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Nitrogen and water management can limit premature ripening of sunflower induced by *Phoma macdonaldii*

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Premature ripening (PR) is one of the most important diseases of sunflower in France since the 90s. Previous results indicated that girdling canker of the stem base, caused by *Phoma macdonaldii* was its primary cause but elucidation of critical environmental factors involved is crucial for better control of the disease. A field study was conducted in three contrasting cropping seasons (2006 2008) and investigated the effect of N fertilization (0, 75 and 150 kg N ha ^{1}) and water regime (rainfed, irrigated) on two cultivars with artificial inoculation (AI) and natural infection (NI). Disease assessment was recorded weekly to calculate the area under disease progress curve (AUDPC) and the final percentage of PR plants. Data showed that high levels of N fertilization led to significantly ($P < 0.05$) more PR than non fertilization. Water deficit conditions were significantly ($P < 0.05$) involved in disease severity, and AUDPC and PR were increased when dry conditions were associated with high N supply. This was true for two cultivars which differed in their susceptibility to the disease but cv. Heliasol RM was significantly ($P < 0.05$) more affected than cv. Melody, partially resistant to PR. Despite contrasting weather patterns, these results demonstrated a clear role of crop management and environmental conditions on the incidence and severity of stem base attacks responsible for the PR syndrome. These findings suggest that sunflower crop husbandry should be adapted to minimize premature ripening induced by *P. macdonaldii*.

1. Introduction

Sunflower (*Helianthus annuus* L.) is a major oilseed crop grown under a wide range of agro ecological conditions worldwide. However, fungal diseases are often considered as severe con straints for its yield stability (Gulya et al., 1997). Among fungal pathogens, *Phoma macdonaldii* Boerema (teleomorph: *Lepto sphaeria lindquistii*) (McDonald, 1964) is one of the most wide spread and detrimental diseases in sunflower production. *P. macdonaldii* has been reported worldwide (Gulya et al., 1997) as the causal agent of black stem disease. In France, the incidence and severity of the disease increased dramatically in the early 90s and the entire sunflower cropping area is now affected by Phoma black stem (Penaud and Pérès, 1994; Pérès and Letof, 1996). Short rotations (mainly sunflower wheat) and simplified soil tillage of wheat crops may have increased inoculum since infected stubble remains on the soil surface (Poisson Bammé and Pérès, 2000).

The disease is mainly characterized by the appearance of black lesions on the stem (McDonald, 1964). With a severe attack, the lesions girdle the stem and, through coalescence of several spots, the whole stem becomes black. Even when *P. macdonaldii* infections result in extensive pith decay, seed yield losses observed in inoculated trials are generally slight, especially if infections occur late in the seed filling stage (Carson, 1991; Penaud, 1996). Lesions at the foliar nodes are not particularly damaging in terms of yield as compared to infection at the stem base. Sackston (1950) first described a wilt and stalk rot of unknown etiology as "premature ripening" (PR), and earlier evidence showed that stem base girdling canker caused by *P. macdonaldii* was its primary cause. Before PR, crops show loss of vigour from mid to late summer, leaves become wilted and necrotic, the stalk turns dark brown to black, and this is followed by senescence and plant death a few weeks before physiological maturity (Donald et al., 1987). In France, the impact of PR on yield has not been clearly assessed, but yield losses up to 1.3 t ha ^{1} have been reported (Pérès et al., 2000).

Few studies have aimed at a better understanding of the etiology of premature ripening of sunflower and the identification of agronomic factors that promote the disease. Although different genotypic susceptibilities to Phoma black stem have been observed

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(Darvishzadeh and Sarrafi, 2007), environmental factors were suggested to play a key role in disease development and PR occurrence (Carson, 1991). Field studies showed that additional nitrogen increased the incidence of *P. macdonaldii* attacks at leaf nodes and stem base (Velazquez and Formento, 2000; Debaeke and Pérès, 2003). However, effects of crop management on PR have never been reported. In this context, there was need for detailed knowledge of agronomic factors involved in sunflower PR. This paper reports experimentation with two cultivars that differed in their susceptibility to PR, under contrasted water and N fertiliza tion levels during three seasons, to assess the effects of water regime and N supply on premature ripening following artificial inoculation or natural infection with *P. macdonaldii*.

2. Materials and methods

2.1. Experimental design and crop management systems

Three field experiments were carried out at INRA, Auzeville, near Toulouse (Haute Garonne, south western France) over 3 years (2006 2008). From preliminary variety screenings, two cultivars were selected, differing in their susceptibility to PR (data not shown): cv. Heliasol RM, susceptible to PR, and cv. Melody, with a higher level of partial resistance.

Prior to sowing, soil cores were taken to 120 cm depth for analysis. The soil was a deep silty clay to clay with a pH of 7.8 8.2 and soil nitrate content ranged from 30 to 37 kg ha ^{1} in 2006, from 57 to 85 kg ha ^{1} in 2007 and from 15 to 28 kg ha ^{1} in 2008. Previous crops were sorghum in 2006, 2007 and 2008 (rainfed plots) and maize in 2008 (irrigated plots).

