Determining the sunflower downy mildew risk by soil analysis

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ABSTRACT

A bioassay using a soil sample was developed for assessing downy mildew risk at the field level. The results were correlated with the rate of infected plants when no other limiting factors were observed. The first tests carried out in fields in 2007 allowed the evaluation of soil infestation and seemed to confirm that inoculum could usefully be reduced by crop rotation. Moreover, the bioassay was used to follow the evolution of soil infestation during the spring. It reached a maximum around April 15th under the French conditions. The test conditions had very little effect on the results, so, hopefully. a large-scale use could easily be developed. For less infested soil, the direct characterization of the pathogen population's virulence profiles was not reliable. However, this protocol allows us to obtain fresh inoculum even when susceptible species are not present in the field, which makes it possible to achieve the characterization of the races after isolate multiplication. The interest of this protocol for the management of control methods is discussed.

Key words: bioassay – downy mildew – epidemiology – *Helianthus annuus* – *Plasmopara halstedii* – risk analysis – soil infestation.

RESUME

Afin d'évaluer le risque «mildiou du tournesol» en parcelles agricoles, un biotest réalisé sur un échantillon de terre a été mis au point. Les résultats obtenus sont corrélés avec les taux de plantes malades observés en absence d'autres facteurs limitants. Les premiers essais de ce protocole en parcelles agricoles en 2007 ont permis d'évaluer le potentiel infectieux du sol et semblent confirmer l'intérêt d'un allongement des rotations pour limiter l'inoculum. Ce biotest a également montré son utilité pour suivre l'évolution du potentiel infectieux durant le printemps. Ce potentiel passe par un maximum qui se situe, dans les conditions françaises, autour du 15 avril. Les résultats obtenus sont relativement peu influencés par les conditions de réalisation du test, ce qui laisse espérer une généralisation aisée. Pour les terres peu contaminées, la caractérisation directe du profil de virulence de la population parasitaire n'est pas fiable. Cependant, ce protocole permet l'obtention d'inoculum frais, même en absence d'espèce sensible sur la parcelle, ce qui permet ensuite de mettre en œuvre la caractérisation des races présentes après multiplication de l'isolat. L'intérêt de ce protocole pour la gestion des méthodes de lutte est discuté.

Mots clés: analyse de risque – biotest – épidémiologie – *Helianthus annuus* – mildiou –*Plasmopara halstedii* – potentiel infectieux

INTRODUCTION

Plasmopara halstedii is mainly a soilborne plant pathogen which can survive as oospores from one year to the next (Tourvieille de Labrouhe et al., 2000). This kind of conservation, which results from the sexual reproduction, allows the survival of the pathogen for several years waiting for a susceptible culture. Among arable crops, only sunflower is susceptible to *P. halstedii*. However, some *Asteraceae* known as weeds could harbor the pathogen and enhance the inoculum reservoir. Under favourable conditions, oospores in the soil can germinate and give rise to a zoosporange which releases mobile zoospores in free water. These zoospores are responsible for the primary infection, which is the most harmful form of the disease. If the level of risk depends on the weather conditions, in parallel, quantitative and qualitative (pathotypes) aspects of the inoculum are essential to explain the severity of attacks. In order to understand the evolution of downy mildew risk and also be able to make a diagnosis

of fields before sowing, we have developed a bioassay based on soil sampling. The principle has already been published (Tourvieille and Walser, 2005) and it has served to show the relationship between the presence of downy mildew in a field and the risk for the next sunflower crops. Moreover, this device seems to be of interest for predicting the behaviour of various sunflower hybrids against the endogenous pathogen population. The article presents experiments using this protocol and whose aims were to specify: i) the link between level of soil infestation and downy mildew risk; ii) the evolution of the infestation level of the soil during spring and iii) the possibility of using this protocol in a regional management of the downy mildew risk. It is not certain that downy mildew finds favourable conditions for its expression because of environmental conditions and/or absence of susceptible plants. For this reason, with a large scale study, on the level of a pilot site, we wanted to know if the protocol of soil bioassay could be a decision-making aid in the management of control methods.

MATERIALS AND METHODS

Plant material: The open-pollinated line Peredovik, without any known resistance gene, was used to quantify the infestation level of the soil or to estimate the disease incidence in fields. The virulence profiles of *P. halstedii* populations were determined using a set of nine international differential host lines (D1 to D9) (Gulya et al., 1998).

