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ABSTRACT

Premature ripening (PR) caused by *Phoma macdonaldii* results in yield damage for sunflower, mainly in the South-West of France, a major production area. The aim of the study was to characterize and identify the effect of crop management systems on PR incidence and severity, in 2006 and 2007. This field study used artificial and natural inoculation to investigate the role of host resistance, N-fertilization and water regime in the Phoma epidemics and aimed to reveal the most critical factors responsible for the disease progress and plant injury. On both years, the susceptibility of the cultivar appeared as a main factor influencing PR. However, the most severe attacks were observed in conditions of high nitrogen nutrition, especially when it was associated with water stress after flowering.

Key words: crop management - disease assessment - *Phoma macdonaldii* - premature ripening - sunflower.

INTRODUCTION

Premature ripening (PR), induced by *Phoma macdonaldii* Boerema, is one of the most severe sunflower (*Helianthus annuus* L.) diseases. The disease increased in the early 1990s and the entire French sunflower cropping area is now affected (Penaud and Pérès, 1995). The term "premature ripening" was first used for sunflower by Sackston (1949) to describe wilt and stalk rot. Evidence suggests that collar girdling canker caused by *P. macdonaldii* is the primary cause of PR. Sunflower premature death is most often characterized by loss of plant vigor during mid- to late summer followed by senescence and death of the plant a few weeks before normal maturity (Gulya et al., 1984). Generally, PR plants have small heads, reduced seed yield, low seed weight, and low oil content (Donald et al., 1987).

The Phoma symptoms generally appear on the petiole, the stem and the collar of the plant (Maric and Schneider, 1979; Gulya et al., 1997). The spot may girdle the stem or the collar and the black to brown lesions may only affect the epidermal layer or penetrate into the pith of the plant. If *P. macdonaldii* is not organ specific throughout the stages of sunflower development (Penaud and Pérés, 1995), sunflower resistance depends on the organ infected and the aggressiveness of the pathogen isolates. Research carried out by CETIOM, INRA and ENSAT since 1998 has highlighted that collar infection is the best way to reproduce prematurely dead plants in the field at flowering stage (Pérès and Poisson, 2000).

Recent investigations revealed an impact of crop management on PR, as N-fertilization and water regime. However, few studies have reported on the effects of crop on the incidence of the disease. The aims of this study are to confirm the role of *P. macdonaldii* in causing PR of sunflower by artificial collar inoculation and evaluate the effects of sunflower crop management on the severity of fungus attacks at collar levels. Both of the factors under investigation in thus study may be of importance in explaining the irregular occurrence of this disease, with a special emphasis on the difference of sensitivity of two cultivars, N fertilization and water regime on PR induced by *P. macdonaldii*.

MATERIALS AND METHODS

Climate and soil: Two field experiments were carried out in Auzeville, near Toulouse (Haute-Garonne, South-West of France) over two years (2006-2007) on the experimental INRA Station. The soil was deep, silty-clay to clay with a pH of 7.8 to 8.2. From the inoculation time to the end of the experiment, the mean relative humidity was 63% and 67% and the temperature between min. 9.7-10 °C and max. 38-36°C in 2006 and 2007, respectively. Seasonal precipitation was 115 mm in 2006 and 307 mm in 2007.

Experiment design and crop management systems: The experimentation was done in a split-plot design with inoculation either artificial (AI) or natural (NI) as the main plot (800 m^2 each). Each main plot was subdivided into 2 water regimes (no irrigation vs irrigated), then 2 levels of nitrogen (0 vs 150 kg /ha) and finally 2 cultivars (cv. Heliasol RM (Semences de France) vs cv. Melody (NK Semences)). This resulted in 24 subplots of 22 m^2 in 2006 and 36 in 2007. The plant population was similar (6.7 plants/m^2). In 2006, because of a dry season, 6 irrigations were applied up to 220 mm while only 2 irrigations were applied in 2007 (80 mm). N-fertilization was applied at sowing and at early flower bud stage. The two cultivars differed by their susceptibility to Phoma black stem, cv. Heliasol being the one most affected by premature ripening.