The experiment was arranged as a 4 factor split plot design with three replicates, with water regime (irrigated or not) as the main plots. N fertilization, the subplot treatment, was applied in strips. Within each N treatment, cultivars, as sub subplots, were arranged at random and were either artificially inoculated (AI), or left subject to natural infection (NI). The crop was sown in April 2006 and 2007, and in May 2008 due to rainy conditions in April (85 mm) (Table 1). Plant density was 6.5 plants m ^{2} after thinning. Each plot consisted of six rows, with a row width of 60 cm. Plot size was 37, 22 and 18 m^2 in 2006, 2007 and 2008, respectively. Since inoculum of *Diaporthe helianthi* Munt. Cvet (Phomopsis stem canker) was regularly present, and strongly competes with *Phoma*, Punch CS was applied in June (0.6 0.8 l ha ^{1} of flusilazole + car bendazim (DuPont)).

In 2006, two levels of N fertilization were applied, and three were made in 2007 and 2008. Unfertilized control plots (N0) and high N rates of 150 kg ha ^{1} (N150) were compared over the 3 years of the experiment. In 2007 and 2008, an intermediate rate of 75 kg N ha ^{1} (N75) was also applied. At sowing, 75 kg N ha ^{1} was applied as urea in N75 and N150, and then a second application of 75 kg N ha ^{1} was made at early bud stage for the N150 treatment. Quantity and timing of sprinkler irrigation were decided according to rainfall and soil water deficit. In 2006, a dry year, six irrigations were applied, giving 220 mm on irrigated plots and 50 mm was applied to rainfed plots for seedling emergence and N incorpora tion. In 2007, 80 mm of irrigation was applied at two periods to satisfy water requirements on the irrigated treatment while the rainfed treatment received no irrigation. In 2008, 120 mm were applied to irrigated plots and 20 mm was applied on rainfed plots for N incorporation. Factors studied and experimental layouts are summarized in Table 2. Over the 3 years of experimentation, individual factors were combined to create contrasting crop management systems. A range from 12 to 24 treatments was assessed by combining two water regime (rainfed vs. irrigated), N fertilization (2 levels in 2006 and 3 in 2007, 2008), and infection (AI and NI) on both cv. Melody and cv. Heliasol RM in 2006 and 2007, and only on cv. Heliasol RM in 2008. In 2006, the treatment N150 rainfed was not tested in AI for either cultivar.

2.2. Phoma isolates and plant inoculation

One *P. macdonaldii* monopycniospore strain was used in these studies, selected for its severe aggressiveness. The strain was isolated from an infected sunflower residue showing severe black stem base lesions in a sunflower field located close to the trial site. Isolation and conservation of the strain was performed according to the method of Roustaee et al. (2000). Inoculum was plated on Petri dishes containing potato dextrose agar (Difco) (39 g l ^{1}, 150 mg of streptomycin, pH 6) and grown at 25 °C for 10 days in the dark. Inoculation of the AI plots was carried out at star bud stage on 25 uniform plants tagged within the two central rows. A 6 mm diameter disk of PDA with mycelium was placed at the stem base and left for 5 days. Drying of the disk was avoided by applying a moist cotton wool plug covered with aluminum foil around the stem base.

2.3. Disease assessment

Development of necrotic areas at the stem base induced by *P. macdonaldii* was assessed from 12, 8 and 7 days after artificial inoculation in 2006, 2007 and 2008, respectively. The disease was scored using a 0 4 scale: 0 = healthy plant, 1 = less than 3/4 of the stem base circumference black, 2 = spots circling the stem base, 3 = all leaves wilted but the stem green, 4 = plant completely dry. A PR plant was thus defined as one completely dry before physiological maturity with necroses circling the stem base. Disease symptoms were observed weekly on the 25 tagged plants for all treatments on AI and NI plots. A few plants affected by other fungal diseases (Phomopsis stem canker, Sclerotinia stalk rot, Verticillium wilt) were ignored and only Phoma was observed on the remaining tagged plants throughout disease scoring. Disease assessment was continued up to 82 days post inoculation (DPI) in 2006 and 2007, and 81 DPI in 2008, 1 week before the onset of normal senescence. At least 13 recordings were taken in 2006 and 11 in 2007 and 2008.

Over the 3 years of experiment, 100% of AI and NI tagged plants were infected by *P. macdonaldii*, equivalent to a disease score of $\geq$2. Disease development was assessed by the area under disease

Table 1
Mean monthly temperature and relative humidity, and total monthly rainfall during the sunflower growing season recorded at a weather station near the experimental site.

Month	Temperature (°C)			Relative humidity (%)			Rainfall (mm)		
	2006	2007	2008	2006	2007	2008	2006	2007	2008
April	11.1	17.8	11.7	58	67	77	0.0	4.5	85.0
May	16.7	15.9	16.5	74	76	78	30.5	113.5	60.5
June	20.9	19.4	19.4	63	71	70	21.0	80.5	36.0
July	25.2	20.5	21.3	62	67	66	21.5	13.5	40.5
August	20.5	20.4	21.1	61	67	68	28.0	57.5	37.5
September	20.3	17.2	18.7	70	68	72	88.5	32.5	36.0
April–September	19.1	18.5	18.1	65	69	72	189.5	302.0	295.5

progress curve (AUDPC) and the final percentage of PR plants. The percentage of PR plants was recorded weekly and used to calculate AUDPC for all experiments. The AUDPC was calculated according to the equation of Campbell and Madden (1990):

$$AUDPC \quad \sum_{i}^{n-1} \frac{yi + yi + 1}{2} \times (ti + 1 \quad ti) \tag{1}$$

where n is the number of evaluations, y the percent of PR and t the DPI of each evaluation. The final percentage of PR plants was that at the last observation.