Test in culture: Experiments were carried out in plots of calcareous clayey loam soil located in Limagne (Centre of France) under a continental moderate climate. To assess the downy mildew risk independently of the climatic conditions, a contamination of plants before emergence was performed ensuring a very important irrigation (≈ 100 mm) when the root of the seedlings reached a size ranging between 0.5 and 1.0 cm length (Vear et al., 2007). The number of infected plants was observed at the stage "appearance of the second pair of leaves". Plants with systemic symptoms of downy mildew resulted from a telluric primary infection.

Soil bioassay: Experimental soils were collected in the seed bed at the sowing period in each field by focusing on low ground locations or headlands. The soil samples were directly placed in pots (30 cm x 30 cm x 6 cm). Two hundred seeds of a trap genotype Peredovik or 10 seeds for each of the nine differentials were sown in each pot, covered by 1 cm of soil and grown at 18°C. After 48 hours, which was the time required for obtaining germs from 0.5 to 1.0 cm in length, each pot was separately immersed in water during 8 to 12 hours. Then the pots were maintained at 18°C with a 16 h photoperiod (12 000 Lux) per day. After 12 days, sporulation was induced by covering the infected seedlings with a plexiglass cap or a transparent plastic bag (PEBD 50µm) for 48 hours to provide a saturated humidity (Tourvieille de Labrouhe and Walser, 2005).

Choice of the fields for the study in a pilot site: Fields were chosen according to 3 factors: i) downy mildew history: the whole of the fields had already expressed downy mildew during the last 3 years or were located in an area where downy mildew was usually observed, ii) there was a delay between two sunflower crops (1 year, 2 years, 3 years and more) and iii) the type of soil (calcareous clay, clayey silt, silt like “boulbène”). Ten fields located in the departments of Gers and Tarn-et-Garonne (South-western France) were finally selected. A soil sampling was performed in each field. In 3 field plots, the same bioassay was carried out in a ring-test between 3 laboratories under variable conditions (Table 1), and in 4 field plots, two methods of sampling were studied: 4 independent samples of 4 liters taken from 4 points in the field were compared with a pooled sample made with 1 liter of soil taken from the same 4 points.

Table 1. Experimental conditions in the different laboratories carrying out the soil bioassay.

	Lab 1	Lab 2	Lab 3
Delay between soil sampling and seed sowing	72 h	48 h	120 h
Light	12 000 lux (neon)	day light	day light
Temperature	18°C ± 1°C	uncontrolled (-)	uncontrolled (-)
Time of immersion	17 h	8 h	8 h
Time of saturated humidity	48 h	66 h	45 h

In addition, we tried to characterize the virulence of the pathogen population by carrying out the bioassay directly with the 9 differential host lines in 4 fields. Results were confronted with the

characterization of the virulence profile according to the classical method using an infected plant collected in the field as the inoculum source (Tourvieille de Labrouhe et al., 2000).

RESULTS

Relationship between the response of the soil bioassay and the disease incidence in the field: On small plots (≈ 70 m²) known to be infested by *P. halstedii,* soil samples were analyzed and downy mildew incidence was observed *in situ* in 2006 and 2007. Observations showed a close relationship between the number of seedlings with symptoms of downy mildew from the soil bioassay and the number of infected plants observed in the year of sampling (Fig. 1).

The relationship between the disease incidence in the field and the rate of infected seedlings as a result of the soil bioassay was quite similar in spite of the differences in the disease pressure, which was 54.5% in 2006 and 16.3% in 2007. The correlation coefficient of the 12 data pairs was highly significant (r=0.917).

