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Results from the trap nursery network in Europe

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SUMMARY
Trap nurseries with potato cultivars with one or more known resistant genes (R-genes) were established in different countries in Europe. The objectives of the trap nursery were to monitor the stability of R-genes in potato cultivars in Europe in space and time, hunt for new genotypes of *Phytophthora infestans* and to test mega cultivars. Late blight severity was regularly monitored and assessed on the differentials throughout the growing seasons in 2017-2019. A system for data collection and analysis was established. In this paper, we discuss the methodology used for analyzing the disease assessment data from trap nurseries. Additionally, we show some results of the trap nursery from Droninglund in Denmark in 2019.

KEYWORDS
Late blight, *Phytophthora infestans,* Trap nursery, Disease progress curves, Apparent infection rate (AIR), area under the disease progress curve (AUDPC)

INTRODUCTION

Late blight, caused by *Phytophthora infestans*, is the most important disease on potato and tomato in the world. The disease has the potential of causing substantial yield loss when not controlled. Fungicides are still one of the major means of controlling late blight. However, the frequent application of fungicides can be expensive, environmentally unfriendly and could result in the development of *P. infestans* isolates that are resistant or insensitive to fungicides. Resistant cultivars provide a more environmentally friendly means of preventing or reducing the impact of late blight. However, R-genes deployed in potato cultivars are easily defeated by new *P. infestans* races, due to the dynamic nature of the pathogen. The best strategy to control late blight is to integrate both host resistance and fungicide within the integrated pest management (IPM) context.

The population of *P. infestans* in Europe is rapidly changing, with the emergence of new clones like EU_36_A2, EU_37_A2 and EU_41_A2. The emergence of these new genotypes of *P. infestans* raises the question of "what is their impact on late blight epidemic, virulence on cultivars and control of late blight in Europe?" These questions were answered in the IPMBlight2.0 project (Corbiere *et al.* this proceeding). As part of the IPMBlight2.0 project, trap nurseries were established from 2017 to 2019 in different countries in Europe (Denmark, Sweden, France, Scotland and Estonia) to serve the following purposes:

1. To monitor the temporal and spatial stability of resistance (R) genes in potato cultivars to *Phytophthora infestans* populations in Europe.

2. To hunt for new genotypes of *P. infestans* as well as phenotyping these new *P. infestans* genotypes in Europe.
3. Test of mega cultivars; that is how to control late blight on cultivars with high level of genetic resistance to late blight with less fungicide.
4. As an input to the IPM Blight 2.0-decision support system (DSS).

The trap nurseries consisted of potato cultivars or breeding lines with one or more known R-genes (See Table 1).

Figure 1. *Example of a trap nursery in Denmark. The trap nurseries are used to do live monitoring of the stability of resistance genes against Phytophthora infestans genotypes in the field*

As stated earlier in this paper, the trap nurseries serve several purposes; however, we shall restrict the focus of this paper to the methodology for analyzing the disease data generated from the trap nurseries and some results from some countries.

Table 1. *Details of the differential set and their resistance gene content (R-gene)*[1]

Differential sets	Genotype for 2018	R-gene content (Zhu *et al.*, 2015)
Black R1	**Craigs Snow White**	R1
Black R2	**Black 1512c (16)**	Unknown
Black R3	**Pentland Ace**	Unknown
DS-R4 / Black R4	**MaR4: CEBECO 4431-5**	R4
DS-5 / Black R5	**MaR5: Black 3053-18**	R1, R2, R3b,
DS-6	**MaR6: Black XD2-21**	R1, R2, R3a
DS-7	**MaR7: Black 2182 ef(7)**	R3a, R4
DS-8	**MaR8: Black 2424 a (5)**	R3a, R3b, R4, R8
DS-R8	3020-018	R8 = Rpi-smra2
DS-9	**MaR9: Black 2573 (2)**	R1, Rpi-abpt1, R3a, R3b, R4, R8, R9
DS-R9	3151-04	R9
DS-10	**MaR10: Black 3681 ad (1)**	R3b, R10
DS-11	**MaR11: Black 5008 ab (6)**	R3b, R10
DS-r½<<<<<	**BINTJE**	None
Sarp Mira	**SARPO MIRA**	R3a, R3b, Rpi-smra1, R4, R8
DS-smira1	3079-08	Rpi-smira1
Alouette	**ALOUETTE**	Unknown
Carolus	**CAROLUS**	Rpi-chc1
INRA 92T118.36	**COQUINE_INRA 92T118.36**	Rpi-*iiii* + QTL's
INRA 95T118.2	**KELLY_INRA 95T118.2**	
INRA 95T141.12	**MAKHAÏ_INRA 95T141.12**	

