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Leaf area dynamics of potato cultivars infected by Phytophthora infestans

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Abstract

The effect of *Phytophthora infestans* on foliage growth and senescence of three potato cultivars was studied in two field experiments. Inoculum or fungicide was applied in different frequencies to establish a range of levels of disease. At weekly intervals leaf numbers were determined as well as vertical canopy profiles of senescent and lesion covered leaf and stem area.

P. infestans reduced appearance of new leaves on the main stem only at the highest level of disease. The cultivars differed more in rate of primary infection of healthy leaves than in the subsequent increase in percentage lesion coverage of the infected leaves. Differences between cultivars in stem lesion coverage resembled the differences for leaf lesions, but in every cultivar stem lesions were most prominent in the top of the canopy, contrary to leaf lesions. *P. infestans* stimulated leaf senescence similarly in the different cultivars.

Additional keywords: late blight, partial resistance, tolerance, senescence, stem lesions.

Introduction

Late blight shortens green leaf area duration (*LAD*) of potato crops, but does not reduce the rate of photosynthesis of green leaves (Haverkort and Bicamumpaka, 1986; Van Oijen, 1990, 1991). Differences between potato cultivars in yield loss thus are only caused by differences in *LAD*. *LAD* is determined by the available leaf area at initiation of the disease, by the capacity of the host to *resist* extension of the pathogen through the foliage, and the capacity to *tolerate* the presence of disease without acceleration of senescence in non-infected leaf tissue. Every cultivar can be characterized by its level of resistance, which can be complete or partial, and its level of tolerance to blight. For breeding purposes it is important to quantify the genetic variation for these characters. However, studies of leaf area dynamics of blighted potato plants have tended to ignore tolerance, and have not taken genotypic differences in available leaf area into account. Leaf appearance, growth and senescence have generally only been studied in disease-free crops, while leaf area loss caused by blight has usually been quantified as rate of increase of percentage diseased foliage, without concurrently quantifying the dynamics of undiseased leaf area (Rotem et al., 1983).

The present study quantifies the effect of partial resistance, tolerance and varietal patterns of foliage growth and senescence on the dynamics of blighted, senescent and

green leaf area in blighted crops of three potato cultivars. The spatial distribution of lesions over different leaf positions and stem internodes is included in the study because the rate of leaf destruction may depend on the position of lesions (Lapwood, 1961; Wenzl, 1967).

Materials and methods

Data were gathered in two field experiments using the cultivars Bintje, Surprise and Pimpernel. Foliage resistance to blight of these cultivars is rated as 3, 7 and 8 respectively (Anonymous, 1988; scaling from 1, very susceptible, to 9, very resistant). Planting dates were April 29, 1987 (Experiment 1) and June 1, 1988 (Experiment 2). Experiment 1 comprised three levels of disease, while Experiment 2 had four. The highest level of disease was established by spraying a suspension of sporangia (100 l ha^{-1}; 20 000 sporangia ml^{-1}) over the plots, on June 23, 1987 and July 27, 1988 ('inoculated'). Disease was absent or low in a treatment where fungicide (maneb/fentin acetate: 34%/11% a.i., 2.25 kg in 400 l water ha^{-1}) was applied weekly until the foliage died ('control'). Intermediate levels of disease were established without the use of inoculum by stopping fungicide application eight or ten weeks after planting ('unsprayed-A' and 'unsprayed-B', the latter only in Experiment 2). Experiment 1 followed a completely randomized design while Experiment 2 had a randomized block design; both experiments had four replicate plots per combination of cultivar and treatment. All plots in Experiment 2 were sprinkler-irrigated on rainless days. Further details of the cultivars, the treatments and the lay-out of the experimental plots, have been reported elsewhere (Van Oijen, 1991).

In each plot, four observation plants were chosen. At various positions along a representative main stem of every observation plant, stem internodes and leaves were tagged. At weekly intervals, after inoculation, the percentages lesion coverage and senescence of each tagged stem internode and leaf were visually estimated. In Experiment 1 these weekly measurements were repeated four times, at three positions along the stem, in the bottom, middle and top of the canopy. In Experiment 2 every third internode and leaf was studied weekly until the leaf had no green area left. With the growth of the plants, newly appeared leaves were tagged and included in the measurements. In both experiments, the number of leaves on the chosen main stems, with distal leaflets longer than 5 cm, was also determined weekly.

