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ORIGINAL PAPER

Host–pathogen interaction between *Phytophthora infestans* and *Solanum tuberosum* following exposure to short and long daylight hours

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Abstract The influence of short and long day length on the expression of qualitative and quantitative resistance to *Phytophthora infestans* in potato was studied. The incompatible interaction was tested for available set of isolates avirulent in greenhouse conditions to potato Black's differentials possessing the genes: *R*2, *R*5, *R*6, *R*8, *R*9, *R*10, and standard potato cultivar Tarpan (no known *R* gene). The avirulent isolates either were completely avirulent regardless of plant growing conditions, or they infected leaflets of these differentials more frequently when plants were exposed previously to short day conditions than to long day conditions. This study highlights the importance of day length, among many other factors which are controlled, in testing the expression of the virulence of *P. infestans* isolates. In compatible interactions, when quantitative resistance was evaluated in differentials with gene *R*1, *R*3, *R*4, *R*7, *R*11, and potato cultivar Craigs Royal (no known *R* gene), stronger infection expressed by lesion growth rate, as well as stronger sporulation, were observed on potato leaflets of plants exposed to short day for 6–7 weeks before inoculation. The analysis of variance revealed a significant contribution to variation in lesion growth rate of day length, genotype, as well as day length by genotype interaction. Significant influence of isolate, and genotype, but not day length, on the expression of the incubation period was found. The results indicate the necessity of evaluating components of partial resistance present in potato lines used in breeding potato resistant to *P. infestans* in destined day length growing conditions.

Keywords Potato late blight · Avirulence · *R*-genes · Photoperiod

Abbreviations

AULEC	Area under lesion expansion curve
GH	Greenhouse
IE	Infection efficiency
IP	Incubation period
LD	Long day
LGR	Lesion growth rate
MGR	Maximal growth rate
SD	Short day
SI	Sporulation intensity

Introduction

The most economically important potato disease is late blight, caused by *Phytophthora infestans*, which results in 16 % of global yield losses and annual costs of global control and damage in more than €10^9 (Haverkort et al. 2008, 2009), as well as in environmental and economical losses due to chemical control. One of the control measures is to cultivate potato varieties resistant to this pathogen. There are two kinds of resistance expressed in potato plants to *Phytophthora infestans*. Qualitative resistance, conferred by *R*-gene(s), according to the gene-for-gene model (Flor 1971), when effector-triggered immunity is activated, resulting in the hypersensitive response (HR) (Kamoun et al. 1999; Jones and Dangl 2006). This kind of interaction is called incompatible, and the isolate avirulent, when *P. infestans* pathogen secreted RXLR effectors that include

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avirulence (AVR) proteins, are targeted by corresponding resistance (R) proteins from wild *Solanum* species (Vleeshouwers et al. 2011). Race-specific *R*-genes were first found in a wild-potato species *Solanum demissum* Lindl., and then introduced into commercial cultivars (Black et al. 1953; Malcolmson and Black 1966). The resistance was quickly overcome for the first four *S. demissum R*-genes in the 1950s (Howatt and Hodgson 1954). The virulence of *P. infestans* is essential to breeders and plant pathologists willing to use control strategies based on the race-specific *R*-genes (Andrivon et al. 2011). Eleven Black's differentials are applied to assess the virulence of *P. infestans* isolates. Studies on *P. infestans* genome revealed a wide spectrum of fast-evolving effector genes localized to a highly dynamic and expanded region of its genome, and may play a crucial role in the rapid adaptability of the pathogen to host plants (Haas et al. 2009). The assessment of virulence is not easy due to many factors influencing the expression of potato *R*-genes: age of plant, position of tested leaflet, source of inoculum, growing media for pathogen maintenance, spores concentration, and range of environmental conditions, such as humidity, temperature and light intensity (Hodgson and Sharma 1967; Victoria and Thurston 1974; Carnegie and Colhoun 1982; Stewart 1990; Sobkowiak et al. 2004; Rubio-Covarrubias et al. 2005). From our previous observations, the virulence of *P. infestans* isolates assessed on differential plants grown in late autumn was more complex than virulence assessed on plants in summer. It is evaluated in qualitative manner, plants can be either infected or not infected, and the isolate virulent or avirulent, respectively.

