COMPONENTS OF QUANTITATIVE RESISTANCE TO DOWNY MILDEW (*PLASMOPARA HALSTEDII*) IN SUNFLOWER (*HELIANTHUS ANNUUS*)

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Abstract: Components of quantitative resistance in sunflower (*Helianthus annuus*) to *Plasmopara halstedii*, the pathogen causing downy mildew, were investigated. Percentage infection, latent period, sporulation density and reduction of hypocotyl length were compared on two sunflower lines showing different levels of quantitative resistance in the field infected with different *P. halstedii* strains of races 100, 300, 710 and 714 in controlled conditions. The inbred line BT, rather susceptible in the field, showed a higher percentage infection, a higher sporulation density, a shorter latent period and less reduced hypocotyl length than inbred line FU, which showed a greater resistance in the field. The very good resistance of inbred line FU observed in the field was confirmed by the measurements of quantitative resistance criteria described in this study. Percentage infection of FU was 1.4% less than BT, latent period of BT was 12.4% less than FU, sporulation density of FU was 22.3% less than BT and reduced hypocotyl length of BT was 15.3% less than FU. It seems that the criteria such as latent period, sporulation density and reduction of hypocotyl length may be used to measure quantitative resistance in sunflower to *P. halstedii*.

Key words: aggressiveness, major gene *Pl*, virulence

INTRODUCTION

Sunflower downy mildew is a common disease in many regions where sunflowers (*Helianthus annuus* L.) are grown. The pathogen, *Plasmopara halstedii* (Farlow) Berles & de Toni, is an obligate parasite. The disease affects young plants when the water content of the soil is high and the maximum temperature is between 15 and 18°C. *P. halstedii* is an Oomycete with asexual multiplication by liberation of zoosporangia produced on the lower surfaces of sunflower leaves and sexual reproduction giving oospores which are found in crop residues (Tourvieille de Labrouhe *et al.* 2000).

P. halstedii has physiological races (pathotypes) capable of infecting a variable range of sunflower genotypes. The nomenclature of these races is based on the reaction of a series of differential lines (Tourvieille de Labrouhe *et al.* 2000). Race specific resistance against it is controlled by major genes, denoted *Pl* (Tourvieille de Labrouhe *et al.* 2000). Pathogen – specific recognition occurs following a gene-for-gene model and tends to produce a disease-free plant (Tourvieille de Labrouhe *et al.* 2000). Since 2000, 8 new *P. halstedii* races (304, 307, 314, 334, 704, 707, 714 and 717) have been reported (Tourvieille de Labrouhe *et al.* 2005), meaning that the reaction of many varieties that were registered as resistant (or re-registered after introduction of new *Pl* genes) varies according to where they are grown.

The alternative is resistance of sunflower which does not depend on host/pathogen interactions and recognition, so that selection pressure on the pathogen populations is much reduced. This type of resistance, usually partial, non-race specific or quantitative resistance. Quantitative resistance of agricultural crops to fungal pathogens is characterized by a continuous variation ranging from very low to moderate levels of resistance (Parlevliet 2002). It reduces the rate of epidemic development in crops and therefore the severity of disease (Geiger and Heun 1989), and it often more durable (Johnson 1984) than qualitative resistance. Recently, the quantitative resistance to downy mildew has been studied in France (Vear *et al.* (2007) and Tourvieille de Labrouhe *et al.* (2008)). It is controlled by minor genes and tends to impact the rate of disease development (rate reducing) rather than produce a disease-free plant (Vear *et al.* 2008). However, this resistance depends on overall symptom expression in the field (% attack) according to Vear *et al.* (2007) and Tourvieille de Labrouhe *et al.* (2008).

In this study, different measurements of quantitative resistance were carried out in controlled conditions to determine the best criteria which would be used to determine the resistance in sunflower against *P. halstedii*. These measurements may reflect expression of pathogen's aggressiveness criteria according to Van der Plank (1968) and Robinson (1976). These are percentage infection, latent period, sporulation density and reduction of length of vegetal materiel (Sakr *et al.* 2008 a,b; 2009). We use terms according to the definitions of Van der Plank (1968). Aggressiveness is used to indicate the quantitative

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component of pathogenicity that is expressed horizontally, irrespective of plant cultivars or species. Virulence is used to indicate the qualitative component of pathogenicity that is expressed vertically.

