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Epidemic fitness of *Phytophthora infestans* in foliage and tubers during growing season and harvest

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Summary
This papers describes laboratory and field experiments studying competition between *P. infestans* isolates A, B and C during an epidemic in three potato cultivars. The aim was to determine whether a trade off exists between aggressiveness towards foliage and towards tubers. Three *P. infestans* genotypes were inoculated (solo and as a mixture) on three potato cultivars during a replicated field experiment. Each cultivar supported the *P. infestans* isolates that were best adapted to the specific cultivar. The *P. infestans* isolates differed in their capability to establish following inoculation, in their competitive ability during the foliar epidemic and in the efficiency of transition to the tuber. In general, the B isolate was best in establishment and the A isolate was best during the foliar epidemic and the transition to tubers. None of the isolates was best at all three capabilities indicating 'room for improvement' and a future direction for adaptation within *P. infestans* populations.
A trade off between aggressiveness towards foliage and tubers was not found.

Keywords
Potato late blight, potato, aggressiveness, fitness.

Introduction

Following the re-introduction of *Phytophthora infestans* in the Netherlands around 1976, genetic variability increased and the sexual cycle was shown to be functional. An increase of aggressiveness and highly complex virulence spectra (Flier *et al.*, 2003; Flier & Turkensteen, 1999) were two of the consequences that caused drastic changes to the daily practice of potato late blight control.
In a genetically heterogeneous population, the various *P. infestans* genotypes are engaged in a struggle for survival through competition for a 'limited' amount of substrate (potato foliage and tubers). Successful genotypes will dominate both, the foliar and tuber population and survive the winter to be an important part of next years (early) population.
Dominance of the foliar population can be achieved through a superior level of fitness as compared to competing genotypes. Dominance in the tuber population can be achieved through an efficient transition to the tuber (production of high numbers of sporangia, washing down of sporangia by rain, survival in the soil and efficient infection of tubers). In both cases high levels of foliar aggressiveness (high infection efficiency (IE), lesion growth rate (LGR), sporulation intensity (SI) and a short latent

period (LP)) are beneficial for a specific genotype.
Once in the tuber however high levels of aggressiveness may not be beneficial for survival to the next season. Highly aggressive genotypes will quickly destroy the tuber and with it their survival capsule to the next growing season. There may thus be a trade off between foliar aggressiveness and aggressiveness in the tubers. In support of this theory, bio-assays aimed at quantifying *P. infestans* aggressiveness towards foliage and tubers may display differences in the level of aggressiveness between both tissue types. In support of the alternative hypothesis (the aforementioned trade off does not exist) are the modern, low temperature, storage regimes and the idea that heavily infected tubers during and shortly after harvest may contaminate much more healthy tubers on their way to the storage facilities leading to high levels of latently infected tubers.
Competition between *P. infestans* genotypes was investigated earlier in conjunction with fungicide resistance (Cohen & Samoucha, 1990; Kadish & Cohen, 1988) and host specificity (Lebreton & Andrivon, 1999). This papers describes laboratory and field experiments aimed at testing the hypothesis that a trade off exists between aggressiveness towards foliage and tubers.

Materials & Methods

Potato cultivars and P. infestans isolates
During the first stage of this project, isolate – cultivar combinations were selected displaying differential compatibility. An overview of the cultivars and isolates selected and their characteristics is given in Table 1 and Table 2. Isolates NL01900, NL01096 and NL00228 will be further referred to as isolates A, B and C respectively.

Table 1. Potato cultivars and their characteristics concerning potato late blight resistance as used in the laboratory and field experiments.

Cultivar	Earliness	Foliar resistance	Tuber resistance	Purpose
Karakter	4	6	5	Starch
Mondial	4.5	4.5	5.5	Ware
Remarka	5	6.5	9	Ware

Table 2. *P. infestans* isolates and their characteristics as used in the laboratory and field experiments.

Isolate	Location of origin	Host of origin	Year of origin	Haplo type[1]	Mating type
NL01900 (A)	Wageningen (NL)	*S. sisymbriifolium*	2001	Ia	A1
NL01096 (B)	Katshaar (NL)	*S. tuberosum*	2001	IIa	A2
NL00228 (C)	Dinteloord (NL)	*S. tuberosum*	2000	Ib	A2

Aggressiveness to foliage and tubers
Aggressiveness of the selected isolates towards the foliage of the selected cultivars was determined in laboratory bio-assays by quantifying compatibility parameters 'infection efficiency' (IE), 'latent period'(LP), 'radial lesion growth rate' (LGR) and 'sporulation intensity' (SI).
Aggressiveness towards tubers was determined in bio-assays by quantifying the infection efficiency on tuber eyes.