Monitoring the occurrence and development of late blight on the differentials

The cultivars or differentials in the traps nurseries were monitored regularly for typical late blight lesions or symptoms until total defoliation or the end of the season. The severity of late blight on each cultivar was assessed as the percentage leaf area covered by late blight lesions. Frequent assessment, as will be discussed later, is key for proper estimation of the epidemiological parameters for comparing the disease development on the differentials. For example, for a reliable estimate of the apparent infection rate (AIR) of late blight on a cultivar, more than two assessment data is required.

Data management and analysis of the disease data

An online data management system was established for handling data generated from the trap nurseries based on the former Eucablight methods (Hansen *et al.* 2007). Country managers of the trap nurseries can upload the disease assessment and other information about the trial (e.g. location, differentials) into the system. Among several others, the system generates a trial site map that shows the location of the trap nurseries (Figure 2) (2), disease progress curves of selected differentials (Figure 3) and calculations of different epidemiological parameters (e.g. area under the disease progress curve (AUDPC), relative area under the disease progress curve (rAUDPC) apparent infection rate (AIR)) (See Table 2).

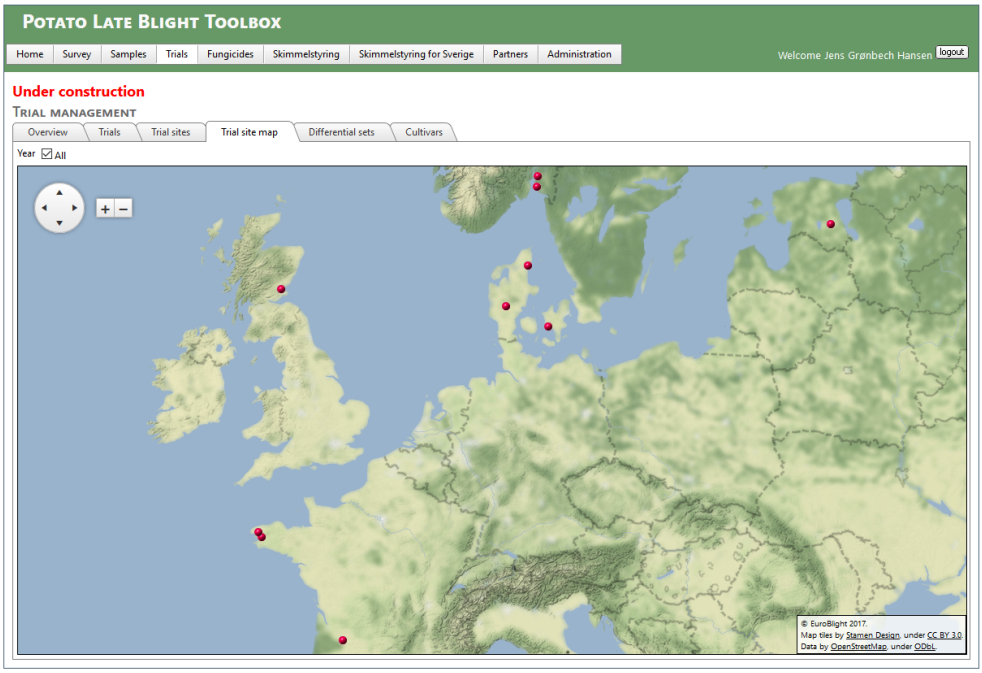

Figure 2. *Trial site map showing where the trap nurseries were established (red dots) in 2017*

