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A laboratory assay for *Phytophthora infestans* resistance in various *Solanum* species reflects the field situation

Vivianne G.A.A. Vleeshouwers[1,3,*], Willem van Dooijeweert[1], L.C. Paul Keizer[1], Luc Sijpkes[1], Francine Govers[2,3] and Leontine T. Colon[1]
[1]*DLO – Centre for Plant Breeding and Reproduction Research (CPRO-DLO), P.O. Box 16, 6700 AA, Wageningen, The Netherlands;* [2]*Laboratory of Phytopathology, Wageningen Agricultural University, Binnenhaven 9, 6709 PD Wageningen, The Netherlands;* [3]*Graduate School Experimental Plant Sciences, The Netherlands;* [*]*Author for Correspondence: Laboratory of Phytopathology, Wageningen Agricultural University, Binnenhaven 9, 6709 PD Wageningen, The Netherlands (Fax: +31 0317-483412; E-mail: Vivianne.Vleeshouwers@medew.fyto.wau.nl)*

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Abstract

Physiological and molecular research on resistance responses of *Solanum tuberosum* cultivars and partially resistant *Solanum* species to *Phytophthora infestans* requires a reliable resistance test that can be used in the laboratory. Laboratory tests performed on detached leaves and intact plants were compared with field tests for similarity of late blight reactions. Detached leaves from field-grown plants were as resistant as detached leaves from climate chamber-grown plants when challenged with *P. infestans*. However, detached leaves incubated in covered trays at high relative humidity were more susceptible than detached leaves kept in open trays or leaves on intact plants. The incubation conditions of detached leaves in covered trays rather than detachment itself appeared to affect the resistance expression. Detached leaves of some wild *Solanum* genotypes became partially infected, whereas intact plants were completely resistant when inoculated. Inoculation of leaves on intact plants, however, resulted in lower infection efficiencies. These limitations should be taken into account when choosing the appropriate inoculation method for specific purposes. For resistance screening, laboratory tests proved to be a good alternative for field tests. The ranking of resistance levels for twenty plant genotypes was similar under laboratory and field conditions.

Abbreviations: ADPC – area under the disease progress curve; IE – infection efficiency; HR – hypersensitive response; LGR – lesion growth rate; LS – lesion size; LSD – least significant difference.

Introduction

Partial resistance to *Phytophthora infestans*, the causal agent of late blight, occurs in several wild *Solanum* species and in some potato cultivars (Colon and Budding, 1988; Colon et al., 1995b). This quantitative type of resistance is influenced by environmental factors (Umaerus, 1969); therefore, experimental conditions for testing levels of partial resistance should be chosen carefully. Although the field tests described by Fry (1978) and Colon and Budding (1988) closely resemble the natural conditions under which late blight resistance is important, field testing can only be performed once a year during the growing season. For large-scale resistance screenings in commercial breeding programs, the limitations of field tests may be accepted in contrast to more sophisticated, costly and time-consuming laboratory tests. However, studies

aimed at unravelling resistance mechanisms at the physiological or molecular levels are best performed under controlled conditions in the laboratory. For these specific investigations, an experimental setup in which a high percentage of successful infections can be assured is a prerequisite.

Several methods have been described to assess foliar late blight resistance. In addition to field tests and whole-plant greenhouse assays (Stewart et al., 1983), laboratory tests on detached leaves (Lapwood, 1961), leaflets (Malcolmson, 1969); Umaerus and Lihnell (1976) or leaf discs (Hodgson, 1961) have been described. Resistance assessed in the field can be expressed as ADPC values (area under the disease progress curve) (Shaner and Finney, 1977), which are considered the best estimates of disease for multi-cycle pathogens like *P. infestans* (Fry, 1978). In laboratory tests, commonly used parameters for resistance assessment are lesion growth rate (LGR), i.e., the rate of necrosis extension, lesion size (LS), which is correlated with LGR, infection efficiency (IE), i.e., the percentage of successful infections, latency period (LP) and spore density (SD) (Birhman and Singh, 1995). Less frequently used, but also suitable as a parameter, is fungal biomass. Its quantification can be carried out either through serology-based tests such as ELISA (Harrison et al., 1990; Beckman et al., 1994) or through GUS assays using a transgenic *P. infestans* strain constitutively producing β-glucuronidase (Kamoun et al., 1998).