The second type of resistance is called as general or quantitative, assumed to be multiple gene-based, and it is evaluated in a quantitative manner, as slower development of the disease resulting in reduced infection efficiency, smaller lesion, lower sporulation and longer latent period (Umaerus 1970; Wastie 1991). The variation of specific components of resistance was revealed among (Colon et al. 1995) and within species (Canizares and Forbes 1995). The importance of infection efficiency (IE), lesion growth rate (LGR) was significant for both *S. tuberosum* and *S. microdontum*, whereas the latent period (LP) was significant for *S. microdontum* and sporulation intensity (SI) for *S. tuberosum*.

The influence of day length on general resistance to *P. infestans* was tested by Mihovilovich et al. (2010). Potato plants were grown in the field in SD or LD conditions, and then evaluated in laboratory assay accordingly in SD or LD conditions. The authors indicated that field resistance to foliar late blight under a given day length depend on the infecting isolate. It was shown that the general resistance expressed as LGR decreased in the short day conditions. The isolate-specific quantitative trait locus that displays interaction with isolate behavior under different day lengths was mapped to chromosome I in the BCT mapping population derived from a backcross of *Solanum berthaultii* to *S. tuberosum.*

To reduce the influence of uncontrolled conditions, we tested the effect of extremely different photoperiod conditions of plant growth on the expression of resistance to *P. infestans*, both the general resistance, after inoculation with three different *P. infestans* isolates, and major gene-dependent resistance after inoculation with a set of 18 isolates. These experiments had following objectives: (i) to test the effect of day length on expression of the *R*-genes in the Black's differentials after inoculation with the set of *P. infestans* isolates, (ii) to evaluate the effect of day length on the expression of general resistance expressed as LGR, IP, and SI to *P. infestans* isolates.

Materials and methods

Plant material

Potato cultivars Craigs Royal and Tarpan, lacking known *R*-gene-mediated resistance to *P. infestans*, the international set of 11 Black's differentials, each possessing a single *R*-gene (*R*1–*R*11) from the Mexican species *Solanum demissum* (Black et al. 1953; Malcolmson and Black 1966), obtained from the in vitro collection at Science and Advice for Scottish Agriculture (SASA), Edinburgh UK) [*R*1, Craigs Snow White; *R*2, 1512(16); *R*3*a*, Pentland Ace (van Poppel et al. 2009); *R*4, 1563c(14); *R*5, 3053-18; *R*6, XD2-21; *R*7, 218ef(7); *R*8, 2424a(5); *R*9, 2573(2); *R*10, 3681ad(1); *R*11, 5008ab(6)].

Phytophthora infestans isolates

The isolates used in these experiments were collected in different locations of Poland from potato to tomato plants in 2004–2007 (Table 1).

Growing conditions of potato plants

Potato plants were produced in the greenhouse from tubers obtained from in vitro plants. 3 weeks after planting, the plants were set into three different conditions. One set of plants remained in a greenhouse (GH), the next two sets were located in two growth chambers with constant temperature 20 °C and different photoperiods: short day (SD) conditions (8 h of light a day), and in long day (LD) conditions (16 h of light a day). The intensity of light was measured at 32 cm of plant height in 10 random places in both chambers with SD and LD light conditions and in the

Table 1 Data on *Phytophthora infestans* isolates used for inoculation

Isolate	Sample date	Place of origin	The coordinates	Host
MP585	2004.08.17	Kurzętnik	53° 24′N 19° 34′E	Potato
MP674	2005.08.05	Boguchwała	49° 59′N 21° 57′E	Potato
MP750	2006.08.30	Wilków	51° 04′N 15° 58′E	Potato
MP832	2007.08.07	Jęcznik	53° 36′N 20° 53′E	Potato
MP833	2007.08.07	Jęcznik	53° 36′N 20° 53′E	Potato
MP834	2007.08.08	Chomętowo	52° 57′N 15° 39′E	Potato
MP840	2007.08.13	Sulęcin	52° 26′N 15° 06′E	Potato
MP842	2007.08.13	Kalsk	52° 07′N 15° 35′E	Potato
MP846	2007.08.16	Boguchwała	49° 59′N 21° 57′E	Potato
MP847	2007.08.16	Boguchwała	49° 59′N 21° 57′E	Potato
MP851	2007.08.29	Krzydlina Mała	51° 17′N 16° 32′E	Tomato
MP866	2007.07.20	Młochów	52° 03′N 20° 47′E	Tomato
MP868	2007.07.27	Bocheń	52° 06′N 19° 48′E	Tomato
MP870	2007.07.27	Strugienice	52° 07′N 19° 47′E	Potato
MP872	2007.08.06	Glinki	52° 00′N 21° 14′E	Tomato
MP873	2007.08.20	Szewce	50° 38′N 21° 38′E	Tomato
MP879	2007.07.20	Łowicz	52° 06′N 19° 56′E	Tomato
MP880	2007.08.17	Radziejów	52° 37′N 18° 31′E	Potato