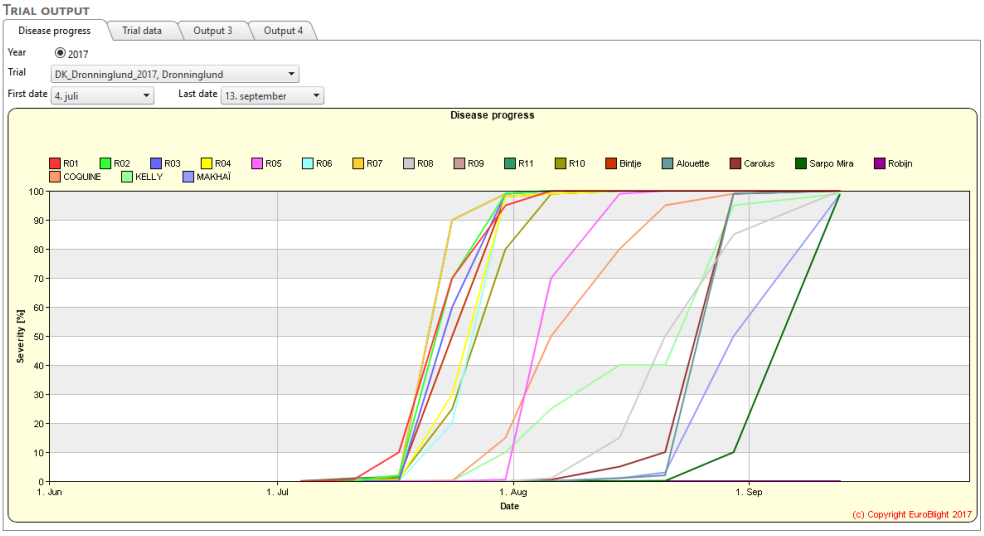

Figure 3. *The development of late blight (Phytophthora infestans) on differential sets in Droninglund in Denmark in 2017*

METHODS AND ANALYSES APPLIED TO THE DISEASE DATA FROM THE TRAP NURSERIES

The disease assessment data taken over time in the season is expressed graphically as disease progress curves (DPC) (Figure 3). DPCs shows the development of late blight on cultivar/differentials during the season and thus provides good grounds for comparison between the differentials. The system estimates or calculates several epidemiological parameters (or curve elements) from DPC of the differentials. For comparing the differentials, we calculate the following curve elements.

1. AUDPC and rAUDPC

Area under the disease progress curve (AUDPC) is an estimation of the total diseased tissues or leaf area covered by the disease for the entire duration of the epidemic. We define the duration of the epidemic as the time elapsed from the onset of late blight until the entire plant is defoliated (100% severity) or the last assessment in cases where 100% severity is not reached.

We calculate AUDPC with the mid-point method (Shanner and Finney, 1977) with the formula below.

$$\mathrm{AUDPC} = \sum_{i=1}^{n-1}\left(\frac{y_i + y_{i+1}}{2}\right)\left(t_{i+1} - t_i\right)$$

Where "y" is the percentage of leaf area covered with late blight at t^{th} assessment date and "n" is the number of assessments.

Generally, AUDPC is not comparable across experiments, especially in cases where the duration of the epidemic differ between experiments or treatments. Therefore, we calculate a standardized version of AUDPC, called relative area under the disease progress curve (rAUDPC). rAUDPC is calculated by first dividing AUDPC by duration of the epidemic, then finally dividing by the "maximum potential AUDPC". The maximum potential AUDPC is the AUDPC a cultivar or differential would have if it had 100% severity at all assessments times.

2. Apparent infection rate (AIR)

The apparent infection rate (AIR), or, the slope of the DPC, represents the rate of disease increase during the epidemic phase of the disease development. To estimate the AIR, the disease assessment data is transformed to the linearized logistic and gompertz models (Campbell and Madden, 2007). A linear regression of the transformed disease assessment (as dependent variable) and time in days (as independent variable) is then fitted. AIR is estimated as the slopes of the regression lines for the logistic (AIR_L) and gompertz (AIR_G) models. The coefficient of determination (R^2 or R-squared) are given for the goodness of fit of each regression is also calculated to allow for model selection. The slope from model with the best fit (high R^2) is chosen. Usually, the logistic model gives the best fit; thus, we use the logistic model estimate AIR.