Several laboratory tests have been compared with field tests. Using ranking studies, the relative resistance levels of several cultivars in greenhouse or laboratory experiments appeared comparable to resistance levels in the field (Hodgson, 1962; Knutson, 1962; Stewart et al., 1983; Dorrance and Inglis, 1997). LS, LP and SD measured in a laboratory assay on detached leaflets appeared well correlated with ADPC values obtained from a late blight field trial (Singh and Birhman, 1994) after multiple linear regression. In these studies, resistance data obtained in laboratory tests were compared with rough resistance scores obtained in completely different field tests. So far, the effect of conditions inherent to laboratory tests on the actual resistance levels has not been described. Recently, Dorrance and Inglis (1997) reported that a greenhouse test with intact plants corresponded better to ADPC values than a laboratory test with leaflets and leaf discs incubated on water agar. However, from a practical point of view, tests with detached leaves, leaflets or discs are more attractive. Additional studies are needed to verify whether resistance observed on detached leaf assays reflects the resistance found in field tests.

The aim of our study was (1) to design a reliable laboratory test for resistance assessment to *P. infestans*, and (2) to compare resistance data obtained in this test with resistance data obtained in field tests. To this end, well-characterized *Solanum* material with a broad range of resistances was used, and experimental conditions and methods were standardized.

Materials and methods

Plant material

The plant genotypes used in this study and their resistance characteristics are listed in Table 1. Material obtained from *in vitro* plantlets was used since the physiological age of tubers has been shown to have an effect on foliage resistance (Stewart et al., 1983). In addition, *in vitro* propagation reduces the chance of virus contamination, and allows rapid multiplication of plant material. *In vitro* plantlets were grown in sterile glass tubes containing MS medium (Murashige and Skoog, 1962) supplemented with 15 gl^{-1} sucrose and 15 gl^{-1} mannitol at 23 °C. For propagation, shoots were cut and transferred to fresh MS medium containing 30 gl^{-1} sucrose. After one week of rooting, the *in vitro* plantlets were transferred into pots of sterilized soil and placed in a climate chamber. To allow a progressive adaptation to lower humidity, the plantlets were initially covered with small transparent containers that were removed the next day. The plants were grown under controlled conditions with a 16 h/8 h day/night regime and 18/15 °C, 65–80% relative humidity (RH); illumination was provided by 400 W Philips-HPIT lamps placed at 50 cm intervals at 150 cm above soil level. For the field tests, plants were grown for one month in the climate chamber, and subsequently transplanted in the field to grow for one additional month before inoculation.

Phytophthora infestans *isolates, maintenance and inoculum preparation*

P. infestans isolates 90128 (race 1.3.4.6.7.8.10.11) and IPO-0 (race 0, kindly supplied by Dr. L.J. Turkensteen, IPO-DLO, Wageningen, The Netherlands) were used

Table 1. Plant genotypes used in this study, known late blight responses (to other isolates) and their levels of resistance to *Phytophthora infestans* isolate 90128 as determined under laboratory conditions (experiment I), intermediate conditions (experiment II) and field conditions (experiment III). Resistance levels are expressed as the mean lesion growth rate (LGR, in mm day^{-1}). The infection efficiency (IE, in percentage) is presented in parentheses