greenhouse. Photosynthetic photon flux density (μmol $m^{-2}s^{-1}$) equal 30.7 in SD, 29.7 in LD, 5.28 in GH (measured on a very cloudy day), 69.2 GH (measured on a cloudy day), 403.4 GH (measured on a sunny day). The plants were acclimatized for 5 weeks before the first assessment. The testing was performed in week intervals.

Inoculum preparation

Cultures of isolates were transferred from rye agar onto leaves of cvs Craigs Royal or Tarpan and multiplied for at least two generations, each of 7–10 days. Inoculum consisted of a sporangial suspension that was prepared as described by Zarzycka (2001) from sporulating lesions of potato leaflets and adjusted using a haemocytometer to a concentration of 50,000 sporangia ml^{-1}.

Detached leaflet assay

Replications were leaflets from different fully developed leaves detached from the middle part of the different plants. The number of tested leaflets is listed in Table 2. Leaflets were placed on wet cellulose wadding in a plastic tray. Each leaflet was inoculated by depositing one 30 μl droplet of the inoculum on the abaxial side of the leaf. The trays with leaflets were covered with glass. The inoculated leaflets were incubated for 7 days at 16 °C with a constant illumination of 11.5 μmol $m^{-2}s^{-1}$. After the first 24 h of incubation, the leaflets were turned abaxial side down.

Methods of evaluation

Three experiments were conducted in 2007. Two different methods of evaluation were applied to assess both qualitative and quantitative resistance to *P. infestans*. In 2008, only qualitative resistance was evaluated with the use of avirulent isolates which were available in IHAR collection of *P. infestans* isolates.

Qualitative resistance

The bioassay comprised three experiments in 2007—where each Black's differential, and potato cv. Craigs Royal was tested with three *P. infestans* isolates, and three experiments in 2008—where each of six Black's differentials, and cv. Tarpan, was tested with 15 *P. infestans* isolates (Table 2.). Potato leaflets were sampled from plants grown 5, 6 and 7 weeks under short day length regime (SD), long day length regime (LD), and a greenhouse. The number of tested leaflets from each potato genotype with each *P. infestans* isolate is listed in Table 2. Symptoms were assessed after 7 days of incubation in two grade scale: 0—lack of symptoms or non-sporulating lesions, 1—presence of sporulating lesions.

The phenotype reaction of an isolate with each differential was defined as the pooled result for each set of 12 or 24 leaflets from plants growing in GH conditions. The isolate, which infected less than half of tested leaflets grown in greenhouse conditions, was defined as GH avirulent.

Quantitative resistance

The bioassay comprised two experiments in 2007—six potato genotypes, which showed symptoms of infection, were evaluated: five Black's differentials *R*1, *R*3, *R*4, *R*7, *R*11, and potato cv. Craigs Royal, each with three *P. infestans* isolates. Four leaflets from each plant acclimatized for 6, and 7 weeks in SD or LD conditions were inoculated (Table 2). Symptoms of infection were measured on five consecutive days starting from the second day after inoculation. Resistance was assessed by estimating three components: infection efficiency (IE), lesion growth rate (LGR) and incubation period (IP). LGR was estimated based on the linear regression of mean diameter of lesion as a function of days after inoculation, $y = ax + b$, where y mean diameter of lesion ($\sqrt{\pi cd/4}$), where c and d are vertical and horizontal diameters of lesion, x number of