For each separate leaf studied in Experiment 2, the data of increasing lesion coverage and senescence were fitted by non-linear regression analysis to logistic functions of time:

$$l = 100 / (1 + \exp(-r_l(t - t50_l)))$$
$$s = 100 / (1 + \exp(-r_s(t - t50_s))) \quad (1)$$

where l is the percentage lesion covered leaf area (%), s is the senesced percentage of the non-lesion covered area of the leaf (%), t is time after inoculation (d), r_l and r_s are the logistic rates of increase of lesion coverage and senescence (d^{-1}), $t50_l$ and $t50_s$ are the inflection points of the curves (d), i.e. the number of days after inoculation when l or s is 50%. The estimates of the parameters r and $t50$ were subjected to a multiple linear regression analysis (Snedecor and Cochran, 1980), with genotype, level

of disease and leaf position as independent variables. Analysis of variance was used to examine cultivar effects on stem lesion coverage, and to examine cultivar and treatment effects on leaf number.

Results

Lesion occurrence. In Experiment 1, all leaves of inoculated 'Bintje' plants showed lesions by the first day of measurement, i.e. seven days after inoculation, whereas fewer leaves with lesions were found on 'Pimpernel' (90%) and 'Surprise' (70%). Leaves of control plants of all cultivars were free of symptoms, whereas on unsprayed plants disease was found on leaves of 'Bintje' (11%) and 'Pimpernel' (2%). At the last assessment date, the percentages for the unsprayed treatment had only slightly increased, while control plants still had no infected leaves.
The first day at which the diseased area of a leaf was one per cent or more, was considered to be its time of primary infection. Primary infection was retarded in the control plants in Experiment 2. However, in spite of the continuous fungicide application most leaves did not escape infection (Fig. 1A). Control plants thus became infected in Experiment 2 alone, probably because of the greater availability of natural inoculum, due to the late planting date, and better infection conditions, as a result of the occasional sprinkler-irrigations. In the unsprayed treatments of Experiment 2 the percentage diseased leaves increased significantly above that in the control plants within two weeks after fungicide application was stopped (Fig. 1A). The differences between cultivars were large (Fig. 1B). More than half the leaves of 'Bintje' already showed disease five days after inoculation, while similar levels of disease were reached by 'Pimpernel' and 'Surprise' only six and seven weeks later, respectively. These cultivar differences partly arose from premature natural infection in this experiment. Two days before the artificial inoculation, the percentage diseased foliage area in 'Bintje', 'Surprise' and 'Pimpernel' was 2.6%, 0.2% and 0.2%, respectively. Many old leaves, at the third and sixth stem node, counted from the bottom, escaped disease because of early, natural senescence (Fig. 1C). However, if we correct for natural leaf senescence by considering the number of diseased leaves relative to the final number becoming diseased at the same leaf position (Fig. 1D), we see that old leaves showed disease symptoms earlier than young leaves.

Lesion coverage. In Experiment 1 the average percentage of the area of leaves covered with lesions in inoculated plants was highest in cv. Bintje (Fig. 2A), followed by 'Pimpernel'. Leaf lesion coverage was most prominent in the old leaves low in the canopy whereas stem lesions were predominantly found in the higher stem internodes (compare Figs 2A and B). Logistic regression analysis was applied to the time courses of lesion coverage in each of the 820 leaves studied in Experiment 2. Of these leaves, 143 died without disease symptoms and two could not be fitted satisfactorily with the logistic curve (r^2, the proportion variation accounted for, being less than 0.5). For the remaining 675 leaves r^2 averaged 0.99.

r_l did not vary strongly between cultivars, treatments and leaf positions (Tables 1 and 2; Fig. 3A). Only 21% of the variation in r_l was explained by differences between these variables (Table 2). $t50_l$, on the other hand, showed more variation, of which 78% was accounted for by differences between cultivars, treatments and leaf posi-

