Low plant density can reduce sunflower premature ripening caused by *Phoma macdonaldii*

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A B S T R A C T

In France, premature ripening (PR) is a widespread damage of a fungal disease of sunflower caused by *Phoma macdonaldii*. Previous results indicated that girdling canker at the stem base, caused by *P. macdonaldii*, was its primary cause. Previous studies have reported the influence of nitrogen and water supply on the incidence and severity of PR but an additional study was required to analyze the effect of plant density on the level of attack for a more comprehensive cultural control of PR.

In a 2-year field study (2008 and 2009) in Toulouse (France), a susceptible cultivar (cv. Heliasol) artificially inoculated at star bud stage with *P. macdonaldii* was grown at three plant densities (4, 6.5 and 9 plants m^{-2}) factorially combined with three N fertilization rates (0, 50/75 and 150 kg N ha^{-1}) and two water regimes (irrigated and rainfed). *P. macdonaldii* symptoms were scored weekly to calculate the area under disease progress curve (AUDPC) and percentage of PR plants. Microclimatic conditions were monitored using thermo-hygrometers within the crop. The fraction of photosynthetically active radiation intercepted by the canopy (fPARi) and leaf area index (LAI) were measured at anthesis. Plant water status during the disease progression was characterized by crop simulation (SUNFLO) and N status at anthesis was assessed from shoot N content (Nm) analysis and N Nutrition Index (NNI) calculation.

Increasing plant density resulted in a greater proportion of PR plants, and this proportion increased further when N was applied at 150 kg ha^{-1}, the highest rate, and the crop was not irrigated. Despite differing canopy development, differences in microclimatic conditions between density levels were too small to explain the PR differences. However plant N concentration and diameter at stem base were closely related to PR incidence. Thin plants (grown at high density) with non-limiting N supply were the most susceptible to premature ripening.

This study opens new avenues for the control of PR through crop management and emphasizes the key role of plant morphology in the development of the disease. Stem base diameter is a morphological trait that could be manipulated through crop management (plant density, N fertilization) and probably breeding in the future when developing integrated disease management systems in sunflower.

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 to be severe constraints for sunflower yield stability (Gulya et al., 1997). Among fungal pathogens, *Phoma macdonaldii* Boerema (McDonald, 1964) (teleomorph: *Leptosphaeria lindquistii* Frezzi) (Frezzi, 1968) is one of the most widespread diseases in sunflower. In France, the incidence and severity of the disease increased dramatically in the early 90s and the entire sunflower cropping area is now affected every year by this pathogen (Penaud and Pérès, 1994; Pérès and Lefol, 1996).

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 many spots the whole stem becomes black. Even when infections resulted in extensive pith decay, Phoma black stem canker reduced yield by 0.2–0.7 t ha^{-1}, especially if infections occur late in the seed-filling stage (Carson, 1991; Penaud, 1996). A girdling canker at the stem base is potentially more damaging and can induce the premature

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ripening (PR) of the plant (Sackston, 1950). From mid- to late summer, leaves become wilted and necrotic and the stalk turns dark brown to black, leading to 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).

Recent studies aimed at a better understanding of the etiology of sunflower PR and the identification of agronomic factors that promote the disease. Residues left on the soil surface that produce ascospores, conidia and mycelium are the main source of *P. macdonaldii* inoculum (Maric et al., 1981; Poisson-Bammé and Pérés, 2000) and PR is primarily due to aerial infections of the stem base and not to direct root attacks (Seassau et al., 2010b). Although different genotypic susceptibilities to Phoma black stem and PR have been observed (Darvishzadeh and Sarrafi, 2007; Seassau et al., 2010a), environmental factors were suggested to play a key role in disease development and PR occurrence (Carson, 1991). Additional nitrogen (N fertilization, soil mineral N residues) increased *P. macdonaldii* attacks at leaf nodes and stem base (Velazquez and Formento, 2000; Debaeke and Pérès, 2003). A combination of high N nutrition and severe post-anthesis soil water deficit resulted in high PR pressure, especially with susceptible cultivars (Seassau et al., 2010a). However, the effects of plant density on PR attributed to *P. macdonaldii* have never been reported so far in the literature, although Debaeke and Pérès (2003) suggested an influence of plant density on stem attacks but with a strong effect of water and nitrogen status.

Plant density is a key factor when designing crop management systems as it influences the dynamics of water use and disease incidence (Mestries et al., 2011). In France, plant density may range from 30,000 to 70,000 plants ha^{-1} in farmers' fields with effects on grain yield and oil content (Champolivier et al., 2011). Therefore there was a need for a more detailed knowledge of the influence of plant density on PR in interaction with other agronomic factors.

This paper reports the conclusions of a 2-year experiment in which differing plant density, N fertilization and irrigation levels were applied to a PR-susceptible cultivar inoculated with *P. macdonaldii*. This study aims at investigating the influence of variations of crop canopy development and plant morphology on PR resulting from different levels of plant density.

2. Materials and methods

2.1. Experimental design and crop management systems

Different sunflower crop management options and two *P. macdonaldii* contamination methods were tested on the cultivar Heliasol RM (Semences de France), a hybrid susceptible to PR (Seassau et al., 2010a). The field experiment was carried out during two consecutive years (2008 and 2009) in adjacent plots at the INRA experimental station in Auzeville (south-western France, 43°36 N, 1°26 E).

