

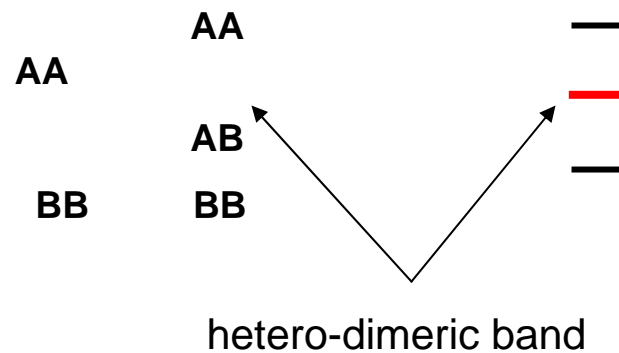
**Natural hybrids of resident and introduced *Phytophthora***

**species proliferating on multiple new hosts in Europe**

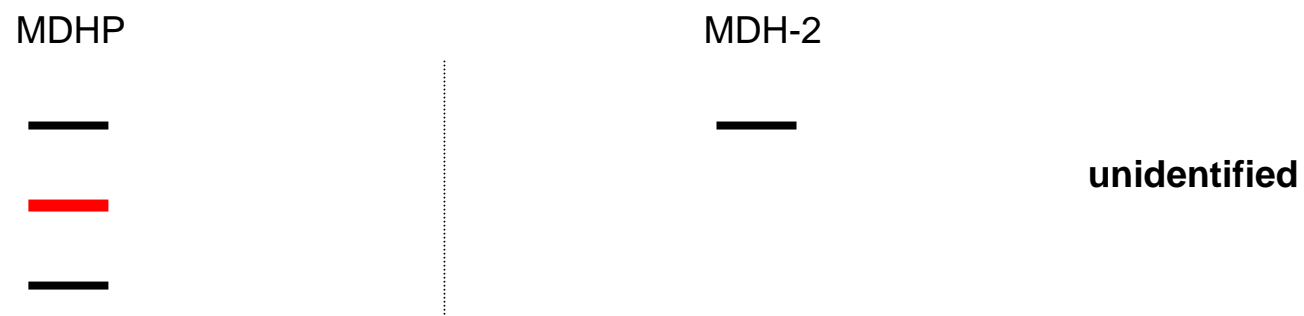
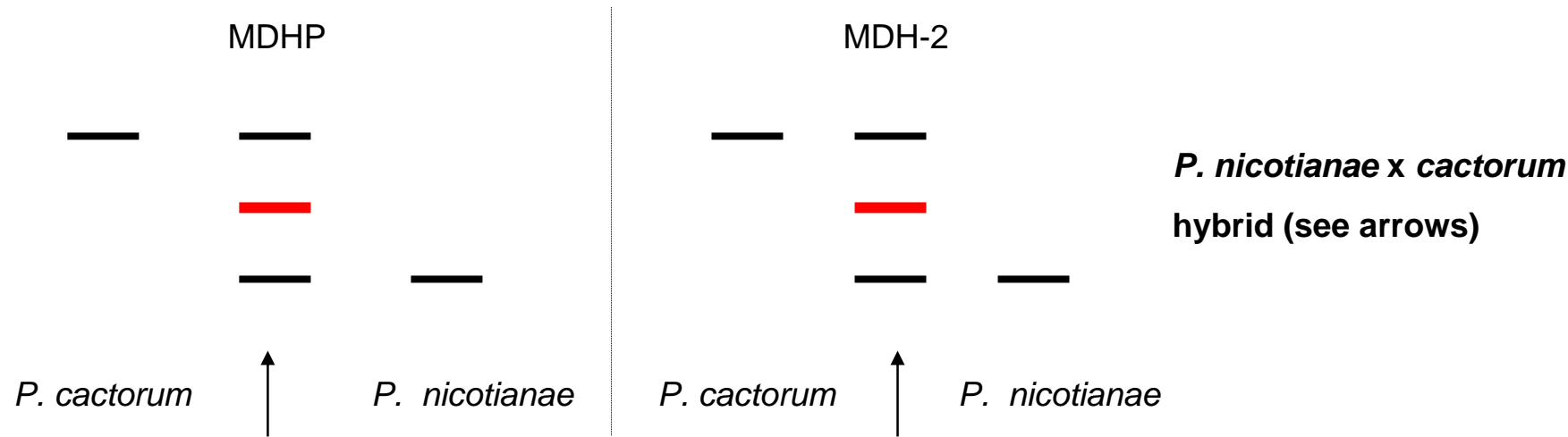
In 1996 some atypical *Phytophthora cactorum* strains were discovered:


