Plant Breeding and Acclimatization Institute in Radzików,
Młochów Research Center, Poland

NEW LABORATORY METHOD OF EVALUATION OF POTATO STEM SUSCEPTIBILITY TO *PHYTOPHTHORA INFESTANS*

A.M. Michalska, S. Sobkowiak and E. Zimnoch-Guzowska

Abstract

A new laboratory method to evaluate the infection degree of potato stems inoculated with *Phytophthora infestans* was developed. Four potato cultivars of different resistance level to late blight were used to define conditions of plant growth, and procedures of inoculation and incubation necessary to obtain reproducible results of tests. Stems of cultivars 'Albina' and 'Irga' were highly susceptible and cultivar 'Meduza' highly resistant in all tests, independently on growth and test conditions. The following testing conditions are recommended for evaluation of potato stem resistance to *P. infestans*: four- to eight-week-old stems (18 cm long), with root fragments, inoculated in the central position with two filter paper discs 6 mm in diameter soaked in inoculum at concentration of 10^5 sporangia per 1 ml. Stems placed on a wet wood-wool in plastic trays at 22°C should be transferred to jars with some water 24–48 h after inoculation and placed in a lighted chamber at 20°C.

Key words: *Solanum tuberosum*, stem resistance, late blight

Introduction

Symptoms of potato stem infection caused by *Phytophthora infestans* can be observed in early stage of epidemics. This kind of infection can be observed earlier than leaf infection, probably due to humidity kept longer at leaf axils than at leaflets (Smith et al. 1988, Turkensteen 1996, Kapsa 2000).

Clayson and Robertson (1956) observed that when high temperature causes dying of infected leaves, the infection spots can develop on stems. Kapsa (2002) stated that isolates originated from potato stems were more resistant to unfavour-

Phytopathol. Pol. 49: 85–96
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ISSN 1230-0462

able conditions and could survive longer during dry and hot weather than isolates collected from leaves. Infected stems may play a significant role in infection of potato plants later on, during the vegetation season and they may be a source of sporangia spread for long distances (Shattock 1988). Affected stems can also be more effective as a source of inoculum for new formed tubers than the infected leaves (Bain et al. 1996, Kapsa 2000).

Screening potato cultivars for resistance to late blight is commonly done under field conditions on inoculated or naturally infected plots (Dorrance and Inglis 1997, Porter et al. 2004, Hansen et al. 2005). The laboratory method of detached leaflet infection (Sujkowski 1986, Dorrance and Inglis 1997, Zarzycka 2001) is also a common way to rate leaf resistance. There were only two trials done under laboratory conditions to assess stem resistance (Dorrance and Inglis 1997, Kapsa 2001) and one with glasshouse grown plants (Porter et al. 2004).

The purpose of this study was to elaborate a new method suitable for testing potato stem reaction to *P. infestans* infection. Such a method should rate potato breeding lines for their stem response independently on plant growth condition and to work out reproducible way of stems testing.

Materials and methods

Nine tests were performed from autumn 2003 to spring 2004 in order to evaluate potato stem infection with *P. infestans*.

Materials

Preliminary tests (2003) on 15 cultivars with susceptible and resistant foliage allowed selecting cultivars for testing stem susceptibility. Two potato cultivars susceptible to *P. infestans*: 'Albina' (official score of foliage resistance: 3 in 1–9 scale, where 1 is the most susceptible) and 'Irga' (scored as 2), and two resistant cultivars: 'Koga' (scored as 7) and 'Meduza' (scored as 8; Charakterystyka... 2004) were used for testing stem infection degree. A highly aggressive MP 324 isolate of *P. infestans* obtained from a stem of cultivar 'Gloria' (1997) was used in all tests of this study.

Methods

Stem preparation for testing

Eye pieces of presprouted tubers harvested in 2003 were planted in pots (10 cm in diameter) filled with compost soil. Potato stems were cut off from plants grown under conditions presented in Table 1, and evaluated in nine experiments.

