



# Population structure of *P. infestans* in Cyprus and a short synopsis on its Mediterranean status

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# The Mediterranean Basin (MB)

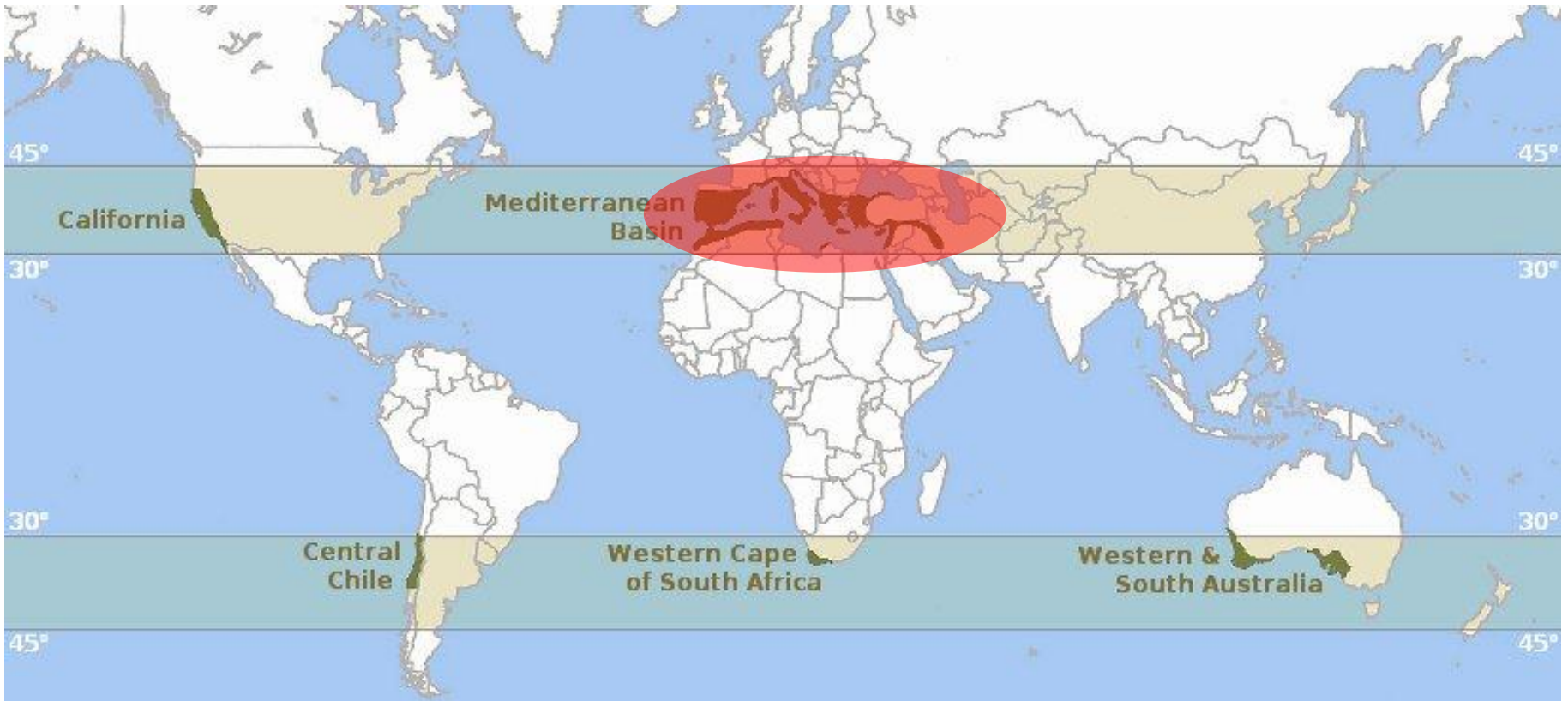


# The Mediterranean Basin (MB)

Twenty two (22) countries have a coastline with the Mediterranean Sea:

- ***European shore:*** Albania, Bosnia & Herzegovina, Croatia, France, Greece, Italy, Monaco, Montenegro, Slovenia and Spain [10]
- ***Asian shore:*** Israel, Lebanon, Palestine, Syria and Turkey [5]
- ***African shore:*** Algeria, Egypt, Libya, Morocco and Tunisia [5]
- ***Island nations:*** Cyprus and Malta [2]

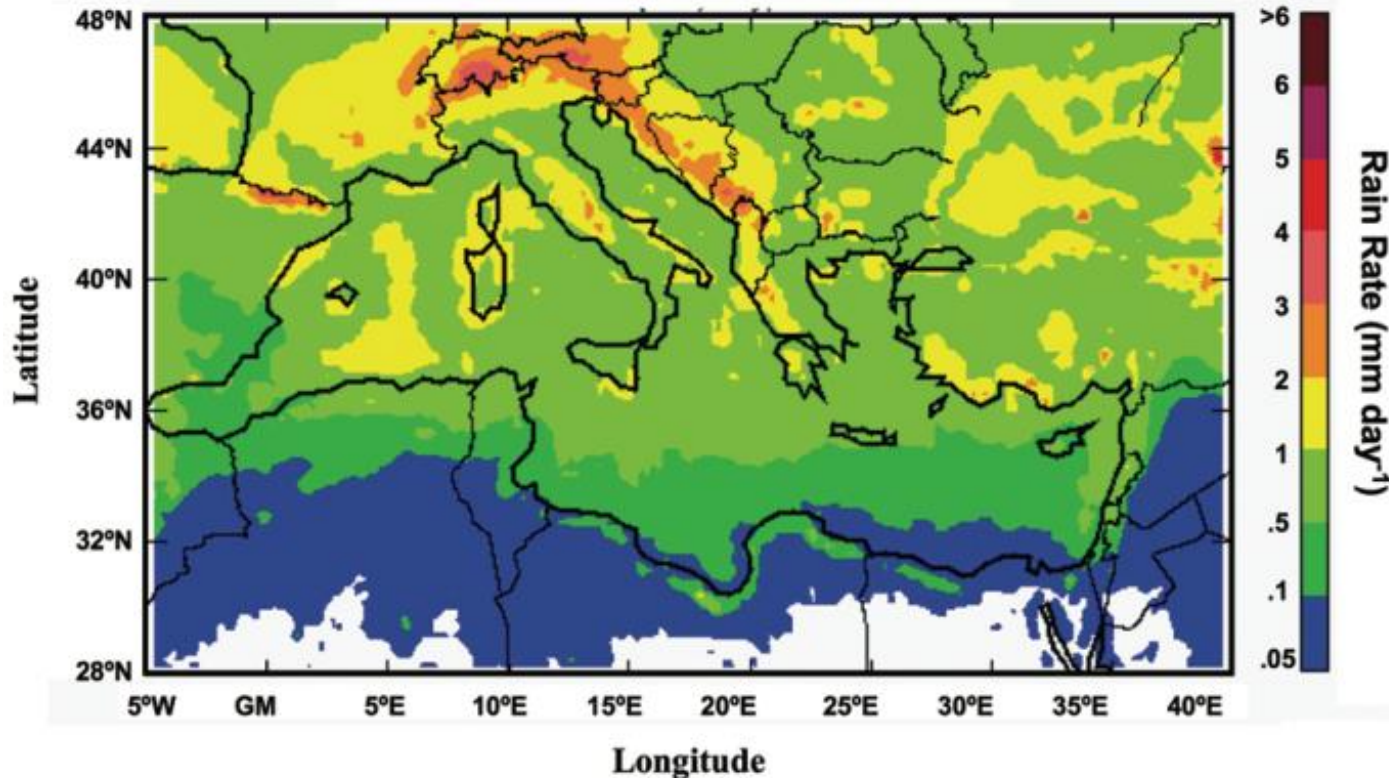
# The climate makes the difference



**Mediterranean-type climates have been defined:**

- (1) Geographically**, as climates similar to those found around the Mediterranean Sea;
- (2) Vegetationally**, as climates where broad-leaved evergreen sclerophyllous shrublands are common or dominant; and
- (3) Climatically**, as regions of summer drought and winter rainfall.