Table 2 Scheme of experiments

Type of resistance to *Phytophthora infestans*/evaluation	Years	Tested potato	*Phytophthora infestans* isolate	Number of leaflets tested from each potato genotype with each isolate								
				I[a] (5 weeks)			II[b](6 weeks)			III[c] (7 weeks)		
				SD[d]	LD[e]	GH[f]	SD	LD	GH	SD	LD	GH
Qualitative/symptoms scored on the 7[th] day after inoculation	2007	*R*1–*R*11, Craigs Royal	MP585, MP674, MP750	4	4	4	4	4	4	4	4	4
	2008	*R*2, *R*5, *R*6, *R*8, *R*9, *R*10, Tarpan	MP832–MP880 [g] (15 isolates)	8	8	8	8	8	8	8	8	8
Quantitative/infection efficiency, lesion growth rate, incubation period	2007	*R*1, *R*3, *R*4, *R*7, *R*11, Craigs Royal	MP585, MP674, MP750	–	–	–	4	4	–	4	4	–
Quantitative/sporulation intensity	2007	*R*1–*R*11, Craigs Royal	MP585	–	–	–	–	–	–	2	2	–

[a,b,c] Tested leaflets detached from potato plants acclimatized in different day length for 5, 6, 7 weeks, respectively

[d,e,f] Plants grown in short day, long day, and in a greenhouse, respectively

[g] *P. infestans* isolates listed in Table 1

days after inoculation, $a = \text{LGR}$ expressed as mean increase of the lesion size in mm day^{-1}. Incubation period (IP), the time after inoculation until initial necroses, was defined as $-\text{b a}^{-1}$. IE expressed as percentage of successful infection. LGR values and IP obtained from the four leaflets of 2 day lengths growing conditions, and of three isolates of *P. infestans*, were subjected to two three factor analysis of variance (ANOVA) using STATISTICA for Windows, Stat Soft, Inc. Tulsa, OK, USA (1997). Variance components were estimated from expected mean squares.

Sporulation intensity (SI) was estimated for all Black's differentials and cv. Craigs Royal after 7 weeks of acclimatization in SD and LD conditions, with the use of the one *P. infestans* isolate—MP585. The area of the infected tissue having an elliptical shape was calculated according to formula $\pi ab/4$ (where a and b are vertical and horizontal diameters of lesion measured on the 6th day after inoculation). Two leaflets per genotype were shaken in 20 ml of water and SI was estimated as a number of sporangia cm^{-2}, based on three to four microscopic counting using a haemocytometer.

Results

Qualitative resistance

Three *P. infestans* isolates, MP585, MP674 and MP750, were virulent to the differentials *R*1, *R*3, *R*4, *R*7, *R*11, and potato cv. Craigs Royal, independent on the growing conditions, in all the three experiments in 2007. Thus, the results for these genotypes are not shown in Table 3.

The *R*5 plants grown in SD, LD, and GH conditions did not show any symptoms of infection after inoculation with 6 *P. infestans* isolates. After inoculation, with other 10 avirulent *P. infestans* isolates, there were observed symptoms of infection more frequently on leaflets originating from SD conditions (69.0 % of leaflets, out of 228 tested, were infected), than from LD, and GH conditions (22.4 and 2.6 % of the leaflets were infected, respectively). This analysis does not include isolates which were not tested in GH conditions. Only in one case, when the *R*5 differential was inoculated with the isolate MP750 different pattern was observed, when 8 leaflets out of 20 from LD conditions were infected, and only 1 leaflet from SD conditions. After inoculation with the isolate MP832 symptoms were observed on leaves from both SD and LD, but not on leaflets from greenhouse plants (Table 3).

No differences in infection were observed for 6 isolates of *P. infestans* and did not infect any leaflets of *R*6. Another 9 avirulent isolates infected, 45.6 % of leaflets from SD (out of 204 leaflets tested), 4.4 % from LD, and 0.1 % from GH conditions.

Ten isolates were avirulent to differential *R*8. Symptoms of infection were observed on 58.3 % of leaflets from SD conditions, 31.4 % from LD conditions and 10.3 % from GH (204 leaflets were inoculated).

Among 18 GH avirulent isolates to differential *R*9, 13 did not infect any leaflets from SD to LD conditions. Out of the 96 leaflets inoculated with 5 isolates, 38.5 % of infected leaflets were grown in SD, 4.2 % in LD, 0.0 % in GH.