2.4. Assessment of crop nitrogen and water status

The N nutrition status for each treatment was quantified using the Nitrogen Nutrition Index (NNI), calculated as follows (Lemaire and Gastal, 1997):

$$NNI \quad \frac{Nm}{Nc} \tag{2}$$

where Nm is the total N concentration measured for all the aerial parts and Nc is the critical total N concentration calculated for the weight of aerial dry matter (ADM) measured *in situ*; Nm and Nc are expressed as % of ADM. Nc is the minimum N concentration needed to obtain the maximum dry matter production by the crop. In a preliminary study, Debaeke and Raffaillac (2006) proposed the following critical dilution curve for sunflower:

$$Nc \quad 5.03 \times ADM^{-0.447} \tag{3}$$

NNI was calculated on 5 plants of cv. Heliasol RM for each treatment over the 3 years of experiment at flowering on AI or NI plots. A value of NNI ≥ 1 indicated a crop with ample N supply (N non limiting); NNI = 1 was optimal N nutrition and below 1, N deficiency.

The crop model SUNFLO developed by Casadebaig (2008) for dynamic simulation of response of sunflower cultivars to a range of soil, weather and management conditions was applied to the different water × nitrogen situations. The model accounted for plant phenology, leaf area index development and stomatal response to soil water deficit in non diseased plants. The output variable used in this study was the ratio of actual to potential evapotranspiration (ET_a:ET_0) over the period from stem base inoculation to physiological maturity, which can be used as an index of plant water stress under healthy conditions. High values of (ET_a:ET_0) indicated an adequate water supply and water stress by (ET_a:ET_0) below 1.

2.5. Statistical analyses

The independent variables AUDPC and the final percent of PR plants were analysed with multifactor analysis of variance procedure of Statgraphics Plus 5.1 statistical software (Rockville, MA, USA). For each analysis of variance, homoscedasticity by Levene's test (confidence level of 0.95) and the normality of the residuals by the Shapiro Wilk's test (confidence level of 0.95) were tested. Prior to ANOVA, square root transformations were carried out to AUDPC. Arcsine transformations were applied to PR percentage data (Gomez and Gomez, 1984). When significant differences were found at $P \leq 0.05$, means were compared using Fisher's protected least significant difference test (95% LSD).

For each year, PR was subjected to variance component analysis to estimate variability of infection, cultivar, water regime and N fertilization.

3. Results

3.1. Effect of climate and crop management on plant water and plant nitrogen status

Climatic conditions in the 3 years differed greatly in temperature, relative humidity and rainfall (Table 2). Mean temperature from April to September 2006 was the highest compared with 2007 and 2008. Temperatures throughout disease development were particularly high in 2006, with a mean of 25.2 °C in July, compared with 20.5 °C in 2007 and 21.3 °C in 2008. Rainfall during the cropping season was 300 mm in 2007 and 2008 but only 190 mm in 2006.

The simulated ET_a:ET_0 ratio for cv. Heliasol RM (from plant inoculation to physiological maturity) was used as a water stress index (Table 3). The ratio varied with irrigation and nitrogen management between years and climatic conditions. In irrigated plots, ET_a:ET_0 was above 0.81 for all levels of N and all years. In rainfed plots, this ratio was always below 0.73. Predicted water stress was higher in fertilized plots, especially under rainfed management. Highly fertilized plots had Nitrogen Nutrition Indices (NNI) from 0.86 to 1.2 (Table 3) whereas values for unfertilized plots were from 0.45 to 0.62, except in 2007 when NNI was 0.77 due to a high nitrogen content in the soil of rainfed plots (20 kg ha ^{1} more than in irrigated treatments). In 2008, an intermediate NNI was observed in N75 treatment. In 2007, NNI in N75 was comparable to N150 in 2007 because of a high initial soil N content. With N150, rainfed plots had lower NNI than irrigated plots.

3.2. Effect of year on AUDPC and PR

Disease severity was analyzed as AUDPC and final PR over the 3 years. These variables were calculated for AI and NI, cultivars, water regimes and N fertilization (Table 4). The highest AUDPC values were observed in 2006 but did not differ significantly from those in 2007 and 2008. Significant differences ($P < 0.05$) in final percent of PR were observed between 2006 and 2007 for all crop management systems taken together. In 2007, PR was highest (47.5%), followed by 2008 (41.9%) and 2006 (39.7%).

3.3. Effect of artificial inoculation and natural infection on AUDPC and PR

AUDPC and final PR were higher after artificial inoculation (AI) than under natural infection (NI) (Table 4). The differences were significant ($P < 0.05$) in 2006 and 2007 but not in 2008. The highest

Table 2
Description of the main aspects of the crop management experiments carried out at INRA Auzeville from 2006 to 2008.