Cut stems with some roots were prepared by cutting off leaves and side shoots to obtain 18-cm-long stems with few roots left at the stem base. They were rinsed with tap water, air dried and placed on wet wood-wool in plastic trays. Roots of

Table 1

Dates of experiments, age of tested plants, and plant treatment

No. of experiment	Date of planting	Date of inoculation	Growing conditions	Plant age (weeks)	Additional treatment
1	9.09.2003	29.10.2003	glasshouse	7	GA[a]
2	9.09.2003	13.11.2003	glasshouse	9	GA[a]
3	19.01.2004	16.02.2004	glasshouse	4	Topsin[b]
4	19.01.2004	24.02.2004	glasshouse	5	Topsin
5	18.03.2004	20.04.2004	glasshouse	5	Topsin
6	18.03.2004	26.04.2004	glasshouse	6	Topsin
7	4.05.2004	28.05.2004	glasshouse	3.5	Topsin
8	4.05.2004[c]	7.06.2004	glasshouse + net-house	5	Topsin
9	4.05.2004[c]	24.06.2004	glasshouse + net-house	7	Topsin
9	27.05.2004	24.06.2004	net-house	4	Topsin
9	28.04.2004	24.06.2004	field	8	Topsin

[a]Tubers before planting were treated with 1 ppm gibberellic acid.
[b]Plants were watered with 0.2% Topsin solution one or two weeks after planting to avoid soil-borne infection.
[c]Plants were transferred from the glasshouse to net-house three weeks after planting.

stems were covered with wet wood-wool. Five stems in three replications were tested in each of nine experiments.

Inoculum preparation and stems inoculation

Before experiments the isolate MP 324 was reproduced several times on leaves of a potato cultivar susceptible to *P. infestans*. Inoculum consisted of a sporangial suspension that was prepared from sporulating lesions of potato leaflets and adjusted (with the hemocytometer) to the concentration of 5×10^4 or 10^5 sporangia per 1 ml. Then inoculum was incubated for 2 h at 7°C. Filter-paper discs (6 mm in diameter) were soaked in inoculum by mixing for 5 min and then used for inoculation. This method was chosen from among three preliminarily tested methods of stems infection.

Conditions of testing

The experiments No. 1 to 8 consisted of basic treatment and other variants. In basic treatment 18-cm-long stem parts with root fragments were inoculated with *P. infestans* inoculum at the concentration of 5×10^4 sporangia per 1 ml. Two filter-paper discs soaked in inoculum were placed on stems: one on lower part (L), 4 to 5 cm above roots, and one on upper part (U), 13 to 14 cm above roots. Plastic trays, with inoculated stems, covered with glass, were placed in a dark climatic chamber at 22°C. After 24 h stems were put into jars with tap water on the bottom and kept in a chamber at 20°C with a constant illumination of about 1400 lx. In the experiments No. 6 to 9 jars were washed and water was changed in them four days after inoculation. The basic treatments were applied in eight tests and compared with additional treatments (Table 2).

Table 2

Treatments applied in tests

No. of test	Age of stems (weeks)	Place of inoculation, No. of discs	Concentration of inoculum sporangia per 1 ml	Time on wet wood-wool (h)	Part of stem (cm)	Liquid in jars
Basic treatment (BT)						
Basic treatment (BT)		L – 1 disc U – 1 disc	5×10^4	24	1–18	water
Additional treatments						
1	7	BT	BT	BT	18–36	BT
2	9	BT	10^5	48	18–36	BT
3	4	BT	BT	48	BT	Quinosol[a]
4	5	BT	BT	48	BT	Quinosol[a]
5	5	BT	BT	48	BT	Oxytetracycline[b]
6	6	BT	BT	48	BT	BT
7	3.5	C – 1 disc C – 2 discs	BT	BT	BT	BT
8	5	C – 2 discs	BT	BT	BT	BT
9	4 7 8	C – 2 discs	10^5	BT	BT	BT

[a]Quinosol solution (8-hydroxyquinoline sulfate) of 0.04%.
[b]Oxytetracycline solution in concentration of 10 mg/l.
L – lower part of stem, 4–5 cm over roots, U – upper part of stem, 13–15 cm over roots, C – central part of stem, 9 cm over roots.