Field experiments
A field experiment was carried out in 2005 and 2006. Each field experiment contained 36 potato plots measuring 10x10.5m organized in four 'isolate blocks'. Each isolate block contained all three cultivars in three replicates. Isolate blocks were separated by bare soil and maize. Each isolate block was inoculated with one of the *P. infestans* isolates or a mixture of all three isolates.
P. infestans was inoculated by spraying nine single plants per plot with the appropriate sporangial

suspension. Potato late blight severity was assessed twice a week in the following weeks.
The composition of the *P. infestans* population in each plot was determined at three points in time during the epidemic: in the foliage at the start of the epidemic following inoculation, in the foliage just before desiccation and in the tubers following harvest. At each of these sampling times, leaf or tuber samples were taken, *P. infestans* was re-isolated and the haplo type was determined to be able to identify the *P. infestans* isolate. All three isolates used in the experiments could be distinguished based on the mitochondrial haplo type (Griffith & Shaw, 1998, Table 2).

Results

Aggressiveness to foliage and tubers in bio-assays
Based on the results of bio-assays quantifying IE, LP, LGR and SI, two composite parameters were calculated as estimates for R_0, the foliar net life time reproduction, and r, the foliar apparent infection rate according to Skelsey *et al.* (2005). Results are given in Table 3. The higher R_0 and r, the more aggressive, or fit, the isolate on this cultivar.

Table 3. Calculated estimates for foliar aggressiveness; the net life time reproduction (number of daughter lesions per mother lesion) "R_0" and the apparent infection rate "r" for a total of nine cultivar – isolate combinations.

Parameter / Isolate	Karakter	Remarka	Mondial
R_0 [-]			
Isolate A	1158	133	1543
Isolate B	231	167	920
Isolate C	63	0	635
r [day^{-1}]			
Isolate A	0.75	0.39	0.78
Isolate B	0.55	0.45	0.68
Isolate C	0.27	0	0.71

Results on infection efficiency on tuber eyes are given in Table 4. In this experiment, a cultivar effect was the only statistically significant factor. Mondial clearly is the most susceptible cultivar after inoculation of tuber eyes. Remarka and Karakter are equally resistant to infection by the isolates included in this study.

Table 4. Cultivar effect on tuber infection after inoculation of tuber eyes with a sporangial suspension. Means followed by the same letter are not statistically different according to an LSD test at P = 0.05.

	Karakter (5)	Mondial (5.5)	Remarka (9)
Tuber infection (%)	2.5 a	26.5 b	2.2 a

Field experiments
In accordance with the laboratory results, analysis of the levels of tuber infection (incidence) in the field experiments resulted in a significant cultivar effect. The average level of tuber infection was found to be 23.8% for Karakter, 14.1% for Mondial and 4.9% for Remarka ($LSD_{0.05}$ = 9.2%). In contrast to the laboratory results however, Karakter tubers are the most susceptible in the field followed by Mondial and Remarka. This discrepancy possibly indicates additional infection sites, such as lenti cells, apart from the tuber eyes which were specifically targeted in the bio-assay.

P. infestans population dynamics
Analysis of *P. infestans* population dynamics focuses on the cultivar Mondial. The time course of the

epidemics on Mondial in both years and for all inocula is given in Figure 1. Differences between the inocula exist within each year but are not consequent between years. Overall, the course of the epidemic is more or less the same for all inocula. In 2006 however the epidemic is delayed by approximately 1 – 2 weeks as compared to 2005. This is due to a warm and dry period in July 2006 which stopped epidemic development for 1 – 2 weeks but did not succeed in killing *P. infestans*.

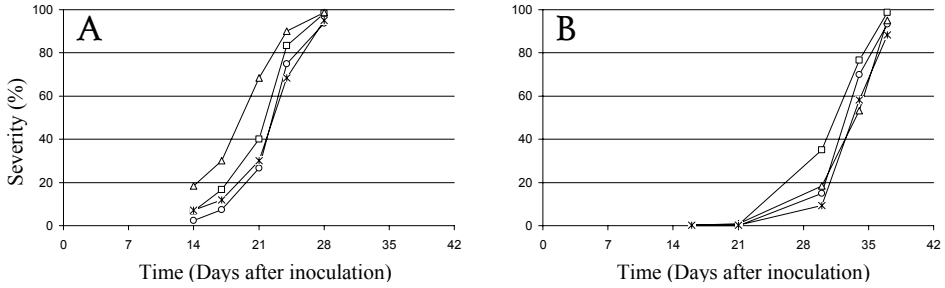

Figure 1. Time course of foliar epidemics in 2005 (A) and 2006 (B) on Mondial for each of the four inocula: A isolate (○), B isolate (∗), C isolate (Δ) en Mixed inoculum (□). The first and second leaf sampling took place on day 7 and day 24 in 2005 en on day 7 en day 34 in 2006.

The result of the competition between *P. infestans* isolates on potato cultivar Mondial are given in Figure 2. The top three rows in this Figure represent the results after inoculation with one of three *P. infestans* genotypes. The fourth and last row of two graphs represents the results after inoculation with a mixture of all three *P. infestans* genotypes. The population composition is given for each of the three sampling times. In general, the genotype used for inoculation dominates the population after inoculation with 1 genotype. Due to incoming inoculum from other isolate blocks, the A isolate is however always able to establish itself and become a significant part of the population during the foliar epidemic, also in plots where it was not inoculated. Although the reverse is true for the B and C isolate, their contribution to the population remains marginal when not inoculated.