Experiment		I[D]		II[C]		III[A]	
Solanum genotype	Known resistance	LGR	(IE)	LGR	(IE)	LGR	(IE)
S. berthaultii-9 (BGRC 10063)	Complete resistance[1]	0.0	(0)	0.0	(0)	0.0	(0)
S. berthaultii-11 (BGRC 10063)	High partial resistance[1]	2.7	(10)	0.0	(0)	0.0	(0)
S. arnezii x hondelmannii-63 (BGRC 27308)	Partial resistance[2]	1.9	(95)	0.2	(7)	0.7	(73)
S. arnezii x hondelmannii-72 (BGRC 27308)	Partial resistance[2]	3.4	(75)	2.2	(31)	1.5	(43)
S. circaeifolium ssp . *circaeifolium*-circ1 (BGRC 27058)	Complete resistance[3]	0.0	(0)	0.0	(0)	0.0	(0)
S. microdontum-167 (BGRC 24981)	Partial resistance[1]	3.3	(45)	1.9	(9)	0.8	(10)
S. microdontum-178 (BGRC 24981)	Partial resistance[1]	2.8	(25)	nd	(nd)	0.0	(0)
S. microdontum var. *gigantophyllum*-265 (BGRC 18570)	Susceptible[1]	5.4	(90)	3.6	(49)	2.2	(63)
S. sucrense-23 (BGRC 27370)	Partial resistance[2]	4.5	(45)	0.0	(0)	0.0	(0)
S. sucrense-71 (BGRC 27370)	Partial resistance[2]	4.1	(100)	0.6	(29)	1.0	(43)
S. vernei-530 (BGRC 24733)	Partial resistance[1]	3.8	(80)	3.2	(36)	2.0	(93)
ABPT (30 × 33)-44[4]	Partial resistance[3]	2.5	(80)	0.0	(0)	1.2	(55)
S. nigrum-SN18	Nonhost resistance	0.0	(0)	0.0	(0)	0.0	(0)
S. nigrum-SN18 × potato cv. Désirée[5]	Resistant[6]	nd	(nd)	0.0	(0)	0.0	(0)
Mirabilis jalapa	Nonhost resistance	nd	(nd)	0.0	(0)	0.0	(0)
S. tuberosum cv. Bintje	Susceptible	5.0	(100)	4.0	(47)	2.3	(53)
S. tuberosum cv. Ehud	Susceptible, R1 specific resistance	4.2	(100)	3.1	(56)	2.2	(63)
S. tuberosum cv. Estima	Susceptible, R10 specific resistance	4.8	(100)	2.5	(24)	1.2	(30)
S. tuberosum cv. Première	Susceptible, R10 specific resistance	3.5	(90)	2.8	(44)	1.3	(55)
S. tuberosum cv. Robijn	Durable, partial resistance[7]	2.9	(100)	1.8	(47)	1.2	(58)
LSD ($P < 0.05$)		1.7	—	0.6	—	0.6	—

[D]Treatment D, Table 2, consisted of detached leaves, grown in climate chambers, tested in the laboratory.
[C]Treatment C, Table 2, consisted of intact plants, grown in climate chambers, tested in the laboratory.
[A]Treatment A, Table 2, consisted of intact plants, grown and tested in the field.
[1](Colon et al., 1995b).
[2](Colon and Budding, 1988).
[3]Colon, personal communication.
[4]Double-bridge hybrid of *S. acaule, S. bulbocastanum, S. phureja, S. tuberosum* (Hermsen and Ramanna, 1973).
[5]A sexual hybrid of *S. nigrum*-SN18 × potato cv. Désirée (Eijlander and Stiekema, 1994).
[6](Colon et al., 1993).
[7](Colon et al., 1995c).

throughout this study. Aliquots of sporangiospore suspensions in 15% dimethyl sulfoxide were preserved in liquid nitrogen. For each experiment, a fresh sample of sporangiospores was plated on rye agar medium supplemented with 20 g l^{-1} sucrose (Caten and Jinks, 1968) and incubated at 18 °C in the dark. After a few days, a plug of mycelium was transferred to a fresh agar plate. One week later, when the plate was covered with mycelium, cold water (4 °C) was added to the sporulating mycelium. The sporangiospore suspension was pipetted into a test tube and incubated at 4 °C. After 1–2 h, zoospores were separated from the sporangiospores by filtration through a 15 µm nylon mesh. The concentration was adjusted to 5×10^4 zoospores ml^{-1} for inoculation.

Inoculation: Conditions and plant material

Eight to ten weeks old climate chamber or field-grown plants were spot inoculated either on detached leaves or intact plants. The third to fifth fully developed leaves (counted from the top) were used. Five leaflets per compound leaf were inoculated (one spot per leaflet) by pipetting 10 µl droplets on the abaxial side.

