SUNFLOWER DOWNY MILDEW RESISTANCE GENE PYRAMIDING, ALTERNATION AND MIXTURE: FIRST RESULTS COMPARING THE EFFECTS OF DIFFERENT VARIETAL STRUCTURES ON CHANGES IN THE PATHOGEN

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Abstract

Changes in the virulence of a *Plasmopara halstedii* population have appeared after three years cropping of a single variety in which several monogenic resistances (*Pl* genes) were pyramided, whereas no new virulences have been observed when resistance genes were alternated in three successive years or when a multi-hybrid with different plants carrying different resistance genes was grown three years running.

Résumé

Le suivi de l'évolution des virulences dans une population de *Plasmopara halstedii*, sous différents systèmes de gestion des gènes *Pl* de résistance monogénique du tournesol vis-à-vis du mildiou, montre une évolution rapide de celles-ci dans les différents cas de pyramidage des gènes dans un seul génotype. Par contre, aucune nouvelle virulence n'a été trouvée après trois cycles de « culture » des gènes en alternance ou en mélange.

Introduction

Downy mildew of sunflower, caused by *Plasmopara halstedii* (Farl.) Berl. and de Toni is controlled by major resistance genes denoted *Pl*. In France the first resistant hybrid grown was Remil, registered in 1973. Most of the sunflower crop was resistant by 1980, with varieties carrying *Pl1* or *Pl2*. In 1974, Zimmer and Fick observed that the downy mildew races present in Europe and in the US were not the same; *Pl1* controlled only race 100 (Europe) whereas *Pl2* controlled both races 100 and 300 (US). In Europe, no changes were reported until 1988, whereas there was continuous evolution of downy mildew in the United States (Gulya et al., 1991). Resistance to *P. halstedii* is monogenic, dominant and complete, so that it appears to be

a typical example of "vertical resistance." Following observations of race changes, research has been done, both in the USA and in Europe, for new resistance genes. About 10 genes have now been reported (Pustovoït et al., 1976; Vranceanu et al., 1981; Miller and Gulya, 1991; Vear et al., 2004) and they are widely used in sunflower varieties. This is true especially in France, where there is quite frequent rainfall in spring, on the best sunflower sowing dates, which would favour downy mildew infection on susceptible varieties (Délos et al. 2000). Since the observation of downy mildew isolates resistant to the only efficient chemical seed treatment (Lafon et al., 1998), use of genetic resistance has become an essential part of sunflower breeding programmes.

An analysis of changes in pathogen races and of the use of different resistance genes in sunflower varieties in France has shown that after a long period of stability, introduction of foreign downy mildew isolates caused a considerable upset in genetic control of the disease (Tourvieille, 2003). Following this introduction, almost exclusive use of two genes (*Pl6* and *Pl7*) appears to have created the conditions which favoured the multiplication of isolates with new virulences. The identification since 2000 of races (for example 304) (Penaud et al., 2003), able to grow on sunflower varieties carrying *Pl* 6 or *Pl* 7 (Table 1), may well lead to the widespread utilisation of one or two other resistance genes (*Pl5, Pl8*, etc.) without forethought for the probable consequence: the selection of more new pathogen races which can overcome these genes.

Table 1. Estimated percentage use of varieties containing *Pl* 6 or *Pl* 7 in the French sunflower crop since 1989 and the number of virulent *Plasmopara halstedii* pathotypes observed on them.