When the plots are inoculated with a mixture of all three genotypes, all three establish and contribute significantly to the total population. During the course of the foliar epidemic however, the A isolate becomes more dominant at the expense of mostly the C isolate.

During the transition from foliage to tuber, the A isolate gains an even more dominant position in the tuber population than was to be expected based on its contribution to the foliar population.

On both other cultivars, Karakter and Remarka, the C isolate does not establish and can be considered non-compatible. The A isolate behaves in a similar way as described above for Mondial. On Remarka and Karakter however, the B isolate is better in establishment following inoculation than the A isolate.

Overall we can thus say that potato cultivars support those *P. infestans* genotypes that are best adapted to a cultivar. Furthermore, the B isolate seems to be better in establishment than the A and C isolate, at least on Karakter and Remarka. The A isolate is a better competitor during the subsequent foliar epidemic and is more efficient than the other two *P. infestans* genotypes during the transition to tubers.

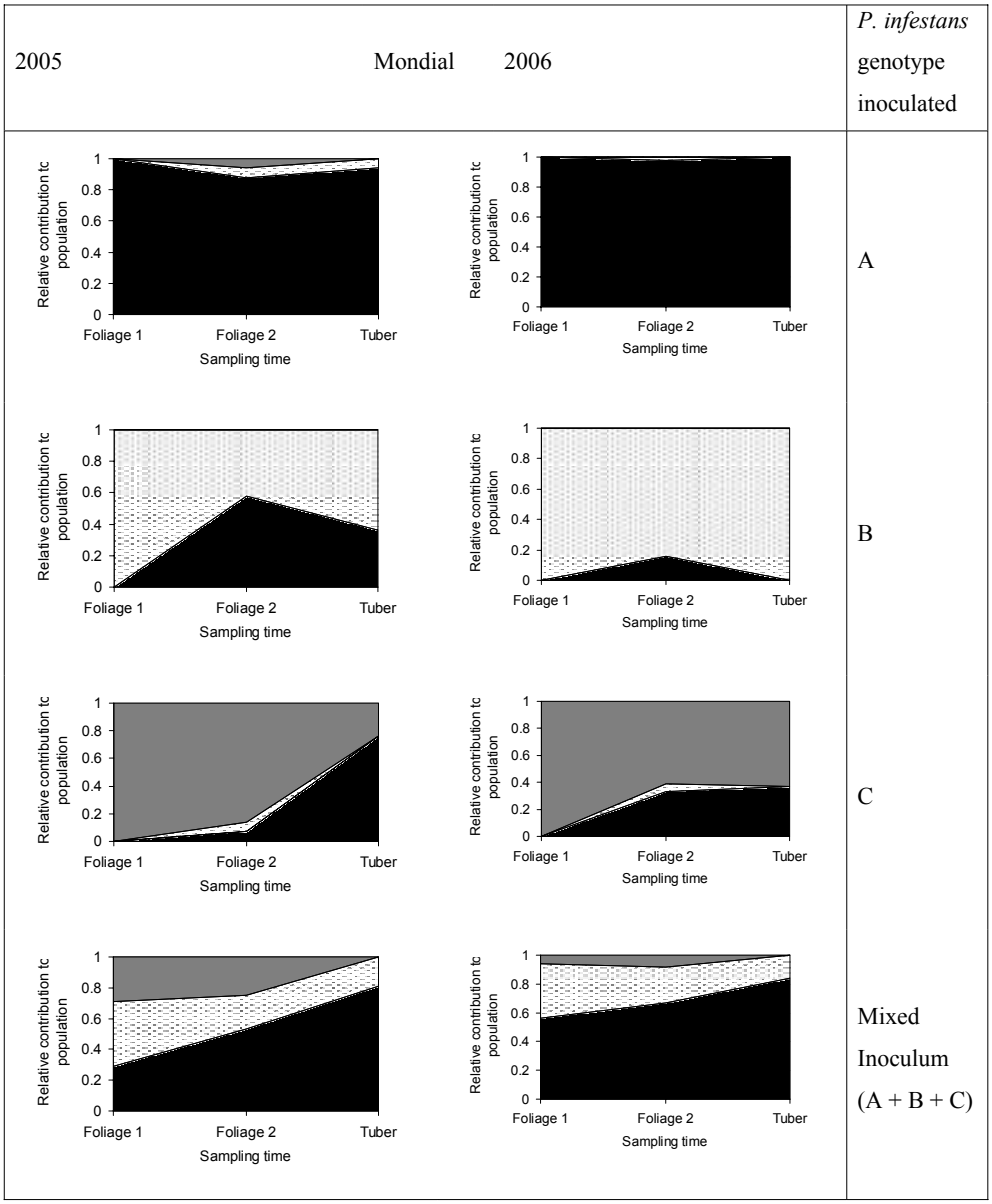

Figure 2. Relative contribution of P. infestans genotypes following solo inoculation and following inoculation with a mixture of the three genotypes. ■ : P. infestans A genotype; ▒ : P. infestans C genotype; ▨ : P. infestans B genotype.