Prior to sowing, soil cores were taken to 120 cm depth for analysis of texture and mineral nitrogen. The soil was a deep silty clay to clay (from 25% to 47% clay, increasing with depth) with a pH of 7.3–8.1 (0–30 cm) and soil nitrate content (0–120 cm) ranging from 15 to 28 kg ha^{-1} in 2008, and from 33 to 52 kg ha^{-1} in 2009. Previous crops were sorghum in rainfed plots and maize in irrigated plots for both years. Since inoculum of *Diaporthe helianthi* Munt.-Cvet (Phomopsis stem canker) was present, and strongly competes with *Phoma*, 0.6 l ha^{-1} of flusilazole + carbendazim was applied in June, this fungicide having a selective action on *D. helianthi*.

The experimental layout was a 4-factor split-plot design with three replicates, with water regime (irrigated or not) as the main plot. N fertilization, the subplot treatment, was applied in strips. Within each N treatment, plant density treatments, as sub-subplots, were arranged at random and plants were either artificially inoculated (AI) or left subject to natural infection (NI). The size of each main plot was 4.6 m × 3 m, arranged in six rows, with a row width of 60 cm.

The sunflower crop was sown on May 5, 2008 and 2009. The three plant densities tested were 4 (D1), 6.5 (D2) and 9 (D3) plants m^{-2} after thinning. Three levels of N fertilization were applied. Unfertilized control plots (N0) and high N rates of 150 kg ha^{-1} (N150) were included in both years of the experiment. Intermediate rates of 75 (N75) and 50 (N50) kg N ha^{-1} were tested in 2008 and 2009 respectively. At sowing, 75 kg N ha^{-1} was applied as urea in N75 and N150, and 50 kg N ha^{-1} in N50. An additional amount of 75 kg N ha^{-1} was applied at early bud stage for the N150 treatment. Quantity and timing of sprinkler irrigation were decided according to rainfall and soil water deficit. To satisfy the water requirements, 120 mm (2008) and 70 mm (2009) were applied on the irrigated treatment. The rainfed treatment received no more than 20 mm for N incorporation around early bud stage.

2.2. Phoma isolate and plant inoculation

A single conidium culture of *P. macdonaldii* (MPH2 strain), selected for its aggressiveness, was used for artificial inoculation. The strain was isolated according to the method of Roustaee et al. (2000) from an infected sunflower stem residue showing severe black stem base lesions collected close to the trial site in 2006 and stored at −80 °C in glycerol. Mycelium was derived from the MPH2 culture. The fungus was plated on Petri dishes containing potato dextrose agar (Difco) (39 g l^{-1}, 150 mg of streptomycin, pH 6) and grown at 25 ± 1 °C for 10 days in the dark. Inoculation of the AI plots was carried out at star bud stage, a growth stage of high receptivity to Phoma collar attack high receptivity stage of sunflower to Phoma collar attack (Delos et al., 1997), 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 of each plant and left for five days. Drying of the disk was avoided by applying a moist cotton wool plug covered with aluminum foil around the stem base. Observations done *a posteriori* showed that natural attacks appeared one week after AI.

2.3. Disease assessment

Development of necrotic areas at the stem base induced by *P. macdonaldii* was assessed weekly from 7 and 16 days after artificial inoculation (DPI) in 2008 and 2009 respectively. In plots left subject to natural infection, the progression of the fungus on the stem base was done from 30 DPI in 2008 and 23 DPI in 2009. The disease was scored using a 0–4 scale: 0 = healthy plant, 1 = less than ¾ of the stem base circumference black, 2 = spots circling the stem base, 3 = all leaves wilted but the stem green, 4 = plant completely dead. A PR plant (scale 4) was thus defined as one completely dry before physiological maturity with necrosis circling the stem base. Over the experiments, 100% of AI and NI tagged plants were infected by *P. macdonaldii*, equivalent to a disease score of ≥2. A few plants affected by Phomopsis stem canker were ignored and only Phoma was observed on the remaining tagged plants throughout disease scoring. Disease assessment was done up to 81 DPI in 2008 and 77 DPI in 2009, ten days before the onset of normal senescence. At least 11 recordings were taken in 2008 and 10 in 2009. In NI, 9 and 8 recordings were taken in 2008 and 2009 respectively to monitor the progression of the fungus and PR expression.

Disease development was assessed by the area under disease progress curve of PR plants (AUDPC) and the final percentage of PR plants. The AUDPC, integrating the proportion of PR plants from their emergence at 64 DPI and 42 DPI still 81 and 77 DPI in 2008

Table 1
Monthly mean temperature (°C), relative air humidity (%) and monthly total rainfall thought the sunflower growing season in 2008 and 2009.

Month	Temperature (°C)		Relative humidity (%)		Rainfall (mm)	
	2008	2009	2008	2009	2008	2009
May	16.5	17.5	78	73	61	34
June	19.4	20.8	70	66	36	35
July	21.3	22.1	66	67	41	28
August	21.1	23.3	68	63	38	24
September	18.7	19.2	72	65	36	27
May–September	18.1	20.6	72	67	212	148

and 2009 respectively, was calculated according to the equation of Campbell and Madden (1990):

$$\text{AUDPC} = \sum_{i}^{n-1} \left[\frac{(y_i + y_{i+1})}{2} \right] * (t_{i+1} - t_i) \quad (1)$$

where n is the number of evaluations, y the percentage of PR and t the DPI of each evaluation. The final percentage of PR was estimated at the end of each disease assessment experiment.

2.4. Environment, microclimate and canopy measurements

Mean air temperature (°C, 2 m height), air relative humidity (%) and precipitation (mm) were recorded daily during the sunflower growing season. The weather station (CE-180, Cimel, France) was located on the edge of the trial.