Six GH avirulent isolates to *R*10 showed infection of 20.8, 13.2, and 0.0 % of leaflets in SD, LD, and GH,

Table 3 Number of infected leaflets with 18 *Phytophthora infestans* isolates of Black's differentials *R*5, *R*6, *R*8, *R*9, *R*10, grown for 3–7 weeks in short (SD), long day (LD), or greenhouse (GH) conditions

Isolate	No of leaflets tested per each combination	Number of infected leaflets														
		*R*5			*R*6			*R*8			*R*9			*R*10		
		SD	LD	GH	SD	LD	GH	SD	LD	GH	SD	LD	GH	SD	LD	GH
MP832	24	24	20	0	24	24	23	23	8	4	20	1	0	24	23	24
MP846	24	22	4	2	2	0	0	22	13	13	0	0	0	24	22	22
MP847	24	22	1	0	16	1	0	24	22	16	0	0	0	24	24	24
MP834	24	19	8	3	4	1	2	9	6	4	0	0	0	24	17	12
MP840	24	19	3	0	13	0	0	21	20	12	0	0	0	23	20	20
MP880	24	17	7	1	11	2	0	24	21	19	0	0	0	24	24	24
MP674	12	12	5	nt	12	12	12	1	0	0	3	0	0	12	9	nt
MP585	12	11	4	nt	12	12	12	5	4	0	12	0	0	12	3	nt
MP842	24	15	4	0	13	2	0	24	24	7	0	0	0	24	24	24
MP833	24	14	0	0	15	0	0	24	17	12	0	0	0	24	24	24
MP851	24	4	0	0	10	3	0	24	10	23	0	0	0	24	16	14
MP750	12	1	4	0	9	0	0	12	5	0	0	0	0	12	6	nt
MP866	24	0	0	0	0	0	0	6	4	2	0	0	0	2	0	0
MP868	24	0	0	0	0	0	0	6	3	0	0	0	0	8	6	0
MP870	24	0	0	0	0	0	0	15	5	0	0	0	0	1	0	0
MP872	24	0	0	0	0	0	0	21	17	17	2	0	0	15	11	0
MP873	24	0	0	0	0	0	0	18	5	4	0	3	0	3	2	0
MP879	24	0	0	0	0	0	0	12	12	15	0	0	0	1	0	0
Sum	396	180	60	6	141	57	49	291	196	148	37	4	0	281	231	188

nt not tested

respectively (144 leaflets tested). Different reaction of R9 and R10 differentials is shown in Fig. 1.

The influence of day length was not observed for the *R*2 plants inoculated with *P. infestans* isolates. The leaflets detached from these plants showed symptoms of infection after inoculation with three virulent isolates and did not show any symptoms of infection after inoculation with 15 avirulent isolates of *P. infestans*.

Quantitative resistance

All virulent isolates infected all inoculated leaflets. IE was equal to 100 % in both SD and LD conditions; that is why, this component was excluded from the analysis. LGR and IP were used for analysis of variance. A significant influence of the growing conditions (SD and LD) explained 93.6 % of total variation. In addition, genotype and genotype by growing conditions interaction, on expression of LGR was revealed (Table 4.). The effect of isolate and the interaction of growing conditions with isolate on the LGR was not found. In general, LGR was higher for plants grown in SD than in LD conditions, the mean of LGR was equal to 9.13 and 5.93 mm day^{-1}, respectively. The analysis of variance for IP revealed statistically significant contribution of isolate (31.7 % of total variation), and of genotype (26.0 % of total variation) on this trait, but not of growing conditions (Table 4).

Tested genotypes differed from each other for LGR, and also different reaction of genotypes was observed depending on growing conditions (Table 5). The correlation coefficient between LGR of plants grown in SD and LD conditions was not significant and equal $r = 0.235$ ($P = 0.654$).

Out of the three isolates tested, the isolate MP674 was significantly faster in infection, which was expressed by shortest IP equal 2.14 days, when for MP585 and MP750 equal 2.25 and 2.29, respectively.

The significant differences were observed for SI for plants grown in different photoperiods. Higher SI was observed on leaflets detached from plants grown in SD conditions for cv. Craigs Royal, *R*2, *R*4, *R*6 and *R*7. For the differentials *R*5, *R*9, *R*10 and *R*11, sporulation was observed only on leaflets grown in SD conditions. No significant differences in SI were observed on both differentials *R*1 and *R*3 grown in SD and LD conditions (Fig. 2).