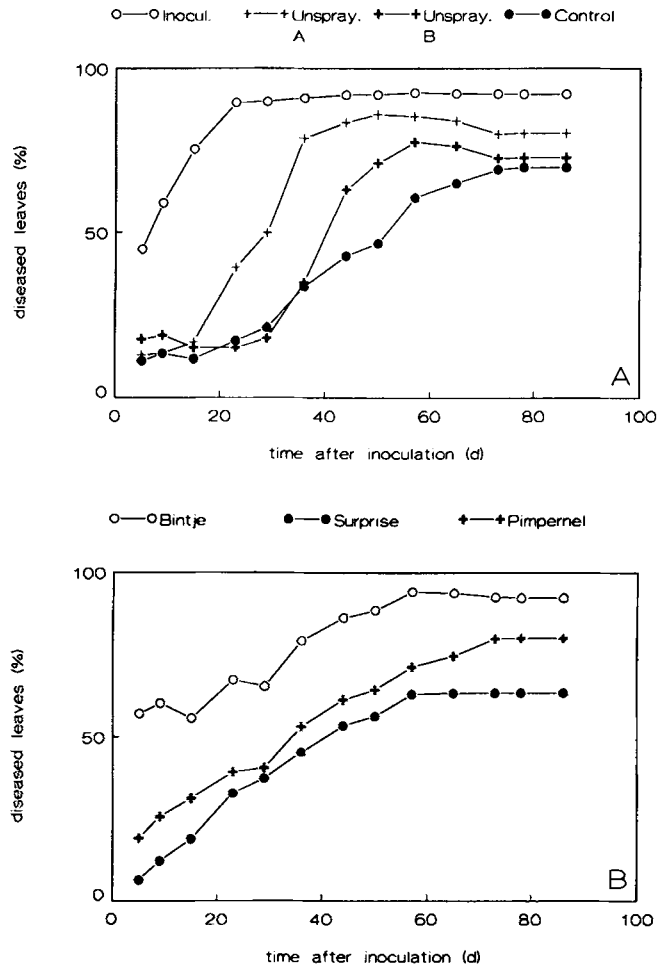

Fig. 1. Time course of the number of diseased leaves (leaves with lesions of *P. infestans*, in per cent of the total leaf number) in Experiment 2. Points refer to estimates taken from a multiple regression analysis on treatment, cultivar and leaf position. A: the effect of treatments; B: the effect of cultivars; C: the effect of leaf position, counted from the bottom; D: as C, but corrected for natural leaf senescence.

tions, and their interactions (Table 2). $t50_l$ was lowest in 'Bintje', while 'Surprise' and 'Pimpernel' did not differ significantly from each other (Table 1). As expected, $t50_l$ increased with longer periods of fungicide application (Table 1). Leaf position affected $t50_l$ in that young leaves reached 50% lesion coverage later than old leaves (Fig. 3A). Late cultivars, like 'Pimpernel', thus have an advantage over early cultivars, in that they longer continue to form new leaves with large $t50_l$ (Fig. 4). Only at very high levels of disease, as in the inoculated treatment, appearance of new leaves may be significantly reduced (Fig. 4; Experiment 1: Van Oijen, 1990).

Leaf senescence. Logistic curves of increasing percentage senescence were fitted

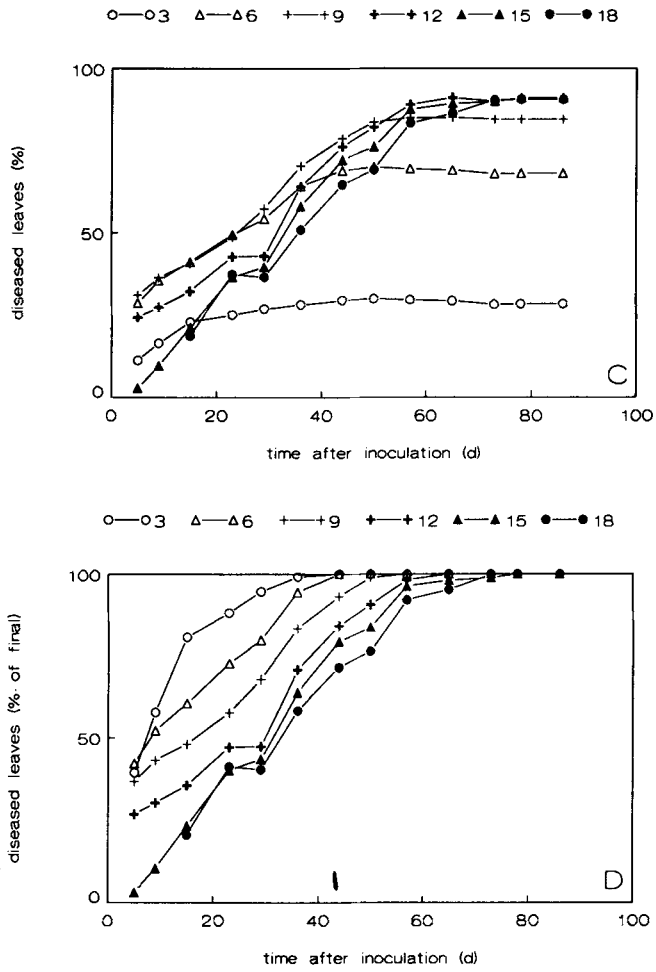

Fig. 1. (Continued).

