Downy mildew of sunflower as affected by nitrogen fertilization

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ABSTRACT

- Downy mildew of sunflower is caused by the obligate parasite *Plasmopara halstedii* Farl. Berl. and de Toni, a soilborne oomycete which infects seedlings systemically and can also affect above ground parts of sunflower, mainly leaves, during the airborne phase of its life cycle causing localized lesions (secondary symptoms). Nitrogen fertilization has proven to be a very positive crop input for increasing yield in sunflower under irrigation in Mediterranean-type climatic regions. However, nitrogen supplies to sunflower are not frequent in farms of Spain, where low crop costs are pursued. Nitrogen fertilizers may have an impact on the airborne phase of sunflower downy mildew, as has been reported on some diseases caused by airborne pathogens. In this work we investigated whether different levels of N fertilization can affect disease development (both primary and secondary lesions).
- An experiment was conducted in 2011 in a naturally-infested field located in Cordoba (Spain). Eight nitrogen treatments (0 to 140 kg N/ha) were applied in a randomised complete block design with four replications. The experimental plots consisted of eleven 13.5 m-long rows, 0.7 m apart, and plants being 0.2 m apart within the row. A sunflower hybrid susceptible to all races of the pathogen was sown on March 24th, and the time course of the incidence and symptomatology of the disease was weekly recorded until June, when plants were in full bloom. At that moment, the percentage of plants showing leaf chlorosis and stunting or shortened internodes was noted as incidence of early primary symptoms (EPS). Secondary symptoms (percentage of affected leaves, location of secondary leaf spots in the plants (based on a 0-6 scale), and leaf severity index [LSI]) were also assessed. Leaf area index (LAI) was measured in samples made of four plants per experimental plot, collected on May 20th. Since the blocks had different water supplies from flowering to harvest, and in order to evaluate the disease occurrence under different doses of N and good water availability, the incidences of late primary symptoms (LPS) on the three central rows (replications) of each experimental plot of block I were noted and analysed following a completely randomised statistical design. Analyses of variance were performed for all the variables and Fisher's protected LSD tests were used for comparisons of treatments.
- The incidence of EPS varied between 12 and 28%, but it did not significantly depend on the dose of nitrogen (P = 0.2155). However, secondary symptoms were highly dependent on the nitrogen treatment (P < 0.0001). Highest values of percentage of leaves showing secondary infections and of location of these leaves in the plants were obtained for 80 and 60 kg N/ha (58% affected leaves and 2.88 respectively), and lowest values were associated to the dose of 0 kg N/ha (27% affected leaves and 1.54). Values of LSI ranged from 0.87 for 0 to 2.06 for 140 kg N/ha respectively, and no significant differences were obtained for doses between 40 and 120 kg N/ha (average 1.85 LSI). Similarly, significant differences of LAI were associated to nitrogen fertilization; values ranged from 1.37 for 0 to 2.94 for 140 kg N/ha, and similar LAIs were measured at doses ranging from 40 to 120 kg N/ha (average 2.24 LAI). At the end of flowering significant differences of LPS were associated to fertilization in block I, with values ranging from 17% for 0 kg N/ha to 50% for 120 kg N/ha.
- *The incidence of EPS caused by *P. halstedii* was not affected by the dose of N. On the contrary, the incidence, location and severity of secondary lesions was favored by high N doses, as has been reported for diseases caused by airborne pathogens, and these high doses were associated to a dense crop canopy. Besides, the increase of the incidence of LPS and significant response to N fertilization as compared to EPS, under good water availability, suggest that some plants initially displaying secondary infections became systemically infected, thus increasing the incidence of LPS.
- The incidence of secondary lesions by *P. halstedii* can be affected by N fertilization and these lesions can turn into systemic infections resulting in primary symptoms in the plants. The effect of moderate increases of N fertilization under different water supplies on the development of primary and secondary symptoms of sunflower downy mildew as compared to their impact on crop yield will be further studied.