Temperature and relative humidity (RH) within the canopy were recorded on AI plots up to 81 and 71 DPI in 2008 and 2009 respectively, using thermohygrometers Rotronic MP100A (Campbell Scientific Ltd., Les Ulis, France) placed in the middle of the inter-row at 0.4 m above the soil. Microclimatic variables were recorded every 30 min and measurements were stored on a Campbell CR10X (Campbell Scientific Inc.) data logger. Selected management treatments representing a range of sunflower canopies were equipped with the sensors in irrigated and rainfed plots: D1-N0, D1-N50/75, D3-N0, D3-N50/75 and D3-N150.

Leaf area index was measured with a LAI-2000 Plant Canopy Analyzer (LI-COR Biosciences, Lincoln, Nebraska, USA) on two replicates per unit plot. The fraction of photosynthetically active radiation intercepted (fPARi) was measured (400–700 nm wavelength) at flowering on both central inter-rows using a hand-held Picqhelios (Aeric, Balma, France) apparatus (Debaeke and Estragnat, 2003). This tool is composed of a 2 m high rod equipped with light sensors at the top (incident PAR) and at the bottom (residual PAR after canopy interception) and a data logger. While walking between adjacent rows, fPARi was measured continuously by the two sets of sensors.

2.5. Plant measurement

The timing of developmental stages was determined weekly using the scale proposed by Cetiom for sunflower (Cetiom, 2004). A given crop phenological stage was considered as having been reached when more than 50% had reached that stage.

Two indicators of the nitrogen nutrition status of the plant were measured at flowering (stage F3.2): the shoot N content (Nm) and the Nitrogen Nutrition Index (NNI). At flowering, five plants were cut at the soil surface on each unit plot. After drying at 80 °C for 48 h, these plants were ground to pass through a 1 mm screen prior to determination of plant N concentration on sub-samples using the Dumas combustion procedure with a Leco CNS 2000 analyzer (LECO Corporation, St. Joseph, USA) (Hansen, 1989). The NNI was calculated as follows (Lemaire and Gastal, 1997):

$$\text{NNI} = \frac{\text{Nm}}{\text{Nc}} \quad (2)$$

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. Debaeke et al. (in press) proposed the following critical dilution curve for sunflower:

$$\text{Nc} = 4.53 \times \text{ADM}^{-0.42} \quad (3)$$

A value of NNI ≥ 1 indicates a crop with ample N supply (N non-limiting); NNI = 1 is optimal N nutrition and NNI < 1 reveals N deficiency.

The crop model SUNFLO developed by Casadebaig et al. (2011) for dynamic simulation of response of sunflower cultivars to a range of soil, weather and management conditions was applied to the different water × nitrogen × plant density 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 ($ET_{actual}/ET_{potential}$) over the period from stem base inoculation to physiological maturity, which can be used as an index of plant water stress (WSI).

The stem base diameter (SBD, mm) was measured with a 150 mm Stainless Steel Digital Caliper on a sample of 10 plants for each replicate of the 18 water regime × nitrogen × plant density combinations. SBD was measured at the end of flowering (stage F3.5/F4+ in 2008 and F4/M0 in 2009) when stem growth in diameter was completed.

2.6. Statistical analyses

The two disease variables (AUDPC and final PR plants), the plant (Nm, WSI and SBD), the microclimate (temperature and RH) and canopy (fPARi) measurements were analyzed by analysis of variance (ANOVA) *via* the general linear model procedure of Statgraphics Plus 5.1 statistical software (Rockville, MA, USA) with replicate as a random variable. 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 applied to AUDPC and SBD values and arcsine transformations to PR percentage to normalize the data distribution (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).

Linear correlations (Pearson's coefficient of correlation) were computed between the final percentage of PR plants or AUDPC and the canopy variables (Nm, NNI, WSI, LAI, fPARi and SBD).

A stepwise regression analysis was computed to describe the relationship between PR or AUDPC and the different previous

Table 2
Nitrogen content (Nm, %) and fraction of fPARi intercepted by the canopy (%) at flowering for plants infected with *P. macdonaldii*, and water satisfaction index (WSI) under three levels of N fertilization (N0, N50/75 and N150) and three plant densities (4, 6.5 and 9 plants m^{-2}) in 2008 and 2009.

Plant density	Nitrogen content (%)						fPARi (%)						WSI[a]			
	2008			2009			2008			2009			2008		2009	
	N0	N75	N150	N0	N50	N150	N0	N75	N150	N0	N50	N150	Irrigated	Rainfed	Irrigated	Rainfed
D1 (4 plants m^{-2})	1.26[ns,b]	1.50[ns]	2.36[ns]	1.45[ns]	1.39[ns]	2.42[ns]	76.9[ns]	84.5 a	85.7 a	77.6[ns]	91.4[ns]	94.3 a	0.87 a	0.74 a	0.84 a	0.63 a
D2 (6.5 plants m^{-2})	1.14[ns]	1.58[ns]	2.10[ns]	1.29[ns]	1.38[ns]	1.62[ns]	78.3[ns]	86.0 ab	87.3 b	83.6[ns]	92.5[ns]	96.1 ab	0.84 b	0.69 b	0.80 b	0.59 b
D3 (9 plants m^{-2})	1.13[ns]	1.36[ns]	2.10[ns]	1.17[ns]	1.30[ns]	2.13[ns]	80.3[ns]	86.7 b	88.6 b	85.7[ns]	95.1[ns]	97.8 b	0.81 c	0.66 c	0.78 b	0.56 b

[a] High values of ET_a/ET_p (WSI) indicated an adequate water supply and significant water stress was expressed by ETa/ET_p below 0.70.
[b] Within each column, means values followed by different letters (a–c) for each of given agronomic factor (N-fertilization and plant density) for each year (2008 and 2009) are significantly different from one another based on $LSD_{0.05}$ and [ns], not significant at 5% level.

canopy variables determined on the 36 treatment combinations (year × water regime × N fertilization × plant density). A probability of $P \leq 0.05$ was used for inclusion in the stepwise analysis.