MATERIALS AND METHODS

Fungal strains

The *P. halstedii* strains used in this study were collected in France and maintained at INRA, Clermont–Ferrand. Manipulation of this quarantine parasite respected European regulations (No 2003/DRAF/70). Strain MIL 001 (race 100) was isolated in 1966 and strain MIL 002 (710) in 1988. Strains DUxxxx were isolated from naturally infected plants. Their races were determined using the method reported by Tourvieille de Labrouhe *et al.* (2000) (Table 1).

Sunflower genotype

To characterise quantitative resistance criteria of sunflower plants infected by *P. halstedii* strains, two inbred lines not carrying any *Pl* gene and known to have different levels of quantitative resistance (Vear *et al.* 2007; Tourvieille de Labrouhe *et al.* 2008) were studied: FU, which has greater resistance in the field and BT, rather susceptible in the field.

Test conditions

All the tests were carried out in growth chambers with 18h light, 18°C±1 and RH of 65–90%. Germinating seed with radicls of 5–10 mm were infected with fresh suspensions of zoosporangia obtained from diseased seedlings of the open pollinated variety Peredovik (susceptible to all known races of *P. halstedii*) maintained for 48 hours at 100% RH. Infection was obtained by soaking the seeds for 3h in a suspension of 1×10^5 zoosporangia per ml, counted with a Malassez cell (Sakr *et al.* 2009). The seedlings were grown in soilless horticultural compost.

Measurement of quantitative resistance criteria

These criteria were used by Sakr *et al.* (2008 a,b; 2009) to study aggressiveness of *P. halstedii* strains. Infection is considered as successful when the seedlings show sporulation on the shoot surface. Observations were made 13 days after infection and expressed as the percentage of seedlings showing sporulation, whatever the plant parts concerned and the amount of sporulation observed. For each strain 60 plants were measured and the experiment was repeated 3 times. Latent period was defined as the number of incubation days necessary to obtain 80% sporulating plants. For measurement, 6 treatments were carried out: sixty infected germinating seeds were planted in 6 pots (10 seeds per pot). Each day, after incubation for 7 to 12 days, one pot was covered with a polythene bag. After 24 hours at 100% RH, the number of seedlings in the pot showing sporulation on the shoot was noted recorded. These observations were combined to give the number of plants expressing symptoms after 13 days incubation, which enabled calculation of the percentage of diseased plants expressing symptoms for each incubation period compared with the total after 13 days. The latent

Table 1. List and virulence of 19 *P. halstedii** strains on sunflower differential lines used in the present study

Strains	Race	Year isolated	Differential lines								
			D1 Ha-304	D2 Rha-265	D3 Rha-274	D4 PMI3	D5 PM-17	D6 803-1	D7 HAR-4	D8 QHP1	D9 Ha-335
MIL 001	100	1960	S	R	R	R	R	R	R	R	R
DU 1842	300	2005	S	S	R	R	R	R	R	R	R
MIL 002	710	1988	S	S	S	S	R	R	R	R	R
DU 1552	710	2005	S	S	S	S	R	R	R	R	R
DU 1555	710	2005	S	S	S	S	R	R	R	R	R
DU 1564	710	2005	S	S	S	S	R	R	R	R	R
DU 1571	710	2005	S	S	S	S	R	R	R	R	R
DU 1635	710	2005	S	S	S	S	R	R	R	R	R
DU 1651	710	2005	S	S	S	S	R	R	R	R	R
DU 1659	710	2005	S	S	S	S	R	R	R	R	R
DU 1670	710	2005	S	S	S	S	R	R	R	R	R
DU 1753	710	2006	S	S	S	S	R	R	R	R	R
DU 1776	710	2006	S	S	S	S	R	R	R	R	R
DU 1777	710	2006	S	S	S	S	R	R	R	R	R
DU 1782	710	2006	S	S	S	S	R	R	R	R	R
DU 1839	710	2006	S	S	S	S	R	R	R	R	R
DU 1845	710	2006	S	S	S	S	R	R	R	R	R
DU 1865	710	2006	S	S	S	S	R	R	R	R	R
DU 1915	714	2005	S	S	S	S	R	R	R	R	S