atypical <i>P. cactorum</i>	<i>P. cactorum</i>
<ul style="list-style-type: none"><li>• Numerous abortive oospores</li></ul>	healthy
<ul style="list-style-type: none"><li>• Amphigynous and paragynous antheridia</li></ul>	paragynous
<ul style="list-style-type: none"><li>• Maximum growing temperature 35 – 36 °C</li></ul>	32 °C
<ul style="list-style-type: none"><li>• Heterozygous isozyme patterns</li></ul>	homozygous

Isozyme analysis of *Phytophthora* species using **dimeric** enzymes has the advantage over other molecular markers that it can detect crossings:



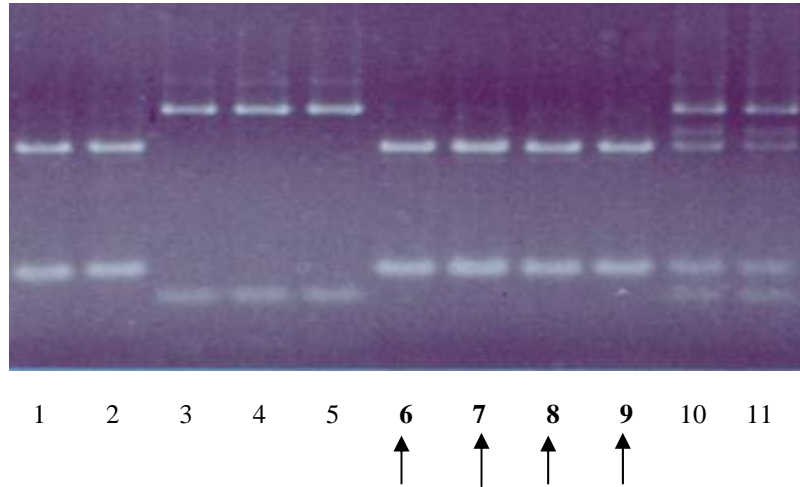
There were two groups of isozyme types:



 = hetero-dimeric band

Hypotheses about unidentified strains:

- intercrosses or backcrosses of hybrids to *P. cactorum*
- chromosome loss



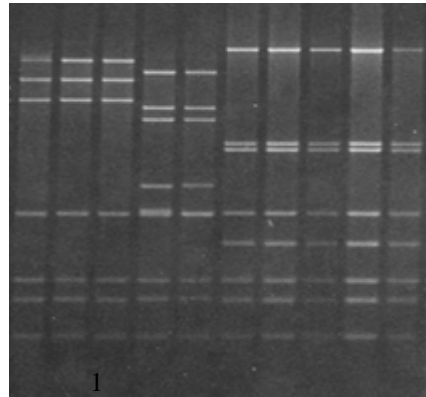
## Restriction enzyme analysis (Alu 1) of ITS.

1,2: *Phytophthora cactorum*

3,4,5: *Phytophthora nicotianae*

6,7,8,9: Unidentified (see arrows)

10,11: *Phytophthora nicotianae* x *cactorum* hybrids



1 2 3 4 5 6 7 8 9 10



Restriction enzyme patterns of mitochondrial DNA of  
*Phytophthora* spp. digested with Hind II

1, 2, 3: *P. cactorum*

4, 5: Unidentified (see arrows)