Year	Cropping data					Treatments			
	Sowing date	Plant density (plants m ^{2})	Emergence date	Experimentation duration (days)[a]	Cultivar(s)	N fertilization (kg ha ^{1})	Irrigation (mm) (rainfed–irrigated)	Artificial inoculation date	Disease assessment duration (days)
2006[b]	19 April	6.5	30 April	121	Heliasol RM and Melody	0 and 150	50–220	8 June	82
2007	12 April	6.5	24 April	126	Heliasol RM and Melody	0 and 75 and 150	0–80	6 June	82
2008	5 May	6.5	16 May	127	Heliasol RM	0 and 75 and 150	20–120	1 July	81

[a] From emergence till final severity estimation.
[b] The N150—rainfed treatment was not tested in artificial inoculated plots for both cultivars in 2006.

Table 3
Actual: potential evapotranspiration ratio (ET_a:ET_0) calculated by the SUNFLO model and Nitrogen Nutrition Index (NNI) values for cv. Heliasol RM under both water regimes (irrigated and rainfed) and three levels of N fertilization (N0, N75, N150) throughout the disease development period after natural infection from 2006 to 2008. High values of (ET_a:ET_0) indicated an adequate water supply and water stress by (ET_a:ET_0) below 1. A value of NNI ≥ 1 indicated a crop with ample N supply (N non-limiting); NNI = 1 was optimal N nutrition and below 1, N deficiency.

Year	Irrigation	ET actual:ET potential (ET_a:ET_0)			Nitrogen Nutrition Index (NNI)		
		N fertilization			N fertilization		
		N0	N75	N150	N0	N75	N150
2006	Irrigated	0.85	–	0.81	0.58	–	1.04
	Rainfed	0.56	–	0.51	0.62	–	0.86
2007	Irrigated	0.85	0.82	0.82	0.45	0.99	0.98
	Rainfed	0.73	0.70	0.70	0.77	1.10	0.95
2008	Irrigated	0.87	0.83	0.83	0.59	0.79	1.20
	Rainfed	0.72	0.68	0.68	0.57	0.69	0.95

percent of PR was observed in 2007 after AI with 52% of dead plants. In 2006, PR was the lowest with NI and increased by a factor of 1.5 with AI. Final PR in 2008 was comparable to the other years, but no significant difference was observed between AI and NI. Variance components analyses showed that the total variance in PR over the 3 years was not explained completely by the 2 types of infection.

3.4. Effects of individual agronomic factors on AUDPC and PR

3.4.1. Cultivar

Cultivar susceptibility was tested in 2006 and 2007. Significant differences ($P < 0.05$) were observed between cv. Heliasol RM and cv. Melody except in 2006, when AUDPC value in NI did not differ significantly from AI (Table 4). Disease was more severe on cv. Heliasol RM than on cv. Melody with both AI and NI. AUDPC was the highest in 2006 with 7.49 for cv. Heliasol RM in AI and 4.64 for cv. Melody. PR was most severe in 2007 with 67.5% for cv. Heliasol RM compared to 36.6% for cv. Melody after AI. Variance components analyses showed that cultivar explained 31% and 27% of the total variance in PR in 2006 and 2007, respectively.

3.4.2. Water regime

In 2006 and 2007, a significant ($P < 0.05$) effect of water regime on the disease development was observed (Table 4). In 2008, no significant differences were observed between irrigated and rainfed plots. Plots under rainfed condition showed higher AUDPC and PR values than irrigated ones, the greatest disease severity being observed in the absence of irrigation in 2006 when climatic conditions were very dry and hot throughout disease development (Table 2). In that year, AUDPC under rainfed management was 2.7 and 3.2 times greater than in the irrigated treatment with AI and NI respectively, and PR was increased three fold. The same pattern was observed in 2007 to a lesser extent, with PR increased by 1.4 and 1.9 times with AI and NI respectively. In 84% of the 72 pairs of plots differing only by water regime, PR was greatest in absence of irrigation (Fig. 1). PR in rainfed plots was above PR in irrigated plots in 100% of cases in 2006, 92% in 2007 and 56% in 2008. From the variance component analyses, water regime in 2006 explained 29% of the total variance in PR, but had no significant effect in 2007 and 2008, 2 years with high rainfall from July to September (104 and 111 mm, respectively) (Table 2). However, over the 3 years, rainfed conditions increased PR by a mean of 20 percentage points (Fig. 1).

3.4.3. N fertilization

Variance component analyses demonstrated that N fertilization was the main factor increasing PR. N supply explained 26%, 62% and 81% of PR in 2006, 2007 and 2008, respectively. ANOVA showed a significant ($P < 0.05$) effect of N fertilization on AUDPC and PR (Table 4) except in 2008, where no significant differences were observed between N0 and N75 for AUDPC values. In 2007 and

Table 4
Mean values of AUDPC and final percentage of sunflowers prematurely ripened (PR) showing effects of two cultivars (cv. Heliasol and cv. Melody), different levels of N fertilization (N150, N75, N0) and two water regimes (irrigated, rainfed) with artificial inoculation (AI) and natural infection (NI) over the 3 years (2006–2008).