*S: – susceptible, R: – resistant (Tourvieille de Labrouhe *et al.* 2000)
Identification of virulence of *P. halstedii* strains was presented by Sakr *et al.* (2008a)

period was read from a quadratic regression curve considering per cent plants sporulating, including the intermediate points and the first day when 100% sporulation was obtained. For each strain, the experiment was repeated 3 times. Sporulation density was defined as the number of zoosporangia produced by one cotyledon. For measurement, the cotyledons of seedlings showing sporulation after a given incubation period, were grouped together in a small container. One ml of physiological sporulation (9 g NaCl/1l permuted water) per cotyledon were added and vigorously shaken before counting (18 observations per sample) under an optical microscope with a Malassez cell. The statistical analysis was performed concerning concentrations measured after 12 and 13 days of incubation, which corresponded to the maximum quantity of spores produced by a cotyledon. Hypocotyl length corresponds to the distance from the stem base to cotyledon. It was measured after 13 days incubation on diseased plants that showed sporulation on the shoot. It was compared with the length measured on the same but uninfected genotype grown in identical conditions. Data are expressed in % of length of healthy plants. For each strain 10 plants were measured, the experiment was repeated 3 times.

Statistical analyses

Statistical analyses of the quantitative resistance data were performed using StatBox 6.7® (GrimmerSoft) software, France. Before statistical analysis, the percentages were transformed using the Arcsinus√ function. A complete randomised design with two factors (*P. halstedii* strain and sunflower genotype) and 3 replications was used for analysis of percentage infection, latent period and reduction of hypocotyl length. A randomized complete block design with two factors (*P. halstedii* strain and sunflower genotype) and 2 blocks corresponding to two incubation periods was used for analysis of sporulation density. The Newman-Keuls test (Snedecor and Gochran 1989) was used to compare the means at p = 0.05. Table 2 presents F tests (**p = 0.01).

Table 2.   Responses of two sunflower inbred lines BT and FU to 19 *P. halstedii* strains

Strains	Aggressiveness criteria							
	percentage infection [%]		latent period [days]		sporulation density (10³ zoosporangia per cotyledon)		hypocotyl length (% of length of healthy plants)	
	BT	FU	BT	FU	BT	FU	BT	FU
MIL 001	100.0	96.0	7.8	9.1	2114	1432	32	34
DU 1842	100.0	99.1	7.8	8.9	2265	1672	30	37
MIL 002	100.0	93.2	8.3	10.8	825	703	30	31
DU 1552	100.0	100.0	8.1	9.7	988	562	39	42
DU 1555	100.0	98.9	7.9	9.1	901	736	36	44
DU 1564	100.0	99.4	8.3	9.3	919	740	36	41
DU 1571	100.0	98.9	8.3	9.3	8.97	774	38	44
DU 1635	100.0	99.3	8.5	8.6	1343	1186	30	43
DU 1651	100.0	100.0	8.8	8.8	1016	947	27	41
DU 1659	100.0	100.0	7.9	8.7	977	965	32	32
DU 1670	100.0	99.4	8.2	8.9	1149	1129	31	45
DU 1753	100.0	100	8.2	8.8	913	566	31	36
DU 1776	100.0	100	8.0	8.8	1021	830	28	42
DU 1777	100.0	100	8.0	8.5	934	721	29	42
DU 1782	100.0	98.9	7.9	9.1	939	714	34	39
DU 1839	100.0	100	7.9	9.1	858	682	33	42
DU 1845	100.0	97.0	8.2	9.6	772	582	33	40
DU 1865	100.0	97.0	8.1	9.4	812	686	41	39
DU 1915	100.0	96.4	8.58	11.06	1118	502	34	31
Mean[z]	100.0 a	98.6 b	8.13 b	9.28 a	1093 a	849 b	33 b	39 a
	F strains: 5.03** Probability: 0.0		F strains: 12.58** Probability: 0.0		F strains: 57.52** Probability: 0.0		F strains: 2.01 ns Probability: 0.01956	
	F genotypes: 77.23** Probability: 0.0		F genotypes: 343.01** Probability: 0.0		F genotypes: 131.06** Probability: 0.0		F genotypes: 46.14** Probability: 0.0	
	F interactions: 5.03** Probability: 0.0		F interactions: 6.8** Probability: 0.0		F interactions: 4.71** Probability: 0.00004		F interactions: 1.53 ns Probability: 0.10218	