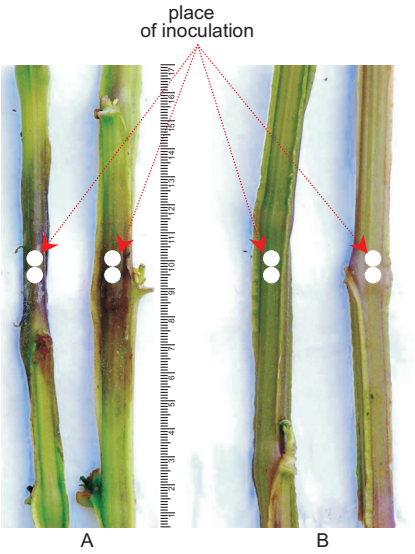
Evaluation of stem infection

The length of infected stem spots was measured seven days after inoculation (Phot. 1). The length of lesions (excluding the inoculation site) was classified according to 1 to 9 grade scale:

0 or necrosis:	9,	11–20 mm:	6,	41–50 mm:	3,
1–3 mm:	8,	21–30 mm:	5,	51–60 mm:	2,
4–10 mm:	7,	31–40 mm:	4,	> 60 mm:	1.

Data were analyzed by ANOVA and the significance of differences was with Tukey's test.

Furthermore, percentages of rotting stems, infected with other than *P. infestans* pathogens, were calculated in the experiments No. 1 to 5. Percentages of weakly infected stems of susceptible cultivars 'Albina' and 'Irga', inoculated at lower and upper parts of the stems, in the experiments No. 1 to 8, were also calculated.



Phot. 1. Reaction of susceptible and resistant stems of potato to infection with *Phytophthora infestans*: A – susceptible stems, B – resistant stems (photo by S. Sobkowiak)

Results

Results of the basic treatment in the experiments No. 1 to 7 are presented in Table 3. Analysis of variance showed that cultivar, place of inoculation and date of test as well as interactions cultivar by date of test and cultivar by inoculation place were highly significant (Table 4).

Means of stems infection of susceptible cultivars 'Albina' (5.1) and 'Irga' (4.9) were significantly lower than means of resistant cultivar 'Koga' (6.9), and cultivar 'Meduza' (8.0; Table 3). Stems of four cultivars inoculated in the upper part (U; general mean: 6.0) were stronger infected than in the lower stem part (L; general mean: 6.6). This difference was significant for susceptible cultivars 'Albina' and 'Irga', but not significant for resistant 'Koga' and 'Meduza'. Average infections of stems in the experiments No. 1, 2 and 4 were significantly weaker than in the others. The greatest variation of results obtained in particular tests was observed for cultivar 'Koga', and the least variation for resistant cultivar 'Meduza'. Considering the interaction of cultivar and date of test, cultivar 'Albina' was the least infected in the experiment No. 4, cultivar 'Irga' in the experiments No. 2, 4 and 7, cultivar 'Koga' in the experiments No. 1 and 4 and cultivar 'Meduza' in the experiment No. 3.

In the experiments No. 7 and 8 additional studies on influence of inoculation place and number of filter-paper discs used to infect four cultivars was investigated (Table 5). Cultivar, date of experiment and inoculation place had highly significant influence on average degree of infection. Interaction cultivar by date of test was also highly significant. Mean infections of upper and central parts of stems inocu-

Table 3

Stem infection degree of four potato cultivars grown in glasshouse in seven experiments applied according basic treatment. Stems were inoculated in upper and lower parts with *Phytophthora infestans* sporangia at concentration of 5×10^4 per 1 ml