Treatment	AUDPC[a]						Final PR (%)[b]					
Year	2006		2007		2008		2006		2007		2008	
	4.83 A[c]		4.14 A		3.60 A		39.7 B		47.5 A		41.9 AB	
	AI[d]	NI	AI	NI	AI	NI	AI[c]	NI	AI	NI	AI	NI
Inoculation method	6.07 A	3.60 B	4.83 A	3.45 B	3.83 A	3.37 A	48.0 A	31.4 B	52.0 A	43.0 B	45.8 A	38.0 A
Cultivar												
Heliasol RM	7.49 a[c]	4.47 a	6.78 a	4.48 a	3.83	3.37	60.9 a	40.4 a	67.5 a	52.6 a	45.8	38.0
Melody	4.64 b	2.73 a	2.87 b	2.42 b			35.1 b	22.4 b	36.6 b	33.3 b		
Water regime												
Irrigated	3.27 b	1.70 b	4.02 b	2.48 b	3.51 a	2.42 a	23.7 b	14.5 b	43.5 b	29.4 b	46.6 a	34.7 a
Rainfed	8.86 a	5.50 a	5.63 a	4.42 a	4.16 a	4.32 a	72.3 a	48.3 a	60.5 a	56.6 a	44.9 a	41.4 a
Fertilizer												
High (N150)	9.0 a	5.10 a	9.17 a	6.96 a	8.55 a	7.17 a	66.3 a	43.2 a	82.6 a	69.5 a	85.3 a	68.3 a
Usual (N75)			4.45 b	2.90 b	2.31 b	2.35 b			58.8 b	48.0 b	43.3 b	33.7 b
None (N0)	3.13 b	2.11 b	0.86 c	0.49 c	0.64 b	0.59 b	29.7 b	19.5 b	14.6 c	11.5 c	8.7 c	12.0 c

[a] Calculated according to Campbell and Madden (1990).
[b] Final PR (%) taken 81 days from the start of epidemic.
[c] For a given treatment, means followed by different capital letters in a row are significantly different from one another, whereas means followed by different small letters in a column are significantly different from one another based on $L.S.D_{0.05}$.
[d] The treatment N150—rainfed was not tested for cv. Heliasol RM and cv. Melody.

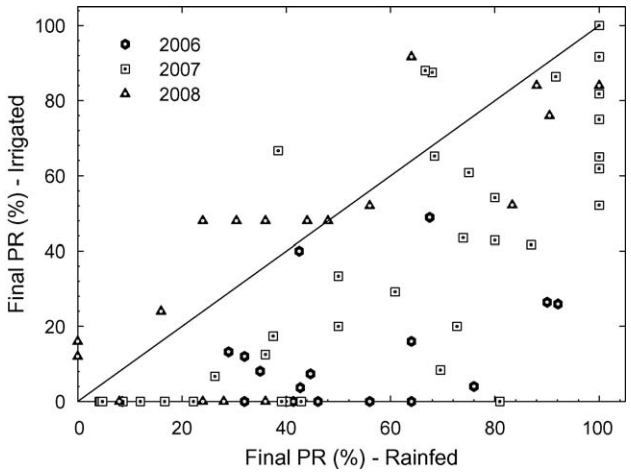

Fig. 1. Relation between the percentage of sunflowers affected by premature ripening (PR) for 72 pairs of plots differing only for irrigation (irrigated, rainfed) from 2006 to 2008.

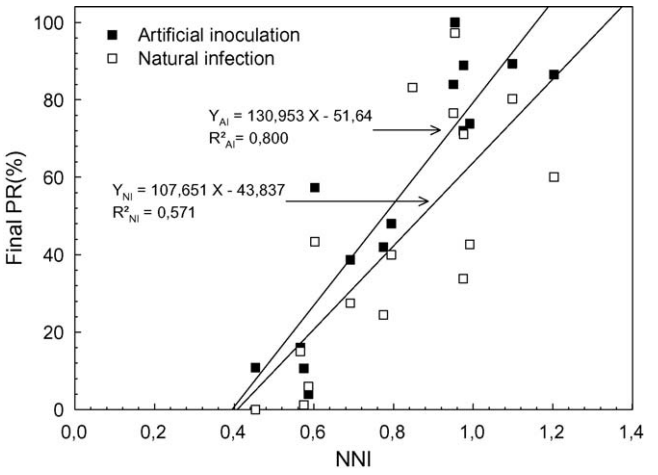

Fig. 3. Relationship between the percentage of sunflowers affected by premature ripening (PR) and the Nitrogen Nutrition Index (NNI) for cv. Heliasol RM in artificial inoculation (AI) and natural infection (NI) in 2006, 2007 and 2008.

2008, AUDPC values were increased 11 14 times under the N150 treatment compared with N0. The final percent of PR was 82.6% and 85.3% for N150 plots with AI in 2007 and 2008, respectively (Table 4). In 52 of the 54 pairs of plots differing only for N150 vs. N0, PR was greater under N150 (Fig. 2). PR in N150 plots was above PR in N0 plots in 89% of cases in 2006, 96% in 2007 and 100% in 2008. The relative plant nitrogen status was indicated by the Nitrogen Nutrition Index (NNI) at flowering. The relationship between PR and NNI for cv. Heliasol RM from 2006 to 2008 is shown in Fig. 3. Significant regressions were observed between NNI at flowering and PR in AI plots (R^2_{AI} 0.80) and NI plots (R^2_{NI} 0.57). Maximal PR values were observed, with N75 and N150 treatments, for NNI values $\geq$0.9. Conversely, low PR was associated with NNI values below 0.6, when N deficiency was severe.