Inoculations in the field were carried out just before nightfall. To obtain a high RH, necessary for good infection, the plants were thoroughly wetted by sprinkling during the afternoon prior to inoculation. During the entire experiment, the field was kept humid by regular overhead sprinkling. Climate chamber-grown plants were transferred to another climate chamber and incubated at a 16 h/8 h day/night photoperiod with fluorescent light (Philips TLD 36W/84o) tubes at 18/15 °C and 95–98% RH for inoculation on intact plants. The next day the RH was lowered to 70%. For detached leaf inoculations, leaves were cut, placed in water-saturated florist's foam (Oasis®) in a tray, and inoculated. The trays were wrapped in transparent plastic bags (covered trays) and incubated in the same climate chamber as the inoculated intact plants.

Estimation and analysis of lesion growth rate (LGR) and infection efficiency (IE)

Lesions were measured three times, usually at days three, four, and five after spot inoculation using an electronic caliper connected to a palmtop computer. The largest length and width (perpendicular to the length) of each lesion were measured, and the ellipse area ($A = 1/4 \times \pi \times \text{length} \times \text{width}$) was calculated. The lesions were divided into two groups, i.e., 'no infection/arrested lesion' (no lesion, or lesion remaining within the size of the inoculum droplet, i.e., $A \leq 16\,mm^2$), or 'growing lesion' (the area is larger than $16\,mm^2$ at least at one time point). The arrested lesions were regarded as unsuccessful infections where the pathogen had been stopped by a hypersensitive response (HR). Therefore, these lesions were not included in the estimation of LGR. From the 'growing lesions' group, the area of the ellipses was square-root transformed, resulting in radius of the lesions. The LGR was estimated by linear regression over time. The infection efficiency (IE) was calculated as the percentage of successful inoculations (i.e., percentage of growing lesions relative to the total number of inoculations) per plant. IE and LGR were estimated and analyzed with ANOVA using Genstat (Genstat 5 Committee, 1987).

Experimental design

As summarized schematically in Table 2, five different treatments can be distinguished. In this design, treatment A represents the field situation, treatment D represents the laboratory test as designed in this paper, treatments B and C are intermediates. Treatment E was included to analyze the effects of leaf detachment and environmental conditions separately.

Treatments A, B, C, and D were compared in a three-factor experiment: within each growing condition (field or climate chamber) the resistance levels of

Table 2. Treatments used to test the effect of growing conditions, inoculation material and environment on resistance of plants to *P. infestans*. Experiments were performed at indicated test locations

Treatment	A	B	C	D	E
Plants grown in	Field	Field	Climate chamber	Climate chamber	Climate chamber
Inoculation on	Intact plants	Detached leaves	Intact plants	Detached leaves	Detached leaves
Environment	Open air	Covered trays	Open air	Covered trays	Open air
Test location	Field	Laboratory	Laboratory	Laboratory	Laboratory

two cultivars (Bintje and Robijn) were determined after inoculation with *P. infestans* isolate 90128 on either detached leaves or intact plants. The same inoculum suspension was used for the plants in the climate chamber and field. Through accurate labelling of the inoculation spots on designated leaves, potential confusion with outside *P. infestans* contaminants was excluded. The field and the climate chamber were each divided into two blocks, which were divided into three sub-blocks. The sub-blocks consisted of two Bintje and two Robijn plants, resulting in 48 plants per experiment. Two experiments were carried out at a one-week interval, and the next summer these were replicated. The four experiments were combined for statistical analyses.

A two-factor experiment was carried out on climate chamber-grown plants, which received treatments C, D, and E (Table 2) in four replications. Bintje and Robijn were inoculated with *P. infestans* isolate 90128. The results from duplicate experiments were combined for statistical analysis.

In a separate experiment, leaves from Bintje and Robijn were detached either one hour or one day prior to inoculation. The detached leaves were inoculated with *P. infestans* isolate 90128 and incubated in covered trays (treatment D, Table 2). Two plants were used per cultivar, per incubation period. LGRs were compared between different times of incubation prior to detachment. Three identical experiments were performed and combined for statistical analysis.