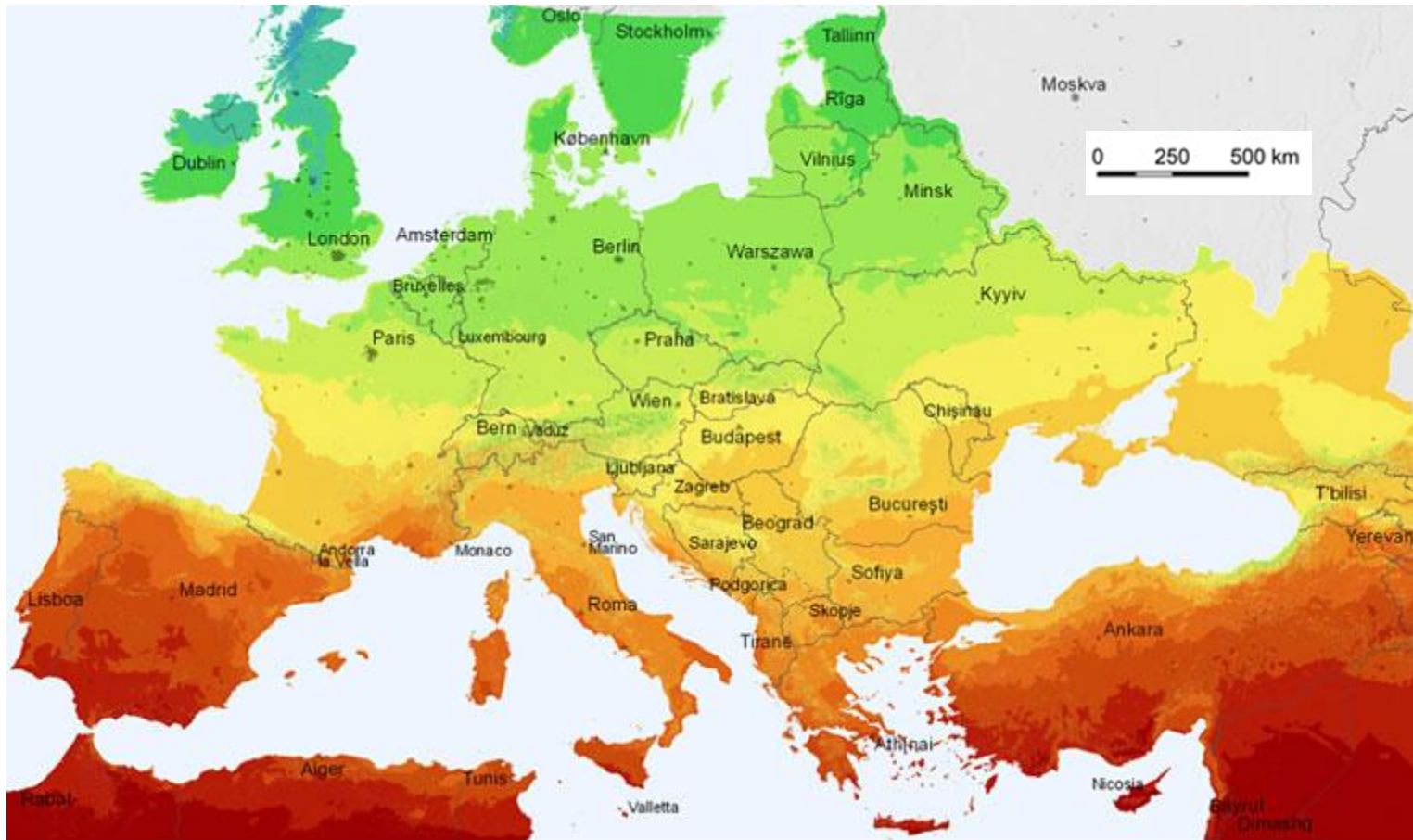
# Climatic conditions within the MB



The MB climate exhibits intricate spatial and temporal characteristics:

- **Main rainy season:** October to March (max rainfall: November - December)
- **Average rainfall:** 275 - 900 mm, of which **at least 65% falls into winter**
- **Max rainfall:** 3-5 mm day<sup>-1</sup>, over the mountainous regions of Europe
- **Min rainfall:** ~ 0,5 mm day<sup>-1</sup>, over North Africa

# Irradiation levels in the MB



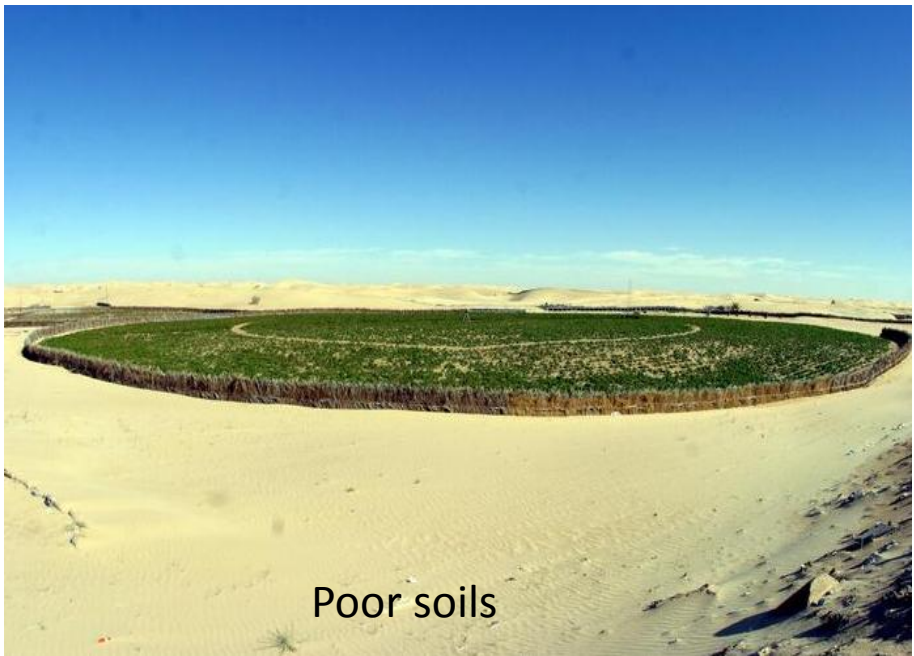
Average annual sum (4/2004 - 3/2010)



Considering the ephemeral lifespan of *P. infestans* airborne propagules, MB irradiance levels should be a limiting factor for later blight epidemics.

# Potato cultivation in the MB

- Generally, farmers in the Mediterranean region plant two crops per year.
- Along the Mediterranean, winter planting occurs from late November through January.
- **Fall planting** takes place in August, with harvest in November or December.
- **Harvest** is generally about **four months after planting**.
- Elsewhere, at higher elevations (500-2,000 m.), **only one crop**, planted in April/May with harvest in September/October.
  - *The limiting climatic factors are the severe winters, as well as the early and late frosts in autumn and spring, respectively.*



Poor soils



Small acreage




Hot and dry environment



Hot and dry environment



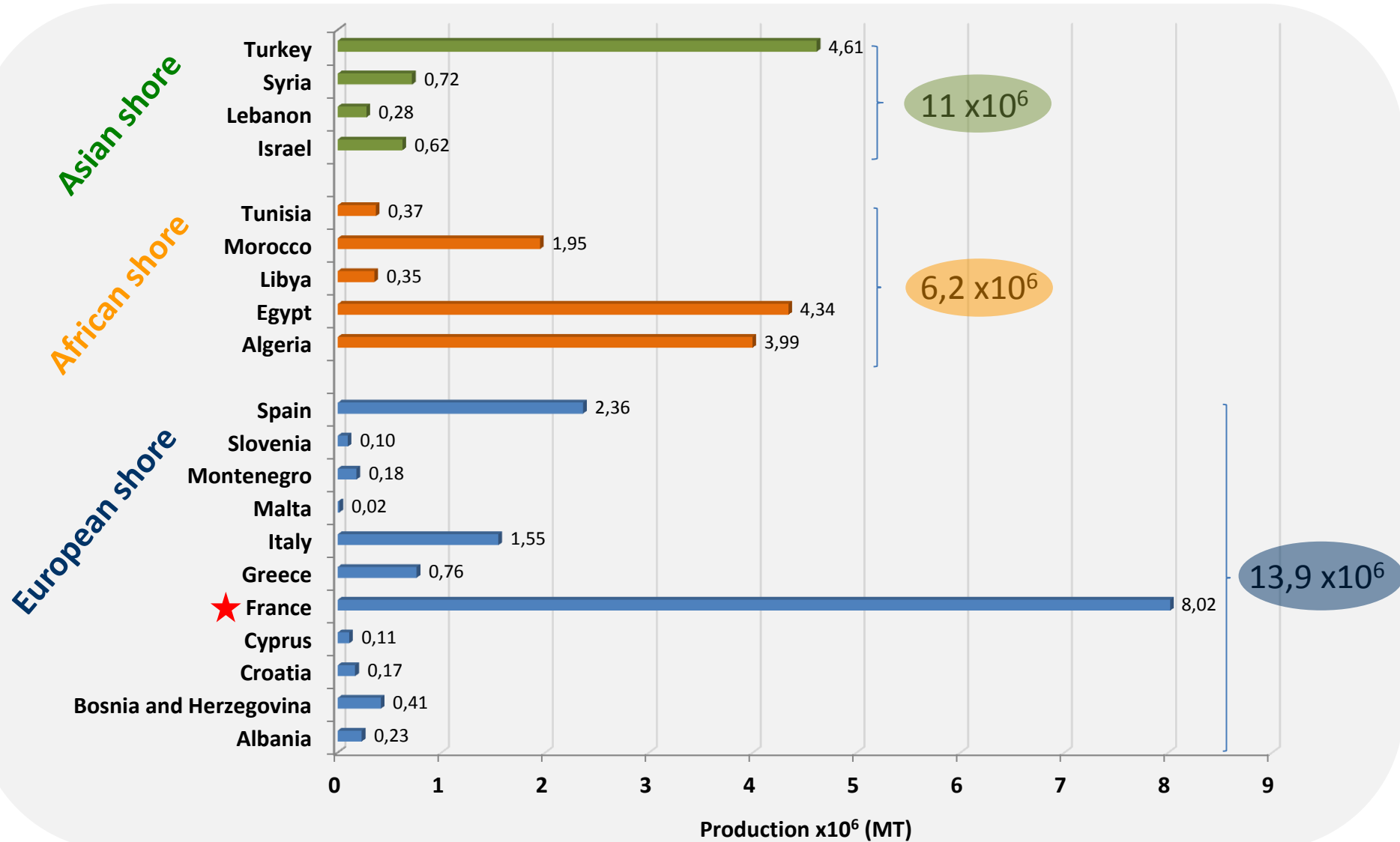
A wide-angle photograph of a potato field in Cyprus. In the foreground, a red tractor with a yellow tank is parked in a row of potato plants. The field extends to the horizon, where the Mediterranean Sea is visible under a hazy sky. A blue text box in the upper right corner contains the text "The Mediterranean sea".

