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New Phytophthora populations: A shift from indirect to direct sporangial germination?

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INTRODUCTION

Phytophthora infestans, the causal agent of potato- and tomato late blight, remains a serious threat for (commercial) potato and tomato production. In North Western Europe, frequent fungicide applications, mostly aimed to prevent infection, form the back bone of potato late blight control.
Modern protectants such as Shirlan (a.i. fluazinam) are highly effective against (germinating) *P. infestans* sporangia and zoospores. Zoospores in particular are so sensitive to low concentrations that the many applications over the past two decades may well have exerted sufficient selection to pressure against the formation of zoospores. Thus, over the years the balance between direct and indirect germination may have shifted towards direct germination.
This hypothesis was investigated at Bayer Crop Science and Plant Research International.

MATERIALS AND METHODS

Plant Research International (PRI)

At PRI, 15 *P. infestans* isolates originating from before 1990 and 20 isolates from after 1990 (including 15 from 2000 or later) were selected to test the hypothesis that zoosporulation is more abundant in isolates from before 1990. In the period before 1990, Shirlan and other fungicides highly active against zoospores were not available whereas the more recent isolates have a history of exposure to fungicides highly active against zoospores of 10 years or more.
P. infestans isolates were grown on potato leaves, cv Bintje and after 7 days incubation at 15°C, sporangial suspensions were obtained by rinsing sporulating leaves in tap water. The sporangial concentration was adjusted to $5 \times 10^4 - 1 \times 10^5$ sporangia/ml.
For the indirect germination assay, 1ml of potato extract was added to 10 ml of sporangial suspension. This suspension was incubated at 10°C for 3 hours to allow for zoospore release. 100µl of this suspension was then examined microscopically for the fraction of empty sporangia (out of 100 sporangia) and the zoospore density in the field of view on a 1 – 5 scale: 1 = no zoospores observed; 2 = a few zoospores are present, … 5 = the field of view is swarming with zoospores.

Germination was defined as a sporangium with a germ tube larger than half the diameter of the sporangium or zoospore.

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Bayer Cropscience (BCS)

To evaluate the potential for direct or indirect germination of different *Phytophthora infestans* samples as related to the country and year of origin and mating type, a methodology was developed at BCS laboratory to determine in parallel for the same strain both the capacity for direct and indirect germination.

60 *P. infestans* isolates were tested for their capacity of releasing zoospores in water and for their direct germination capacity in potato dextrose broth (PDB). *P. infestans* strains were grown on potato leaflets and after 6 days, sporangia were washed off either in PDB or in demineralized water. Sporangial concentrations were adjusted to 4 x 10^4 sporangia/ml and four 20µl droplets of each suspension were deposited on silanized microscope slide (fig.1.).

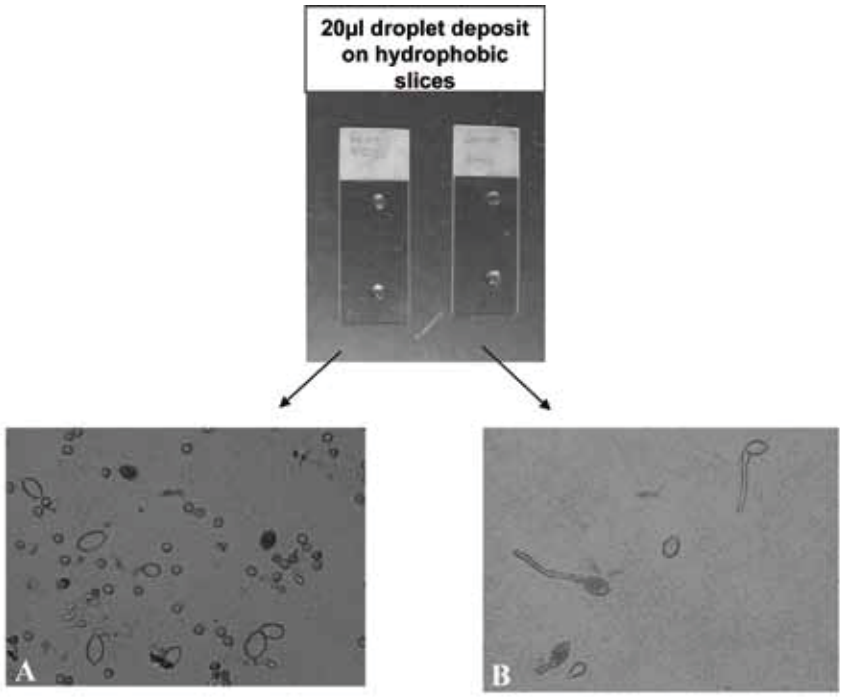

Figure 1. In vitro germination of sporangia A: % of indirect germination in water B: % direct germination in PDB

These slides were then incubated at 16-18°C for 18 hours. Germination was assessed microscopically as described above. The % of empty sporangia and germinated sporangia are used as indicators for the potential for indirect and direct germination respectively.