No. of test	Plant age (weeks)	'Albina'			'Irga'			'Koga'			'Meduza'			Mean		
		U	L	mean	U	L	mean	U	L	mean	U	L	mean	U	L	mean
1	7	–	–	–	4.6	5.1	4.9	7.5	8.3	7.9	8.1	8.2	8.2	6.7	7.2	7.0
2	9	–	–	–	5.8	5.1	5.5	7.1	7.1	7.1	8.1	8.3	8.2	7.0	6.8	6.9
3	4	4.6	5.6	5.1	3.1	4.9	4.0	7.2	6.8	7.0	7.1	7.3	7.2	5.5	6.1	5.8
4	5	5.4	6.6	5.9	5.3	6.2	5.7	7.7	7.8	7.5	8.3	8.3	8.3	6.7	7.2	6.9
5	5	4.7	5.2	5.0	4.3	4.9	4.6	6.7	7.1	6.9	8.5	8.4	8.4	6.0	6.4	6.2
6	6	3.5	5.9	4.7	2.9	4.6	3.7	6.4	7.1	6.7	8.2	8.1	8.1	5.3	6.4	5.8
7	3.5	3.5	5.7	4.6	5.5	6.1	5.8	4.3	5.9	5.1	7.8	7.7	7.8	5.3	6.3	5.8
Mean		4.4	5.8	5.1	4.5	5.3	4.9	6.7	7.1	6.9	8.0	8.0	8.0	6.0	6.6	6.3
$LSD_{0.05}$ for		factors			cultivar: 0.458											
					inoculation place: 0.246											
					date of test: 0.697											
		inter-actions			cultivar by date of test: 0.989											
					cultivar by inoculation place: 0.423											

U – upper part of stem, L – lower part of stem.

lated with one disc were similar (in experiment No. 7 in average 5.3 and 5.4, respectively), while the lower part of the stem was less infected (average 6.3). In the test No. 8 differences between the upper and lower parts were not so clear. The two discs used for inoculation increased the infection degree of all cultivars except cultivar 'Koga' in the experiment No. 8. On average, stems of cultivar 'Albina' (5.0)

Table 4

Analysis of variance of stem infection with *Phytophthora infestans* for four potato cultivars evaluated in the experiments No. 1–7

Source	Degree of freedom	*F* value
Date of test	6	7.892**
Cultivar	3	143.923**
Inoculation place	1	24.175**
Date of test by cultivar	11	4.106**
Date of test by inoculation place	6	1.337
Cultivar by inoculation place	3	4.335**
Date of test by cultivar by inoculation place	16	0.907
Error	104	

**Significant at $P < 0.01$.

Table 5

Stem infection degree of four potato cultivars inoculated with *Phytophthora infestans* sporangia at concentration of 5×10^4 per 1 ml, depending on the place of inoculation and the number of paper discs soaked in inoculum used for inoculation in the experiments No. 7 and 8

Place on stem and number of discs used for inoculation	'Albina'		'Irga'		'Koga'		'Meduza'		Mean		Total mean
	7	8	7	8	7	8	7	8	7	8	
Upper – 1 disc	3.5	6.2	5.5	6.3	4.3	7.1	7.8	8.7	5.3	7.1	6.2
Lower – 1 disc	5.7	6.9	6.1	6.6	5.9	7.4	7.7	8.7	6.3	7.4	6.9
Central – 1 disc	4.2	–	5.5	–	4.3	–	7.8	–	5.4	–	–
Central – 2 discs	2.9	4.9	4.6	5.2	3.4	7.3	7.3	8.3	4.5	6.4	5.4
Mean	4.1	6.4	5.4	6.3	4.5	7.0	7.7	8.5	5.4	7.0	
	5.0		5.8		5.6		8.0				
$LSD_{0.05}$ for	factors	cultivar: 0.566									
		date of test: 0.305									
		inoculation place: 0.571									
	interaction	date of test by cultivar: 0.524									

7 – test No. 7 (3.5 weeks old plants).
8 – test No. 8 (5 weeks old plants).

were infected most while stems of 'Irga' and 'Koga' cultivars were significantly less infected (5.8 and 5.6, respectively), and the least infected ones were those of cultivar 'Meduza' (mean 8.0).

In the experiments No. 2 to 6, the infection of stems lying on wet wood-wool for 24 and 48 h was not significantly different. Two different concentrations of inoculum, 5×10^4 and 10^5 sporangia per 1 ml were applied in the experiment No. 2, but the difference of stems infection was not significant.

The influence of three factors on stem infection degree was investigated in the experiment No. 9. There were cultivars, inoculum concentration and physiological state of three groups of plants, each of them at various age and grown under different conditions (Table 1, Fig. 1). All those factors had significant influence on stem infection. Stems of cultivars 'Albina' and 'Irga' (means respectively 4.4 and 4.0) were significantly more infected than those of cultivars 'Koga' and 'Meduza' (means respectively 7.8 and 8.2). Stems inoculated with inoculum of 10^5 sporangia per 1 ml were significantly stronger infected than in the case of 5×10^4 sporangia per 1 ml (means respectively 5.5 and 6.7).

