Phytophthora infestans 13_A2, diagnostic and monitoring in 2009 and 2010

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Introduction

Concurrently to the base line monitoring and sensitivity studies carried on since 2001 to optimize an effective anti resistance strategy for fluopicolide, fenamidone and propamocarb based products, molecular methodologies have been investigated since 2009 to characterize the genotypes of the EU populations (ex.13_A2). Confirmation of mating types A1 & A2 and resistance to metalaxyl-M was also included in the characterization of the strains.

Materiel & methodes

Sampling:

Samples with potato late blight symptoms originated from different commercial locations of all important European regions were collected in 2009 (108) and in 2010 (120) and tested for their sensitivity to metalaxyl M, fluopicolide , fenamidone and propamocarb. Fourty eight isolates out of 108 and fourty six out of 120 were respectively selected for genotyping and/or sexual mating type survey (fig.1)

Molecular characterization:

13_A2 genotyping was conducted with the two main primers G11 and D13 directly from infected potato leaves based on D.E.L. Cooke technology (fig.2).Then this primary diagnostic was validated at the SCRI on a range of primers.

A1/ A2 sexual mating type determination:

Sexual mating type diagnostic is based on the observation of oospores when reference types A1 or A2 are confronting with the unknown strain: suspensions of sporangia were mixed by pair for all combinations and 10µl droplets of each were distributed on potato leaf discs surviving on agar medium in Petri dishes. After 5 or 6 days of incubation in a climatic chamber at 16°C foliar discs are discolored by calcium hypochlorite and after washing finally stained with calcofluor and observed under fluorescent microscope (fig.3).

Figure 3 : oospores formation determining A1/A2 sexual mating type

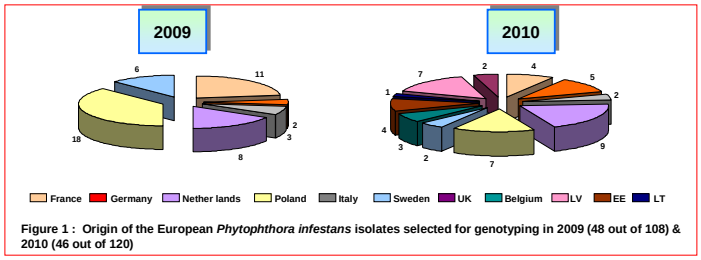


Figure 1 : Origin of the European *Phytophthora infestans* isolates selected for genotyping in 2009 (48 out of 108) & 2010 (46 out of 120)

	Initials Conditions D.Cooke	Bayer method
Biological material	Potatoes leaves infected by *Phytophthora infestans*	
1° step : Sample preparation	Mycelium purification	Leaf symptom : Freeze-drying and grinding
2° step : DNA extraction	Quick&Easy Protocol	DNeasy plant mini kit QIAGEN protocol
3° step : PCR	PCR Kit : GoTaq Flexi PROMEGA	Kit PCR : Taq DNA polymerase Q-Dio Gene
4° step : Capillary electrophoris	System : ABI system (Applied BioSystem)	System : GexP

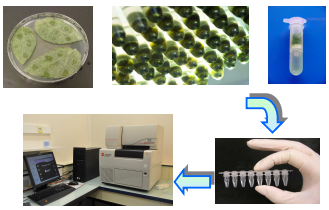


Figure 2 : 13_A2 genotyping from infected potato leaves compared with the protocol used on purified mycelium

Results

No shift of Potato Late blight sensitivity was detected in 2009 & 2010 monitoring in Europe for the 3 actives fluopicolide, fenamidone and propamocarb when resistance to metalaxyl-M is largely present in all the countries as described for a long time.

All the strains characterized for being 13_A2 genotype were exclusively resistant to metalaxyl-M.
There were isolates with the D13 alleles 136/154 that were NOT 13_A2.This illustrates the danger of relying on a single locus to test for clonal lineages. Only very few 6_A1 isolates were detected compared to the UK situation where it is mentioned more prevalent (D.E.L. Cooke com.).

Some tendency for the regression of the 13_A2 genotypes was observed from 63% of the metalaxyl-M resistant strains in 2009 to around 25% in 2010 random monitoring.
In parallel, A1 mating type seems to significantly progress.

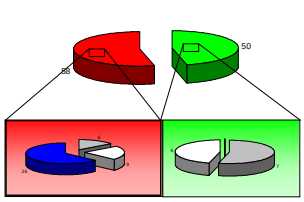
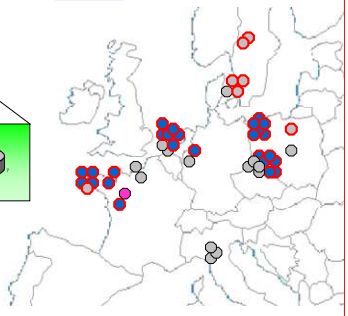


Figure 4 : 2009 breakdown of 41 resistant & 13 sensitive isolates to metalaxy-M according to sexual mating type A1/A2 and 13_A2



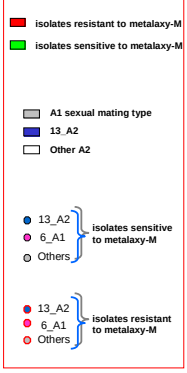


Figure 5 : 2009 geographic distribution of 6_A1, 13_A2 and other genotypes linked to resistance to metalaxyl M

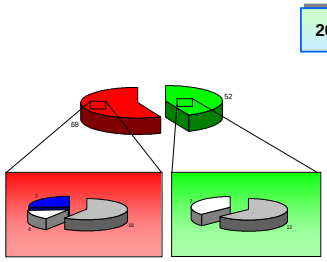


Figure 6 : 2010 breakdown of 27 resistant & 19 sensitive isolates to metalaxy-M according to sexual mating type A1/A2 and 13_A2

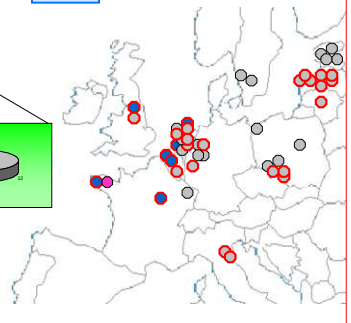


Figure 7 : 2010 geographic distribution of 6_A1, 13_A2 and other genotypes linked to resistance to metalaxyl M

Conclusion

Some tendency for the regression of the13_A2 genotypes resistant to metalaxyl-M was observed from 2009 and 2010. This evolution could be the consequence of changes in the fungicides applications with the limitation or absence of metalaxyl-M selection pressure in some countries such as NDL. The competition with other more aggressive genotypes such as 6_A1 is not relevant in these limited random monitoring.