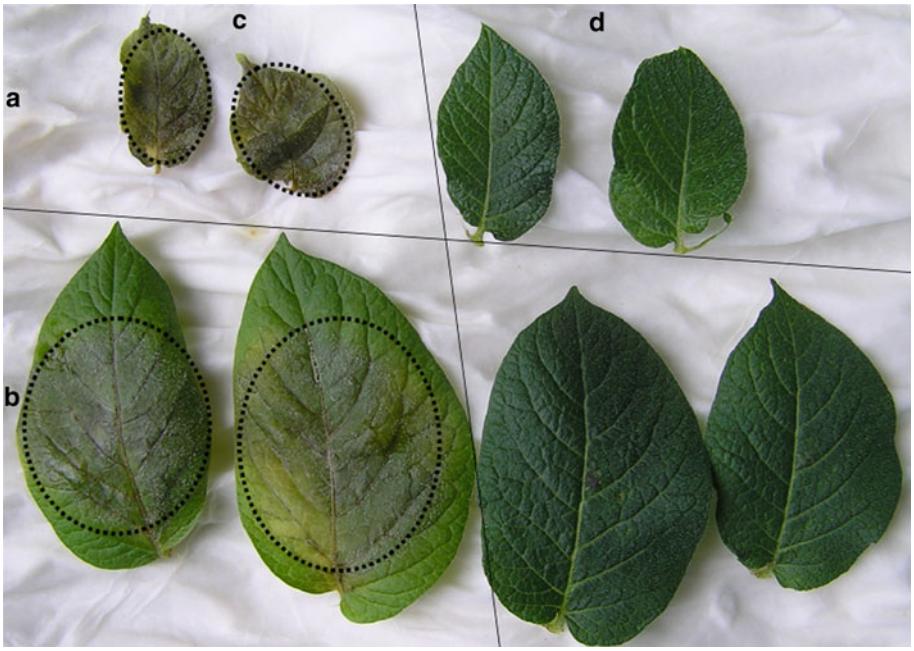

Fig. 1 Bioassays demonstrate the different recognition of the isolate MP585 by the Black's differential *R*9 and *R*10 on the 6th day post-inoculation: **a** the *R*9 differential, **b** the *R*10 differential, **c** leaflets from plants grown in short day conditions (spreading sporulating lesions), **d** leaflets from plants grown in long day conditions (resistant, lack of infection)

Table 4 Analysis of variance on the lesion growth rate (LGR) and incubation period (IP) of potato leaflets of Black's differentials *R*1, *R*3, *R*4, *R*7, *R*11, and potato cultivar Craigs Royal, grown for 6–7 weeks in short or long day conditions, inoculated with three *Phytophthora infestans* isolates

Source	*df*	Analysis of variance mean squares	
		LGR (mm day^{-1})	IP (day)
Growing conditions (GC)	1	202.35***	0.018
Isolate (I)	2	1.95	0.151**
Genotype (G)	5	4.18**	0.124**
GC × I	2	2.95	0.047
GC × G	5	2.70*	0.047
I × G	10	0.67	0.051
GC × I × G	10	0.50	0.011
Error	36	0.94	0.027

Significant at * $P = 0.05$, ** $P = 0.01$, and *** $P = 0.001$, respectively

Discussion

In this study, it was demonstrated that there is a difference in reaction of potato differentials grown in different day length regimes to *P. infestans* isolates. For the first time, it was shown that several isolates avirulent on plants grown in GH and LD conditions appeared to be virulent on plants grown in SD conditions. Determining virulence towards race-specific resistance genes is a prerequisite to understand the response of pathogen populations to resistant cultivars, and therefore to assess the durability of these resistance genes and the performance of resistance management strategies (Andrivon et al. 2011). One of the main activities in potato breeding for resistance to late blight is collecting *P. infestans* isolates representing local populations. These isolates are characterized for phenotypic and genotypic traits, among them for virulence against differential set of potato genotypes having 11 late blight resistance (*R*) genes originated from *Solanum demissum*. The number of methods and different conditions of assessing the virulence of *P. infestans* isolates did not allow comparing the data across laboratories/countries, to have a better understanding on the changes and evolution of this pathogen. One of the goals of the European Concerted Action on Blight EUCABLIGHT was to collate and review existing methods for assessing variation in populations of *P. infestans*, and to test, standardize and publish agreed methods, to create a database accessible via the web of *P. infestans* European isolates. A joint protocol for virulence testing in detached leaf assay was established and a blind ring test involving 12 laboratories and 10 European isolates of the pathogen was conducted. A high level of consensus in the determination of virulence to *R*1, *R*3, *R*4, *R*7, *R*8, *R*10 and *R*11 was achieved among the collaborators, but virulence to *R*2, *R*5 and *R*9 was detected more frequently in some laboratories, essentially from northern Europe and it was concluded that these genes are seems to be highly sensitive to host and environmental conditions (Andrivon et al. 2011). Among many factors that were controlled in the joint protocol day length was not considered. From many years of virulence evaluation, we have noticed that the isolates evaluated in November and December, when the days are shortest during a year in Europe, the pathotypes of some isolates are more complex. For the first time, it was observed in *P. infestans* that a