In the experiment No. 9 inoculum concentration had a highly significant influence on infection of susceptible cultivars, but not on the resistant ones (Fig. 1). Concentration of 10^5 sporangia per 1 ml caused significantly higher infection of cultivars 'Albina' and 'Irga' stems (means 3.2 and 3.0, respectively) than concentration of 5×10^4 sporangia per 1 ml (means 5.6 and 4.9, respectively), whereas the average stem infection of cultivars 'Koga' and 'Meduza' did not differ significantly depending on inoculum concentration.

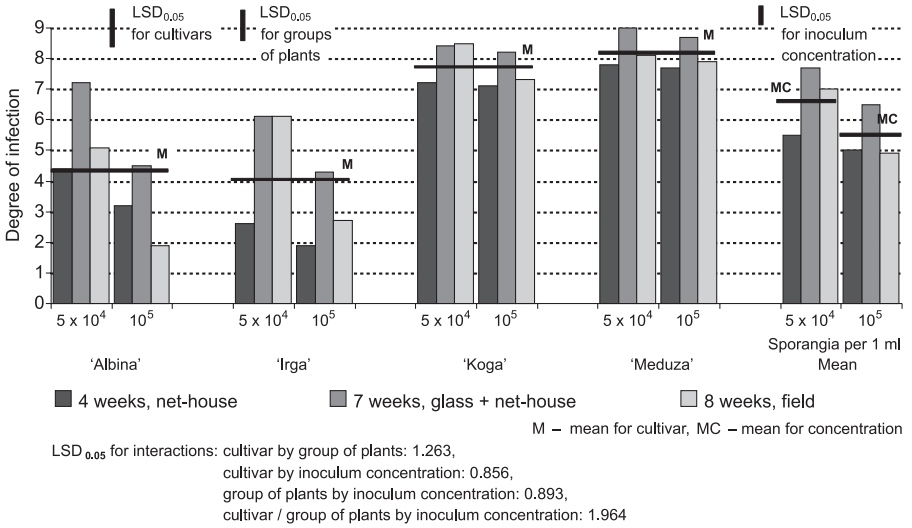


Fig. 1. Stem infection in 1–9 scale, where 1 is the most susceptible, of four potato cultivars at three groups of plants, inoculated with *Phytophthora infestans* at two inoculum concentrations: of 5×10^4 and 10^5 sporangia per 1 ml in the test No. 9

In the experiment No. 9 age of plants caused significant differences of infection degree of stems cut off from different groups of plants. Stems from four-week-old plants grown in the net-house were the most infected (mean 5.2). Weaker stem infection was observed for field grown, eight-week-old plants (mean 5.9).

The stems cut from seven-week-old plants grown in a glasshouse and later in the net-house (mean 7.1) were least infected.

Higher concentration of inoculum influenced significantly stem infection of older plants from the net-house and field but did not differentiate the youngest stems infection (Fig. 1). Moreover, stronger infection degree of 'Albina' and 'Irga' cultivars was observed when inoculum at concentration 5×10^4 sporangia per 1 ml was applied for young stems of plants grown in the net-house, while two older groups of stems from the field and growing in glasshouse + net-house were weaker infected (Table 1, Fig. 1).

In many combinations of the experiments No. 1 to 6 rotting stems infected with pathogens other than *P. infestans* were observed and counted. Those stems were excluded from observations of *P. infestans* infection, and therefore not used for statistical analysis. Percentage share of these stems in all the tested ones was calculated for older (with roots) and younger (without roots) parts of stems (Table 6). The highest number of rotting stems was observed in younger parts of inoculated stems, especially for cultivars 'Irga' and 'Koga' (30.8% and 24.6% of tested stems, respectively).

Treatments with Quinosol or oxytetracycline solutions did not solve the problem of stem rotting. Rotten stems were not observed, when in the experiments No. 7, 8 and 9 more root fragments were left, water was changed and jars were washed.