The Mediterranean sea

Cyprus

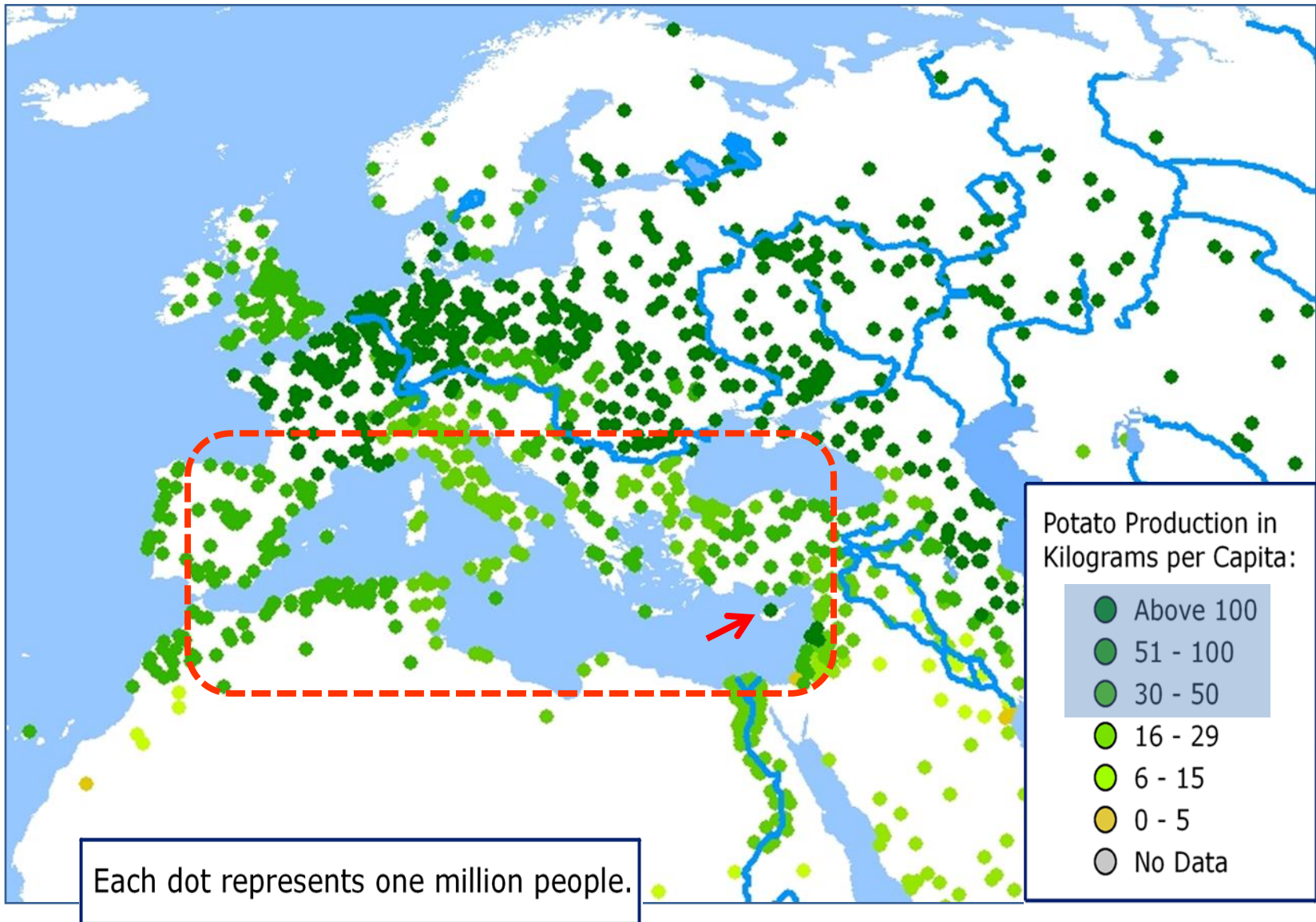
Potato cultivation by the sea

# Mediterranean Potato Production - 2011

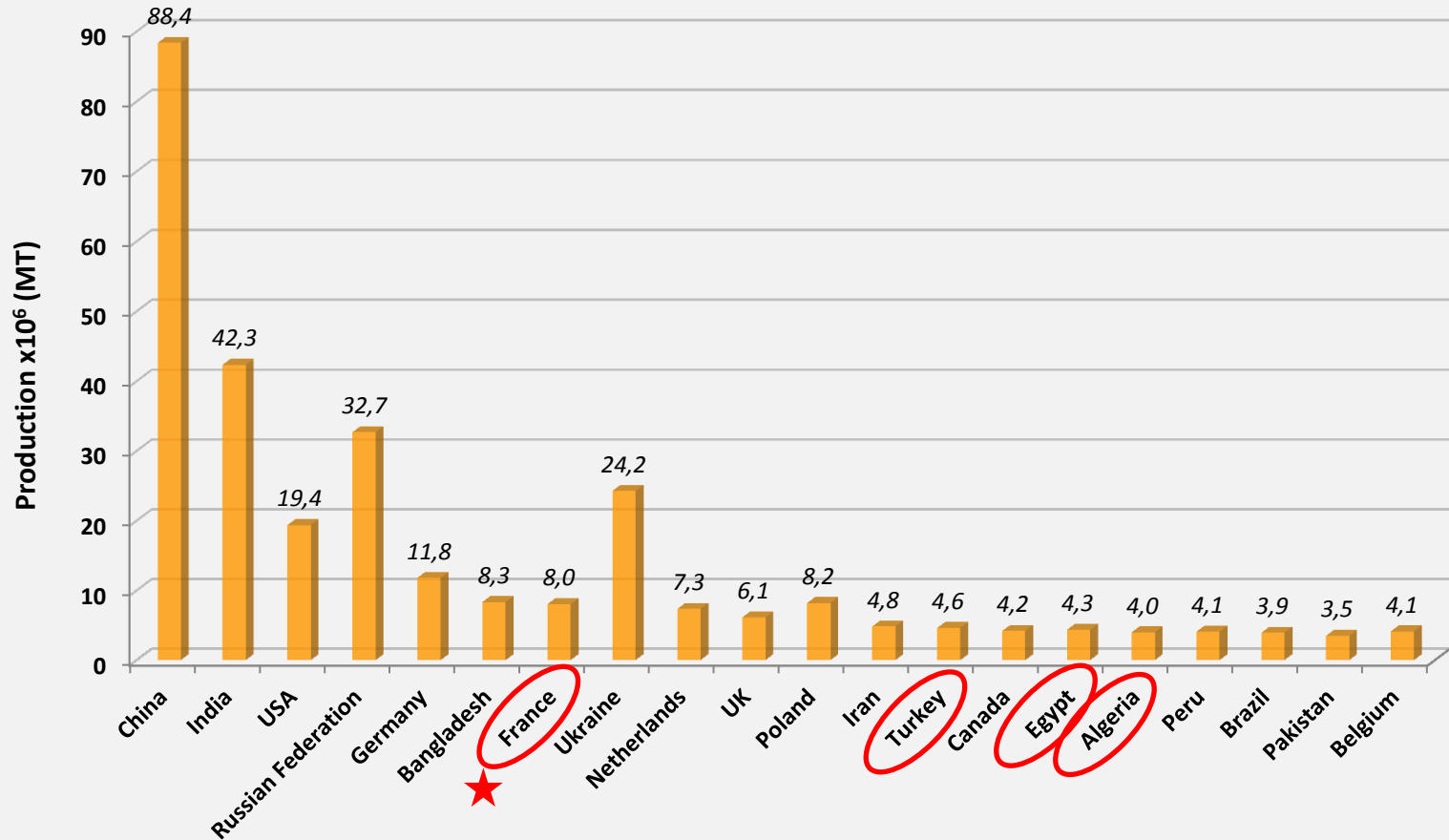


**Total Mediterranean potato production: 31.12 x10<sup>6</sup> MT**

# Population by per capita potato production



# Top 20 potato production countries- 2011



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## Literature search on Scopus

- Refereed journals
- English language
- **Key words:** (1) *Phytophthora infestans*  
(2) Late blight  
(3) Country name

# Documentation of late blight in the MB

| Asian shore countries | Refereed articles | Population structure | Data  |
|-----------------------|-------------------|----------------------|---|
| <b>Israel</b>         | 11                | 3 (2002, 1996, 1989) | A1/A2, oospores, metalaxyl-R, mtDNA/allozyme data |
| <b>Lebanon</b>        | 0                 | 0                    | -   |
| <b>Syria</b>          | 0                 | 0                    | -   |
| <b>Turkey</b>         | 3                 | 1 (2007)             | A1/A2, US1&US8                                    |

| African shore countries | Refereed articles | Population structure |  |
|-------------------------|-------------------|----------------------|--|
| <b>Algeria</b>          | 1                 | 1 (2007)             | 13_A2  |
| <b>Egypt</b>            | 8                 | 1 (1997)             | A1   |
| <b>Libya</b>            | 0                 | 0                    | -  |
| <b>Morocco</b>          | 6                 | 3 (2005, 2002, 2000) | metalaxyl-R, A1/A2(pot.), A1(tom.)           |
| <b>Tunisia</b>          | 1                 | 1 (2013)             | A1(low)/A2, IA haplotype, all tomato were A1 |

# Documentation of late blight in the MB

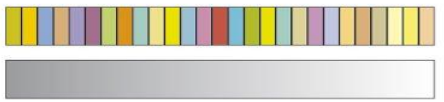
| European shore countries | Refereed articles | Population structure                                    |                            |
|--------------------------|-------------------|---|----------------------------|
| Albania                  | 0                 | 0   | -                          |
| Bosnia & Herzegovina     | 0                 | 0   | -                          |
| Croatia                  | 0                 | 0   | -                          |
| Cyprus                   | 2                 | <i>under preparation</i>                                | A1/A2, 13_A2, metalaxyl -R |
| France                   | 20                | 11 [2010, 2009, 2007(3), 2002, 1999, 1998(2), 1994 (2)] | A1/A2, metalaxyl-R, 13_A2? |
| Greece                   | 0                 | 0   | -                          |
| Italy                    | 4                 | 1 (1997)  | A1/A2                      |
| Malta                    | 0                 | 0   | -                          |
| Montenegro               | 0                 | 0   | -                          |
| *Serbia                  | 6                 | 2 (2007, 2003)  | <i>A1/A2, IA haplotype</i> |
| Slovenia                 | 1                 | 0   | -                          |
| Spain                    | 0                 | 0   | -                          |

# Summarizing

- No available information on potato late blight from 10/22 Mediterranean countries.
- 24 refereed publications to *P. infestans* population structure (genotypes) – 11 of French origin.
- Major players in the potato production industry, **Algeria**, **Egypt** and **Turkey** are under represented in the international literature.
- Most of the genotyping is based on different markers – mainly mtDNA/RFLP and allozymes – **a common genotyping language (probably SSR's) is in need.**
- **Cooperative efforts** should be put together in order unravel the genotype status at the Mediterranean basin countries.