3.5. Effect of combined water and nitrogen factors on PR

Annual disease progress curves of PR integrating the effects of N fertilization and water regime for both cultivars with AI and NI (Fig. 4) show significant effects of crop management. N150 plots had the highest percent of PR, except in 2006 when PR was greater in rainfed N0 than in irrigated N150 conditions. The first PR plants appeared in rainfed N150 treatment, and the final percent of PR was the highest in these plots for both cultivars and infection methods. Next highest levels were obtained in irrigated N150, rainfed N0 and irrigated N0 crop management in 2007, 2008 and for cv. Heliasol RM in AI in 2006. In 2007, 100% of PR plants were observed in rainfed N150 plots and 0% in irrigated N0 ones with cv. Heliasol RM (Fig. 4b). The time course of PR differed between cultivars: for all years and treatments, cv. Heliasol RM was affected by PR earlier, and to a greater extent, than cv. Melody (Fig. 4).

A stepwise linear regression was calculated integrating NNI and ET_a:ET_0 (Table 3) aimed at explaining PR values of cv. Heliasol after AI and NI (Eq. (4)):

$$\begin{array}{ll} \mathrm{PR(AI+NI)} & 27.8 + 118.3\mathrm{NNI} \quad 102.1(\mathrm{ET_a} : \mathrm{ET_0}) \\ R^2 & 0.787 \end{array} \tag{4}$$

PR increased significantly with increased N (NNI) ($P < 0.05$) and decreased with increased water availability (ET_a:ET_0).

The stepwise linear regression of PR for AI plots (Eq. (5)) explained more variance than that for NI plots (Eq. (6)):

$$\begin{array}{ll} \mathrm{PR(AI)} & 33.8 + 103.2\mathrm{NNI} \quad 102.7(\mathrm{ET_a} : \mathrm{ET_0}) \\ R^2 & 0.904 \end{array} \tag{5}$$

$$\begin{array}{ll} \mathrm{PR(NI)} & 61.5 + 104.6\mathrm{NNI} \quad 138.4(\mathrm{ET_a} : \mathrm{ET_0}) \\ R^2 & 0.809 \end{array} \tag{6}$$

However, nitrogen and water regime had a similar impact on PR in both AI and NI, so the difference may come from the fact that artificial inoculation permitted control infection date, pathogen strain and quantity of inoculum.

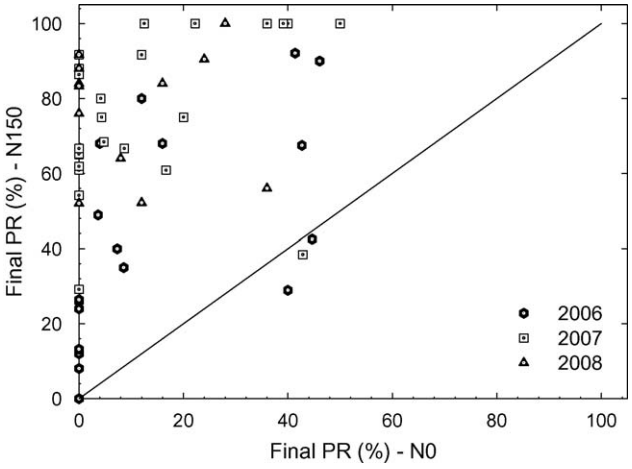

Fig. 2. Relation between the percentage of sunflowers affected by premature ripening (PR) for 54 pairs of plots differing only in the quantity of N applied, from 2006 to 2008. Low nitrogen: 0 kg N ha ^{1}; high nitrogen: 150 kg N ha ^{1}.

4. Discussion

This research was conducted to determine how agronomic factors such as nitrogen fertilization and the water regime can influence occurrence and the severity of sunflower premature ripening. Our results with artificial inoculation and natural infection indicated that high levels of nitrogen fertilization and absence of irrigation were the main factors increasing PR emergence for both susceptible and partially resistant cultivars.

Artificial inoculation on the surface of the stem base confirmed the involvement of *P. macdonaldii* in sunflower PR and gave reproducible disease symptoms. With natural infection, the timing of disease development and pathogen aggressiveness were not