In the experiments No. 1 to 8 the percentage of weakly infected stems (degree 7 to 9), inoculated in upper (U) and lower (L) parts of a stem, was calculated for sus-

Table 6

Percentage and number of rotting stems of four potato cultivars infected with *Phytophthora infestans* in the experiments No. 1–6

Part of stem	'Albina'		'Irga'		'Koga'		'Meduza'	
	%	No.	%	No.	%	No.	%	No.
Older[a]	16.3	147	6.0	215	3.3	215	1.4	215
Younger[b]	–	–	30.8	65	24.6	65	0.0	65

[a]Parts of stems with roots (0–18 cm).
[b]Parts of stems 18–36 cm above roots.

ceptible cultivars 'Albina' and 'Irga' (Table 7). In all treatments weak infection was observed more often in lower parts of stems (mean 54.3%) than in upper parts (29.9%). It was independent on how long stems were kept on wet wood-wool (24 or 48 h). In the experiments No. 7 and 8 inoculation with two discs in central (C) part of a stem caused strong stem infection of cultivars 'Albina' and 'Irga' (Table 7).

Table 7

Percentage and number of weakly infected stems (with grades 7 to 9) of two susceptible potato cultivars 'Albina' and 'Irga', depending on places of inoculation in the experiments No. 1–8

Time on wet wood-wool	U		L		C	
	%	No.	%	No.	%	No.
24 h	32.6	337	52.5	337	0.0	60
48 h	24.6	171	57.9	171	–	–

U – upper part of stem, one disc, L – lower part of stem, one disc, C – central part of stem, two discs.

Discussion

The significant role of potato stem infection in late blight epidemics (Smith et al. 1988, Turkensteen 1996, Kapsa 2000) was the reason of interest in stem resistance to the disease. Elaboration of a reproducible testing method is a necessary condition to start such a study.

Based on field observations and preliminary laboratory tests conducted at IHAR (Plant Breeding and Acclimatization Institute), Młochów Research Center, four cultivars differing in stem resistance were selected for this study to elaborate procedures and to assess factors influencing tests results.

Some procedures preliminary evaluated in tests were then chosen as basic treatments for this study. Results of tests confirmed stable resistance of cultivar stems 'Meduza' and significant differences between cultivar 'Meduza' and susceptible cultivars 'Albina' and 'Irga'. Cultivars 'Albina' and 'Irga' proved good standards of susceptibility and cultivar 'Meduza' – of high resistance. The variable reaction of cultivar 'Koga' was too high to use it as a standard in testing stem resistance.

Additional treatments were applied to find procedures allowing better reproducibility of tests and to avoid false data like lack of infection of susceptible cultivars, or infection by pathogens other than *P. infestans*.

Stems infected according to procedures of basic treatment reacted differently after inoculation of lower and upper part. All stems of cultivar 'Meduza' showed very weak reaction to infection in both places. Stems of susceptible cultivars inoculated in two places showed different reaction depending on place of inoculation. In lower part (L) many stems were weakly infected (7 to 9 grades) while in upper part (U), weak infection was less frequent. When two discs were applied one by one in the centre of stem, differences between particular stems were smaller and weak infection of susceptible cultivars did not happen.

The effectiveness of infection with two discs applied in the central position of stems was confirmed by results of experiment No. 9 with three groups of plants (varying in age and place of growing) and with two concentrations (5×10^4 and 10^5 sporangia per 1 ml). In all combinations of this test cultivar 'Meduza' was weakly or not infected. Susceptible cultivars were particularly strongly infected at the concentration of 10^5 sporangia per 1 ml, but infection of older stems (seven- and eight-week-old) of these cultivars was significantly weaker when inoculum at the concentration of 5×10^4 sporangia per 1 ml was applied. Inoculum at the concentration of 10^5 sporangia per 1 ml is therefore recommended for stems inoculation despite the lack of significant differences in the test No. 2.

Weak infection of the stems originated from plants transferred from glasshouse to net-house, in comparison with younger plants (the experiments No. 8 and 9) might have been caused by process of plants transplantation to more dry and hot conditions. This suggests that growing conditions and/or age of plants are important factors influencing infection. Lack of infection, when older stems collected from fields were tested in July and August 2002 and 2003 (not published), and weak infection of low (L) part of stems of susceptible cultivars (Table 7) support this opinion. Taking into consideration these results we can suggest performing tests on four- to six-week-old glasshouse grown plants and six- to eight-week-old plants from fields.