Resistance tests with a set of Solanum *genotypes*

Average LGRs and IEs were determined on nineteen *Solanum* genotypes and on the nonhost *Mirabilis jalapa* (Table 1) under different experimental conditions (treatments A, C, and D, Table 2) after inoculation with either *P. infestans* isolate IPO-0 or 90128. A randomized block design was applied, with 3 or 4 blocks, depending on the experiment. LGRs could not be determined for genotypes that were completely resistant (lesion size remains 0) or displaying a HR, such as *S. berthaultii*, *S. circaeifolium*, *S. nigrum*, and *M. jalapa* (Table 1). Genotypes displaying no symptoms at all were considered more resistant than genotypes predominantly exhibiting HR. To include these highly resistant genotypes in the resistance rating, the average LS at day 6 was calculated.

Correlations between different experimental conditions

To test whether the resistance levels of *Solanum* genotypes were comparable under the different experimental conditions, separate experiments were compared to each other. Per individual experiment, the LGRs and LSs at day 6 from the plant genotypes were ranked in decreasing resistance, and Spearman's rank correlation test was applied to pairs of experiments.

Results

Comparisons between laboratory and field tests

When comparing the various experimental conditions (Table 2) late blight lesions extended always significantly ($P < 0.001$) more rapidly on Bintje than on Robijn (Table 3). During incubation, plants in the field encounter other environmental conditions than plants in the climate chamber. Thus, for intact plants, the effects of growing conditions and inoculation material (detached leaves vs. intact plants) are unavoidably interwoven. Therefore, analysis of the effect of growing conditions was carried out with the LGRs estimated on detached leaves (treatment B vs. D, Table 2) from Bintje and Robijn. The effect of growing conditions on LGR was not significant ($P = 0.65$) but there was a significant interaction between growing conditions and cultivars ($P < 0.001$). These results suggest that plants grown in the climate chamber are as resistant as field-grown plants. The effect of inoculation on intact plants vs. detached leaves was analyzed for climate chamber-grown plants (treatment C vs. D), and

Table 3. Effect of four different treatments (Table 2) on lesion growth rates (LGR, in mm day^{-1}) after inoculation with *Phytophthora* infestans isolate 90128, on potato cultivars Bintje and Robijn ($n = 360$ inoculation spots)

	Bintje		Robijn	
	Intact plants	Detached leaves	Intact plants	Detached leaves
Field	1.88[A]	3.81[B]	0.63[A]	1.87[B]
Climate chamber	2.87[C]	3.94[D]	0.75[C]	1.65[D]

LSD = 0.12 ($P < 0.05$).
[A]Treatment A, Table 2, representing the field test.
[B]Treatment B, Table 2.
[C]Treatment C, Table 2.
[D]Treatment D, Table 2, representing the laboratory test.

a highly significant ($P < 0.001$) effect of the inoculation material was found, with an interaction between inoculation material and cultivar ($P = 0.029$).

The table of means from the total experiment is presented in Table 3. The LGR on intact plants was lower in the field (A) than on intact plants in the climate chamber (C). IEs showed a similar pattern as LGRs (data not shown). In general, climate chamber-grown plants appeared to have the same resistance level as field-grown plants, while detaching the leaves significantly reduced the expression of resistance. This suggests that either the environmental conditions in a covered tray, or leaf detachment, affects resistance expression.

Effect of leaf detachment

To discriminate between the effects of environmental conditions and leaf detachment on LGR, three treatments (C, D, and E) were compared for Bintje and Robijn. Incubation conditions ($P < 0.001$) and cultivars ($P < 0.001$) significantly influenced LGRs. Lesions extended significantly faster on Bintje than on Robijn in all treatments (Table 4). The LGRs on detached leaves from both cultivars were significantly higher in covered trays (D) compared to open trays (E). However, there was no significant difference between the LGRs on intact plants (C) and detached leaves in open air (E). These data suggest that the decreased resistance of detached leaves is caused by environmental conditions, rather than by leaf detachment.