# Cyprus: the southern most European country



Member states of the European Union  
Candidate countries

# Potato crop production in Cyprus



**Kokkinochoria region**

**Area:** 9,521 km<sup>2</sup>

**Highest point:** Mount Olympus (1,952 m)

**Population:** 803,147 (as of 2010)

# Specifics of Cyprus climate

- Hot and dry summers (*mid May – mid September*)
- **Mean summer temperature: > 30 °C**
- Mild winters (*mid November – mid March*); 60% of annual precipitation
- **Mean winter temperature: 10 °C**
- Mean annual precipitation (1991-2001): 453 mm  
*(following a downward trend)*

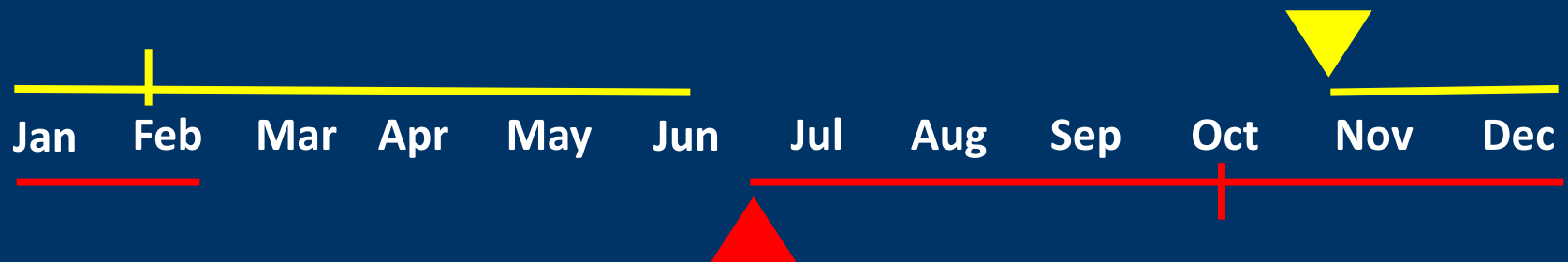
# Cyprus potatoes



- Cyprus potatoes are renowned for their great flavour and firm texture.
- Cyprus potatoes are easily distinguishable by the reddish colour of their skins, derived from the fertile red soil they are grown mainly in the Kokinochoria region (red-soil villages), at the Eastern region of the island.

# Potato crop periods in Cyprus

- **Spring crop** (Acreage: 4,000 hectares; Production: 70,000 - 80,000 tons) – *mainly exported*
  - **Planting:** November until early February
  - **Harvest:** March - mid June
- **Winter crop** (Acreage: 1,200 - 1,500 hectares; Production: 25,000 tons)
  - **Planting:** June/July - mid October
  - **Harvest:** November - end of February



# Potato production in Cyprus

A wide-angle photograph of a lush green potato field in Cyprus. The plants are densely packed and appear to be in the middle of their growth cycle. The background shows a clear horizon with some distant trees and a bright sky.


- **> 60% of the annual potato production is exported mainly to European Union countries; estimated exports around €50 millions (Data 2008).**
- **Almost 50% of Cyprus total “agriculture-based” exports exchange results from potato exports.**

# Potato late blight

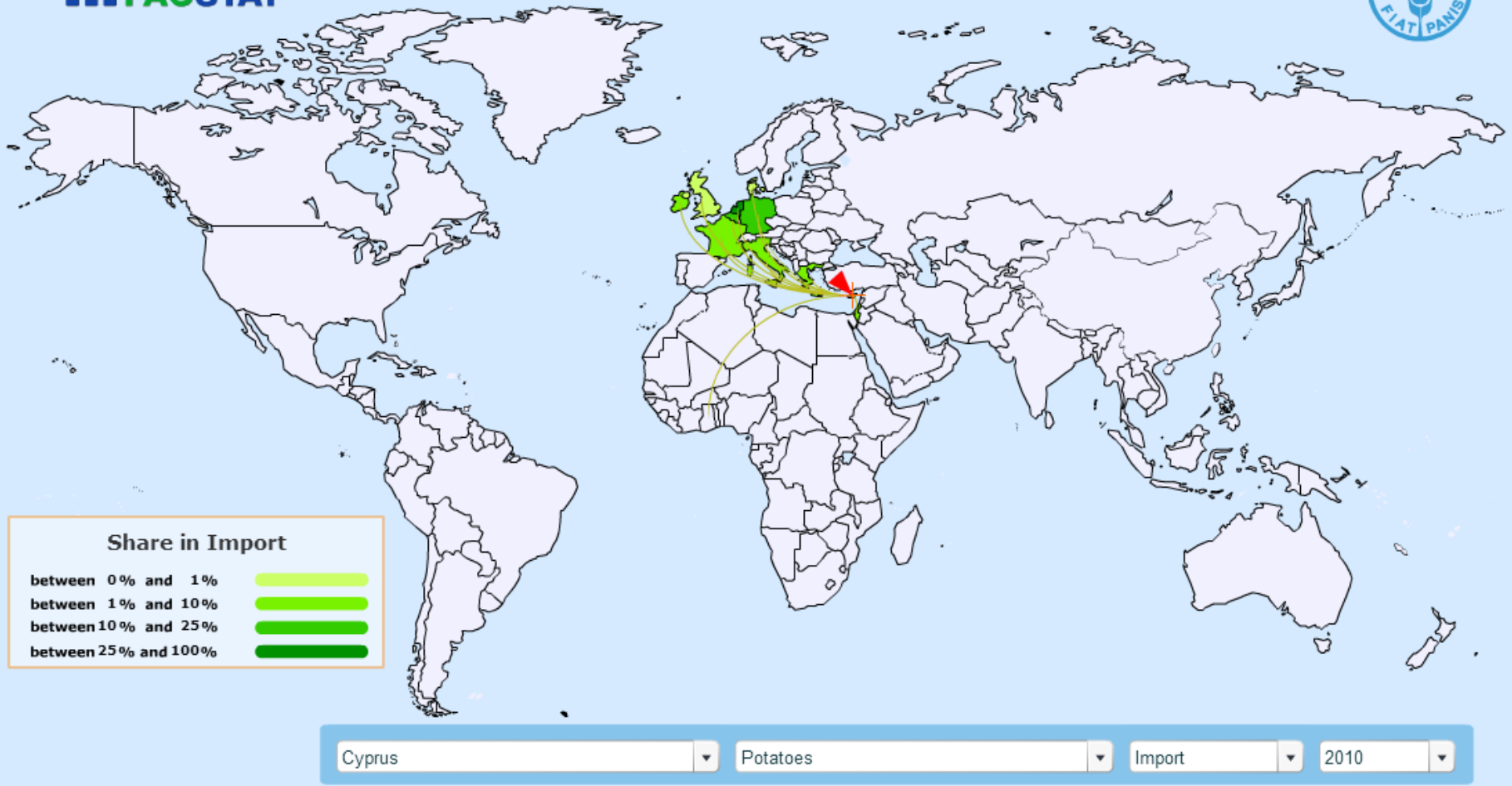


- Despite the hot and dry climate that characterizes Cyprus weather, late blight **can cause significant crop losses.**
- The winter crop is mainly affected (*rainy period*) by *Phytophthora infestans*.
- Disease management is predominately based on routine fungicide applications – *no DSS in practice.*

# More commonly used potato cultivars in Cyprus

- 
- Spunta (S)
  - Nicola (MR )
  - Diamant (MR-S)
  - Cara (MR-R)
  - Timate (MR)
  - Marfona (R-MR)
  - Filea (S)
  - Markies (MR-S)
  - Carrera (MR)
  - Annabelle (S)
  - Sieglinde (S)
  - Charlotte (MR)
  - etc.....