F tests (**p = 0.01), ns not significant
[z]values in the line with a letter in common are not significantly different according to Newman-Keul (p = 0.05)
Values of latent period, sporulation density and hypocotyl length of 15 strains DUXXXX of race 710 were presented by Sakr *et al.* (2008a) and values of latent period and sporulation density of strains MIL 001 and MIL 002 were presented by Sakr *et al.* (2009)

RESULTS

Results are presented in table 2. Mean percentage infection of sunflower inbred line BT (100%) was significantly (probability = 0.0; variation coefficient = 4.11%) greater than on sunflower inbred line FU (98.6%). Percentage infection of sunflower inbred FU was 1.4% less than on sunflower inbred BT. *Mean latent period* of sunflower inbred BT (8.13 days) was significantly (probability = 0.0; variation coefficient = 3.56%) shorter than on sunflower inbred FU (9.28 days). Latent period of sunflower inbred BT was 12.4% less than on sunflower inbred FU. Mean sporulation density (10^3 zoosporangia per cotyledon) on sunflower inbred BT (1093 10^3) was significantly (probability = 0.0; variation coefficient = 9.69%) greater than of sunflower inbred FU (849 10^3). Sporulation density on sunflower inbred FU was 22.3% less on than sunflower inbred BT. Diseased plants hypocotyls were only one third of mean lengths of healthy plants that reached 92 mm and 90 mm on sunflower inbred lines BT and FU respectively whatever was the strain of *P. halstedii*. Mean reduced hypocotyl length of sunflower inbred BT (33%) was significantly (probability = 0.0; variation coefficient = 14.72%) less than on sunflower inbred FU (39%). Reduced hypocotyl length on sunflower inbred BT was 15.3% less than on sunflower inbred FU. For all criteria studied (except for reduction of hypocotyl length), there was significant interaction between the 2 factors: *P. halstedii* strain and sunflower genotype.

DISCUSSION

In this paper, quantitative resistance components were determined in controlled conditions. The four components: percentage infection, latent period, sporulation density and reduction of hypocotyl length were analysed on two sunflower inbred lines showing different levels of quantitative resistance in the field (Vear *et al.* 2007; Tourvieille de Labrouhe *et al.* 2008) infected with different *P. halstedii* races. Quantitative resistance in sunflower genotypes to *P. halstedii* depends on percentage of diseased plants in the field infected naturally with one or two races. It is difficult and expensive for performing and it needs a large cultivated area and appropriate equipments (Vear *et al.* 2007; Tourvieille de Labrouhe *et al.* 2008). The criteria described in this study are easy for testing sunflower genotypes against different *P. halstedii* races. For biotrophic pathogens such as rusts and powdery mildews, Geiger and Heun (1989) and Denissen (1993) found that major components of quantitative resistance are infection efficiency, latent period and duration of sporulation.

Differences in percentage infection could be due to differences in rate of maturation of zoosporangia or in capacity to penetrate healthy host tissue (Delanoe 1972). Very high percentages of infection can be explained by the test conditions that were very favourable to the parasite: germinating seeds were placed in direct contact with a suspension containing 10^5 zoosporangia/ml and Meliala (2001) showed that in similar conditions, 60% of the zoosporangia liberated zoospores within 20 minutes. Latent period measured as the number of days of incubation necessary to obtain 80% of sporulating plants could pro-vide accurate measurement in this study. It corresponded to rate of appearance of symptoms on infected plants. Latent period has been found to be an important factor of quantitative resistance in most host-pathogen interactions (Kolmer and Leonard 1986; Denissen 1993; Browne and Cooke 2005; Yan *et al.* 2006). The number of spores produced by a diseased plant will determine the quantity of inoculum that can infect neighbouring plants. But they also indicate the interaction between the host plant and the parasite, since the quantity of spores produced depends on the aggressiveness of the parasite and the level of host quantitative resistance (Robinson 1976). It also reflects the capacity of the fungus to invade host tissues during the incubation period. Dwarfing is a symptom characteristic of plants systemically infected by *P. halstedii* and is explained by a decrease in the concentration of growth hormone (Iodole Acetic Acid) in infected tissue (Cohen and Sackston 1974). This decrease in size can be observed at a very early stage.