Phoma isolates and plant inoculation: Single pycnidiospore isolates of *P. macdonaldii* derived from sunflower fragments with severe black collar lesions were used in the experiment. The isolation and conservation of *P. macdonaldii* monopycnidiospore strains (MP6 and MPH2) was done following the method described by Roustaee et al. (2000). Artificial inoculation in the field was carried out using mycelium of the fungus (vegetative part). To allow mycelium growth, single pycnidiospore isolates were transferred to Petri dishes containing potato dextrose agar (Difco) (39 g/l, pH 6) and incubated for 10 days at 25°C in the dark. The inoculation on the AI plot was carried out at star bud stage on 25 homogenous plants on the 2 central rows of 6-row plots. A disc of mycelium (6 mm diameter) was placed for 5 days at the plant collar using MP6 as single pycnidiospore in 2006 and MPH2 in 2007. Previous tests had suggested that the latter was more aggressive. Desiccation of the disc was avoided by applying a damp cotton wool plug and aluminum around the collar.

A*ssessments of Phoma macdonaldii incidence and severity:* A disease assessment method was used to evaluate *Phoma macdonaldii* incidence (proportion of necrotic areas of infected collars) and final severity (proportion of early ripened plants). The first evaluation was performed 5 days after inoculation. Severity ratings were assessed weekly on the 25 tagged plants for all treatments from 59 DAE (days after emergence) and 52 DAE until harvest in 2006 and 2007, respectively, at least 15 assessments were performed during the experiment. The disease rating scale used was proposed by Cetiom: (0) healthy plant, (1) black collar in less than ¾ of the stem diameter (2) coalescent spots on collar, (3) all the leaves are wilted but the stem is still green, (4) the plant is totally dry.

The weekly monitoring of disease progress was used to calculate the area by a disease progress curve (AUDPC) and the value was standardized by dividing it by the total number of epidemic days. The AUDPC was calculated according to the equation of Campbell and Madden (1990):

$$AUDPC = \sum_{i}^{n-1} (y\mathrm{i} + y\mathrm{i}+1)/2 * (t\ \mathrm{i}+1 - t\mathrm{i})$$

where n is the number of evaluations, y the severity or incidence of the disease and t the thermal time of each evaluation. (t,y) = (441, 0) is included as the first evaluation date, approximately 1 week before AI. Daily thermal time (DTT) was calculated using daily mean air temperature. from emergence to 120 DAE (equivalent to 1930 DTT) and 126 DAE (1776 DTT) in 2006 and 2007, respectively, the last observation date where disease rating scale might be attributed to PR and not to natural senescence. The threshold temperature was taken at 4.8 °C (Granier and Tardieu, 1998).

Statistical analysis: Final severity ratings and AUDPC values were subjected to one-way and multifactor analysis of variance (ANOVA) both in 2006 and 2007. ANOVA was performed by comparing the impact of water regime*N-fertilisation*cultivar for each inoculation treatment (AI, NI) on AUDPC values. Where significant differences were found at $P \leq 0.05$, means were compared using Fisher's protected least significant difference test (95% LSD). Analysis was performed using Statgraphics Plus 5.1 statistical software (Rockville, MA, USA).

RESULTS

Disease incidence measured by AUDPC values between the two experiments (2006 and 2007) did not differed significantly on AI plots (P = 0.08), whereas AUDPC on NI was significantly higher in 2007 (P=0.00*). According to the mode of inoculation (AI and NI) for each year, disease severity was measured by the final fraction of PR plants and the AUDPC did not vary significantly, except in 2006

when it was higher in AI than in NI plots (Table 1). This lack of significant effects on AUDPC and PR suggests that AI reproduces NI correctly.

Table 1. AUDPC values and percentage of premature ripened sunflower plants for artificial (AI) and natural (NI) inoculation with *Phoma macdonaldii* in 2006 and 2007 as a function of the cultivar, N-fertilisation and water regime.

	AUDPC[1]				PR plants (%)			
	2006		2007		2006		2007	
Treatment	AI	NI	AI	NI	AI	NI	AI	NI
	3860 a	2177 b	3755 a	3792 a	38 a	34 a	49 a	40 a
Heliasol	4141 a	2325 a	3843 a	3927 a	69 a	43 a	60 a	48 a
Melody	3787 b	2028 b	3665 b	3657 b	39 b	26 b	37 b	33 b
0-N	3812 b	2101 b	3627 b	3617 b	34 b	23 b	15 b	11 b
150-N	4116 a	2253 a	3919 a	3882 a	74 a	45 a	83 a	69 a
Unirrigated	4125 a	2184 a	3800 a	3968 a	83 a	52 a	55 a	50 a
Irrigated	3804 b	2169 a	3708 b	3616 b	25 b	17 b	42 b	31 b

1Calculated according to Campbell and Madden (1990).
Means were calculated using Fisher's protected LSD test a $P \leq 0.05$. The effect of cultivar, nitrogen and water regime was tested separately for AI and NI. Letters to the right of each value refer to differences between values. Means with the same letter do not differ significantly.