The following are the steps that are taken to estimate AIR of each differential.

1. The disease severity data are converted from percentage (%) to a 0-1 scale by dividing the severity data in percentage by 100.
2. Only disease severity data from 0.005 to 0.99 (or 0.5%-99%) are used.

3. Only the first observation in 95-99% is included in the calculation of AIR.
4. The disease severity data in 0-1 scale are transformed to ln(y/1-y) and –ln(-ln(y)) for the logistic and gompertz transformations, respectively
5. If the severity value just prior to the first assessment of 100% is below 90%, then the first assessment of 100% is lowered to 99%. For example, disease assessment data 0, 0.1, 5, 40, 100, 100, 100, will be changed to 0, 0.1, 5, 40, 99, 100, and 100. In this example, the original data have only 2 assessments between 0.5 and 99% (5 and 40%) and AIR will not be calculated. When a situation like this happen, it will be indicated by a star in the result table. After change of the first assessments of 100% to 99%, the total result for AIR will be calculated.

As a rule of thumb, the total result for AIR for a cultivar is excluded if there are less than three observations between 0.005-0.99 of any of the replicates, the slope (AIR) of the fitted regression line is less than zero, or the coefficient of determination of the regression fit is less than 0.5 in any of the replications.

3. Days until 1% and 5% disease

Based on calculations of AIR (slope) and the intercept from regression of the logistic linearized equations we calculate the number of days until 1% disease and 5% disease from first observation in the trial. AIR and the days until 1% and 5% disease severity for a cultivar are excluded if there are less than three observations between 0.005-0.99 in any of the replicates or the slope (AIR) of the fitted regression line is less than 0 or the coefficient of determination of the regression fit is less than 0.5 in any of the replications.

Determination of the type of resistance via the Delta method

The observed field resistance of potato cultivars to late blight could either race-specific (RS), race-non-specific (RNS) or both RNS and RS (Vander plank, 1968). The type of resistance has a huge epidemiological significance and disease control for that matter. RS is governed by the gene for gene relationship and controlled by single or few genes. Usually RS provides a complete resistance against avirulent races of the pathogen. However, upon the arrival of virulent races of the pathogen, RS breaks down and the plant becomes very susceptible. In other words, RS type of resistance delays the onset of epidemic until the arrival or emergence of virulent races. In contrast, RNS does not delay onset of epidemic, but rather slows down the development of the disease. The performance of RNS depends on the aggressiveness a trait that is generally postulated to be polygenic and hence evolve slowly. Consequently, RS is normally highly efficient but short lived, whereas RNS is providing partial, but more lasting protection.

To determine the type resistance in the cultivars or differentials, we use the Delta method. Succinctly, the delta method calculates the difference between the AIR (Delta a) or time to reach 1% (Delta t) of a test cultivar and that of a reference susceptible cultivar. **Delta t** is as the difference between Days until 1% for the cultivar tested and Days until 1% for Bintje. **Delta a** is the difference between AIR for the cultivar tested and AIR for Bintje.

A graph is generated with Delta a on the x-axis and Delta t on the y-axis (See Figure 4). Type of resistance identified via Delta plot are as follows:

- **Susceptible cultivars:** Cultivars with the same or a higher AIR and less number of Days until 1% disease than Bintje. These cultivars will be located in the lower right corner in the graph

- **Race-specific cultivars (RS):** Same or a higher Air and more Days until 1% disease than Bintje. Upper right corner in the graph
- **Race-non-specific cultivars (RNS):** Lower Air and the same or less number of Days until 1% disease than Bintje. These cultivars will be located in the lower left corner in the graph
- **RS + RNS (or RS not overcome):** Lower AIR and more Days until 1% disease than Bintje. These cultivars will be located in the upper left corner in the graph