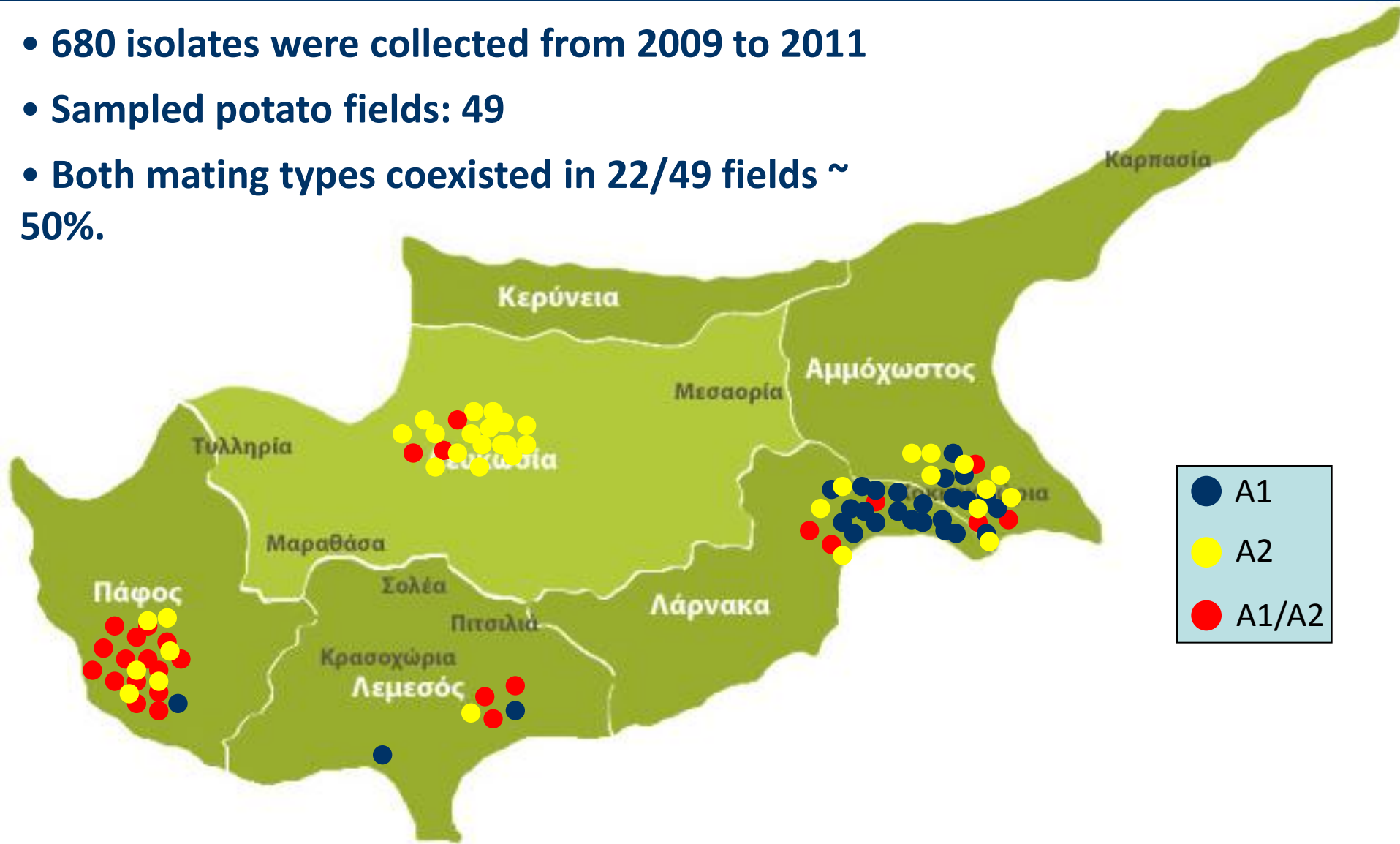




Cyprus imports ca. 6,000 tons/year of potato seed, mainly from N. European countries – the Netherlands, France, Germany, Belgium and UK.

# Mating types

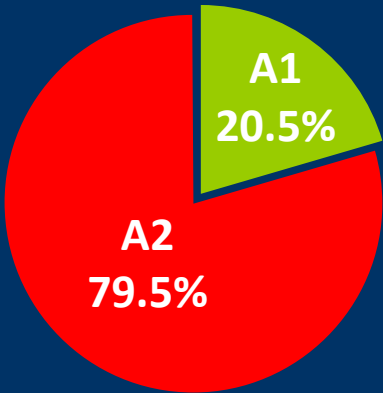
- 680 isolates were collected from 2009 to 2011
- Sampled potato fields: 49
- Both mating types coexisted in 22/49 fields ~ 50%.



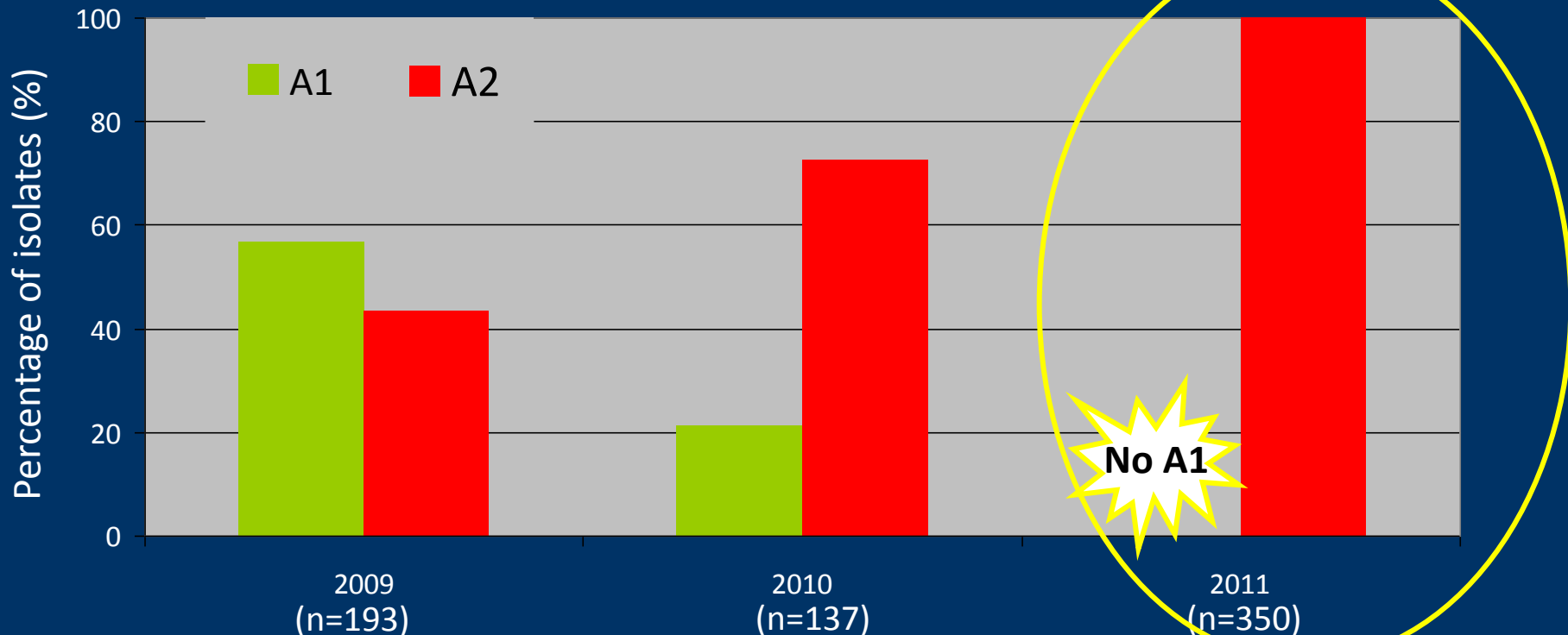
Nevertheless, oospores have been detected under field conditions

# Mating types

Cyprus 2009-2011 (n=680)

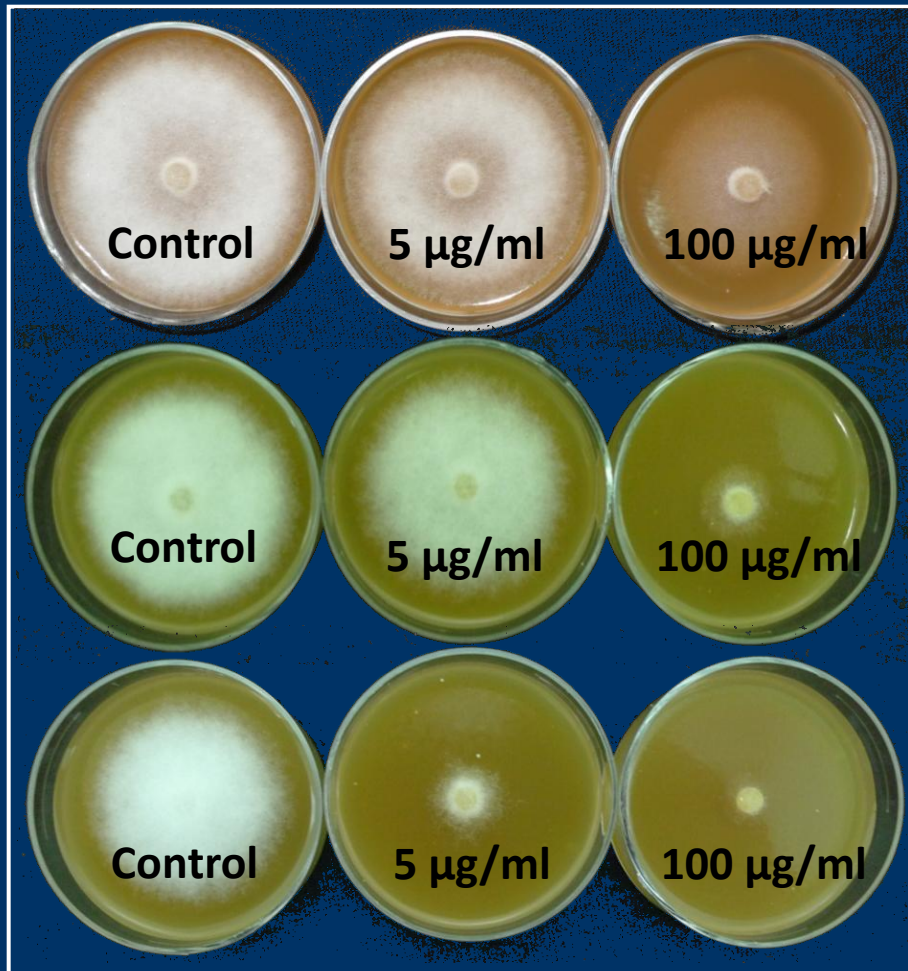


- 2009 and 2010 national coverage (n=330).
- 2011 concentrated sampling at the municipality of Paphos (n=350).
- A2 mating type is high and increasing.