In AI and NI plots, sunflower crop management through cultivar, N amount and water regime increased the proportion of plants infected by *P. macdonaldii* ($P < 0.01$). All the tagged plants had a score of 2 (black coalescent spots on collar), but the evolution into PR plants (score 4) was only induced by the crop management. Cv. Heliasol was systematically more susceptible than cv. Melody to Phoma attacks whatever the season and the mode of inoculation ($P < 0.01$).

AUDPC values and PR (%) differed significantly between 150-N and 0-N and between rainfed and irrigated management for AI and NI ($P< 0.01$ for N and water) both in 2006 and 2007. High N-fertilisation and water stress (resulting from rainfed management) increased the proportion of plants infected by *Phoma macdonaldii* (at stem and collar level) and especially the percent of PR plants.

Two examples of disease progress curves for AI in 2007 are shown in Fig.1.

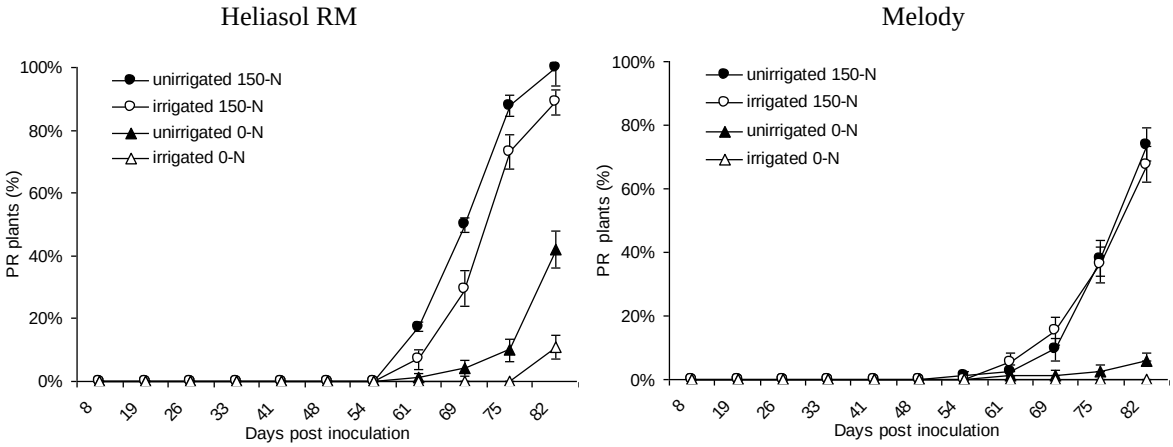

Figure 1: Disease progress curves of premature ripening plants after artificial inoculation in 2007 for cv. Heliasol (susceptible) and cv. Melody (tolerant) for contrasted crop management systems combining nitrogen and water applications.

The susceptibility of the cultivar was the main factor influencing the final rate of PR plants, cv. Heliasol being more attacked than cv. Melody. Nitrogen fertilisation appeared as the second factor affecting disease progress for both cultivars. Water stress seems to amplify nitrogen effect and may be considered as the third factor stimulating the disease progress. High N fertilization combined with rainfed management resulted in the highest plant injury: up to 100% of PR plants were observed for cv.Heliasol (in AI and NI treatments) on 150-N and no irrigation. Effects of cultivar x nitrogen interaction were observed in 2006 for NI and for AI in 2007 ($P<0.01$).

DISCUSSION

This study intended to point out the most influencing factors of sunflower premature ripening attributed to *Phoma macdonaldii*. A preliminary investigation into the possible role of a different fitness among the fungus isolates did not clearly reveal differences in disease severity. In 2006 and 2007, no significant differences in AUDPC were found in AI plots with two different monopycnidiospores. This suggests that differences in disease severity observed in the field could not be only attributed to isolate aggressiveness but more to environment and crop management.

As expected, the proportion of PR plants was higher in 2007 than in 2006. Higher precipitation and air relative humidity in 2007 resulted in a favourable environment for natural Phoma infection. Weeraratne and Priyantha (2003) observed that HR > 80%, temperature ranging from 25 to 30°C and cloudy weather favour the development of the disease under tropical conditions. Stem injury resulting from *Phoma macdonaldii* infections at collar level probably increased because the microclimate in the lower part of the sunflower stand was more favourable to fungus development and activity. Similar conclusions were drawn by Debaeke and Pérès (2003) on stem and collar attacks. However, if disease injury is influenced by the climatic and microclimatic environment, the PR syndrome relies strongly on cropping system management.