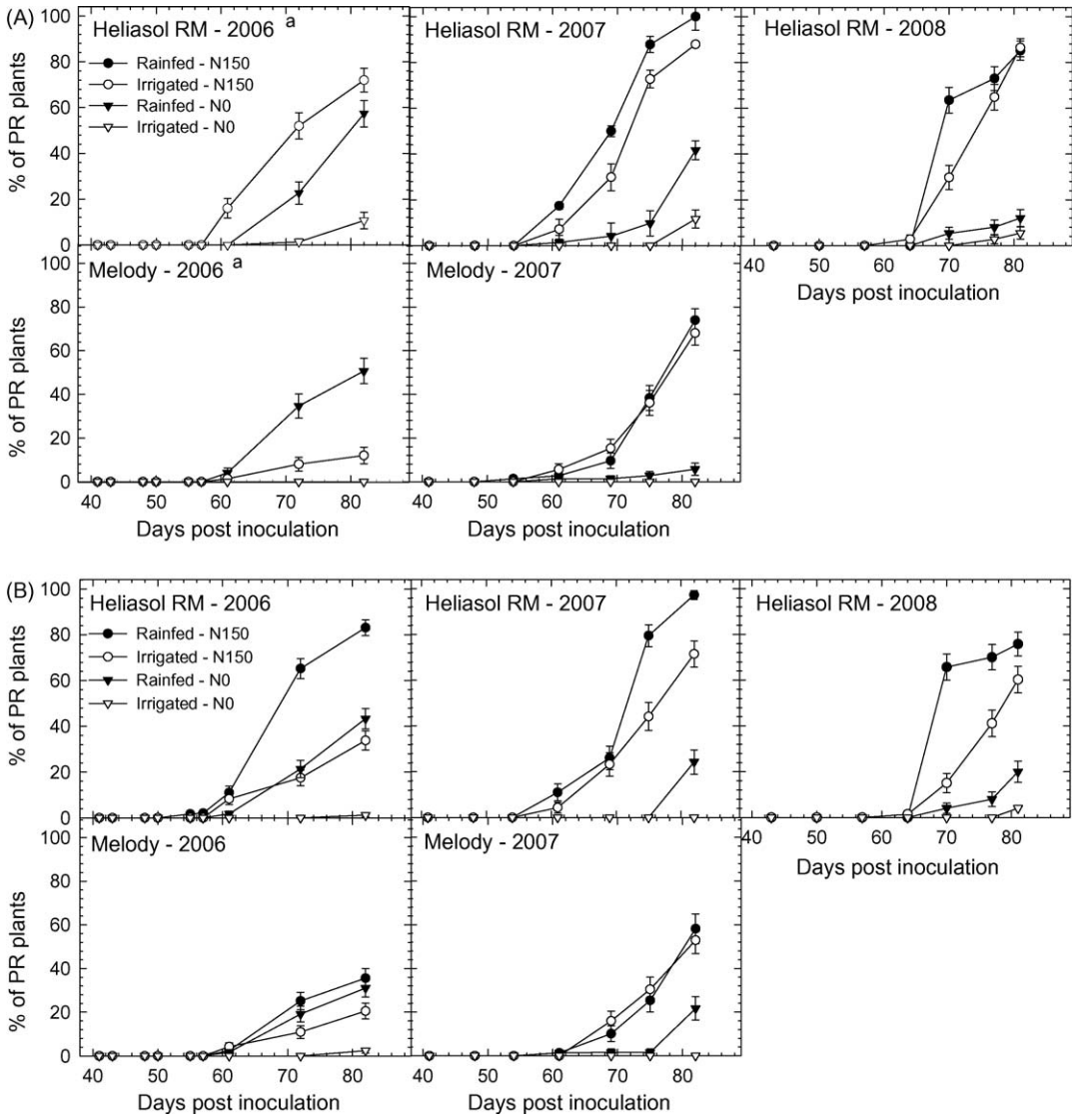

Fig. 4. Annual disease progress curves of premature ripening (PR) plants on both sunflower cultivars (cv. Heliasol RM and cv. Melody) for non-limiting (N150, irrigated) and stressed (N0, rainfed) nitrogen supply and water regime in artificial inoculation (A) and natural infection (B). [a]The treatment N150–rainfed was not tested in artificial inoculation in 2006.

controlled. AUDPC and PR were higher with AI than NI for all cultivar and crop management conditions. Diversity between *P. macdonaldii* strains for aggressiveness (Abou Al Fadil, 2006) could partially explain differences between NI and AI for percent PR. However, differences with AI between years were observed which suggests that climatic conditions probably affected pathogen development and the resulting PR. Pathogen growth rate is largely dependent on temperature and relative humidity (RH), with optimum growth between 20 and 30 °C and RH above 80% (Roustaee et al., 2000; Weeraratne and Priyantha, 2003). Final percent of PR was the lowest in 2006 when high temperatures and low rainfall may have hindered the first stages of progression of *P. macdonaldii* in the stem base (data not shown) and thus slightly reduced PR. Conversely, high rainfall and RH in 2007 and 2008 may have been favourable for pathogen growth and PR. Observations carried out on a hundred farms in south western France showed the same year effect on PR. In 2007, 85% of farms were affected by PR, compared with 71% and 67% in 2006 and 2008, respectively (data not shown).

As reported in the literature, nitrogen is by far the commonest element affecting plant disease (Huber and Thompson, 2007). This study emphasized the negative effect of N fertilization on sunflower PR. A high level (N150) increased significantly the level of crop susceptibility giving high AUDPC and PR values. Conversely, poor host nutrition in the form of deficiency or unbalanced applications of N was less damaging for the crop in agreement with earlier reports on Phoma black stem (Gulya et al., 1997; Debaeke and Pérès, 2003). An increase of N supply has also been shown to enhance disease severity of foliar disease pathosystems and *Fusarium* head blight wheat pathosystems leading to premature death of the spikelet (Lemmens et al., 2004). The microclimate resulting from dense canopies, induced by high N fertilization, may have constituted a major climatic parameter in disease epidemiol ogy and successful infection (Huber and Gillespie, 1992). An experiment carried out in 2008 aimed at investigating the role of microclimate on PR by testing three plant densities (4, 6 and 9 plants m^{-2}). At all densities, 100% of the plants were infected by *P. macdonaldii* but the highest percent of PR plants was observed at the highest density (data not shown). Thus, microclimatic conditions within the canopy were probably not limiting for infection at the stem base in contrast to fungal infection of leaves such as with *Diaporthe helianthi* (Phomopsis stem canker). The

