Thirteenth EuroBlight workshop
St. Petersburg (Russia), 9-12 October 2011

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

M.P. LATORSE[1], Y. TARRIOTTE[1], V. BROZEK[1], H. YADJIA[1], S. VELOSO[1], D. COOKE[2]

[1] Bayer S.A.S., research center la Dargoire, 14 impasse P. Baizet 69009 LYON, France
[2] The James Hutton Institute Invergowrie, Dundee, DD2 5DA3

SUMMARY
Some tendency for the regression of the 13_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.

KEYWORDS
Potato late blight, *Phytophthora infestans*, monitoring sensitivity, fluopicolide, propamocarb, fenamidone, competitors, mefenoxam, genotyping, blue 13, sexual mating type A1/A2

INTRODUCTION

In order to answer EPPO, monitorings have been performed since 2001 with *Phytophthora infestans* covering all important European regions. Concurrently to the base line monitoring and sensitivity studies to optimize an effective anti resistance strategy for fluopicolide, fenamidone and propamocarb based products, molecular methodologies have been investigated since 2009 and conducted in parallel at the Scottish Institute (David Cooke) to characterise the genotype of the EU populations. Confirmation of mating types A1 & A2 and resistance to metalaxyl-M was also included in the characterisation of the strains.

MATERIALS AND METHODS

Sampling

Samples originated from France (Northern France, Champagne and Brittany), the Netherlands, Germany, UK, Belgium, Sweden, Baltics (EE, LT, LI) Poland and Italy were collected during in the season according the respective weather conditions.

Around ten leaves with potato late blight symptoms were sampled from each location. For the transport a sandwich with healthy leaves was made and each sample was then wrapped up in newspaper and posted in a paper bag to avoid rotting during transport.

The 108 samples collected in 2009 and the 120 in 2010 were tested for their sensitivity to metalaxyl M, fluopicolide, fenamidone and propamocarb. Fourty eight isolates out of 108 and fourty six out of

PPO-Special Report no. 15 (2012), 223 - 224

120 were respectively selected for genotyping and/or sexual mating type survey.

Molecular 13_A2 characterisation
An in house methodology was developed based on D. Cooke technology and primers to characterise 13_A2 (Blue 13) genotype directly from infected potato leaves to avoid fungal purification which takes time and is a human resources consumer task. The protocol is summarized in the poster.

A1/ A2 sexual mating type characterisation
Sexual mating type diagnostic is based on the observation of oospores when reference types A1 or A2 are confronting with the unknown strain:
Sporangia suspensions of *P. infestans* are calibrated for the two standards A1 and A2 strains and the unknown one. Then these suspensions are mixed by pair for all combinations and 10µl droplets of each are distributed on 30 potato leaf discs surviving on agar medium in Petri dishes. Petri dishes are then placed in a climatic chamber at 16°C and 90% humidity for 5 or 6 days.
After 5 or 6 days of incubation, discs are discolored by calcium hypochlorite during about 30 minutes until there's no more chlorophyll, washed in, at least, one water bath for 10 minutes and finally stained with calcofluor and observed under fluorescent microscope.
If oospores are visible in the positive standard confrontation (A1XA2) but not in the 2 negative ones (A1XA1 and A2XA2), the test is considered valid.
The presence of oospores on discs resulting from the mixture between the unknown strain and one of the A1 or A2 reference leads to the conclusion that the unknown strain is from the opposite sexual mating type. In the case oospores are formed in confrontation with the two different mating types, we could say that the two mating types are present in the population tested.

RESULTS
No shift of Potato Late blight sensitivity was detected in 2009 & 2010 monitoring in Europe for the 3 actives fluopicolide, fenamidone and propamocarb.

This monitoring was carried on to validate A2_13 genotypes which showed resistance to metalaxyl-M. We could observe that the resistance to mefenoxam is largely present in all the European 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.

CONCLUSIONS
No shift of Potato Late blight sensitivity was detected in 2009 and 2010 monitoring in Europe for actives fluopicolide, fenamidone and propamocarb.

Some tendency for the regression of the 13_A2 genotypes was observed from 75% of the metalaxyl resistant strains in 2009 to 60% in 2010.
In parallel, A1 mating type seems to significantly progress, may be due to some genotypes' aggressiveness such as A1- pink 6