The susceptibility of the cultivar was shown throughout both experiments in AI and NI plots. The response of cv. Heliasol and cv. Melody differed significantly towards the progression of the disease and the final incidence of the inoculation by *P. macdonaldii*. The significantly lower AUDPC values and percent of PR plants for cv. Melody indicate possible differential genotypic susceptibility to premature ripening. Such differences were already noted by Penaud (1994) and Dechamp-Guillaume et al. (2000) on stem attacks. If the susceptibility of the cultivar is one of the main factors inducing PR, host nutrition was responsible for different patterns of epidemics in the experimentation.

As suggested in the literature, mineral nutrition can exert a profound effect on disease development, with fertiliser application increasing development of the disease. The mechanisms leading to these nutrient-induced changes in disease development are complex and multifarious. These mechanisms include the effects of the mineral nutrients directly on the pathogen, on plant growth and development, and on resistance mechanisms. According to Gulya et al. (1997), high N fertilisation increased the proportion of collars infected by *P. macdonaldii*. Conversely, nitrogen deficiency did not predispose plants to PR. The role of N-fertilisation clearly demonstrated in this study should be better explained at a process level. Our investigations did not reveal if the nitrogen had an impact on the sunflower culture that could favour pathogen development or was trophic for the pathogen. Large nitrogen supplies influence the size of the canopy. Dense canopy, observed in the experimentation for a given stand density, especially in 2007, induced a microclimate which might promote inoculum production and create conditions conducive to successful infections. Spore germination of *P. macdonaldii* might be sensitive to this high relative humidity microclimate, constituting a major climatic parameter in disease epidemiology and field infection (Roustaee et al., 2000). The other approaches to the effect of N-fertilisation on the percent of PR plants may concern the possible N sources available by plant pathogenic fungi. Assimilation of N sources, depending on the tissue being colonised, would include nitrate, ammonium, amino acids, amine and protein (Snoeijers et al., 2000). In addition, available N sources may also depend on the mode of nutrition of the pathogen. *P. macdonaldii*, which is a necrotrophic fungus, could have access to a wide range of N sources. The present study did not allow us to determine clearly the role of N-fertilisation. Both nitrogen effects may act on the disease progression, and one probably more than the other. Further investigations will be set up to better understand the N-fertilisation impact on the microclimate by varying crop density of sunflower. The difficulty is that in most plant-pathogen interactions, very little is known about the N content and subsequent colonisation by fungi. This is an area that requires further investigation (Walters and Bingham, 2007).

Previous data showed that infested plants which did not get irrigation presented a higher AUDPC and final percent of PR plants compared to those which were irrigated. If its effect seems to be less influential compared to the susceptibility of the cultivar and high nitrogen supply, rainfed plots receive a significant impact from the severity of the disease. This impact is not clearly demonstrated but this effect might be more linked to the physiological status of the plants. A predisposition to disease is often observed in host plants during water deficiencies. According to the literature, there are no studies in which the biochemical and biophysical causes of predisposition to disease during water deficiencies are known with any certainty. When the plant is infected by the pathogen, changes in host plants may alter their interactions with other organisms and suggest possible mechanisms of susceptibility. Boyer (1995) proposed two mechanisms to explain how water stress increases the susceptibility of plants to attacks from pathogens : (1) reduced photosynthate production induced by drought eliminates the plants' ability to defend

themselves against pathogens and/or (2) plant growth is reduced without reducing the pathogen's ability to reproduce, thus allowing further progression and increased symptom severity in the host. These two mechanisms may be an approach to a better understanding of the water deficiency effect on disease progression in the plant leading to PR as a nitrogen effect.

This study has attempted to identify the most crucial elements of PR induced by P. macdonaldii infestation. Our data revealed that sunflower epidemiology efficiency is mainly influence by Phoma and crop management. The susceptibility of the cultivar, high N-fertilization and rainfed management have a strong impact on the disease. Nitrogen input and water stress enhance PR. The interaction of both may act to favour pathogen infection in the plant and reduce expression in host defence mechanisms. Deployment of resistant lines in combination with an integrated disease management framework is suggested as suitable tools for reducing inoculum pressure and PR plants induced by *P. macdonaldii*.

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