



**HAL**  
open science

## Development of sandwich ELISA and PCR-based assays for diagnosis of downy mildew in sunflower seed

Sandrine Bouterige, Veronique Says Lesage, Denis Tourvieille de Labrouhe, Valérie Molinéro-Demilly, J.P. Bouchara, P. Nicolas, Patricia Roeckel-Drevet, Raymond Robert

### ► To cite this version:

Sandrine Bouterige, Veronique Says Lesage, Denis Tourvieille de Labrouhe, Valérie Molinéro-Demilly, J.P. Bouchara, et al.. Development of sandwich ELISA and PCR-based assays for diagnosis of downy mildew in sunflower seed. 26th International Seed Testing Congress, Jun 2001, Angers, France. hal-04664151

**HAL Id: hal-04664151**

**<https://hal.science/hal-04664151v1>**

Submitted on 16 Aug 2024

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

See discussions, stats, and author profiles for this publication at: <https://www.researchgate.net/publication/283257145>

# Development of a sandwich ELISA and a PCR –based assays for the diagnosis of downy mildew in sunflower seed

Conference Paper · June 2001

CITATIONS

0

READS

25

9 authors, including:



**Véronique S Lesage**

French National Institute for Agriculture, Food, and Environment (INRAE)

19 PUBLICATIONS 215 CITATIONS

[SEE PROFILE](#)



**Denis Tourvieille de Labrouhe**

French National Institute for Agriculture, Food, and Environment (INRAE)

256 PUBLICATIONS 3,806 CITATIONS

[SEE PROFILE](#)



**Valérie Molinero-Demilly**

French National Institute for Agriculture, Food, and Environment (INRAE)

15 PUBLICATIONS 109 CITATIONS

[SEE PROFILE](#)



**Jean-Philippe Bouchara**

University of Angers

336 PUBLICATIONS 11,305 CITATIONS

[SEE PROFILE](#)

# Development of a sandwich ELISA and a PCR -based assays for the diagnosis of downy mildew in sunflower seed

Bouterige S.<sup>1</sup>, Says-Lesage V.<sup>2</sup>, Meliala C.<sup>3</sup>, Tourville de Labrouhe D.<sup>2</sup>, Molinéro-Demilly V.<sup>2</sup>, Bouchara J.P.<sup>1</sup>, Nicolas P.<sup>2</sup>, Roedel-Drevet P.<sup>2</sup>, Robert R.<sup>1</sup>

\*The contributions of the two first authors are equivalent.

## Introduction



Downy mildew repartition in France during the year 1999.

(Source SRPV Midi-Pyrénées)

■ Race 710   
 ■ Races 710 and 703   
 ■ Race 703

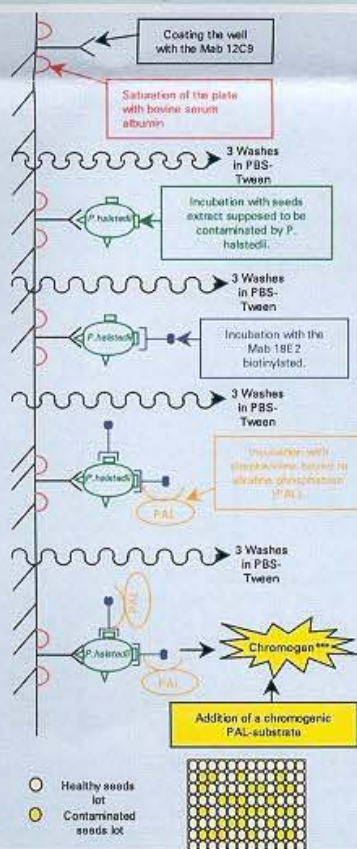
The downy mildew of sunflower, caused by *Plasmopara halstedii* (Farlow) Berlese et de Toni, is a widespread and destructive pathogen known to occur in almost all areas of intensive sunflower cultivation. Typical symptoms include chlorosis of the leaves, often associated with severe stunting of systemically infected plants, and various types and degrees of leaf distortion. Infected plants were shown to have higher respiration rate and higher enzyme activity as compared with healthy plants. The effect of this pathogen has considerable economic implications. Owing to the risk of propagation of the disease by infected seeds and to the regular description of metalaxyl-resistant isolates of the fungus, there is a greater need for efficient methods to detect the fungus in seeds or plant tissues. Therefore, we developed two detection methods, one based on monoclonal antibodies, the other on Polymerase Chain Reaction.