3. Results

3.1. Weather conditions

Weather conditions, namely monthly mean temperature (°C) and relative air humidity (%), and monthly total rainfall (mm), for the two years are presented in Table 1. Mean temperature throughout the growing season but more specifically during disease development (July and August) was higher in 2009 (20.6 °C) than in 2008 (18.1 °C). More rainfall was recorded in 2008 (212 mm) than in 2009 (148 mm). Consequently, the air relative humidity was higher in 2008 (72% in 2008 and 67% in 2009). In both years the weather was favorable to sunflower development. Temperature and relative humidity were optimal for infection with *P. macdonaldii* in 2008 and 2009 but low rainfall in July and August 2009 was potentially more favorable to the expression of sunflower PR.

3.2. Characterization of crop canopy development for different forms of crop management

3.2.1. Nm, fPARi and WSI

The sunflower canopy was characterized by three indicators: the shoot N content (Nm), the fraction of photosynthetically active radiation (fPARi) intercepted by the canopy and the water satisfaction index (WSI). Nm is an indicator of plant N status, fPARi of leaf area index and plant growth, and WSI of the intensity of water stress. As irrigation was generally applied just before or after anthesis, no significant differences were observed between rainfed and irrigated plots for Nm and fPARi measured at anthesis.

Contrasted crop management (N fertilization, plant density and water regime) resulted in different values of Nm, fPARi and WSI. Nm was significantly ($P < 0.005$) different between N fertilization treatments, being twice as high in N150 as in N0 (Table 2). Although the shoot N content of plants grown at low plant density was 7–19% higher than Nm at high density, this difference was not significant. fPARi values were higher in 2009 than in 2008 and significantly ($P < 0.0001$) increased with N availability up to 18% in N150 for D3 (Table 2). fPARi increased with plant density for all N levels and both years. WSI changed with irrigation, nitrogen and density levels and growing season. WSI was always significantly ($P < 0.0001$) higher with irrigation (Table 2). In irrigated plots, the mean value of ET_a/ET_p was 0.84 in 2008 and 0.80 in 2009. In rainfed plots, this ratio was below 0.74 in 2008 and below 0.63 in 2009. Simulated water stress was significantly ($P < 0.05$) higher at high plant density, especially under rainfed management and in 2009, a drier year.

3.2.2. Stem base diameter

The SBD values for the three plant densities for different levels of N fertilization varied significantly ($P < 0.0001$) in 2008 and 2009. In 2008, SBD was larger than in 2009 ($P < 0.0001$) (Table 3). Plants grown at high density were thinner than those at low density. Crops with NNI above 1 were more vigorous than those with NNI around 0.6 and had thicker stems. SBD increased significantly for low plant density (+6 mm in D1 in 2008, +4.3 mm in 2009) and high N (+6 mm in N150 in 2008, +4.8 mm in 2009) compared to D3 and N0 respectively. The difference between the minimum and the maximum diameter in D1 was 15.8 mm in 2008 and 13.6 mm in 2009, and 10.8 mm in D3 for both years.

3.2.3. Characterization of the canopy microclimate

Measurements of temperature and relative humidity in 2009 showed that the canopy microclimate was drier than in 2008. In July and August 2009, the temperature was above 25 °C for 9 days and RH was above 80% for 11 days, as against 0 and 31 days respectively in 2008. The difference in microclimate between D1 and D3 was tenuous (Fig. 1). Temperatures in irrigated plots were slightly higher in D1 than in D3 with maximum differences per day of 1.4 °C in 2008 and 0.7 °C in 2009. Conversely, temperatures in rainfed plots were lower in D1 than in D3, and differences fell to 1.7 °C. However, this variable did not differ significantly between densities in 2008 ($P = 0.59$) and 2009 ($P = 0.88$). The difference in RH between the two densities was greater in 2008 than in 2009, sometimes reaching 8%. Throughout disease development, RH was significantly ($P = 0.003$) higher in D1 than in D3, but only in 2008. Mean RH was 80.2% for D1 and 78.9% for D3 in 2008. Corresponding values in 2009 were 74.8% (D1) and 75.0% (D3).

3.3. Effects of plant density on disease incidence

The progression of the disease was recorded in AI and NI plots to assess the effect of crop management. At 30 DPI in 2008, 100% of AI plants had a girdling black necrosis at the stem base (scales 1 and 2), as against 90% in NI, at all plant densities. A delay in the expression of the disease was observed in 2009. At 35 DPI in 2009, only 27% (D1) and 47% (D2) of NI plants presented girdling necrosis *vs.* 72% (D1) and 79% (D2) of AI plants. AI and high plant density both increased Phoma incidence at stem base.

The date of appearance of PR, delayed in 2009 (60 DPI) compared with 2008 (45 DPI), was the same between AI and NI. Annual disease progress curves of PR in AI clearly showed significant effects of crop management (Fig. 2). In both years, the development of PR differed between plant densities. The first PR plants were observed in D2/D3 and a seven-day delay was observed in D1. Both N fertilization and limiting water supply significantly ($P < 0.001$) exacerbated PR. PR expression was earlier in D2 and D3 for N150, rainfed plots than in D1. The latest PR appeared in D1 for N0, irrigated plots. In 89% of cases in 2008 and 2009, the final PR was greater in D3 than in D1. In general, high plant density (D3) increased PR by 26–29% compared

Table 3
Mean stem base diameter (SBD, mm) at flowering for the three plant densities (4, 6.5 and 9 plants m^{-2}) for different levels of N fertilization (N0, N50/75 and N150) in 2008 and 2009.