6, 7, 8: *P. nicotianae* x *cactorum* hybrids

9, 10: *P. nicotianae*

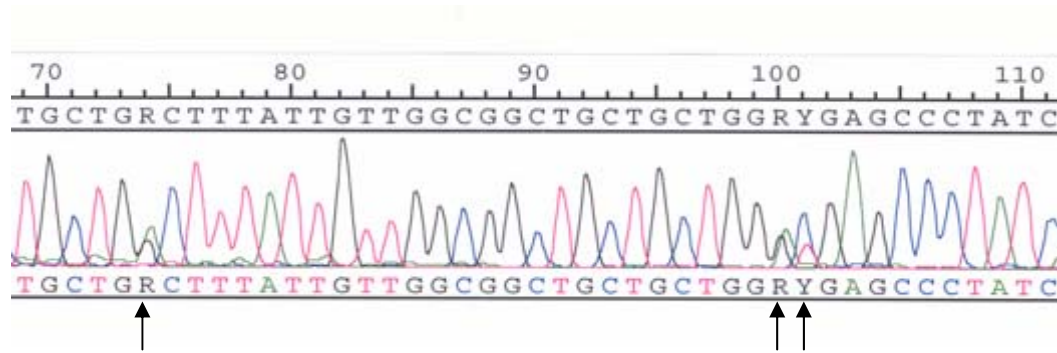
## Conclusion:

The unidentified *Phytophthora* is highly likely

related to *P. cactorum*,

but **not** to

*P. nicotianae* nor to *P. nicotianae* x *cactorum* hybrids.



Electropherogram of part of the ITS of the unidentified *Phytophthora* strains showing double bases at three positions (see arrows)

The ITS differed at the four positions, containing the double bases, from

*P. cactorum* and from the sequence of an unknown isolate.

This isolate was later described as *P. hedraiandra*

ITS variable positions

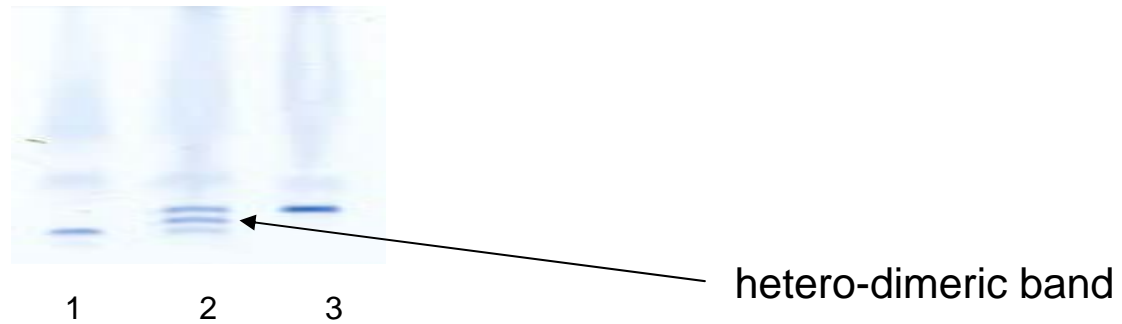
	74	100	101	686
<i>P. cactorum</i>	G	G	T	T
unidentified	R	R	Y	K
<i>P. hedraiandra</i>	A	A	C	G

$$R = A + G$$

$$Y = C + T$$

$$K = T + G$$

Cloning and sequencing of ITS indeed demonstrated the presence of sequences identical to *P. cactorum* and *P. hedraiandra*.