satisfactorily for 466 leaves (r^2 averaged 0.96). r_s, like r_l, was relatively constant over cultivars, treatments and leaf positions (Table 2). Effects on $t50_s$ strongly parallelled those on $t50_l$ (Table 1, Fig. 3), and the two parameters were closely correlated: $t50_s = 0.93 \times t50_l + 1.75$ ($r^2 = 0.95$, $n = 338$). The disease thus accelerated leaf senescence to the same extent in all cultivars.

Discussion

Effects of resistance, tolerance and foliage size on loss of green leaf area. *P. infestans* causes yield reduction in potato by reducing *LAD* (Haverkort and Bicamumpaka, 1986; Van Oijen, 1991). In the present experiments, *LAD* was reduced mainly by the coverage of leaves by lesions. Leaf lesion coverage started at the bottom leaf layers and gradually spread to the top of the canopy (Fig. 3A). The process was described very

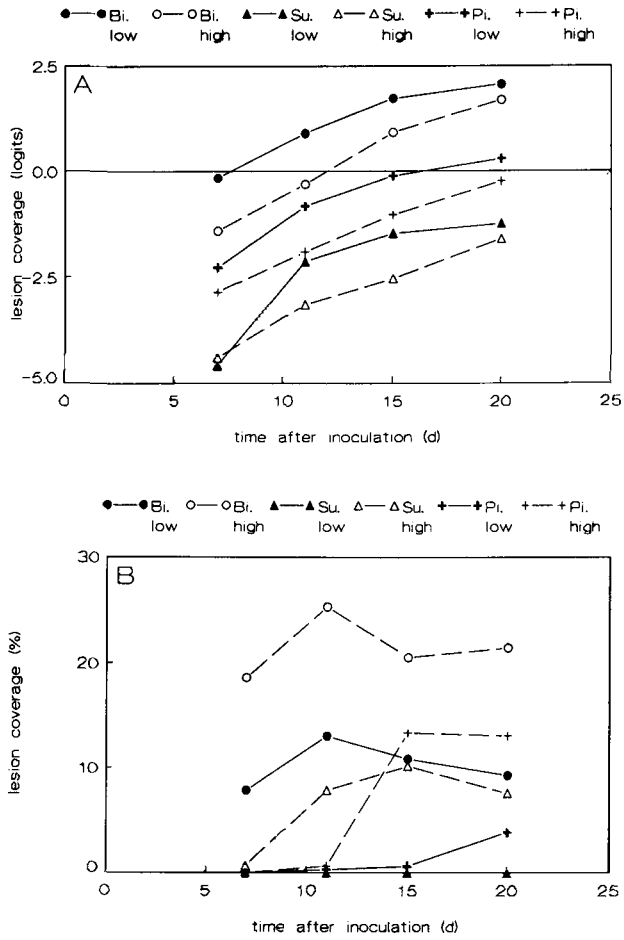

Fig. 2. Percentage lesion coverage in leaves and stems of inoculated plants of cultivars Bintje (Bi), Surprise (Su) and Pimpernel (Pi) in Experiment 1, at two levels (high and low) in the canopy. A: leaves (percentages transformed to logits: ln(percentage/[100-percentage])); B: stem internodes.

well, for individual leaves, by logistic curves, characterized by r_l and $t50_l$. In spite of the widely different resistance ratings of the cultivars, r_l hardly depended on cultivar and level of disease (Table 2). $t50_l$ was the main parameter explaining genotypic differences, and corresponded well with cultivar resistance ratings. Therefore $t50_l$ might be useful as selection criterion in resistance breeding programmes. *LAD* was also reduced because *P. infestans* accelerated leaf senescence, but no genotypic differences in tolerance were detected: $t50_s$ approximately equalled $t50_l$ for all cultivars irrespective of treatment and leaf position. Fortunately such tolerance may not be needed, since only a small proportion of the yield loss was accounted for by the observed acceleration of senescence (9% and 12% in Exps. 1 and 2, respectively; Van Oijen, in prep.). A third process reducing *LAD* was the reduction of new leaf area

Table 1. Experiment 2. Inflection time ($t50$) and logistic rate (r) of increasing lesion coverage (l) and senescence (s) of leaves of three cultivars exposed to four treatments; data are averages over leaf positions. Standard errors of inflection time and logistic rate were less than 2.0 d and 0.05 d^{-1}, respectively, unless otherwise indicated.