In the first method, we produced two monoclonal antibodies (MAbs) by immunization of mice with a partially purified extract of *P. halstedii* race 100. Using ELISA technique, both MAbs detected all races of *P. halstedii* present in France, but not the other molds pathogenic to sunflower or encountered as saprophytes on the seeds. The specificity of these MAbs for *P. halstedii* and their recognition of distinct epitopes on the fungal antigens were used to develop a sandwich ELISA detection system. Incubation of seed or tissue homogenates from infected plants in wells coated with MAb 18E2 resulted in the trapping of *P. halstedii* antigens that were identified with biotinylated MAb 12C9.

The second method using PCR was developed using specific primers designed from the nucleotide sequence of one cloned random amplified polymorphic DNA fragment. Agarose gel electrophoresis stained with ethidium bromide revealed a 1038-bp amplification product for various races of *P. halstedii*. No staining was seen with DNA from other saprophytic or pathogenic molds, nor with the host plant DNA.

We analyzed thirty seed lots: fifteen were checked for the presence of *P. halstedii* and fifteen others for the absence of the pathogen with optical microscopy. The aim of this study was to compare the immunological and the PCR-based techniques for the detection of downy mildew in sunflower.

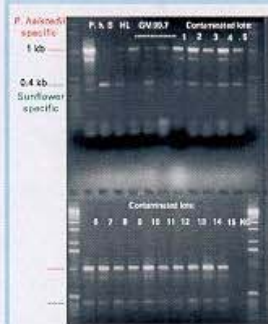
## ELISA procedure



## PCR procedure

DNA was extracted from 500 mg of whole seed, with a combined

method using Dellaporta (Plant Mol Biol Rep, 1983) and Amersham (RPN8511) procedures, and a further purification step to eliminate PCR inhibitors present in the seed. Thirty amplification cycles were conducted with an annealing temperature of 60 °C, and two sets of primers: one set was specific of all races of *P. halstedii*, the other one was sunflower specific, for an internal amplification control.



P. h.: *Plasmopara halstedii*  
 S: Sunflower (healthy leaves)  
 HL: Healthy seed lot  
 GM: contaminated lot as positive control  
 NC: Negative Control (water)

For PCR, results showed two amplified fragments for all the contaminated lots. The highest, around 1 kb long, and the lowest, around 400 bp, were *P. halstedii* and sunflower specific respectively. The 400-bp fragment was the only one amplified both in healthy seeds and leaves. The negative control showed no amplification.

## Results of the two methods

Seed lots	ELISA Test		PCR Test	
	DO value	Results	Results	Results
Contaminated lots				
Lot 1	0,279	+	++	++
Lot 2	0,382	+	+++	+++
Lot 3	0,353	+	++	++
Lot 4	0,261	+	+++	+++
Lot 5	0,570	+++	++	++
Lot 6	0,556	++	++	++
Lot 7	0,508	++	++	++
Lot 8	0,510	++	+	+
Lot 9	0,416	++	++	++
Lot 10	1,613	+++	+++	+++
Lot 11	0,554	-	-	-
Lot 12	0,573	-	+	+
Lot 13	1,234	+++	+	+
Lot 14	1,074	+++	++	++
Lot 15	0,402	++	+	+
Healthy lots				
15 lots	< 0,195	-	-	-
Negative control	0,195	-	-	-

For the ELISA test, the results showed that the lots corresponding to healthy plants presented a negative response (inferior to the negative control). Conversely, the lots corresponding to contaminated plants showed, for all of them, a positive response (superior to the negative control). The statistical study, performed by the non-parametric test of Mann-Whitney, showed that the difference obtained between lots, coming from infected and healthy plants, are highly significant for  $p < 0.01$ .

## Discussion

that we can expect in commercial seed lots. We have made, therefore, further PCR studies on various lots provided by the phytosanitary administration of the Midi-Pyrénées region. Downy mildew was detected in lots containing 10 % of seeds taken from plants showing downy mildew symptoms. In conclusion, these methods may be used to guarantee the phytosanitary quality of seeds, and therefore may help reduce the incidence of seed-borne disease transmission.

Finally, both methods allowed the detection of the fungus in all infected seed lots. However, contamination in these lots varied from 70 to 100 %, which was probably much higher than

<sup>1</sup> Groupe d'Étude des Interactions Hôtes-Parasites  
Laboratoire d'Immunologie-Parasitologie  
Faculté de Pharmacie, 16 Bd Daviers, 49100 Angers

<sup>2</sup> INRA - Université Blaise Pascal  
UMR Amélioration et Santé des Plantes  
24 Avenue des Landais, 63177 Aubière, France

<sup>3</sup> GEVES-SNES  
Station Nationale d'Essais de Semences  
rue G. Morel, BP 24, 49071 Beaucazoué Cedex, France