Plant density	Stem base diameter (mm)							
	2008				2009			
	N0	N75	N150	Mean	N0	N50	N150	Mean
D1 (4 plants m^{-2})	23.3[a] a	25.0 a	26.2 a	26.2 a	19.4 a	21.6 a	22.5 a	21.1 a
D2 (6.5 plants m^{-2})	20.3 b	22.0 b	22.2 b	22.2 b	17.6 b	17.9 b	18.8 b	18.1 b
D3 (9 plants m^{-2})	18.1 c	19.7 c	20.2 c	20.2 c	15.7 c	17.0 c	17.7 c	16.8 c

[a] Within each column, mean values followed by different letters are significantly different from one another based on $LSD_{0.05}$.

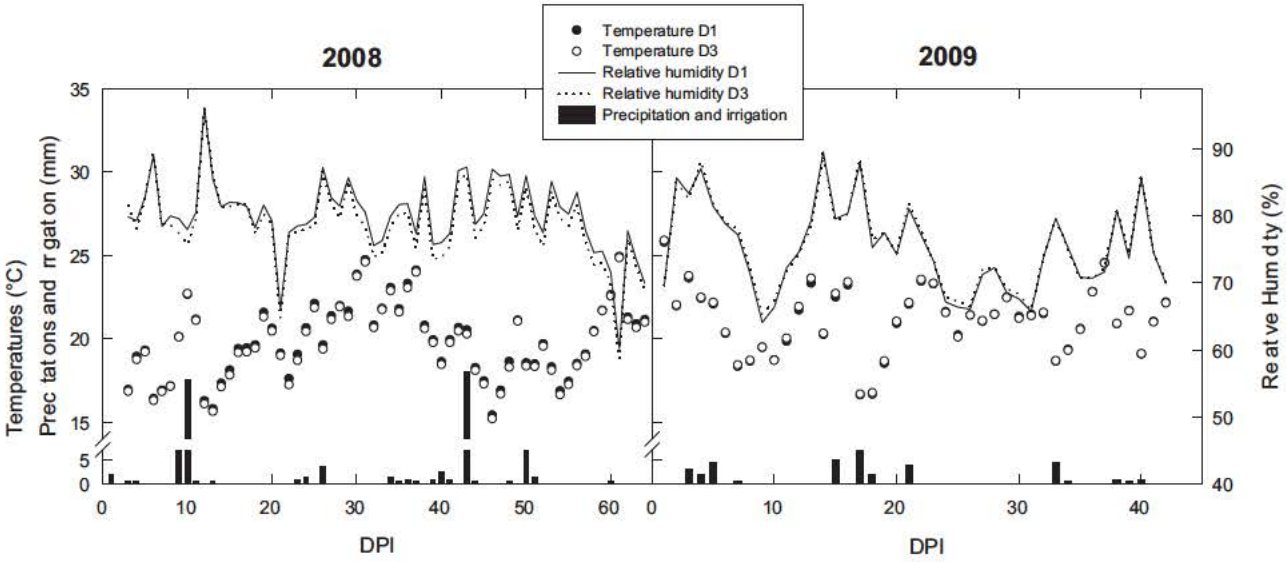

Fig. 1. Daily mean temperature, relative air humidity within the sunflower canopy and cumulated precipitations and irrigations for plant densities D1 and D3 (4 and 9 plants m^{-2}) from inoculation to PR emergence at 64 DPI in 2008 and 42 DPI in 2009 (DPI = days post artificial inoculation).

with low densities (D1) (Table 4). At 81 DPI in 2008, 10% (D1) and 37% (D2) of NI plants were prematurely ripened, against 37% (D1) and 59% (D2) at 77 DPI in 2009.

The disease severity index (AUDPC) integrating the progression of PR in AI plots, differed significantly ($P < 0.005$) for the three plant densities under comparison, whatever the N fertilization, the water regime and the year (Table 4). For plant density ≥6.5 plants m^{-2}, the value of AUDPC increased strongly and was 1.5 times greater than at 4 plants m^{-2} in 2008 and 2009.

Table 4
Mean value of AUDPC and final percentage of sunflower prematurely ripened plants (PR, %) artificially infected with *P. macdonaldii* at stem base for the three plant densities (4, 6.5 and 9 plant m^{-2}) in 2008 and 2009, whatever Nitrogen applied (N0, N50/N75 and N150) and water regime (irrigated, rainfed).

Plant density	AUDPC[a]		Final PR (%)[b]	
	2008	2009	2008	2009
D1 (4 plants m^{-2})	2.37 b[c]	5.76 b	33.6 b	50.4 b
D2 (6.5 plants m^{-2})	3.84 a	8.39 a	45.8 a	68.1 a
D3 (9 plants m^{-2})	3.98 a	8.55 a	45.6 a	71.5 a

[a] The area under disease progress curve of premature ripening plants (AUDPC), based on the percent of plants totally wilted before physiological maturity, was calculated according to Campbell and Madden (1990) from 64 to 81 days post inoculation (DPI) in 2008 and 42 to 77 DPI in 2009.
[b] The final percent of PR plants were taken 81 and 77 days from the start of epidemic in 2008 and 2009 respectively.
[c] Within each column, mean values followed by different letters are significantly different from one another based on $LSD_{0.05}$.