# Response to metalaxyl-M

- 285 isolates of *P. infestans* were tested *in vitro*
- Using discriminatory doses (5 and 100 µg/ml)



**Resistant**

100 µg/ml  $\geq$  40% control



**Moderately resistant**

5 µg/ml  $\geq$  40% control

100 µg/ml  $<$  40% control

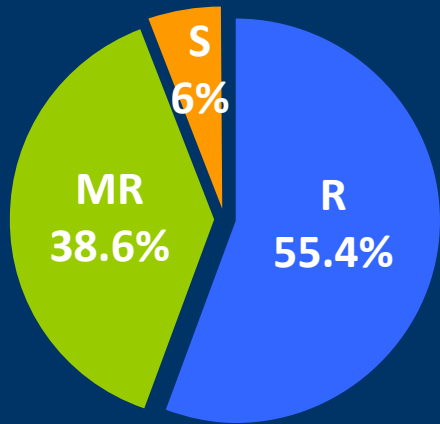


**Sensitive**

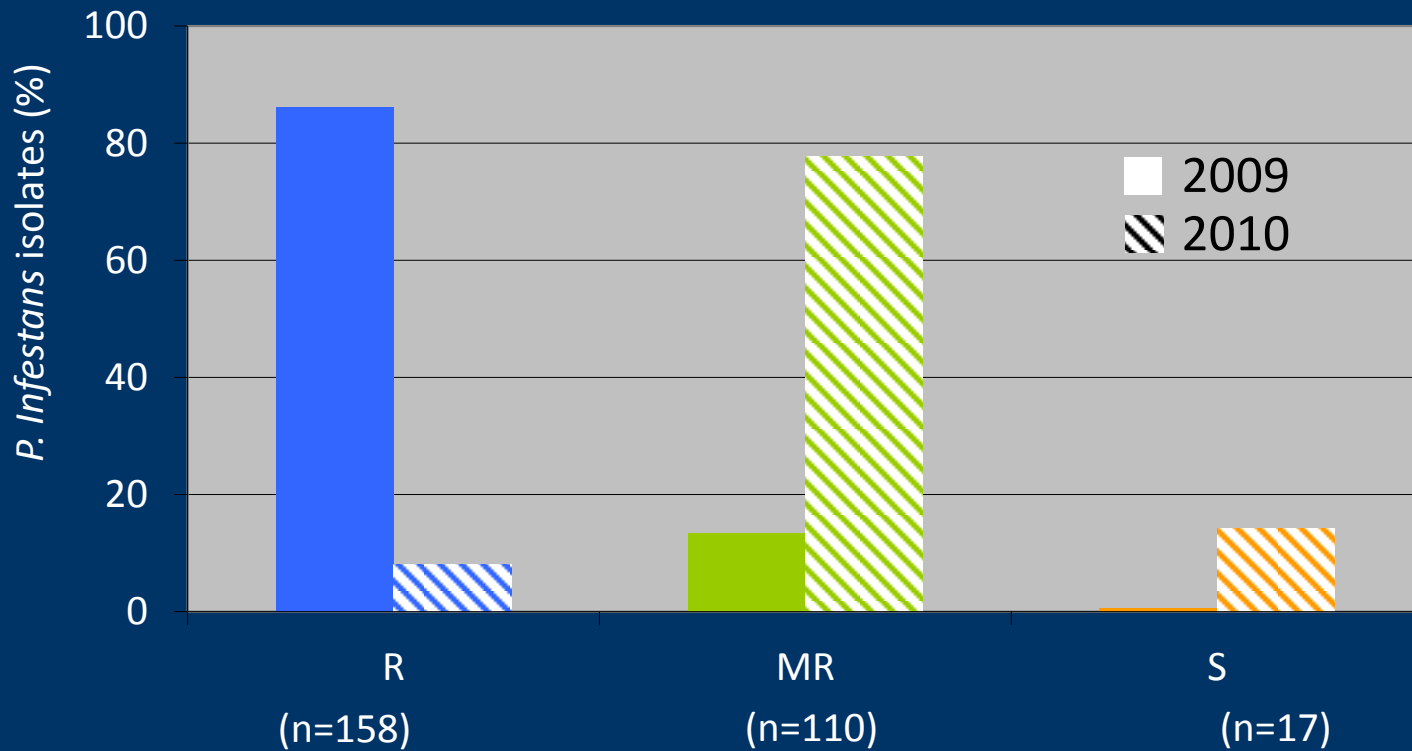
5 µg/ml  $<$  40% control

# Response to metalaxyl-M

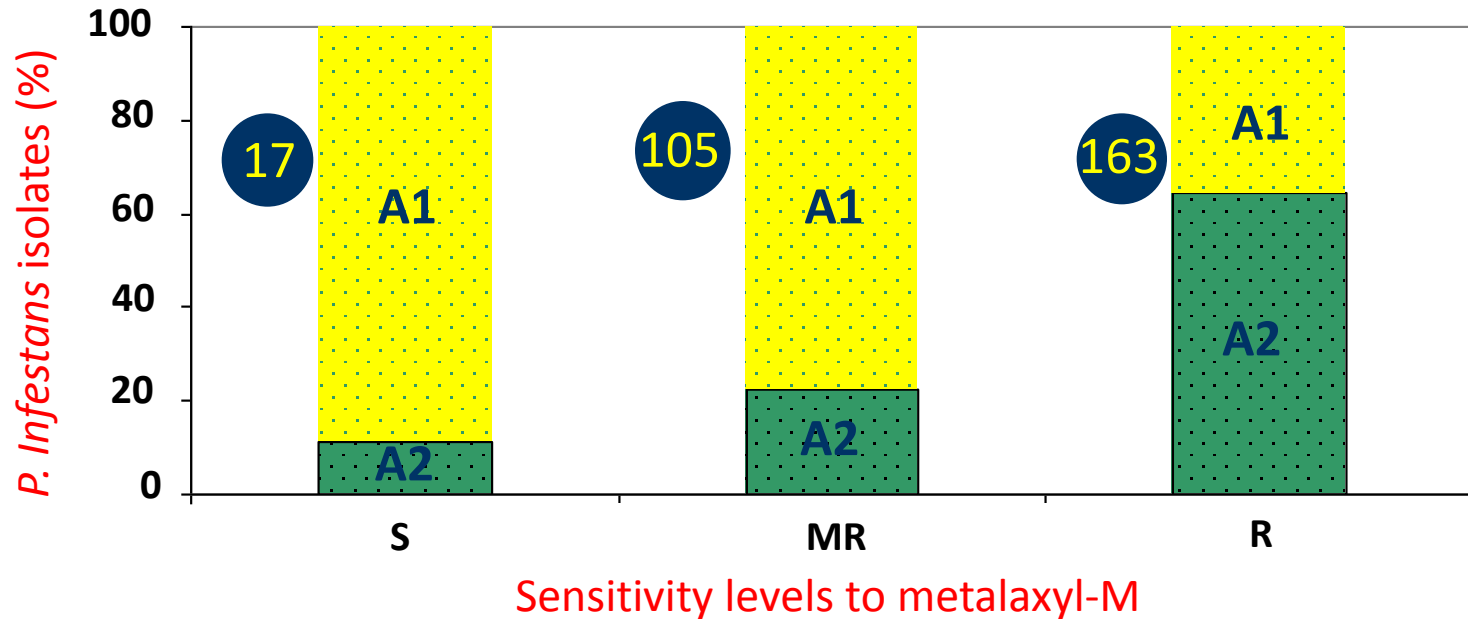
Cyprus 2009-2010 (n=285)



- High levels of isolates with reduced sensitivity to metalaxyl-M (> 90%).
- Observed metalaxyl-M resistance fluctuations (*probably due to different sampling periods between years*).
- **CONCLUDING:** Reduced contribution of metalaxyl-M to the management of the local population of *P. infestans*.



# Relationship between mating types and metalaxyl-M sensitivities

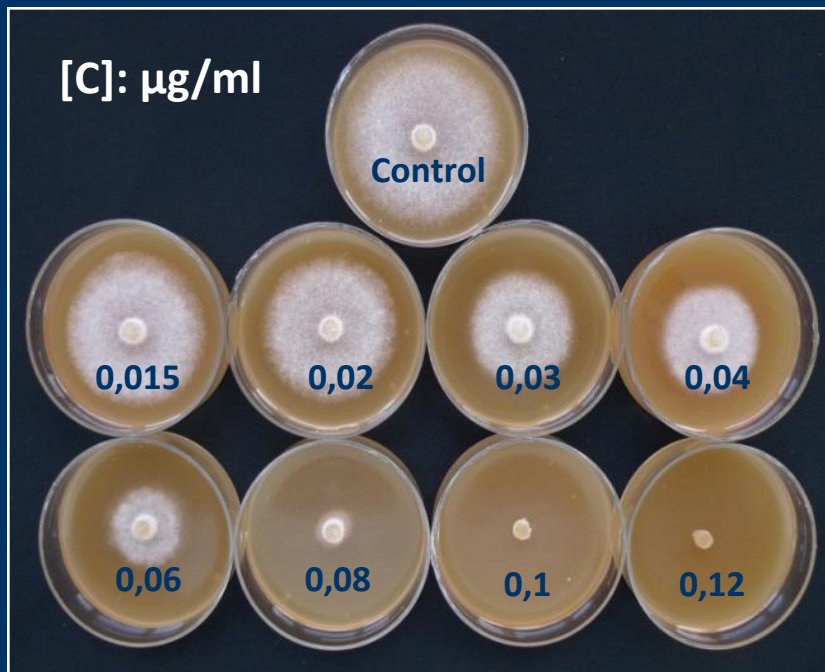


$$\chi^2 (2, N = 287) = 54,4 ; p = <0,0001$$

- A2 > A1 within the metalaxyl-M resistant isolates.
- A1 > A2 within the sensitive and the moderately resistant isolates of *P. infestans* to metalaxyl-M.

# Sensitivities of *P. infestans* isolates to fungicides commonly used in Cyprus

- EC<sub>50</sub> values of 70 isolates were estimated (*in vitro* and *in vivo*) for **cymoxanil**, **mandipropamid** and **propamocarb-HCl**.
- cymoxanil και propamocarb-HCl have been widely used in Cyprus, while mandipropamid was recently introduced in the Cyprus market (2010; baseline sensitivities).



# What's the current status?

| Formulation/<br>Active ingredient (% a.i.)       | Label dose | Label dose<br>( $\mu\text{g/ml}$ ) | Estimated $\text{EC}_{50}$<br>( $\mu\text{g/ml}$ ) |
|--|------------|------------------------------------|--|
| Antiperon WP/<br>cymoxanil (5,92%)               | 230g/100L  | 13,6 ✓                             | 0,23 (X59)   |
| Revus 25 SC/<br>mandipropamid (25%)              | 50g/50L    | 250 ✓                              | 0,014 (X1666)                                      |
| Ridomil Gold Plus 42,5 WP/<br>metalaxyl-M (2,5%) | 300g/100L  | 75 ?                               | <i>Discrimination dose</i><br>100                  |
| Previcur N/<br>propamocarb-HCL (72,2%)           | 120g/100L  | 870 ✓                              | 579 (X1.5)   |

Estimated sensitivities ( $\text{EC}_{50}$  values) of the Cyprus population of *P. infestans* underline that:

- Effective action of cymoxanil, mandipropamid and propamocarb-HCl.
- Compromised effectiveness of metalaxyl-M.