For Bintje, high IEs were reached in all treatments (Table 4), contrasting with the situation on partially resistant Robijn, where a high IE was achieved only on detached leaves in covered trays (92%). IE was significantly lower on detached leaves in open trays (70%), and lowest on intact plants (52%).

To test whether incubation in the trays has an effect on resistance expression, Bintje and Robijn leaves were detached either 1 h or 24 h prior to inoculation. The effect of the incubation period was not significant ($P = 0.393$), the cultivar effect was highly significant ($P = 0.003$) and there was no interaction ($P = 0.979$) between incubation period and cultivar (Table 5). The same pattern was found for the IEs (data not shown). This supports the hypothesis, that leaf detachment *per se* does not affect resistance expression.

Resistance assessment of Solanum *genotypes*

Resistance tests on a set of nineteen *Solanum* genotypes and the nonhost *M. jalapa* revealed that, in general, the LGR was the highest on detached leaves (D), intermediate on intact plants in the climate chamber (C), and the lowest in the field (A) (Table 1). Occasionally, some genotypes that were resistant when intact plants were inoculated became partially infected when detached leaves were used (*S. microdontum*-178, *S. berthaultii*-11, and *S. sucrense*-23). Although detached leaves of *S. sucrense* appeared exceptionally susceptible compared to intact plants, in general the differences between LGRs were usually proportional to each other. However, the frequency of successful infections on intact plants was very low, especially in the climate chamber (Table 1). Even on susceptible genotypes (e.g., Bintje, Ehud, and *S. microdontum*-265) of which

Table 4. Effect of different incubation treatments (Table 2) on lesion growth rates (LGR, in mm day^{-1}) and infection efficiency (IE, in percentages) of *Phytophthora infestans* isolate 90128 on potato cultivars Bintje and Robijn ($n = 120$ inoculation spots)

	Bintje		Robijn	
	LGR (mm day^{-1})	IE (%)	LGR (mm day^{-1})	IE (%)
Detached leaves in covered trays[D]	3.55	100	2.51	92
Detached leaves in open trays[E]	2.92	95	1.40	70
Intact plants[C]	3.19	93	1.41	52

$LSD_{LGR} = 0.54$ ($P < 0.05$).
$LSD_{IE} = 17$ ($P < 0.05$).
[D]Treatment D, Table 2.
[E]Treatment E, Table 2.
[C]Treatment C, Table 2.

Table 5. Lesion growth rates (LGR, in mm day^{-1}) of *Phytophthora infestans* isolate 90128 on leaves of cultivars Bintje and Robijn detached 1 h and 24 h prior to inoculation ($n = 90$ inoculation spots)

	Bintje	Robijn
Detached 1 h prior to inoculation[D]	3.35	2.25
Detached 24 h prior to inoculation	3.07	1.96

LSD = 0.90 ($P < 0.05$).
[D]Treatment D, Table 2.

Table 6A. Spearman's rank correlations between three resistance experiments with *Solanum* genotypes performed under different experimental conditions. Values represent correlations calculated for lesion growth rates (LGR) and lesion size (LS) at day 6 after inoculation with *Phytophthora infestans* isolate 90128. The number of *Solanum* genotypes is shown between parentheses

Experiment[1]	Parameter	I[D]	II[C]
II[C]	LGR	0.76 (17)*	
	LS	0.90 (17)*	
III[A]	LGR	0.72 (18)*	0.94 (19)*
	LS	0.76 (18)*	0.85 (19)*

*$P < 0.001$.
[1]Resistance data from these experiments are presented in Table 1.
[D]Treatment D, Table 2, consisted of detached leaves from climate chamber-grown plants.
[C]Treatment C, Table 2, consisted of intact, climate chamber-grown plants.
[A]Treatment A, Table 2, consisted of intact plants in the field.

detached leaves were completely infected, the IE on intact plants barely reached 50%.