3.4. Statistical relationships between PR and canopy variables

The crop management options resulted in clear differences in Nm, NNI, WSI, LAI, fPARi and SBD values (Tables 2 and 3) but not in microclimate conditions (Fig. 1).

To better analyze the influence of the different canopy descriptors on PR and AUDPC, Pearson's correlation coefficients and stepwise analyses of regression were computed on AI plots only for both years. The final percentage of PR plants was significantly and positively correlated to AUDPC, fPARi, LAI, Nm and NNI (Table 5). AUDPC was significantly and positively correlated to fPARi, Nm and NNI. Moreover, Nm, NNI, SBD, WSI, fPARi and LAI were positively correlated among themselves while fPARi and LAI were negatively correlated to WSI. PR and AUDPC were negatively correlated to SBD and WSI. On highly fertilized plants, the more SDB increased, the more the percentage of PR was reduced (Fig. 3).

Stepwise regression explained PR better than AUDPC. A model associating positively fPARi and NNI explained 88% and 77% of the variability of PR and AUDPC respectively.

$$PR = -270.48 + 3.37 \times fPARi + 38.19 \times NNI \quad (R^2 = 0.876, n = 36, P < 0.05) \tag{4}$$

$$AUDPC = -44.33 + 0.52 \times fPARi + 5.46 \times NNI \quad (R^2 = 0.769, n = 36, P < 0.05) \tag{5}$$

when the model was run on PR with 4 variables describing leaf area (LAI), stem diameter growth (SBD), N shoot content (Nm) and water

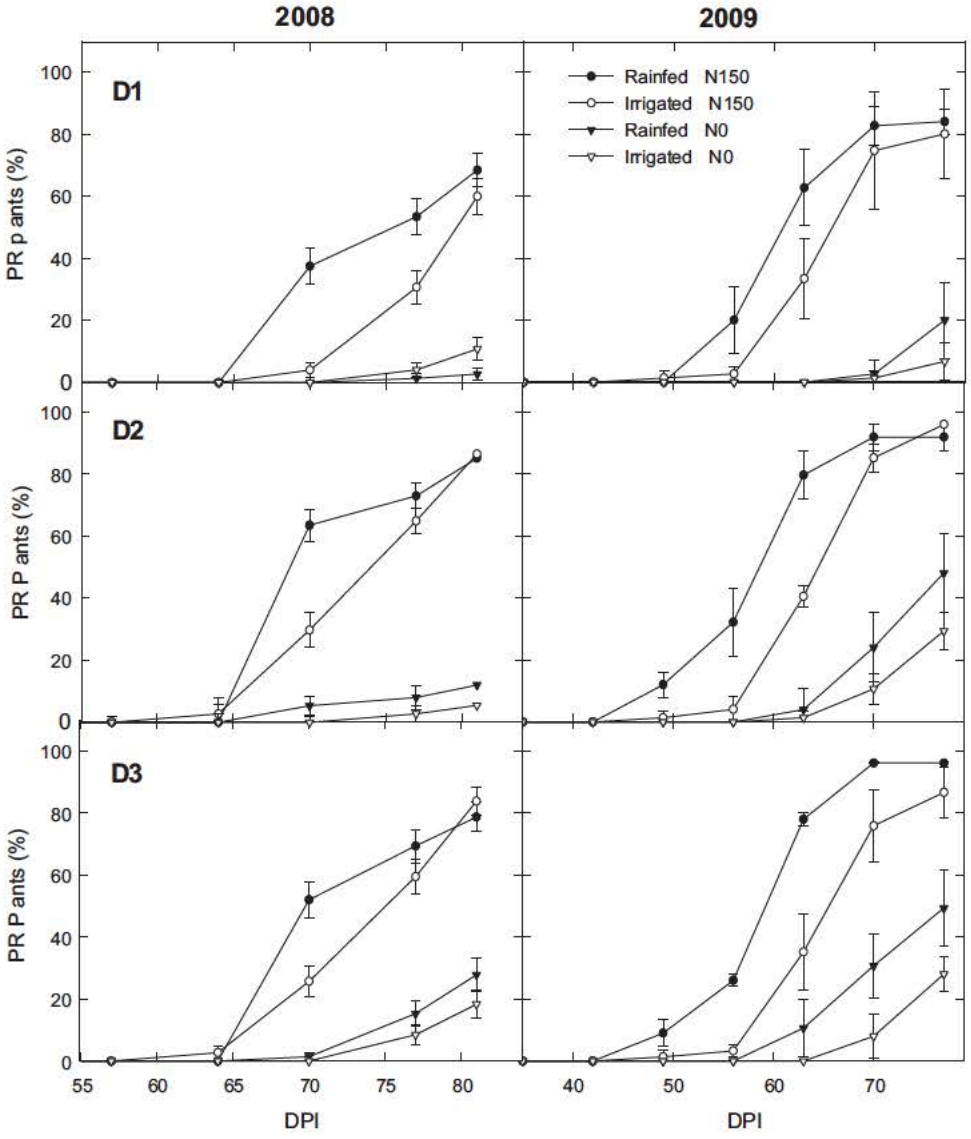

Fig. 2. Annual disease progress curve of premature ripened (PR) plants after artificial inoculation with *P. macdonaldii* for two plant densities D1 and D3 (4 and 9 plants m^{-2}) with non-limiting (Irrigated, N150) and stressed (N0, rainfed) nitrogen and water supply in 2008 and 2009.

Table 5
Correlation coefficients among AUDPC, final percentage of premature ripened plants (PR, %), and canopy development variables (SBD, fPARi, Nm, NNI, WSI and LAI) of sunflower in 2008 and 2009.