Table 5 Resistance to *Phytophthora infestans* expressed as the lesion growth rate (LGR) and incubation period (IP) of potato leaflets of Black's differentials *R*1, *R*3, *R*4, *R*7, *R*11, and cultivar Craigs Royal, grown for 6–7 weeks in short day (SD) or long day (LD) conditions, inoculated with three *Phytophthora infestans* isolates

Growing conditions	Potato genotypes	LGR (mm day^{-1})	Means ranking*	IP (day)	Means ranking
LD	*R*11	4.88	a	2.13	abc
	*R*1	5.36	a	2.36	d
	*R*7	5.75	a	2.20	bcd
	*R*3	5.95	a	2.26	bcd
	*R*4	6.12	a	2.12	ab
	cv. Craigs Royal	7.51	b	2.18	bcd
SD	*R*11	8.55	bc	1.95	a
	*R*7	8.70	bcd	2.26	bcd
	*R*3	9.25	cde	2.35	cd
	cv. Craigs Royal	9.32	cde	2.34	cd
	*R*4	9.85	de	2.24	bcd
	*R*1	10.02	e	2.30	bcd

* Means of LGR or IP followed by the same letter do not differ significantly using Duncan's range test at $P = 0.05$

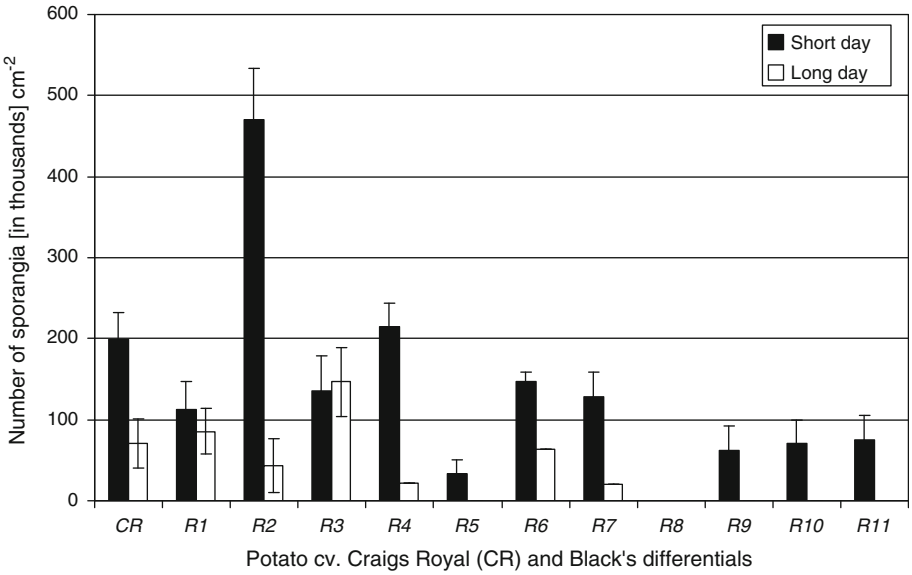

Fig. 2 Sporulation intensity on infected leaflets at 6th day after inoculation with the *Phytophthora infestans* isolate MP585 of potato cv. Craigs Royal and Black's differentials, exposed before inoculation for 7 weeks to short day or long day conditions (the mean ± SD of 3–4 samples)

group of GH avirulent isolates on *R*5, *R*6, *R*8 (seldom but also on *R*9, and *R*10) can behave as virulent on leaflets detached from plants grown in SD conditions, while they remain avirulent or virulent only sporadically for leaflets from LD conditions. Similar effect was reported by Ward and Buzzel (1983) with regard to the light influence on the interaction between soybean genes for resistance and *Phytophthora megasperma* f. sp. *glycinea*. In their studies, several *Rps* genes incompatible with race 4 did not expressed resistance to this race in intact etiolated hypocotyls.