Cultivar	Treatment	$t50_l$ (d)	r_l (d^{-1})	$t50_s$ (d)	r_s (d^{-1})
Bintje	inoculated	12.5	0.70	17.2 [1]	1.08
	unsprayed-A	27.7	0.73	29.2	0.94
	unsprayed-B	31.9	0.63	36.9 [2]	0.90
	control	39.9	0.84	39.0	0.95
Surprise	inoculated	26.5	0.86	24.5	1.02
	unsprayed-A	40.0	0.91	36.4	0.88
	unsprayed-B	46.2	0.87	42.4	0.84
	control	47.5	0.87	44.3	0.89
Pimpernel	inoculated	24.3	0.75	23.4 [3]	0.97
	unsprayed-A	36.4	0.89	35.1	0.82
	unsprayed-B	46.3	0.80	40.1	0.79
	control	56.2	0.81	51.7	0.84

[1] Standard error is 4.3 d.
[2] Standard error is 2.1 d.
[3] Standard error is 2.3 d.

Table 2. Experiment 2. Percentage of variation in parameters of increasing lesion coverage and senescence, accounted for by adding terms in a multiple regression analysis, and level of significance (P) [1] of each addition. Dependent variables: inflection time ($t50$) and logistic rate (r) of leaf lesion extension (l) and leaf senescence (s). Independent variables: block effects and main and interaction effects of cultivar, treatment and leaf position.

Added term	$t50_l$ [2]		r_l [2]		$t50_s$ [3]		r_s [3]	
	%	P	%	P	%	P	%	P
Block	1.0	**	0.1	n.s.	0.6	*	0.7	n.s.
Cultivar (C)	13.2	**	5.4	**	2.4	**	2.2	**
Treatment (T)	40.7	**	2.3	**	25.9	**	5.0	**
Leaf position (P)	15.4	**	1.5	*	37.6	**	6.2	**
C * T	2.0	**	2.3	**	2.2	**	0.7	n.s.
C * P	0.4	n.s.	3.3	**	1.3	*	1.1	n.s.
T * P	4.2	**	2.8	n.s.	3.9	**	1.2	n.s.
C * T * P	1.4	n.s.	3.3	n.s.	1.4	n.s.	3.4	n.s.
Total	78.3		20.9		75.2		20.5	

[1] ** = $P < 0.01$, * = $P < 0.05$, n.s. = not significant.
[2] $n = 675$
[3] $n = 466$