Trait	PR (%)	SBD (mm)	fPARi (%)	Nm (%)	NNI	WSI	LAI
AUDPC	0.89***	−0.17	0.84***	0.50**	0.57***	−0.32	0.29
PR (%)	–	−0.10	0.88***	0.55***	0.62***	−0.24	0.47**
SBD (mm)	–	–	−0.18	0.40*	0.22	0.17	0.36*
fPARi (%)	–	–	–	0.32	0.40*	−0.44**	0.38*
Nm (%)	–	–	–	–	0.93***	0.31	0.28
NNI	–	–	–	–	–	0.33*	0.41*
WSI	–	–	–	–	–	–	−0.04

* Correlation coefficient is significant at the $P = 0.05$ level.
** Correlation coefficient is significant at the $P = 0.001$ level.
*** Correlation coefficient is significant at the $P = 0.0001$ level.

satisfaction rate (WSI), 73% of the variability of PR was explained: PR increased with LAI and Nm, and decreased with SBD and WSI.

$$PR = 105.79 + 45.33 \times Nm - 103.04 \times WSI - 5.00 \times SBD + 12.35 \times LAI \quad (R^2 = 0.731, n = 36, P < 0.05). \quad (6)$$

4. Discussion

Understanding the PR level in a given field and the year-to-year and between-field variation is a complex problem as weather and

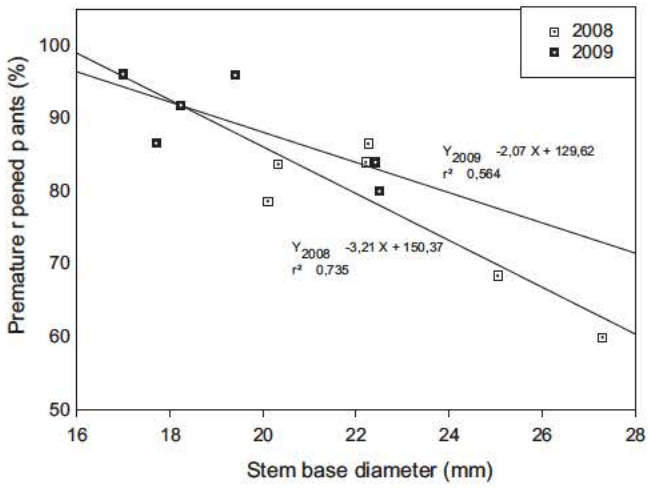

Fig. 3. Relationship between the final percentage of sunflowers affected by premature ripening (PR) and the stem base diameter (SBD, mm) measured at flowering for highly N fertilized plants at 4, 6.5 and 9 plants m^{-2} in 2008 and 2009.

soil conditions, crop practices, cultivar tolerance and field environment are all involved in this syndrome (Bordat et al., 2011). To evaluate the effect on PR of plant density in interaction with N rate and water regime, a thorough characterization of crop canopy and disease progression was necessary under artificial inoculation. Under natural infection, the timing of disease development and diversity for aggressiveness among Phoma stains (Abou AlFadil, 2006) were less controlled. This could partially explain the higher incidence of premature ripening in AI than NI plots for all crop densities.

The occurrence and severity of fungal diseases are closely linked to specific atmospheric conditions affecting the life cycle of the fungus (Bourke, 1970; Duthie, 1997). Low air temperature and high rainfall in 2008 may have been more favorable to spore emission (Delos et al., 1998) and pathogen spread around the collar. In 2009, hotter and drier conditions may have hindered the first stages of the progression of *P. macdonaldii*. Although the influence of temperature on spore emission has not been demonstrated (Bordat et al., 2011), *in vitro* pathogen growth rate is known to be largely dependent on temperature and relative humidity, with optimum growth between 20 and 30 °C and RH above 80% (Roustaee et al., 2000; Weeraratne and Priyantha, 2003).

Changes to crop management modify the microclimate (temperature, relative humidity, and leaf wetness) within the crop canopy (Huber and Gillespie, 1992). Increase host-density and N supply, in the absence of severe water deficit, result in higher values of leaf area index, less air movement and higher relative humidity within the canopy (Stanojevic, 1985). Under these conditions, ascospore germination, infection and disease incidence can potentially be accelerated (Burdon and Chilvers, 1982; Masirevic and Gulya, 1992; Debaeke et al., 2000). For leaf and stem diseases, incidence and severity of Phomopsis stem canker (*Diaporthe helianthi*) and Phoma black stem primary infections are closely related to canopy development (LAI) and microclimatic conditions which are significantly affected by plant density (Gulya et al., 1997; Debaeke and Moinard, 2010). Increasing plant density and N fertilization, in conditions where water is limited, resulted in earlier infection and a greater proportion of girdling stem and collar lesions (Debaeke and Pérès, 2003) which was confirmed in this study. Despite a denser canopy in D3 (as expressed by fPARi), the lack of significant microclimatic differences between low (D1) and high (D3) densities suggests that canopy development, in these environmental circumstances, was not important for fungal spread or PR expression. The effect of the microclimate would have been certainly more conclusive if this study had been focused on NI and on the first stages of the disease.