RESULTS

PRI

Zoosporulation was quantified by the percentage empty sporangia and the zoospore density in the microscopes field of view on a 1-5 scale. Results are given in Figure 2. Both parameters do not

support the hypothesis that the ability for zoospore formation has decreased since 1990.

Bayer Cropscience

In addition to the 59 *P. infestans* strains tested for their capacity for zoospore release in water and capacity for direct germination in PDB, reference strains were included in each experiment. Repeated results on the reference strains were excluded from the statistical analysis to avoid a bias in

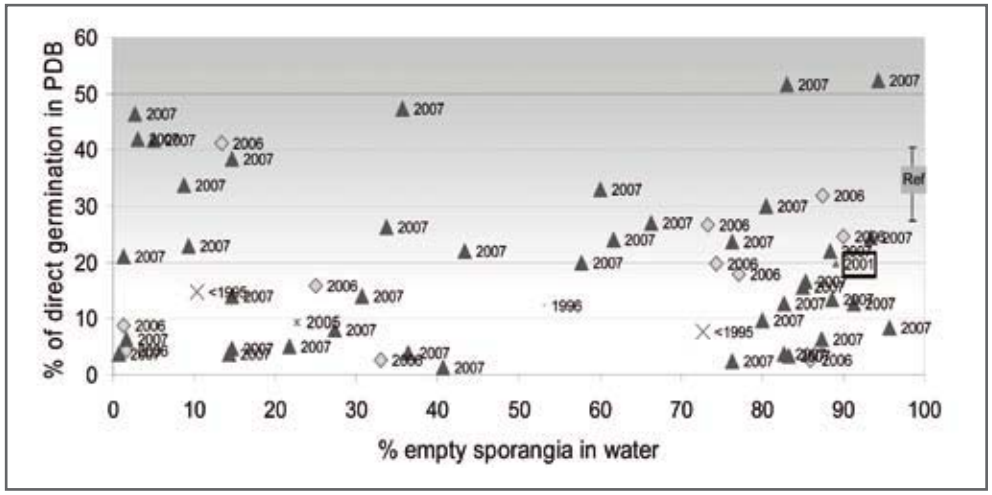

Figure 2. Direct versus indirect germination for individual isolates as influenced by the year of origin of the isolate.

the results caused by the reference isolates.

Results were statistically analysed for the effects of year of origin, country of origin and mating type. As the values studied are percentages, they do not follow a normal distribution and a the non parametric Kolmogovov-Smirnov tests (two samples test) was used to statistically analyze the results.

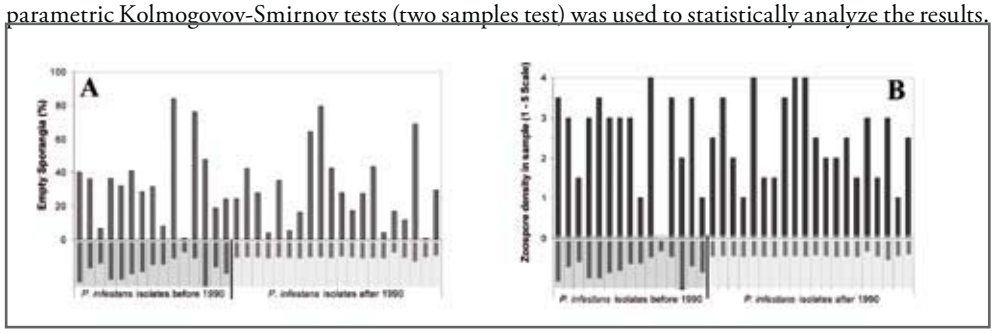

Figure 3. Indirect germination of P. infestans sporangia as influenced by the period of origin of the isolate, before and after 1990. A: the percentage of empty sporangia, as a measure for zoospore release. B: the zoospore density in the microscopes field of view on a 1-5 scale, 1 = no zoospores in field of view, 5 = field of view is swarming with zoospores.