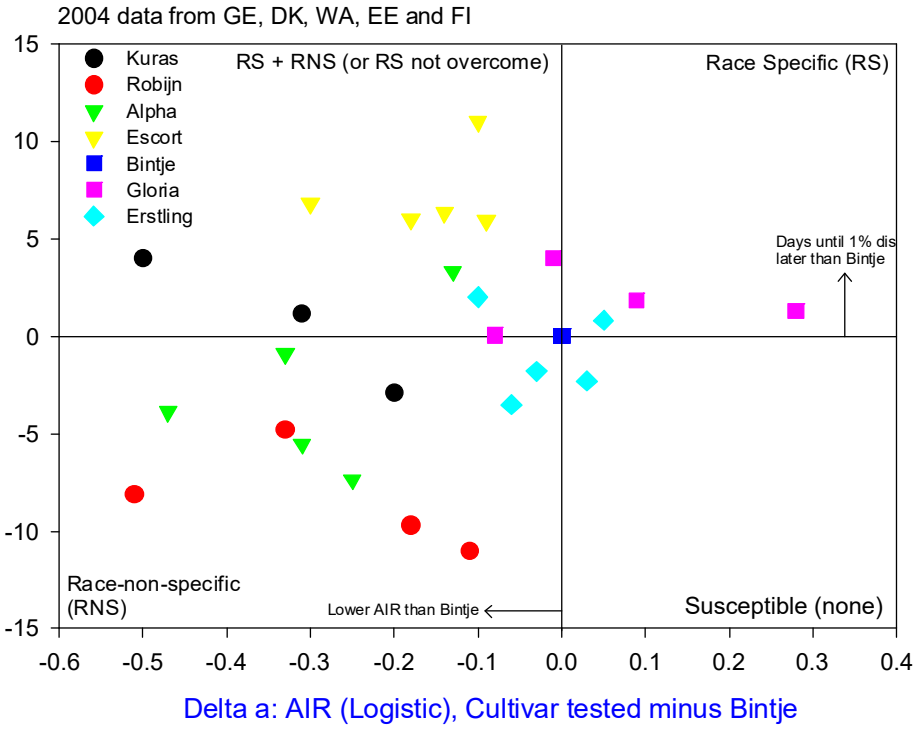

Figure 4. *Example of Delta plot showing the type of resistance of different cultivars*

RESULTS FROM A TRAP NURSERY AT DRONINGLUND IN DENMARK IN 2019

Here, we present some results from the trap nurseries from Droninglund in Denmark in 2019.

The disease progress curves showing the development of late blight on the differential sets and some cultivars are shown in Figure 6. Except for Sarpo Mira, PL11-0111, Nofy, Avito and Ardeche, late blight developed successfully to reach 100% severity on the other cultivars/differentials (Figure 6).

A summary of the estimated or calculated curve elements (AUDPC, rAUDPC and AIR) of the differentials from the trap nursery at Droninglund in 2019 are also shown in Table 2. Except DS-9, which had AUDPC and rAUDPC of zero, all the differentials/cultivars had AUDPC and rAUDPC

greater than zero (Table 2). The zero AUDPC and rAUDPC of DS-9 was because no late blight was observed on this differential at all assessment dates (Figure 5).

As stated earlier in this paper, the AIR are shown for cultivars with more than two assessment values between 0.5 and 99%, and AIR greater or equal to zero and R-squared of at least 0.5 (see Table 2).