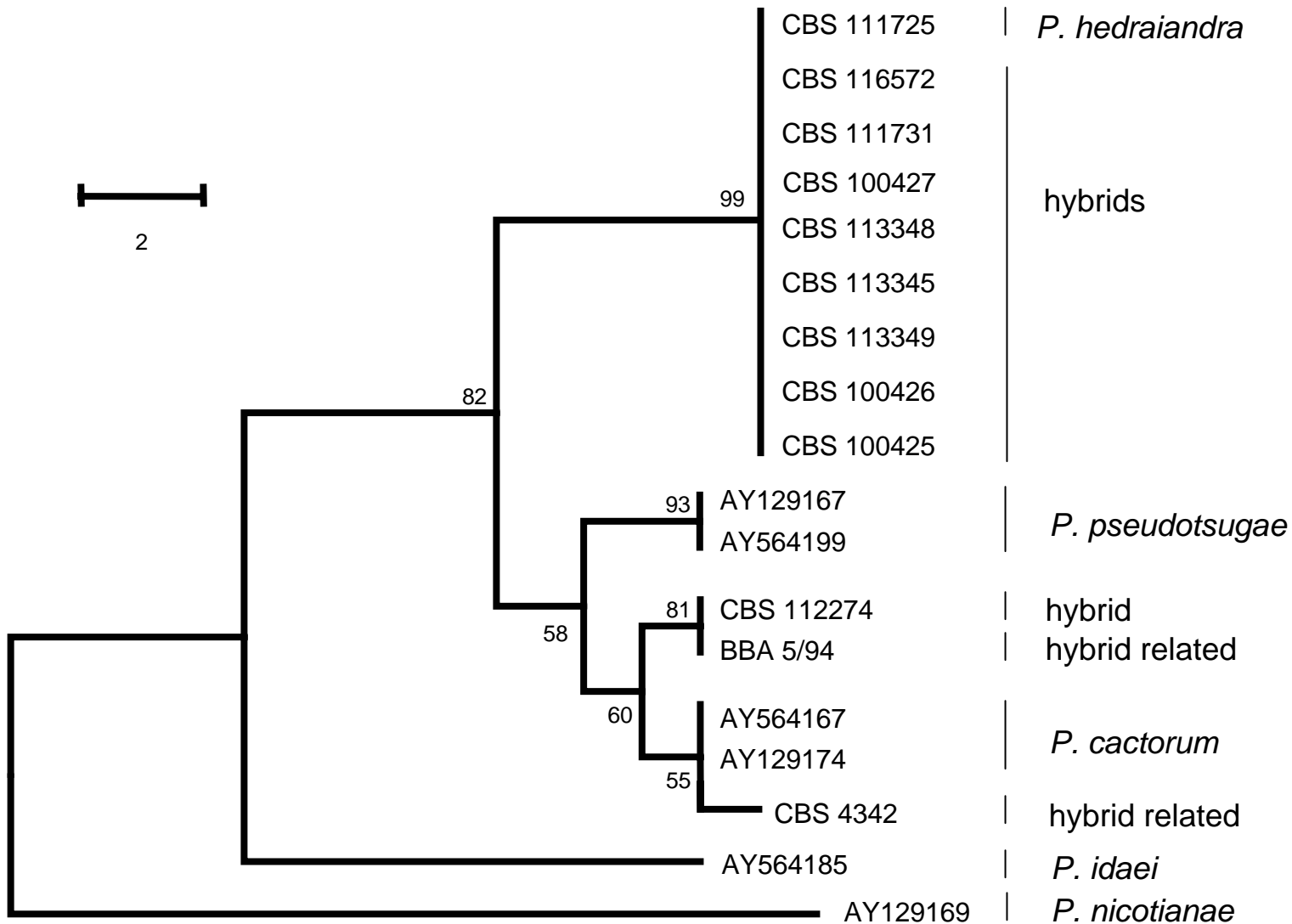
Isozyme patterns of malic enzyme (MDHP)  
generated by *Phytophthora* spp.