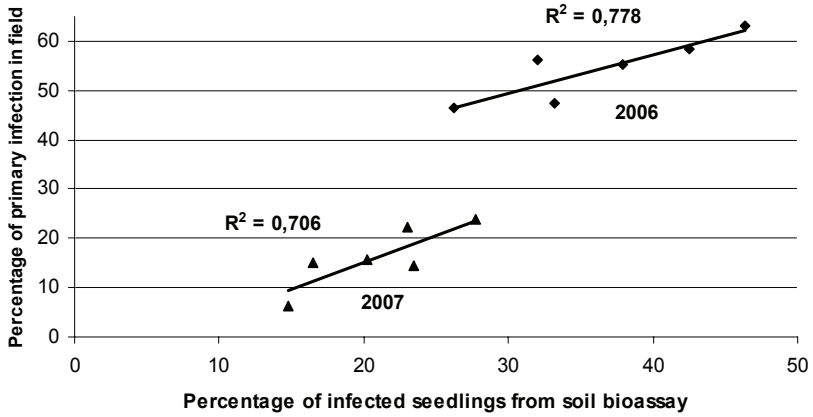

Fig. 1. Relationship between primary infection of downy mildew in the field and infection of seedlings from the soil bioassay.

Infestation of a field plot according to the farming past: On small plots followed for many years, the rate of infected seedlings given by the bioassay was correlated with the number of infected plants observed the previous years. The best correlation was obtained when infected plants were grown the previous year (y-1) or 2-years before (y-2) (Fig. 2).

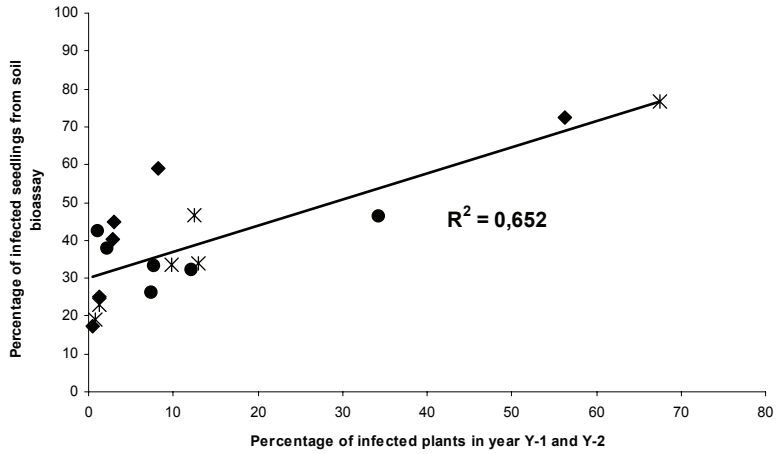

Fig. 2. Relationship between downy mildew incidence in the field observed 1 and 2 years before the soil bioassay and the rate of infected seedlings from the soil bioassay (◆ 2004, ❄ 2005, ● 2006**).**

The rates of infected seedlings assessed by the soil bioassay varied from 14.8% to 76.8% and were on average 43.0% in 2004, 38.7% in 2005 and 36.4% in 2006. These rates were highly correlated with the

downy mildew incidence observed in the field the two previous years (y-1 and y-2). The downy mildew incidences varied from 0.4% to 67.5% and were on average 12.0% in 2004, 17.5% in 2005 and 10.8% in 2006. So the relationship between the soil infestation measured by the soil bioassay and the presence of infected plants the previous years is confirmed in this experiment.

Use of the soil bioassay for measuring the evolution of the soil infestation: To appreciate the evolution of the soil infestation during the whole period of sunflower sowing, soil samples were collected once per week, from March to May. This experiment was carried out in the site of Clermont-Ferrand in 2006 (April-May: mild and humid conditions with soil average temperature ~15°C and sum of precipitations = 178 mm) and in 2007 (April-May: warm and dry conditions). Ten micro-plots were analyzed weekly. Results and the adjusted curves are presented in Fig. 3.

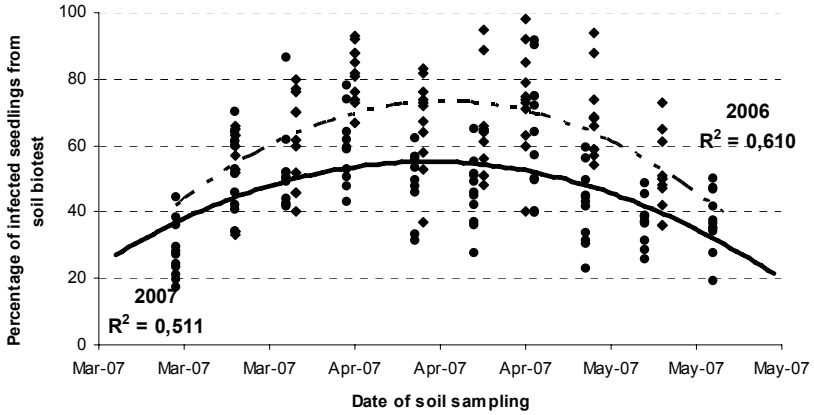

Fig. 3. Evolution of the proportion of infected seedlings given by the soil bioassay according to the date of soil sampling (10 samples per date) in 2006 (◆) and 2007 (●).

