaluation of late blight infection in the potato leaflet tests. The results of these tests were consistent with field observations. The logistic key divides a resistant part of population into subgroups, thus making it possible to distinguish small differences among resistant lines. No other key used allows for such a division. The application of this key showed that field differences between WV63 and WV700 can be confirmed in laboratory studies.

CONCLUSIONS

1. Evaluation of the tomato leaf resistance to *P. infestans*, using the seedlings test, ranks the standards in the way consistent with the field observations.

2. The infection degree of the resistance standards was as follow: Moneymaker≥New Yorker>West Virginia'63>West Virginia 700=Ottawa 30 irrespectively of test conditions or isolate applied.
3. The results of testing depended on concentration of the liquid medium applied for seedlings growth, on isolate used and its spore concentration. Diluted medium and lower spore number results in lower infection.
4. Aggressiveness of the isolates was different while no difference in the virulence of isolates was observed.

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