Our results are in accordance with our previous analyses on the comportment of sunflower inbred lines FU and BT in controlled conditions (Sakr *et al.* 2008 b; 2009). These results showed that there is an interaction between *P. halstedii* and host plant. However, the sunflower inbred line FU, which shows greater resistance in the field, enabled better to characterize the components of quantitative resistance in controlled conditions in comparison with sunflower inbred line BT, rather susceptible in the field.

Data presented in table 2 showed that difference in percentage infection between sunflower inbred lines FU and BT had a low value (1.4%) and did not present an important criterion. However, these differences ranged from 12.4% in latent period, 15.3% in reduction of hypocotyl length to 22.3% in sporulation density. It seems that these criteria may be used to measure quantitative resistance in sunflower genotypes to *P. halstedii*. On the other hand, in pathosystem *Erysiphe pisi* on pea, Viljanen-Rollins *et al.* (1998) found that infection efficiency was an important component of quantitative resistance and the length of latent period did not differ between cultivars.

The inbred line BT showed a higher percentage infection, a higher sporulation density, a shorter latent period and less reduced hypocotyl length than inbred line FU (Table 2). This observation suggests that in inbred line FU, the development of the pathogen was slowed, and might be due to resistance mechanisms expressed by accumulation of QTL in sunflower cultivars (Vear *et al.* 2008). Studying of the reaction of two inbred lines to 19 strains of several *P. halstedii* races showed differences in their behaviour. A very good resistance of inbred line FU observed in the field was confirmed by the measurements of quantitative resistance criteria in controlled conditions. These methods can be used to characterise quantitative resistance in sunflower genotypes. A high level of resistance would be associated with long latent period, low sporulation density and high infected hypocotyl length. Such growth chamber tests would make it possible to confirm the quantitative character of resistance since, in contrast with field observations, it would be easy to analyse the reaction of sunflower lines to many *P. halstedii* races.

ACKNOWLEDGEMENTS

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POLISH SUMMARY

ELEMENTY OCENY ILOŚCIOWEJ ODPORNOŚCI SŁONECZNIKA (*HELIANTHUS ANNUUS*) NA MĄCZNIAKA RZEKOMEGO (*PLASMOPARA HALSTEDII*)

Badano części składowe ilościowej odporności u słonecznika (*Helianthus annuus*) na *Plasmopara halstedii*, sprawcy choroby mączniaka rzekomego. Porównywano w kontrolowanych warunkach polowych procent porażenia, okres infekcji ukrytej, gęstość zarodnikowania i redukcję długości kolanka podliścieniowego dwóch linii słonecznika wykazujących różny poziom odporności ilościowej na porażenie różnymi szczepami *P. halstedii* zaliczonymi do ras 100, 300, 710 i 714. Linia wsobna BT, raczej wrażliwa w warunkach polowych, wykazywała wyższy poziom porażenia, wyższą gęstość zarodnikowania, krótszy okres infekcji ukrytej i mniejszą redukcję długości kolanka podliścieniowego, niż linia wsobna FU, która wykazywała wyższą odporność. Bardzo dobra odporność linii wsobnej FU została potwierdzona wynikami pomiarów elementów odporności ilościowej opisanymi w pracy. Porażenie FU było niższe o 1,4% w porównaniu do BT, okres infekcji ukrytej u BT był krótszy o 12,4%, niż u FU, gęstość zarodnikowania była mniejsza na FU o 22,3% niż u BT, a długość kolanka podliścieniowego BT była krótsza niż u FU o 15,3%. Wydaje się, że kryteria, takie jak okres infekcji ukrytej, gęstość zarodnikowania i redukcja długości kolanka podliścieniowego, mogą być wykorzystane do mierzenia odporności ilościowej słonecznika na *P. halstedii*.