Year	**Percent use of *Pl6*/*Pl7***	**Number of virulent pathotypes**
1989	< 1 %	0
1990	< 1 %	0
1991	2 %	0
1992	3 %	0
1993	6 %	0
1994	9 %	0
1995	28 %	0
1996	38 %	0
1997	49 %	0
1998	58 %	0
1999	58 %	0
2000	67 %	1
2001	82 %	2
2002	86 %	5
2003	88 %	5

In the face of this evolution, it appeared important to determine whether it would be possible to use resistance genes in such a way as to improve the duration of their efficiency. To obtain durable control of downy mildew, the question may be asked as to whether it would be preferable to combine a maximum number of *Pl* genes in one hybrid, to use forms of the hybrid with different resistance genes in alternation or to grow a "multi-hybrid" with different plants of one crop containing different resistance genes. To provide an answer, a multi-location experiment with these three varietal structures and a well identified pathogen population was undertaken in 2000. We plan to continue the experiment until 2005, but the

significant results obtained in 2003 suggested that it would be useful to present an intermediate report at this stage.

Materials and Methods

Plasmopara halstedii Isolates. Two isolates, one of race 710 and the second of race 100 were used to infect the plots in 2000. These isolates had been characterised by their virulence patterns (Tourvieille, 1999) and their molecular profiles (Roeckel-Drevet et al., 1997) and were chosen for their genetic difference (Roeckel-Drevet et al., 2003).

Sunflower Hybrids. Four forms of a hybrid variety (registered in the French Catalogue) which differed only for the *Pl* genes they carried have been studied. They were developed by backcrossing the parental lines to introduce different *Pl* genes. Their characteristics are presented in Table 2.

Table 2. Characteristics of the 4 almost-isogenic forms of a sunflower hybrid.

Hybrid	*Pl* genes source	Race 100	Race 710
H1	A	Resistant	***Susceptible***
H2	A + B	Resistant	Resistant
H3	A + C	Resistant	Resistant
H4	A + B + C	Resistant	Resistant

A = source RHA 274 (USDA line); B = source Ha 335 (USDA line); C = source RHA 340 (USDA line)

Race Determination. To determine the virulence pattern of the isolates collected during the experiment, the method of Gulya et al, (1998) has been used, with tests in growth chambers on differential sunflower lines infected by samples of the pathogen. In addition to the 9 differential sunflower lines these authors proposed (D1 - D9), 3 other inbred lines were tested since they permit additional race distinction: YVQ, a line bred by INRA (France) which is resistant to race 710 but susceptible to race 100. This line thus makes it possible to demonstrate the presence of race 100 when it is mixed with race 710; XRQ, a line bred by INRA (France) which carries *Pl5*. This line distinguishes a US isolate of race 330 from one of Spanish origin, also denoted 330; and RHA 340, a line bred by USDA (USA) which carries *Pl8*, used in hybrids H3 and H4 (Table 1). This gene gives resistance to all the downy mildew races so far identified. The downy mildew tests were carried out in confined growth chambers officially accepted for quarantine parasites. Permit No. 2003/DRAF/70.

Experimental Plan. The order of cropping of the different forms of the sunflower hybrid is presented in Table 3. The multi-hybrid was made up of equal parts (25%) of each form. Each hybrid form was grown under insect-proof net cages (one cage for each form) in two locations (Clermont-Ferrand and Versailles). A permanently saturated humidity was maintained by a misting system controlled by humectostats (Tourvieille et al., 1987). Sowing and observation density was 10 plants/m sq. The plots were infected the first year only, with two types of inoculum: infected soil-less compost was spread over the rows before sowing and then the emerged seedlings were sprayed with suspensions of the two *P. halstedii* isolates of races 710 and 100. In the following years, in order to obtain conditions favourable for natural downy mildew infection, an irrigation of 100mm was provided five days after sowing. In the final year (2005) a completely susceptible hybrid (Airelle) will be grown on all the plots to make it possible to collect plants with all the different profiles of downy mildew which may have developed.

Table 3. Cropping of the four forms of a sunflower hybrid (H1, H2, H3 and H4) in the study of the impact of different combinations of *Pl* resistance genes on evolution of sunflower downy mildew.