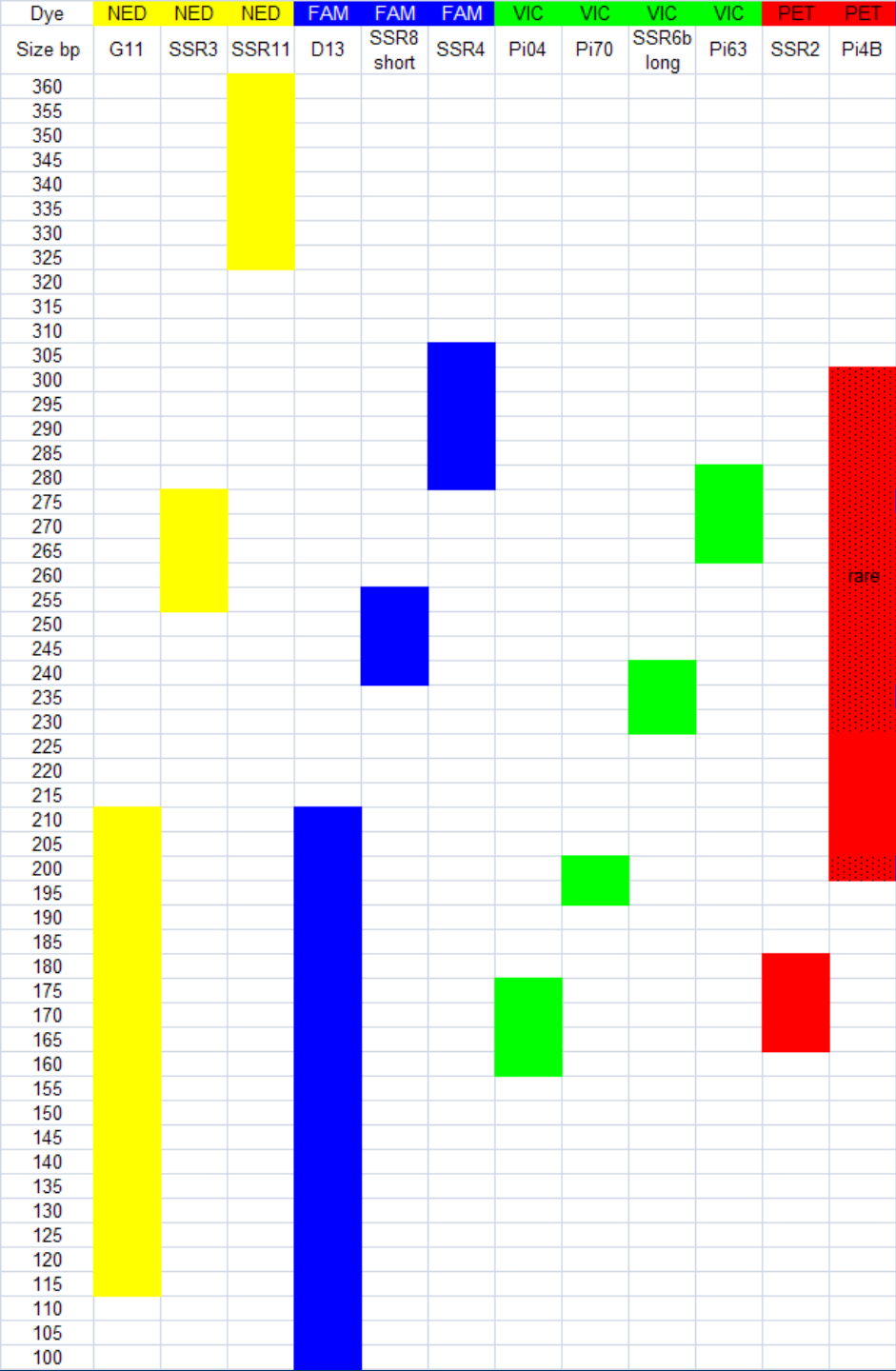
# Genotypic population structure

The knowledge of the genotypic population structure is of great importance.

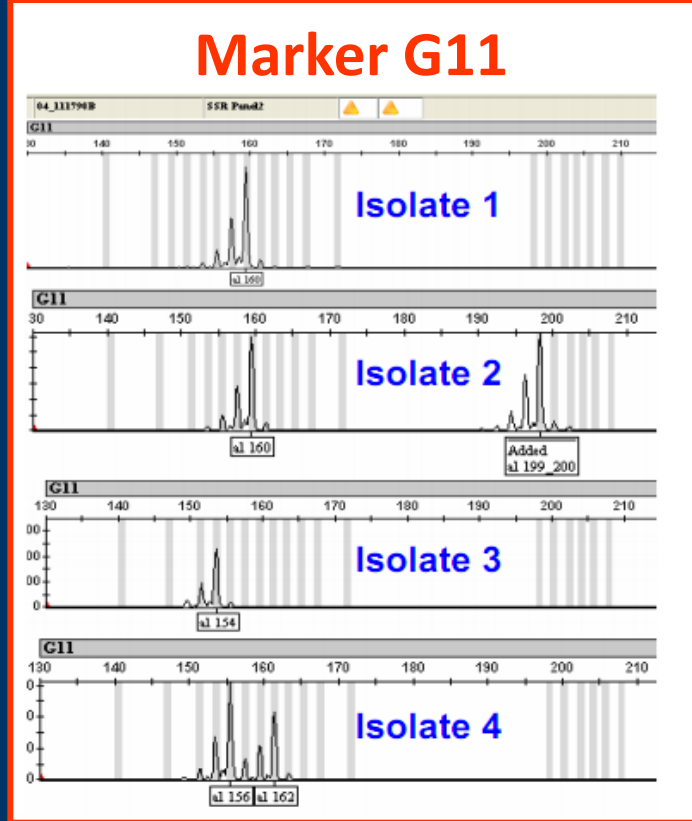
- Useful information regarding the epidemiology of the pathosystem.
- Understanding of the changes of the aggressiveness and other phenotypes of the microorganism.
- Check of the potato propagation material towards the discovery of resistant biotypes/cultivars.
- Emergence of novel disease management strategies.

- (a) high levels of potato seed imports to Cyprus,
- (b) financial importance of the crop for the country, and
- (c) location specifics of Cyprus

**Important scientific and applied interest**



## ABI 3130 Genetic Analyzer



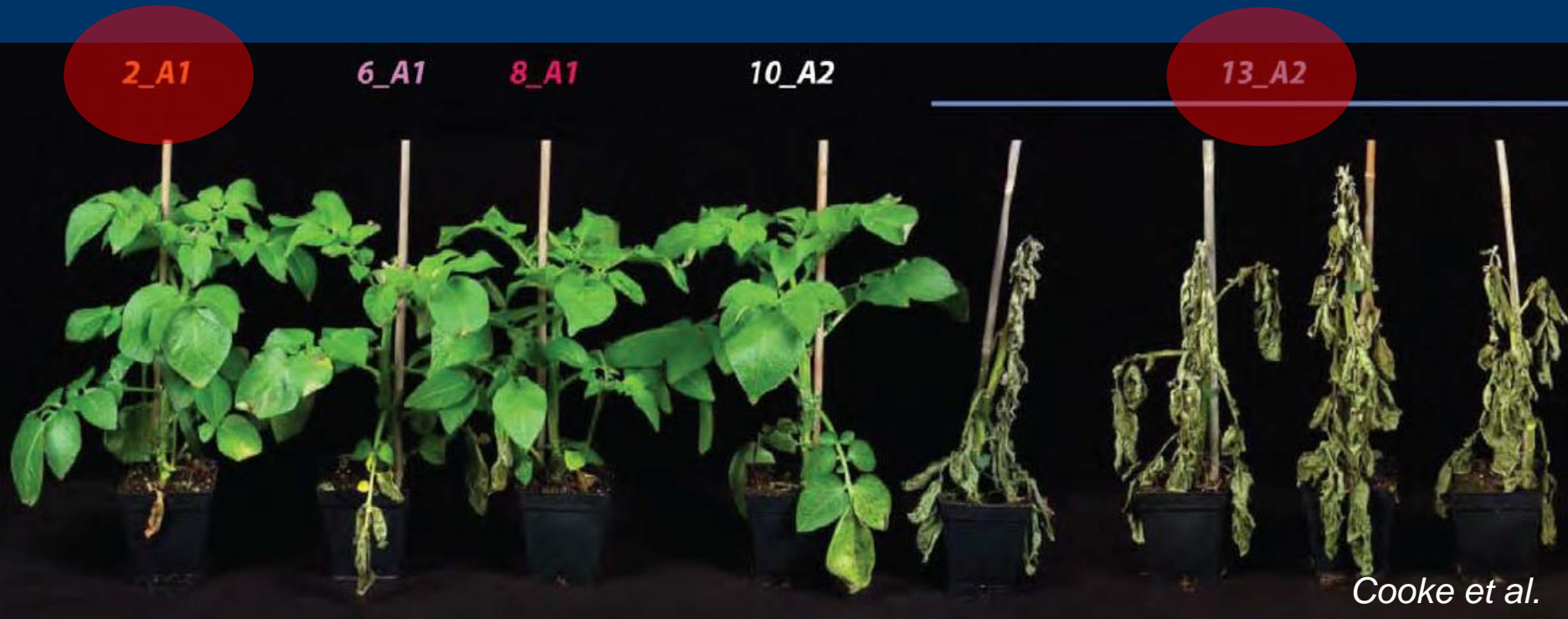
# Genotypic characterization of the Cyprus population of *P. infestans* using SSR's

|  | Cyprus (CUT)                  |
|--|-------------------------------|
| <b>No. of isolates</b>                     | 530                           |
| <b>Municipalities / locations / fields</b> | 5 / 17 / 49                   |
| <b>Isolates/year</b>                       | 2009: 120 ( <i>national</i> ) |
|  | 2010: 55 ( <i>national</i> )  |
|  | 2011: 355 ( <i>Paphos</i> )   |