Influence of the year of origin

Isolates were assigned to 3 classes according to their year of origin:
A: isolates collected before 2001 (including references)
B: isolates collected in 2005 and 2006 (only 1 strain from 2005)
C: isolates collected in 2007

Figure 3 illustrates the results by plotting direct versus indirect germination for each isolate in a scatter graph. In general, direct germination does not exceed 50% whereas indirect germination varies between almost 0% and 100%. All possible combinations between high and low levels for direct and indirect germination occur.
The results of the statistical analysis are given as two box plots (fig 4a and 4b) indicating that the

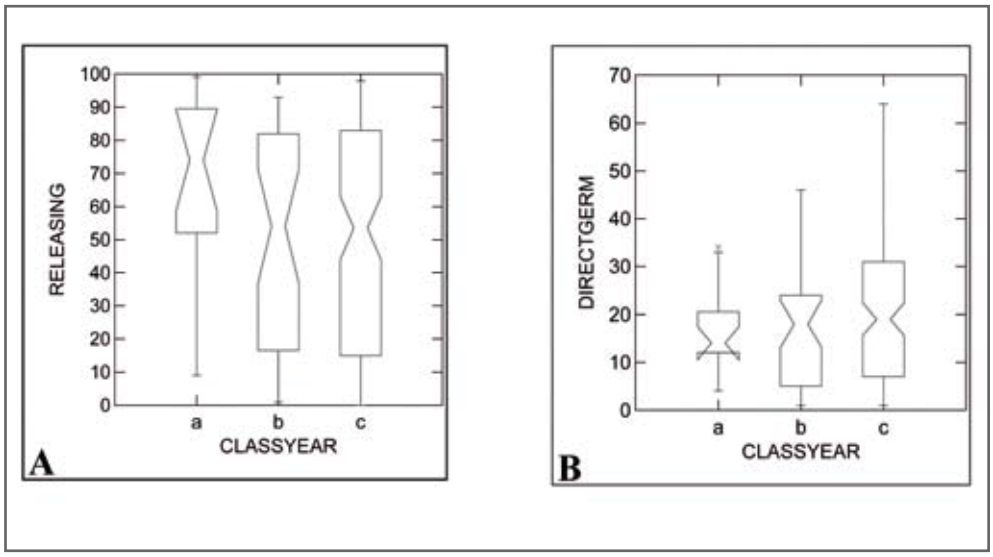

Figure 4. Results from the Kolmogorov-Smirnov two samples test demonstrate that the percentage of indirect germination of sporangia in water (A) and the percentage of direct germination of sporangia in PDB (B) is not significantly influenced by the year of origin of the isolate

year of origin does not significantly influence the isolates capacity for direct or indirect germination.

Influence of the country of origin

Although some countries were much more represented than others, figure 5, the strains seem to distribute at random according to the two axes. Unfortunately, a statistical analysis could not be carried out due to the unbalanced composition of the isolate collection with respect to the country of origin.

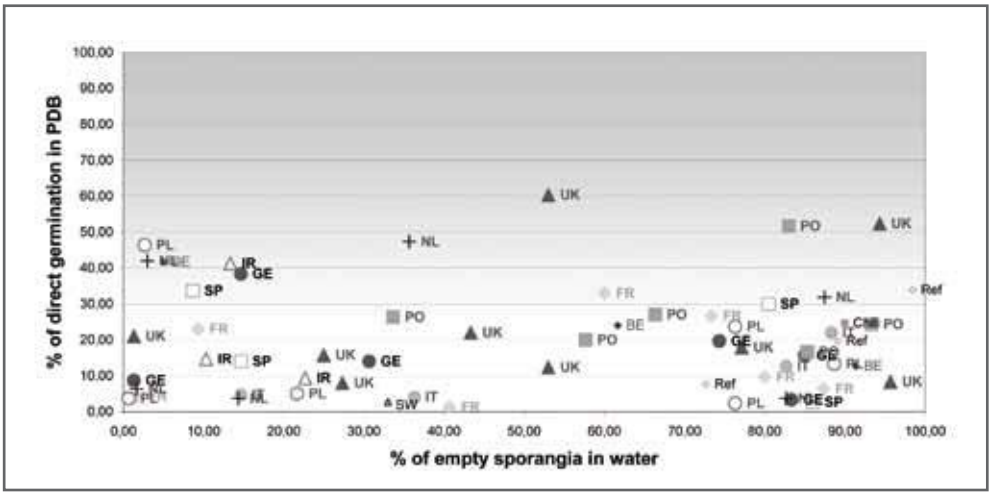

Figure 5. Distribution of European Phytophthora infestans isolates according their ability to germinate directly and indirectly.