Year	Check	Pyramid 1	Pyramid 2	Pyramid 3	Alternation	Multi-hybrid
1	H1	H2	H3	H4	H1	H1+H2+H3+H4
2	H1	H2	H3	H4	H2	H1+H2+H3+H4
3	H1	H2	H3	H4	H3	H1+H2+H3+H4
4	H1	H2	H3	H4	H4	H1+H2+H3+H4

Observations of plants showing symptoms were made once a week and the percentages of primary (root) and secondary (shoot) infections calculated. A representative sample of the downy mildew spores was collected in each cage, its virulence pattern determined in the growth chamber and a sample frozen at -80C for molecular analyses.

To avoid the development of volunteer plants from dropped seed, all the capitula were removed at flowering. Plants with symptoms were retained until maturity (or death) to provide natural inoculum for the following years.

Results

The data for percentage downy mildew attack in the first three years of the experiment at Clermont-Ferrand are presented in Table 4.

Table 4. Percentage of plants showing downy mildew symptoms in the experiment at Clermont-Ferrand in the first three years in the cages with different combinations of *Pl* genes.

Year	Check	Pyramid 1	Pyramid 2	Pyramid 3	Alternation	Multi-hybrid
1	71 %	3 %	3 %	0 %	75 %	14 %
2	42 %	1 %	1 %	0 %	1 %	7 %
3	75 %	5 %	5 %	1 %	2 %	10 %

The check was continuous cropping of the hybrid form carrying source A of *Pl* genes which gives resistance only to race 100. The high levels of attack observed indicate that conditions were favourable for downy mildew infection. All the isolates collected in this cage were of race 710.

Pyramid 1. This combination was continuous cropping of the hybrid form with resistance sources A and B, which gave resistance to both downy mildew races initially infected. However, elsewhere in France, this combination of genes has already been overcome by new races (304) (Penaud et al., 2003). The percentages of plants showing symptoms was always low, and, at least for the first two years, was probably explained by the presence of a few impurities. For years 1 and 2, only race 710 was identified but in the third year (2003) some of the isolates collected appear to have new virulences: race 714. Hybrid H2 is susceptible to this new race.

Pyramid 2. A monoculture of the hybrid form with sources A and C, giving resistance to both races 100 and 710. This combination has never been reported to have been overcome. Observations showed the same results as for pyramid 1: low levels of attack but new virulences in year 3, showing wide diversity: Races 314, 704 and 714. Hybrid H3 is susceptible to races 704 and 714.

Pyramid 3. A monoculture of the hybrid form carrying resistance sources A, B and C. This combination of genes should give resistance to all known races. No diseased plants were observed in the first or second years but in the third year a few symptoms were observed and the isolate appears to have a virulence different from 710 and 100: race 304. However, since hybrid H4 is resistant, the three plants showing symptoms in this cage were probably impurities.

Alternation. Each year the hybrid form grown was different. In year 1 the level of attack was the same as that in the check cage, sown with the same genotype, H1, susceptible to race 710. In the following years the amount of attack dropped sharply, when hybrid forms resistant to race 710 were grown. All the isolates collected were 710.

Multi-hybrid. The same mixture of four forms of the hybrid was grown each year. There were significant downy mildew attacks in the conditions very favourable for the pathogen. All isolates were of race 710. Since 25% of the plants carried only resistance source A (H1), the level of attack can be multiplied by 4 to make a comparison with the check cage which was entirely H1. This gives 56% in year 1; 28% in year 2 and 40% in year 3, percentages much lower than the levels when H1 was grown alone.

Conclusions

At this stage after 3 years of the experiment, some conclusions can already be drawn. First, the experimental method of growing sunflower under irrigated netting cages is satisfactory for obtaining significant levels of natural downy mildew attack. Second, a multi-hybrid with a large proportion of resistant plants appears to have a protective effect against leaf infections on those plants which are susceptible to the downy mildew race present. Third, use of combinations of *Pl* genes by alternation or multi-hybrids may reduce the frequency of appearance of new races compared with gene pyramids.

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