It was revealed that in potato the *R*5, *R*6 and *R*9 differentials posses also the *R*1 gene (Trognitz and Trognitz 2007), and the *R*3 differential contains two closely linked genes, *R*3a and *R*3b (Huang et al. 2004). However, in these studies, *P. infestans* isolates avirulent to the *R*1 and *R*3 were not used; therefore, the obtained results were not biased by these additional genes.

The incompatible interaction of *P. infestans* isolates to Black's differentials *R*1, *R*3, *R*4, *R*7, *R*11 was not tested, because corresponding avirulent isolates were not found in IHAR's collection of the pathogen. Three virulent isolates to all these five *R*-genes were tested, and all these isolates caused symptoms of disease on 20 leaflets of each differential from both short and long day conditions.

The resistance of potato to *P. infestans* depends on many factors. Among them, long day has a strong resistance enhancing effect on potato plants (Trognitz et al. 2009; Colon 1994; Victoria and Thurston 1974). The photoperiod is a significant factor in the expression of quantitative

resistance to *P. infestans* of some potato genotypes, but not all genotypes of potato react in the same way (Mihovilovich et al. 2010). These findings were confirmed for the resistance to *P. infestans* expressed as the LGR in this study for six different potato genotypes. In studies of Trognitz et al. (2009) blight was significantly ($P < 0.001$) reduced on LD pre-treated, genetically resistant cultivars, whereas the susceptible cultivar Linzer Delikatess developed severe disease symptoms under all conditions (Trognitz et al. 2009).

The ANOVA of LGR values in detached leaf assay showed no significance for isolate, and isolate by day length interaction, in contrast to studies of Mihovilovich et al. (2010), in which the interaction of isolate and day length in the LGR variation played a significant role. The authors performed detached leaflet assay in both SD and LD conditions, in contrast to our test, where the conditions of detached leaflet assay were the same, so only plants, not isolates were exposed to SD and LD conditions. It might be assumed that there is a different effect of day length on the aggressiveness of *P. infestans* isolates (not investigated in our studies). LGR and SI could be enough parameters to assess isolates aggressiveness based on the results of Carlisle et al. (2002) and Flier and Turkensteen (1999). They found that correlations between all combinations of all parameters, area under lesion expansion curve (AULEC)/maximal growth rate (MGR), infection frequency, LP and SI, were significant at $P < 0.05$ up to $P < 0.001$ (with exception of correlation between AULEC/MGR and SI). In our studies, both LGR and SI were significantly favored by SD growing conditions (with exception of SI on differentials *R*1 and *R*3, where the influence of growing conditions was not observed). IE was not influenced by growing conditions and all leaflets were infected by all three isolates used. IP was not influenced by growing conditions, but by isolate and genotype.

The influence of SD and LD conditions was not observed for the infection of the *R*2 Black's differential with all virulent or avirulent *P. infestans* isolates. However, surprisingly many avirulent isolates caused symptoms of infection on leaflets of differentials *R*5, *R*6, and *R*8 grown in SD conditions. Further studies are needed to understand the role of light conditions in qualitative resistance of potato to *P. infestans*. In general, SD growing conditions of potato plants are in favour to stronger infection, expressed as higher LGR, and the stronger SI, but not the IP, after inoculation with virulent *P. infestans* isolates. Potato genotypes differ in sensitivity to different photoperiods. The evaluation of *P. infestans* isolates for their virulence should be conducted on leaflets from plants grown in LD conditions. If this evaluation is being done in late autumn, the day length should be extended with an artificial light. The same should be applied for screening potato parental breeding lines and breeding clones for general resistance to *P. infestans*.

Author contribution Renata Lebecka designed research, conducted research, analyzed data and performed statistical analysis, wrote paper, had primary responsibility for final content. Sylwester Sobkowiak: isolated and provided *Phytophthora infestans* isolates.

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