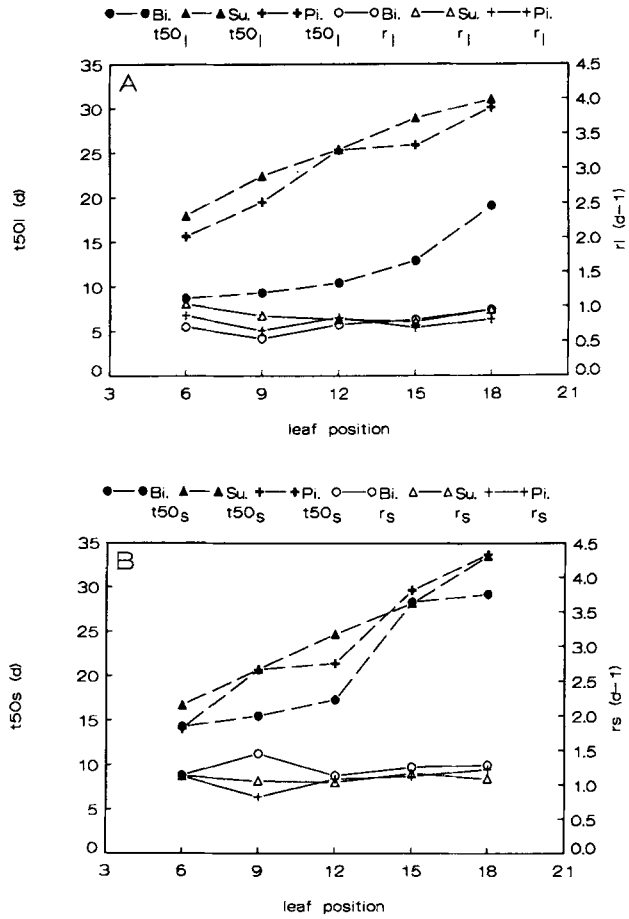

Fig. 3. Parameters of increasing leaf lesion coverage and leaf senescence as functions of leaf position, of cultivars Bintje (Bi), Surprise (Su) and Pimpernel (Pi) in the inoculated treatment of Experiment 2. Results of logistic curve fitting, characterized by the logistic rate parameter r and the inflection point $t50$. A: lesion coverage; B: senescence.

formation during the epidemic. Appearance of new leaves on the main stem was hampered at high levels of disease (Fig. 4). This reduction of leaf area due to reduced leaf appearance and growth is probably unimportant compared to the effect of lesion coverage, as *late* blight generally appears at a late stage of crop development when further leaf area expansion is restricted anyway. However, in potato-growing regions with high natural levels of initial inoculum, such as central Africa and Mexico, the disease may appear earlier (L.J. Turkensteen, pers. comm., 1990). Apart from differences between cultivars in the rate of reduction of green leaf area, the amount of host leaf area at disease initiation should be considered. Late cultivars, as Pimpernel, form more leaves than early cultivars (Fig. 4; Taylor, 1953). Therefore late cultivars can longer maintain green leaf area in the top of the canopy, while the disease spreads from the lower leaf layers upward.

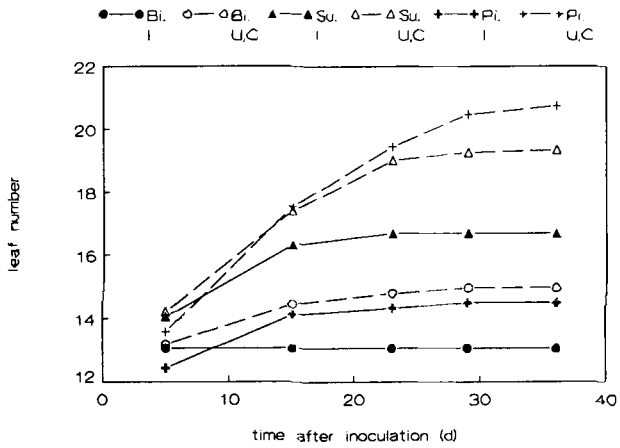

Fig. 4. Time course of average total number of leaves per main stem in inoculated (I) and unsprayed or control (U, C) plants of cultivars Bintje (Bi), Surprise (Su) and Pimpernel (Pi) in Experiment 2. Points for inoculated and non-inoculated plants refer to averages over 16 and 48 plants, respectively.

The role of stem lesions. Primary infection of fully developed leaves did not often originate from lesions on sustaining stem internodes, since the disease was most prominent in old leaves, low in the canopy, where stem lesions were almost absent (Fig. 2). The prominence of stem lesions in the plant tops may have been caused by the artificial inoculation, sprayed drops of inoculum being intercepted mainly in the axils of leaves high in the canopy. Leaf infection, on the other hand, may be more dependent on the higher humidity around leaves at lower positions. This is consistent with the finding that old leaves showed disease earlier than young leaves, while the subsequent extension of lesion coverage occurred at similar rates, lesion growth being less affected by the microclimate than infection efficiency.

The role of epidemiological components of resistance. In both experiments, differences between cultivars in lesion coverage became apparent early after inoculation, but did not increase much afterwards (Figs 2A and 3A). Since in the early stages of the epidemics all disease originated from the first infection cycle after inoculation, the large but unchanging differences between the cultivars can only be explained by differences in infection efficiency and early lesion growth. Differences in latent period or sporulation may have contributed only very little. The method of inoculation used in the present study (commonly used in resistance breeding trials) provides a high and uniform level of initial inoculum, and may therefore be inadequate for cultivars of which the level of partial resistance depends mostly on a long latent period or a low rate of sporulation. Because of the low percentage of variation in r_1 accounted for by differences between cultivars, treatments and leaf positions (Table 2), differences between cultivars in growth of individual lesions may be more important, in selection procedures, than the total increase in lesion covered area within leaves.

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