Under the environmental conditions of the Centre of France, the best sampling date for assessing the soil infestation appeared to be in mid-April. If the levels of infection seemed to be dependent on the weather conditions of the year (65.1% on average in 2006 and 46.7% on average in 2007), the data corresponding to the maximum of primary infection appeared to be fairly constant in both years.

Application of methodology in a pilot site: The 10 field plots of the pilot site can be classified in 3 classes (Table 2):

- slightly infested: Les Mariettes, Le Carascau and Sarrault with less than 10% of infected seedlings.
- moderately infested: Utaut and Janicaut with less than 25% of infected seedlings.
- strongly infested: La Poëte, Le Rauy, Bordeneuve and La Plèche with more than 30% of infected seedlings.

The comparison of the results from different laboratories showed a very good repeatability for 2 field plots, “La poëte” and ‘Utaut”. In contrast, the two analyses of the plot "Le Rauy" appeared to be rather contrary (Table 2). Moreover, information on the soil of the field plot "Les Barbès" could not be given due to the absence of the emergence of sunflower during the bioassay.

In the 4 field plots where two methods of sampling were tested, the levels of response varied from slightly infested (Le Rauy) to very strongly infested (La Poëte). Differences between the individual samples suggest variability in the soil infestation of the field plot (Table 3). It must be noted that the pooled sample did not correspond to the mean of the 4 independent samples and the mean rate was always the weakest.

Table 2. Percentage of seedlings of a susceptible genotype presenting symptoms of downy mildew in soil bioassay according to the location of sampling and analysis

Location	Type of soil	Laboratories		
		Lab 1	Lab 2	Lab 3
Les Barbes	Silt «Boulbène»	?	-	-
Les Mariettes	Silt clay	6.8%	-	-
La Poëte	Calcareous clay	59.4%	65.6%	62.6%
Utaut	Calcareous clay	21.4%	21.6%	22.2%
Le Rauy	Silt clay	37.7%	8.5%	-
Bordeneuve	Calcareous clay	31.3%	-	-
Le Carascau	Calcareous clay	6.3%	-	-
La Plèche	Calcareous clay	36.4%	-	-
Sarraut	Calcareous clay	3.6%	-	-
Janicot	Calcareous clay	14.3%	-	-

Table 3. Percentage of infected seedlings from soil bioassay according to the method of sampling

Location	Pooled sample	Point 1	Point 2	Point3	Point 4
Les Mariettes	2.4%	2.4%	13.8%	12.1%	3.1%
La Poëte	33.3%	67.7%	80.1%	50.0%	82.1%
Utaut	16.7%	18.8%	22.2%	14.3%	39.3%
Le Rauy	3.8%	12.8%	5.6%	15.4%	5.1%

When the virulence profile was determined by using the soil bioassay, rates of infected seedlings were very low, although the results were not in contradiction with results given by the classic test (Table 4)

Table 4. Characterization of virulence profile of the population of *P. halstedii* in the soil (soil bioassay = a) and of a sample taken on an infected plant (classic test = b).

Location	Test	For each differential: Number of infected seedlings / Number of emerged seedlings									Profile
		D1	D2	D3	D4	D5	D6	D7	D8	D9	
Les Barbes	a [(1)]	*0/2*	*0/3*	*0/2*	*0/1*	*0/0*	*0/8*	*0/2*	*0/0*	*0/1*	?
	b	**7/10**	**7/10**	**8/10**	*0/10*	*0/10*	*0/10*	**5/10**	**7/10**	*0/10*	703
Utaut	a	**8/19**	**16/29**	**1/19**	*0/16*	*0/22*	*0/18*	**2/20**	**1/19**	**14/29**	707 [(2)]
	b	**3/10**	**7/10**	1/10	*0/10*	*0/10*	*0/10*	*0/10*	*0/10*	**2/10**	304 ?
La Poëte	a	**6/18**	**26/30**	**4/21**	*0/22*	*0/19*	*0/20*	**10/31**	**9/29**	**11/20**	707 [(2)]
	b	**1/9**	**6/10**	*0/10*	*0/10*	*0/10*	*0/10*	*0/10*	*0/10*	**4/10**	304
Janicot	a	**3/12**	**2/11**	**2/13**	*0/11*	*0/10*	*0/12*	**1/12**	**3/10**	*0/10*	703
	b	**9/9**	**10/12**	**6/13**	*0/10*	*0/8*	*0/2*	**5/7**	**3/6**	*0/5*	703