To test whether different experimental conditions have an effect on the genotype ranking order for resistance, three resistance tests in which *P. infestans* isolate 90128 was used for inoculation (Table 6A), and five tests in which isolate IPO-0 was used (Table 6B), were compared. The experiments with isolate IPO-0 that were performed under the same standard laboratory conditions (treatment D, Table 6B, experiments IV, V, VI, VII) were highly correlated with each other (average correlation coefficient 0.84 for LGR, 0.85 for LS, $P < 0.001$). When resistance data obtained from experiments with isolate 90128 under different growing conditions were compared, similarly high correlations were found (0.94 and 0.85, for LGR and LS respectively, Table 6A). Therefore, no significant differences in resistance ranking could be found between plants grown in the climate chamber compared to those grown in the field.

In experiments with isolate 90128, the correlation coefficients between resistance ratings obtained with different inoculation material (treatment C vs. D) were slightly lower (0.76 for LGR, 0.90 for LS, Table 6A), but still highly significant ($P < 0.001$). In experiments with isolate IPO-0, the correlations (experiment VIII, with IV, V, VI, VII, Table 6B) were clearly lower, i.e., on average 0.57 for LGR and 0.62 for LS. In general, the lower correlations indicate that there might be differences in the level of resistance expression between detached leaves and intact plant inoculation.

Table 6B. Spearman's rank correlations between five resistance experiments with *Solanum* genotypes performed under different experimental conditions. Values represent correlations calculated for lesion growth rates (LGR) and lesion size (LS) at day 6 after inoculation with *Phytophthora infestans* isolate IPO-0. The number of *Solanum* genotypes is shown between parentheses

Experiment	Parameter	IV[D]	V[D]	VI[D]	VII[D]
V[D]	LGR	0.83 (17)***			
	LS	0.88 (17)***			
VI[D]	LGR	0.83 (17)***	0.85 (15)***		
	LS	0.87 (17)***	0.88 (15)***		
VII[D]	LGR	0.87 (17)***	0.78 (15)***	0.87 (18)***	
	LS	0.71 (17)***	0.68 (15)**	0.75 (18)***	
VIII[C]	LGR	0.57 (18)*	0.59 (16)*	0.46 (18) ns	0.67 (18)**
	LS	0.60 (18)**	0.77 (16)***	0.59 (18)**	0.51 (18)**

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.
[D]Treatment D, Table 2, consisted of detached leaves from climate chamber-grown plants.
[C]Treatment C, Table 2, consisted of intact plants, grown in climate chambers.

This effect was also found between field and laboratory tests (treatment A vs. D) performed with isolate 90128, where Spearman's coefficients of rank correlation were 0.72 and 0.76 for LGRs and LS, respectively. Although the rank correlations between field and laboratory tests are still considerable and highly significant ($P < 0.001$), the decrease in correlation coefficients compared to repeated experiments (IV, V, VI, VII, Table 6B) under the same standard conditions indicates that environmental conditions in a laboratory affect the resistance response. Since no effect of growing conditions has been found, these results suggest that the difference between the field situation and laboratory may be caused by inoculation of detached leaves instead of intact plants, confirming the results obtained with Bintje and Robijn.

Discussion

A reliable laboratory test is essential for studying plant-pathogen interactions at the physiological or molecular levels. In this paper, we compared the suitability of a laboratory test for *P. infestans* resistance in *Solanum* species with a field test. Our data indicate that resistance expression is similar for field- and climate chamber-grown *Solanum* plants. Colon et al. (1995a) compared field- and greenhouse-grown potato leaves and found that the latter appeared more resistant. They hypothesized that greenhouse-grown plants might exhibit induced resistance due to heat and drought stress. Leaves derived from climate chamber-grown plants, as described here, did not show differences in resistance expression compared to leaves derived from field-grown plants.

LGRs found on Bintje and Robijn plants that were inoculated and incubated in the field (A) were lower than those found on plants inoculated and incubated in the climate chamber (C) (Table 3). Since the effect of growing conditions was not significant, the lower LGR values in the field probably were due to differences in environmental conditions after inoculation. Although the average outside temperature and humidity during the field experiments were comparable to those in the climate chamber, many fluctuations occurred during the day in the field. In addition, the light conditions in the field and in the climate chamber are different, both qualitatively and quantitatively.