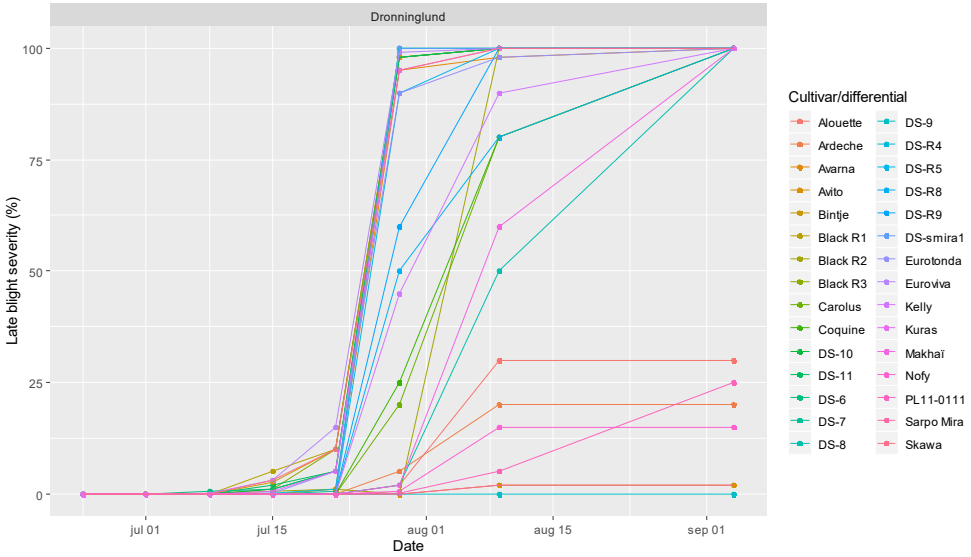

Figure 5. *Disease progress curve (DPC) of the differential sets from the trap nurseries in Denmark (Droninglund) in 2019*

Table 2. *The area under the disease progress curve (AUDPC), relative area under the disease progress curve (rAUDPC), apparent infection rate (AIR), the number of observations between 0.5 and 99%, coefficient of determination (R^2)*

Differential/Cultivar	Observation	AUDPC	rAUDPC	AIR	R-squared
DS-9	0	0	0	-	-
Sarpo Mira*	2	63	0.009	-	-
Avito*	2	63	0.009	-	-
PL11-0111*	2	419.3	0.058	-	-
Nofy	3	477	0.066	0.08	0.53
Ardeche	3	675	0.094	0.03	0.53
Alouette	3	963	0.134	0.07	0.53
DS-8	3	2243	0.312	0.22	0.97
Makhaï	3	2428	0.337	0.22	0.94
Carolus	3	2960	0.411	0.15	0.97
Coquine	3	3005	0.417	0.15	0.98
Black R2	3	3160.5	0.439	0.42	0.96
DS-R8	3	3230	0.449	0.12	1.00
Kelly	3	3370	0.468	0.12	0.95
DS-R9*	2	3690	0.513	-	-
Eurotonda	3	3959.75	0.550	0.36	0.84
DS-R5*	2	3960	0.550	-	-
Avarna*	2	3975	0.552	-	-
Kuras	3	4043.5	0.562	0.59	0.94
DS-smira1*	1	4050	0.563	-	-
DS-7*	2	4053.5	0.563	-	-
DS-R4*	2	4058.4	0.564	-	-
DS-10	3	4074	0.566	0.61	0.89
DS-11	3	4081	0.567	0.56	0.84
DS-6	4	4095.5	0.569	0.45	0.79
Skawa	3	4096	0.569	0.46	0.89
Black R3	3	4109	0.571	0.61	0.94
Bintje	3	4119.5	0.572	0.54	0.89
Black R1	3	4137	0.575	0.49	0.83
Euroviva	3	4167.42	0.579	0.58	0.90

**on a differential/cultivar indicates that for that differential/cultivar, the apparent infection rate (AIR) value was not calculated for one or more of the following reasons. (1) Less than three observations between 0.5 and 99%. (2) AIR was less than zero. (3) Coefficient of determination (R-squared) was less than 0.5*

CONCLUDING REMARKS

We have given a review of the methodology employed to analyze the trap nursery data. The dynamic nature and the ability of *P. infestans* to overcome deployed R-genes makes the establishment of trap nurseries to monitor the stability of R-genes an attractive exercise. Even though R-genes are an important component of integrated pest management (IPM) of late blight, the ability of *P. infestans* to defeat or overcome R-gene is of huge concern. Therefore, it is important regularly to establish trap nurseries to monitor the evolution of the pathogen within and between growing seasons. This would aid scientists to know when and which R-gene has been defeated quickly and growers or advisors to adapt their fungicide application plan.

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