[(1)] asphyxia. [(2)] or mixture of pathotypes (304 + 703).

DISCUSSION

Results obtained on small plots clearly showed the interest of analysing the soil infestation using a soil bioassay since its response was well correlated with downy mildew risk observed in the absence of limiting weather factors. The soil bioassay also allowed us to confirm the close relationship between the mildew history of a field plot and the level of infestation. This is easily explained by the fact that the pathogen is maintained from one year to the next by oospores, which are produced in infected tissues of sunflower (Sackston, 1981). The quantity of oospores is therefore directly related to the number of infected plants. Consequently, short crop rotations are prohibited by recommended measures for control of downy mildew, especially when the presence of the disease is detected (Moinard et al., 2006).

The soil bioassay also allowed us to follow the evolution of soil infestation during the sunflower sowing period in spring. It was demonstrated that soil infestation reached a maximum in mid-April under French conditions. This evolution has to be connected with the weather conditions. These become favourable to the pathogen at the end of winter, inducing a break in soil infestation due to the short lifetime of the zoospores after germination (Goossen and Sackston, 1968). These results could lead to two interesting prospects: i) sowing as soon as possible so that sunflower emergence can escape the favourable periods to downy mildew infection; but this would mean selecting hybrids resistant to cold temperatures and ii) carrying out all experiments of selection (Vear et al., 2007) or screening of molecules (Délos et al., 2000) in April under conditions favourable to the pathogen.

It is always difficult to assess soil infestation of a field plot. When it was possible to analyze either several samples of the same plot or a pooled sample representative of the plot, the pooled sample was always less infected than independent samples. This could be explained by the quantity of sampled soil.

Indeed, this quantity from each sampling point for a pooled sample is less important than the soil quantity which is necessary for an independent analysis. Also, in the first case, soil is not always taken from the whole horizon corresponding to the seed bed. But it is also possible that the lifetime of inoculum could be very low in the upper layer of the soil where more drastic climatic conditions could occur. This leads to recommending sampling soil from the -2cm to -8cm horizon for a more effective soil analysis.

In 2007, the use of resistant sunflower hybrids in the field plots did not enable us to confirm the relation between soil infestation and disease incidence, despite quite favourable weather conditions for downy mildew. Neither did we notice links between type of soil and level of infestation. Nevertheless, it was demonstrated that the protocol was not adapted in the case of silt loam like "Boulbène" because immersion caused a packing of soil and a lack of seed germination. In this case, it would be possible to recover inoculum by percolation and to use this more or less infested water to perform the watering of seeds in uninfested substrate. For the other types of soil, bioassay results indicated that the different test conditions in the three laboratories had little influence, but this should be confirmed under less favourable conditions (e.g. higher temperatures).

Direct characterization of the virulence profile has been seen to have its limits. Its sensitivity depends on the level of soil infestation, which must be high enough to guarantee the infection of susceptible differential hosts. Its specificity is also limited because it uses only nine differentials, which is not enough to determine the virulence profile of a mixture of pathotypes. Moreover, variability in a field plot could only be measured by numerous independent analyses. However, the soil bioassay could potentially be used to investigate the downy mildew risk of the variety to be sown in the field plot.

In the framework of risk management, the soil bioassay shows potential interests:

- determining soil infestation of a field plot in a given year,
- achieving virulence profiles present in the field plot, even in the absence of susceptible sunflower,
- following the evolution of pathogen population in quantitative and qualitative terms in comparison with the means of disease control,
- finally, adapting the means of genetics and chemical control according to the risk in the field plot.

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