Resistance data for Bintje and Robijn revealed that detached leaves exhibited significantly less resistance than intact plants. In the detached-leaf test, the leaves were incubated at a RH that was usually higher than the RH used with intact plants. By incubating detached leaves in open trays, significant differences in LGR between treatments were no longer observed. This suggests that the environmental conditions in the trays, rather than detachment *per se*, have an effect on the expression of resistance. In addition, LGRs on freshly detached leaves and on leaves detached at 24 h prior to inoculation were compared. If detachment would play a role, it is expected that during incubation in trays, resistance would either be (partly) lost or enhanced, e.g., due to stress. No significant differences in LGR were found between leaves that had been incubating in trays for different time periods prior to inoculation, confirming the hypothesis that detachment *per se* does not affect resistance.

From the previous results, we conclude that the lower expression of resistance in the detached leaf tests is due to differences in environmental conditions. The constant highly favorable environment the pathogen finds in the closed trays apparently enhances infection by the zoospores, as visualized by a high IE. Once the requirements for successful infection are established, hyphae can feed on the plant cells and a high growth rate of the lesions can be measured. The quantitative nature of *P. infestans* resistance in potato is described as the competition between mycelium growth and HR of invaded cells (Umaerus, 1969). In the open air, the physical requirements for HR may be more optimal than in the closed trays. The fact that similar LGR values were found on uncovered detached leaves and intact plants suggests that the use of uncovered detached leaves may be a good alternative for intact plants. Unfortunately, considerably lower IEs were found on uncovered leaves, despite the use of humidifiers. For research in which a high amount of successful infections are favored, e.g., in cytological, molecular biological studies, the detached-leaf test in closed trays can be recommended. However, when the IE is to be used as a parameter for resistance, e.g., in resistance testing of germ plasm, a different methodology may be chosen, e.g., incubation of detached leaves in open trays, or intact plants in climate chamber or field.

Ranking for resistance of twenty plant genotypes gave significant correlations between experiments performed with inoculation of intact plants vs. detached leaves (Tables 6A and B). Although significant, these correlations were lower than those found between replications of identical experiments. This suggests that

the type of inoculated plant material has an effect on resistance expression. Testing a diverse set of *Solanum* species provided a wide range of resistance levels and showed that individual species may respond differently under different experimental conditions. An example is *S. sucrense*, of which detached leaves are much more susceptible than intact plants. In other *Solanum* species, this difference was less pronounced.

Our conclusion that for late blight assessment, laboratory tests are significantly correlated with field tests, is in agreement with conclusions drawn by Hodgson (1962), who found that the relative resistance of eight potato cultivars in a laboratory test on leaf discs correlated with the resistance score in the field. In contrast, Stewart et al. (1983) did not find satisfactory correlations in resistance scores when they compared glasshouse and field tests for resistance to foliar blight. However, in their experiments two completely different tests were compared, and even the inoculation procedures were different. Knutson (1962) found that the relative resistance of the cultivars Pontiac, Sebago and Ostbote was consistent between different tests, but obtained contradictory results with cultivar Cobbler, which appeared susceptible in the field but resistant in the laboratory. It is not clear whether Knutson (1962) used the same isolates in field and laboratory experiments, and hence, the occurrence of race-specific resistance cannot be excluded.

From our studies we conclude that different growing conditions do not significantly affect the resistance levels to *P. infestans*, thus allowing late blight testing on *Solanum* plants grown in climate chambers. Although using detached leaves in resistance tests does not have a significant effect, incubating detached leaves in closed trays appears to decrease resistance expression. The ranking of resistance levels for a set of *Solanum* genotypes with different types and levels of resistance was generally consistent across different types of experiments, but occasionally discrepancies were noted for some resistant wild *Solanum* species. Therefore, a suitable experimental condition has to be chosen depending on the aim of an experiment. When the expression of resistance is to be examined on detached leaves, the reduced level of resistance should be weighed against the low infection frequency inherent to intact plants. Inoculation of intact plants is preferred, but in most cases the inoculation of detached leaves incubated in covered trays appears to be an adequate alternative.

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