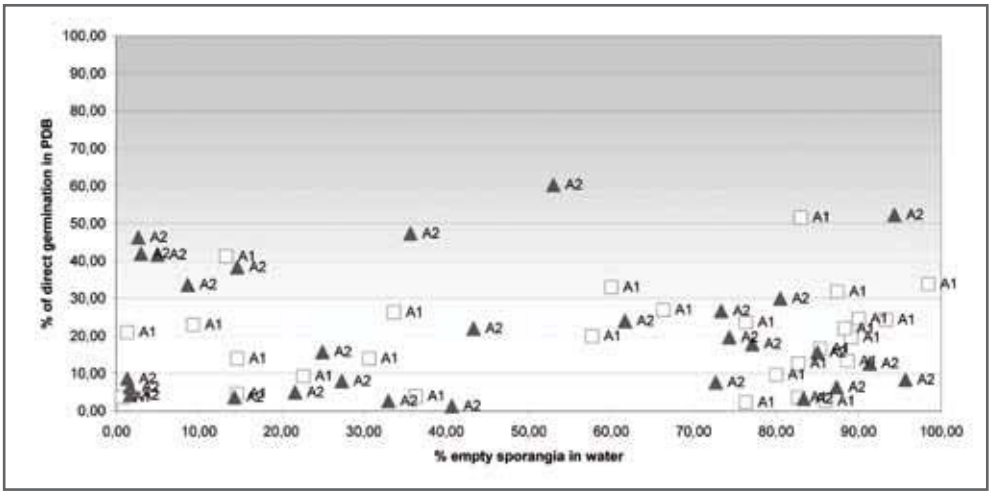

Figure 6. Distribution of the mating type according their ability to germinate directly or indirectly.

Influence of mating type:

The capacity for direct and indirect germination for twenty one strains of A1 mating type and twenty one strains of A2 mating type was determined. The results are given in figure 6 and figure 7. The percentage of direct germination of sporangia in PDB was not significantly different according to the mating type (Kolmogorov-Smirnov two samples test, figure 7A)

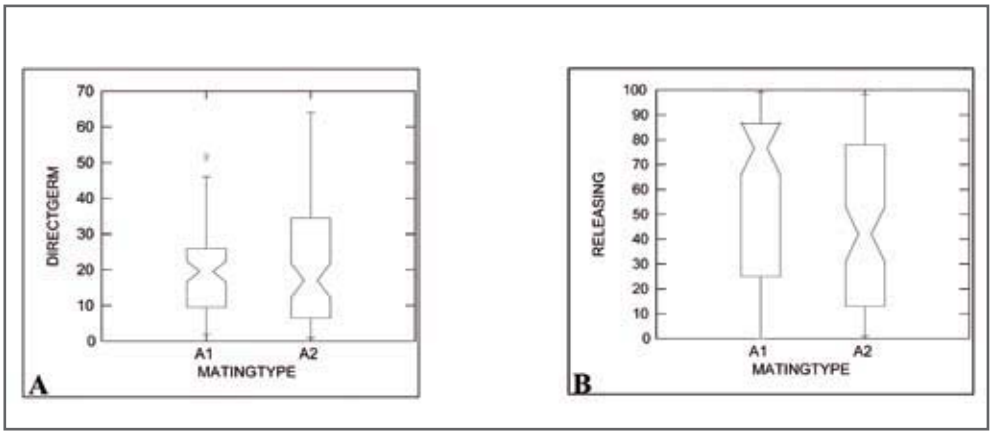

Figure 7. Influence of sexual mating type on direct (A) and indirect (B). Direct germination is not significantly influenced by mating type. Indirect germination on the other hand is significantly influenced by mating type with the A1 mating type displaying a higher capacity for zoospore formation.

Indirect germination on the other hand was significantly influenced by mating type with A1 superior to A2 strains (figure 7B).

Discusssion- conclusions

In this study, the capacity for direct and indirect germination of individual *P. infestans* isolates was studied as related to their period of origin (pre 1990 versus post 1990), the year of origin, the country of origin and the mating type.

At isolate level, the results demonstrate no significant influence of the geographical origin, the year of origin and the period of origin on the isolates ability to germinate directly or indirectly. Within the current set of isolates, A1 mating type isolates were however shown to be slightly better in zoospore formation than the A2 isolates. Thus, at first sight, the hypothesis that modern *P. infestans* isolates may have experienced selection pressure against indirect germination due to the general use of fungicides highly active against zoospores over the past two decades cannot be substantiated.

At population level however Europe has seen a shift from an A1 dominated population before the 1980's to a strongly A2 dominated population at present. On average, the current population may thus have a reduced capability for indirect germination. The cause behind this shift is likely to be related to the driving forces behind the domination of *P. infestans* genotype " blue 13" elucidated elsewhere in this volume.