1: *Phytophthora hedraiandra*

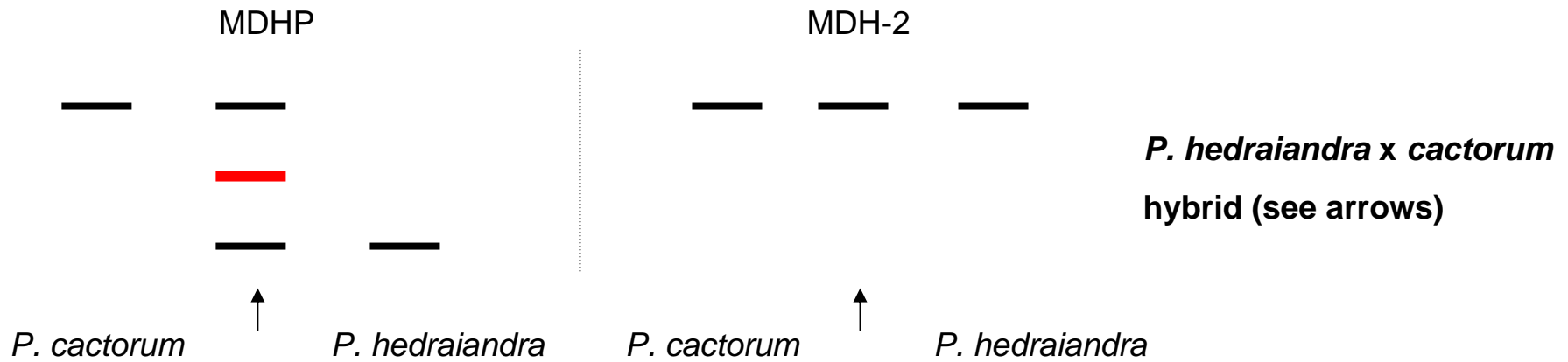
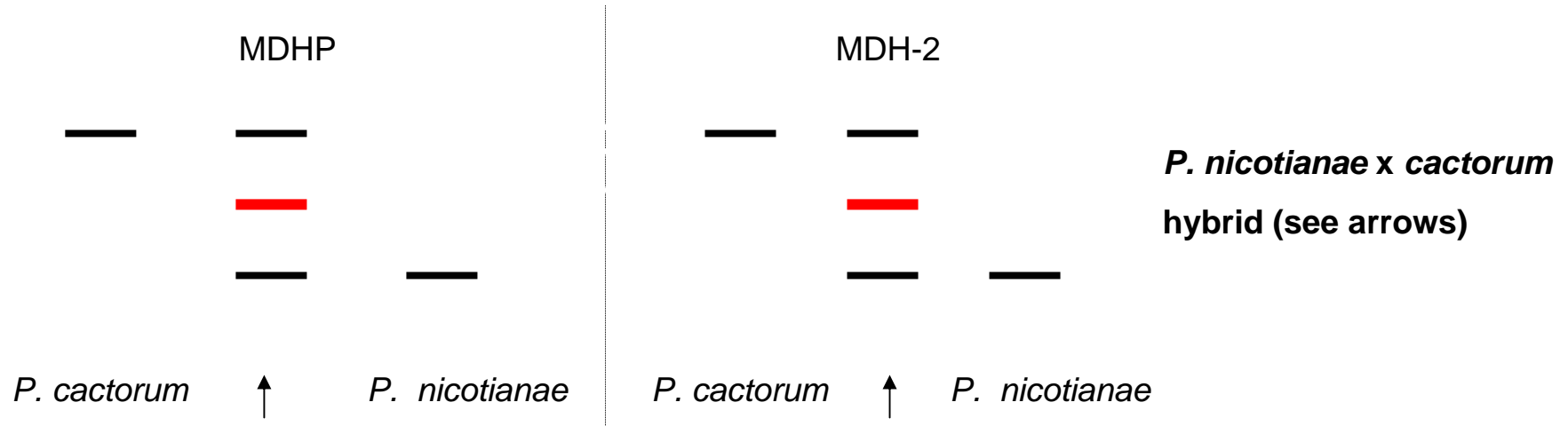
2: unidentified

3: *Phytophthora cactorum*

The atypical *Phytophthora* is a hybrid between *P. cactorum* and *P. hedraiaandra*



Phylogenetic tree, based on the cytochrome oxidase I (*Cox I*) gene of *Phytophthora hedraiandra* x *cactorum* hybrids and related species. Scale bar indicates 2 nucleotides.



 = hetero-dimeric band

*Phytophthora hedraiandra* is an **introduced** species:

- *P. hedraiandra* is never seen before 2001 in the Netherlands
- *P. hedraiandra* and *P.cactorum* have a common host in Europe, *Rhododendron*, and hence they can not have diverged in the same geographical area.
- There is no plausible explanation why the one resident species suddenly would start to hybridize with another “resident” species in the beginning of the 1990s

- The present hybrids are evolving.
- These hybrids are proliferating on multiple new hosts in Europe:

*Allium cepa*

*Penstemon*

*Allium porrum*

*Kalmia latifolia*

*Idesia*

- These hybrids are more successful than *P. hedraiandra* in the Netherlands:
  - ~ 30 hybrids and two *P. hedraiandra* strains
- Since 1996 no *P. cactorum* is found on *Rhododendron*, only hybrids



*Penstemon* suffering from stembase rot caused by the present hybrid

(courtesy Monica Heupel)

The present hybrids are found in:

Netherlands	Switzerland
Belgium	Slovenia
Germany	

*P. hedraiandra* is also found in:

Italy	USA
Spain	Australia
Slovakia	Japan
United Kingdom	

These hybrids can be expected in these countries in the future.

Arthur de Cock

Richard Summerbell

Willem Man in 't Veld