Virulence and fungicide sensitivity of the most prevalent genotypes collected in Belgium in 2017-2018

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Summary
A total of 119 isolates of Phytophthora infestans were collected in the southern part of Belgium (Wallonia) in several potato fields, volunteers, dumps and field trials during the years 2017 and 2018. The isolates were analyzed using standardized 12plex Euroblast SSL genotyping (Euroblast monitoring). Most of isolates were tested for several phenotypic characteristics, such as virulence and fungicide resistance.

Materials and Methods
Phytophthora infestans isolates: Single-hockey isolates were obtained by placing pieces of infected tissue on tuber slices of a susceptible potato cultivar (Bintje). Pure cultures were obtained by transferring small pieces of mycelium growing on the upper side of the potato slice on pea agar medium.

Genotyping: The isolates were analyzed using standardized 12plex Euroblast SSL genotyping. Genotypes were determined by comparing fragment sizes with isolates previously genotyped (Euroblast monitoring).

Virulence: virulence was determined using Black’s differential set of potato clones, each having one of the RI-R11 resistance genes. Virulence was also determined by detached leaf assay on several commercial varieties which have a good rating about resistance in field (rated 1 [very sensitive] to 9 [resistant]). Each isolate was inoculated with 10 ul droplet of sporangial suspension and incubated in humid chambers under controlled conditions (18°C). After seven days of incubation, the sporulation was evaluated (high sporulation, low sporulation and no sporulation).

Fungicide resistance: Metalaxyl resistance was assessed using a floating leaf disk method (leaf of potato cv. Bintje). Leaf disks were floated abaxial side up in Petri dishes each containing water or metalaxyl at concentrations of 0 to 100 μg/mL. Each disk was inoculated with 10μl droplet of sporangial suspension. After seven days of incubation, isolates sporulating on the disks floating on water containing 100 μg/mL metalaxyl were rated resistant. Fluazinam resistance was evaluated on potato leaf discs (Bintje) by mixing fungicide at different concentrations (0.1 - 1 - 10 - 30 and 100 μg/mL) with the sporangial suspensions. After seven days of incubation, the sporulation was evaluated and isolates sporulating with 100 μg/mL fluazinam were rated resistant and isolates sporulating with 30 μg/mL were rated intermediate.

Results
Until 2018, 3 clonal lineages dominated in Belgium: the most prevalent was EU_13_A2 clone which made up 50% of the population. The two others were EU_1_A2 and EU_6_A1 and found at low frequency. In 2017 and 2018, monitoring highlighted the emergence of two new clonal lineage, named EU_36_A2 and EU_37_A2. They have displaced other lineages genotypes and are now dominant in Belgium. The genetically diverse "Others" samples comprised 30% of the sampled population in 2018.

All known virulence genes were found in Wallonia isolates. EU_13_A2 had a more complex virulence profile (1-2-3-4-6-7-8) (9-10-11) than other genotypes. EU_36_A2 and EU_37_A2 had the same virulence profile (1-3-4-6-7-10-11).

Significant differences were observed between genotypes regarding their virulence or resistant varieties. EU_13_A2 was the most virulent genotype. EU_36_A2 and EU_37_A2 were virulent on the same varieties.

EU_13_A2 was resistant to metalaxyl whereas other genotypes were sensitive. EU_37_A2 and some EU_36_A2 were resistant to fluazinam whereas other genotypes were sensitive. Some EU_36_A2 were intermediate.

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