severity of PR appears more likely to be determined by physiological and anatomical changes that could affect host susceptibility to the pathogen, such as alteration of cell wall constituents such as lignins, reduction of cell wall thickness and a decrease in phytotoxic phenolic compounds or their toxicity (Jensen and Munk, 1997). The susceptibility plants may thus vary without changing the facility with which they can be colonized by *P. macdonaldii*. Moreover, a high nitrogen supply could be trophic. This is a necrotrophic fungus which may have access to a wider range of N sources than biotrophic pathogens (Walters and Bingham, 2007).

Water stress occurring during the infection period induced significantly more PR than under irrigated conditions. This was observed in 2006 and 2007 where rainfed conditions were contrasted with irrigated management. High rainfall in 2008 resulted in an absence of significant differences between rainfed and irrigated treatments for both AUDPC and PR. This differs from an earlier report, based on field surveys, which indicated a lack of consistent relationships between water availability and PR (Pérès et al., 2000). A predisposition to disease is often observed in host plants during water shortage. Boyer (1995) proposed two mechanisms to explain how water stress increases susceptibility of plants to pathogens: (i) reduced photosynthate production induced by drought reduces defence mechanisms and/or (ii) a reduction plant but not pathogen growth would give more rapid disease spread and increased symptom severity in the host. Nardini and Salleo (2005) reported that water stress during active sunflower growth reduced plant size and that the diameter of the widest xylem vessels was narrowed by 20% as an adaptation to the risk of occurrence of vascular embolisms (Mepsted et al., 1995). However, under water stress, vascular wilt pathogens are involved in xylem vessel blockage (Beckman, 1964; Robb et al., 1981) leading to the death of the plant. *P. macdonaldii* hyphae were observed in the xylem of 10 day old seedlings (Abou Al Fadil et al., 2009) and of PR plants of cv. Heliasol RM (data not shown). The same strong vessel plugging capacity observed for *Fusarium oxysporum* and *Verticilium* species (Put and Clerkx, 1988) could be involved in sunflower PR. Rainfed conditions may have reduced hydraulic conductance and PR may have occurred by vessel clogging, and embolism of xylem elements. Thus, water stress may be an agronomic factor which increases rather than induces PR, contrary to nitrogen fertilization.

The study emphasized the individual effects of N fertilization and water regime on disease severity. However, integrating the two factors modified significantly AUDPC and PR expression in the field. A high nitrogen supply associated with post anthesis water stress was most damaging for the crop, whereas no fertilization and full irrigation was less damaging especially for the partially resistant cultivar. Whatever the water regime, fertilized plots had more PR plants than unfertilized ones. Haefele et al. (2008) reported that high N fertilizer rates increased the drought risk in water limited field situations as a result of greater biomass development and leaf area index, and the related high transpiration. This phenomenon, called "haying off" in cereals (Taylor, 1965), was expressed by the ratio of actual to potential evapotranspiration (ET_a:ET_0). An excess of nitrogen results more rapidly in soil water exhaustion and transpiration drop so it is necessary to manage the canopy for an effective control of sunflower PR. Avoiding excessive N fertilization by a soil N budget method can significantly minimize disease severity. The Nitrogen Nutrition Index (NNI) can be used as an indicator of plant N status and of the related risk of PR. The correlation between the final PR estimate and NNI at flowering was significant when plotting all the crop management treatments. However, for a reliable evaluation of this indicator, additional experiments should be performed including more cultivars in multi local experiments. In addition, N supply should be adapted to cultivar susceptibility: the N optimum for a susceptible cultivar should be lower than for a partially resistant one. Since genotypes with partial resistance to *P. macdonaldii* have been described (Abou Al Fadil et al., 2009), the choice of the cultivar should be taken into account to limit PR in the future. In this study, the susceptibility to the disease was tested only on two cultivars, but significant differences were confirmed between them. Cv. Heliasol RM, more susceptible to PR, always showed higher AUDPC and PR than cv. Melody, whatever the growing conditions and biotic pressure. Such differences provided evidence of the key role of host susceptibility in PR and its cropping management.

5. Conclusion

This study attempted to identify the most crucial agronomic elements of the sunflower premature ripening disease induced by *P. macdonaldii* in south western France. The combination of high nitrogen fertilization and rainfed conditions resulted in high disease pressure every year. Additionally, differences in suscept ibility of sunflower cultivars could be exploited more instead of developing fungicide protection. Cropping of resistant varieties in combination with appropriate nutrition and other cultural practices could reduce inoculum pressure and PR. Promising cultivars should therefore be screened at high N supplies and under water limited conditions, a procedure which could be used in resistance tests during breeding programmes. It is now important to develop flexible fertilizer management advice for farmers, adjusted to the highly variable and diverse rainfed environments.

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