Preliminary attempts of potato stems inoculation with drops of inoculum suspension and keeping them on wet wood-wool for seven days (according to Kapsa 2002), were not successful. Drops of inoculum flowed down quite often and high humidity in plastic trays favored rotting of tested stems, so that evaluation was not possible. Filter paper pieces soaked in inoculum suspension, previously applied by Strömberg and Brishammar (1991) and Dorrance and Inglis (1997), was the most effective way of stems inoculation, out of the three methods tested.

Results obtained by Dorrance and Inglis (1997) and Kapsa (2001), cannot be compared with ours due to various methods and materials used. Moreover, methods applied by the former investigators have some deficiencies. Kapsa (2001) did not publish both test results and reproducibility was not shown; in the paper of Porter et al. (2004), the high value of $LSD_{0.05}$ suggested a large error of experiments. In Dorrance and Inglis (1997) paper some cultivars fall into resistant or susceptible groups in various tests and authors stated: "there is considerable vari-

ability of the individual rank for each cultivars". Our method was reproducible enough to recommend it for studies of potato stem resistance.

The most effective variants of the tests described above (see "Conclusions") are used in Młochów to compare stem and foliage reactions to *P. infestans* infection in cultivars and advanced breeding lines. The results will be published soon.

Conclusions

1. The recommended way for testing potato stem resistance is inoculation of central part of young stems (four- to eight-week-old) using two filter paper discs 6 mm in diameter, soaked in *Phytophthora infestans* inoculum at the concentration of 10^5 sporangia per 1 ml.

2. Cultivar 'Meduza' is a good standard for high resistance and cultivars 'Albina' and 'Irga' – for susceptibility to late blight of stems.

3. The test is reproducible and suitable to evaluate potato stem sensitivity to *P. infestans*.

Acknowledgements

The authors wish to thank Leszek Domański for his statistical guidance.

Streszczenie

NOWA METODA LABORATORYJNEJ OCENY PORAŻENIA ŁODYG ZIEMNIAKA PRZEZ *PHYTOPHTHORA INFESTANS*

Opracowano nową metodę oceny stopnia porażenia łodyg ziemniaka zakażanych w laboratorium zawiesiną zarodników *Phytophthora infestans*. We wszystkich doświadczeniach zastosowano cztery odmiany różniące się poziomem odporności na zarazę ziemniaka. Celem pracy było znalezienie takich warunków wzrostu i wieku roślin oraz sposobu inokulacji i inkubacji, które zapewniałyby powtarzalność testów odpornościowych.

Łodygi odmian 'Albina' i 'Irga' były bardzo podatne na porażenie, a łodygi odmiany 'Meduza' były wysoce odporne, niezależnie od warunków, w jakich rosły rośliny, oraz sposobów prowadzenia testów. W celu zapewnienia dużych różnic między porażeniem łodyg odmian podatnych a porażeniem łodyg odmian odpornych oraz dla powtarzalności wyników testów łodygi o długości 18 cm z fragmentami korzeni powinny być wycinane z roślin w wieku od czterech do ośmiu tygodni. Łodygi należy zakażać za pomocą dwóch krążków z bibuły filtracyjnej o średnicy 6 mm, nasączonych zawiesiną o koncentracji 10^5 sporangiów w 1 ml

i położonych na środkowej części łodygi. Zakażone łodygi, umieszczone w kuwetach na wilgotnej ligninie w temperaturze 22°C, po 24–48 h od inokulacji należy przenieść do słoików z niewielką ilością wody i umieścić w oświetlonej kamerze w temperaturze 20°C. Ocenę stopnia porażenia przeprowadza się siedem dni po inokulacji.

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Authors' address:
Dr. Anna Maria Michalska, Sylwester Sobkowiak M.Sc., Prof. Dr. hab. Ewa Zimnoch-Guzowska, Plant Breeding and Acclimatization Institute in Radzików, Młochów Research Center, ul. Platanowa 19, 05-831 Młochów, Poland,
e-mail: e.zimnoch-guzowska@ihar.edu.pl

Accepted for publication: 14.09.2008