Despite stem base girdling necrosis appearing later than in 2008, PR emergence was observed earlier in 2009. The only cropping factor differing between years was water supply. Pearson's correlation coefficient and stepwise linear regression showed that WSI reduced PR. Thus, low rainfall in July and August 2009, resulting in water stress (low WSI), was more favorable to PR. This supports a previous report on sunflower PR showing that under conditions where water satisfaction was limiting after anthesis, PR severity was increased (Seassau et al., 2010a). The effect of drought

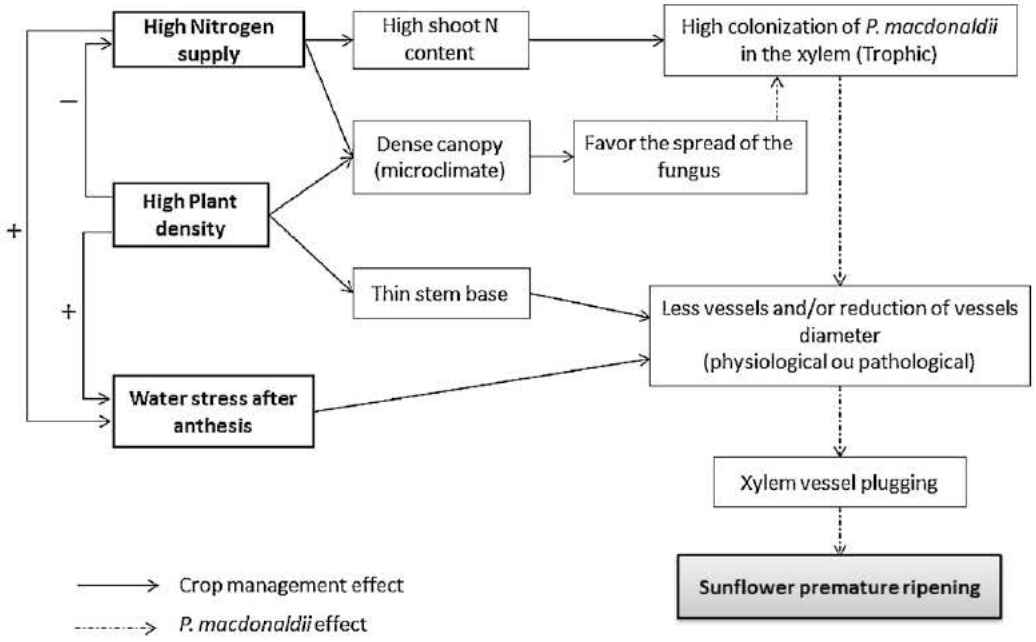

Fig. 4. Potential effect of crop management (water deficit, high Nitrogen supply and plant density) on crop canopy development (architecture, morphology) inducing favorable conditions for the spread of *P. macdonaldii* in the plant (xylem) leading to premature ripening.

conditions on PR may have been reinforced by crop management. High N fertilizer rates and plant density, characterized by high Nm, NNI, LAI and fPARi values, both increased the crop exposure to drought in unirrigated conditions as a result of greater biomass development and leaf area index, and the related high transpiration (Haefele et al., 2008). Water stress may be an agronomic factor which makes the plant more susceptible to PR when the fungus has colonized the stem while N fertilization plays on disease development and plant density facilitates the complete stem colonization.

High N supply and plant density characterized by an increase of Nm, NNI, fPARi, LAI and a reduction in SBD, significantly increased AUDPC, the time course and the final percentage of PR plants. These physiological and anatomical changes that could affect host susceptibility to the pathogen and could be involved in the harmful expression of the disease. For non-limited N supply, dense canopy development may have increased the drought risk (Haefele et al., 2008). A predisposition to disease is often observed in host plants during water shortage. A reduction of xylem vessels diameter under water stress (Nardini and Salleo, 2005) as an adaptation to the risk of vascular embolisms (Mepsted et al., 1995; Hacke et al., 2001) could be involved. Presence of hyphae of *P. macdonaldii* in the xylem of PR plants (Seassau et al., 2010b) could lead to vessel blockage and plant death (Beckman, 1964; Robb et al., 1981; Put and Clerkx, 1988). This phenomenon could be increased with high Nm in the plant, which could be trophic for the fungus, accelerating its development within the plant (Walters and Bingham, 2007). Plants at high density, more susceptible to girdling attacks and lodging, could be weakened which could explain the higher proportion of PR plants in D3 than in D1 for both years. Conversely, stem thickening throughout plant growth would reduce the susceptibility to PR of plants grown at low density.

5. Conclusion

This study was intended to identify the effect of sunflower crop management on PR induced by *P. macdonaldii*. The effect of nitrogen and water supply on PR, previously observed, was confirmed. The effect of plant density on disease expression was studied in interaction with these two factors. Field experiments demonstrated that the combination of high plant density and nitrogen fertilization in rainfed conditions after anthesis resulted in high disease pressure in two contrasting growing seasons. From the characterization of the canopy by agronomic indicators related to N shoot status, plant growth and architecture, stem base morphology, plant water satisfaction rate and microclimate, we can propose a conceptual framework of the effect of plant density on the expression of the disease (Fig. 4). The microclimate apparently has a moderate effect on disease epidemiology and PR, unlike the other variables that directly affect PR. High leaf area index (due to high N fertilization and high plant density) results more rapidly in soil water exhaustion and a drop in transpiration after anthesis. Avoiding excessive N fertilization by using the soil N balance method could significantly reduce disease severity. Also, manipulating the stem diameter, mainly through planting density and N supply, could be exploited more, instead of resorting to fungicide use. This is probably a morphological trait that breeders could exploit in the future. Indeed genetic tolerance to PR should be evaluated in order to achieve complete non-chemical control (Bordat et al., 2011). Promising cultivars, with thick stems, should therefore be tested at high density and N supply under water-limited conditions, a procedure which could be used in resistance tests during breeding programmes for an effective control of sunflower PR.

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