# Genotypic characterization of the Cyprus population of *P. infestans* using SSR's

|  | Cyprus (CUT)            |
|--|-------------------------|
| <b>No. of molecular markers</b>              | 12                      |
| <b>Polymorphic markers</b>                   | 12                      |
| <b>Mean number of alleles/marker (range)</b> | 4,25<br>(2-10)          |
| <b>No. of genotypes</b>                      | 43                      |
| <b>Most polymorphic markers</b>              | D13 > G11 > SSR3, SSR4  |
| <b>Least polymorphic markers</b>             | Pi04, Pi70, SSR2, SSR11 |

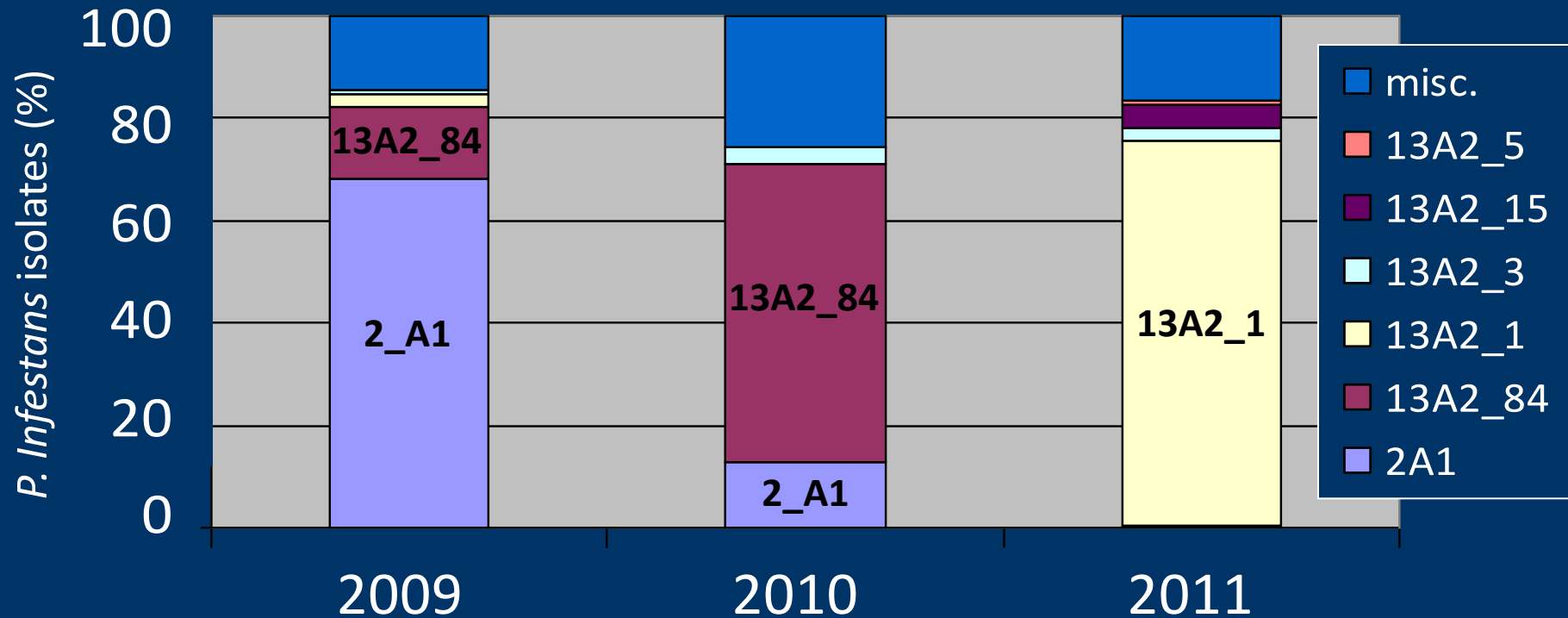
# Predominant genotypes of *P. infestans* in Cyprus



**13\_A2:** The most aggressive current genotype.  
First appeared in 2007 and sovereigns the majority of the European countries.

**2\_A1:** Less aggressive. Appeared in Europe around 1981.

# Genotypic characterization of the Cyprus population of *P. infestans* using SSR's

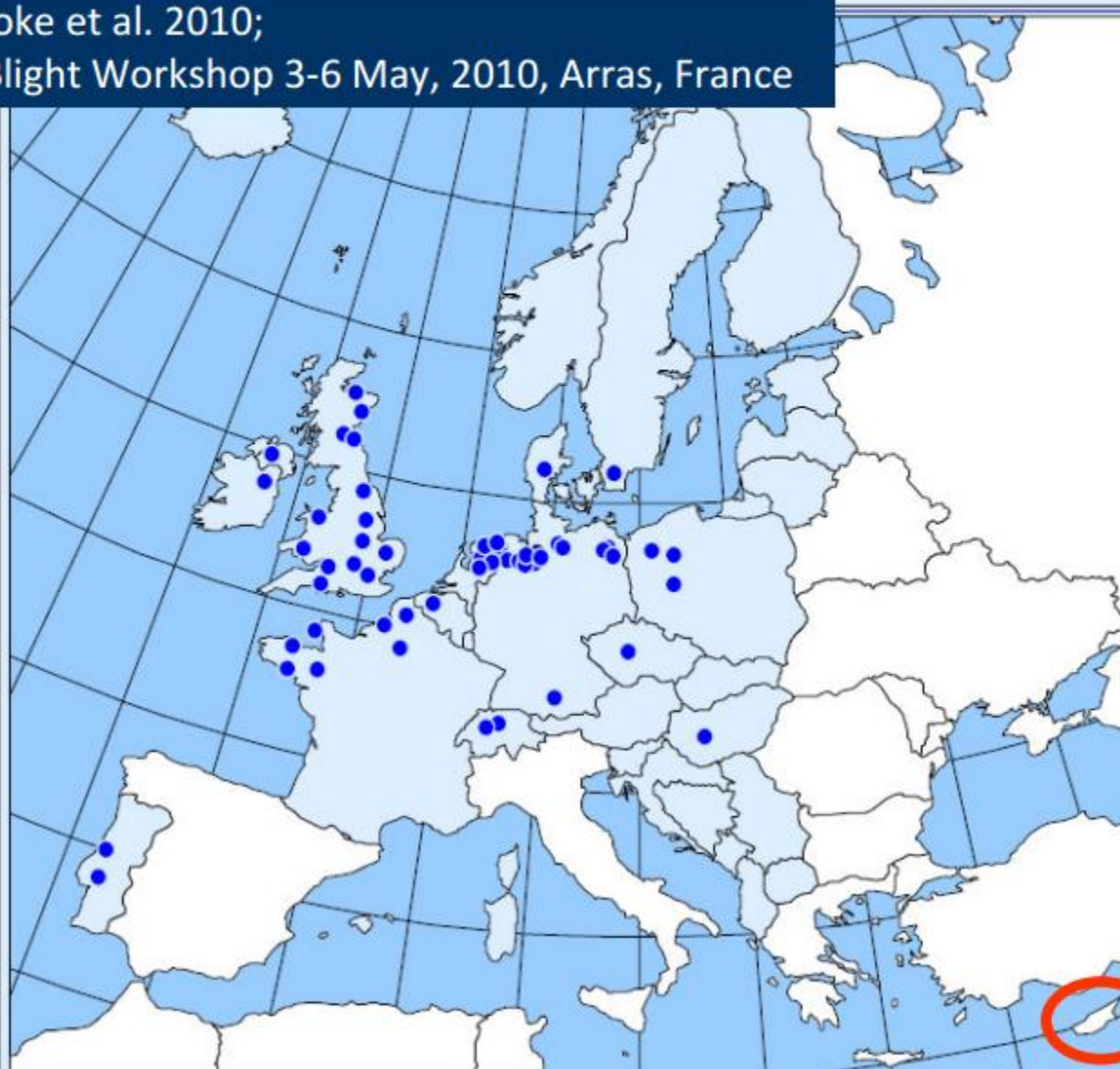


- 2009: 2\_A1; > 65% (old clonal lineage)
- 2010: 13A2\_84; >55% (a 13\_A2 variant)
- 2011: 13A2\_1; >75%

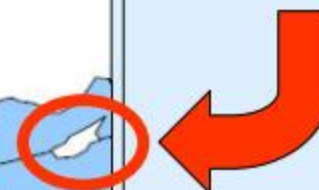
**Other genotypes: 23\_A1 and variants of 13\_A2 were also detected**



D. Cooke et al. 2010;  
EuroBlight Workshop 3-6 May, 2010, Arras, France



• New entry: CY







# Genotypic characterization of the Cyprus population of *P. infestans* using SSR's

- **2009 – 2010 sampling**

- 175 isolates
- Genotypes 2\_A1 and 13\_A2\_84 were predominant
- Six (6) variants of 13\_A2 were detected
- All the sensitive to metalaxyl-M isolates were of the 2\_A1 genotype

- **2011 sampling**

- 355 isolates (*Paphos*)
- Genotype 13\_A2\_1 was the predominant
- Twenty five (25) variants of 13\_A2 were detected

# Conclusions

- Both mating types exist in Cyprus.
- Compromised effectiveness of metalaxyl-M on Cyprus *P. infestans* populations.
- Two main genotypes were detected in Cyprus (2\_A1 and 13\_A2), 70-80% of the local population of *P. infestans* – **Low variability**.
  - **Lack of sexual recombination???**
- The highly aggressive genotypes of 13\_A2 constitute the main proportion of the local population.
- High variability within the 13\_A2 genotype (n=28) – **an interesting phenomenon**.

# Acknowledgements

## **The James Hutton Institute, UK**

- Dr. David Cooke
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## **Ministry of Agriculture, Natural Resources and Environment, CY**

- Dr. George Neophytou
- Pashalis Fellas



**Thank you for listening and  
welcome to Cyprus**



## **Cyprus University of Technology**

Faculty of Geotechnical Sciences and Environmental Management  
Department of Agricultural Sciences, Biotechnology and Food Science