Key words: ammonium nitrosulfate - crop canopy - *Helianthus* annuus L.- oomycetes - *Plasmopara halstedii* - secondary infections.

INTRODUCTION

Downy mildew of sunflower is caused by the biotrophic pathogen *Plasmopara halstedii* Farl. Berl. and de Toni, a soilborne oomycete that infects seedlings in the early stage of germination causing systemic primary symptoms. The pathogen can also affect above ground parts of sunflower, mainly leaves, during the airborne phase of its life cycle, causing localized lesions (secondary symptoms) (Gulya et al., 1997).

Nitrogen fertilization has proven to be a very positive crop input for increasing yield in sunflower under irrigation in Mediterranean-type climatic regions (Muriel et al, 1980). Yield increases to nitrogen fertilizer up to 120 kg N/ha or higher have been reported, but, overall, increases depend on the plant-available nitrogen in the soil and on soil water and rainfall (Blamey et al., 1997). Nitrogen supplies to sunflower are not frequent in farms of Spain, where low crop costs are pursued and high doses of fertilizer are in many cases not economically feasible.

The increased disease incidence after nitrogen fertilizer application above the recommended doses has been demonstrated for some biotrophic fungal pathogens (Mascagni et al., 1997), but, in general, the effect of nitrogen on the disease development depends on the pathogen and on the range of nitrogen fertilizer that is considered (below, within or above the recommended doses) (Walters, 2009). It is commonly thought that application of nitrogen fertilizer can increase disease severity via effects on crop canopy development. Thus, large canopies with high shoot densities may be more conducive to spore transfer and pathogen infection than sparse canopies (Walters, 2009).

In this work we investigated whether different levels of nitrogen fertilization were associated to systemic (or primary) and localized (or secondary) symptoms of sunflower downy mildew (SDM) and how nitrogen fertilization affects vegetative growth of sunflower when SDM occurs in the field.

MATERIALS AND METHODS

An experiment was conducted in 2011 in a naturally-infested field located in Cordoba (Spain). Eight nitrogen treatments (0 to 140 kg N/ha) were applied in a randomised complete block design with four replications. The experimental plots consisted of eleven 13.5 m-long rows, 0.7 m apart, and plants being 0.2 m apart within the row. One irrigation of 25 mm was applied immediately after sowing. Thereafter, each block had a different irrigation management, block I having the highest water supply. A total amount of 238 mm of water were applied to it by means of six irrigations, from the 25th of May (flowering) to the 13th of July (harvest).

A sunflower hybrid susceptible to all races of the pathogen was sown on March 24th, and the time course of the incidence and symptomatology of the disease was weekly recorded until late May, when plants were in full bloom. At that moment, the percentage of plants showing leaf chlorosis and stunting or shortened internodes was noted as incidence of early primary symptoms (EPS). Early secondary symptoms (ESS) were simultaneously assessed considering: percentage of affected leaves, location of secondary symptoms in the plants (based on a 0-6 scale, where 0 = no affected leaves and 6 = leaf spots affecting the terminal bud), and leaf severity index (LSI, calculated as LSI= percentage of affected leaves*location of secondary symptoms/100). Leaf area index (LAI) was measured in samples made of four plants per experimental plot, collected on May 20th. At the end of flowering and in order to evaluate the disease occurrence under different doses of N and good water availability, the incidences of late primary symptoms (LPS) on the three central rows (replications) of each experimental plot of block I were noted and analysed following a completely randomised statistical design.

Analyses of variance were performed for all the variables, previously transformed, and Fisher's protected LSD tests were used for comparisons of treatments.

RESULTS AND DISCUSSION

Incidence of EPS (leaf chlorosis and shortened internodes) varied between 12 and 28% in the experimental plots, but it did not significantly depend on the dose of nitrogen ($P = 0.2155$) (Table 1). However, secondary symptoms, i.e. percentage of affected leaves, location of these leaves in the plants, and LSI, were highly dependent on the treatment ($P < 0.0001$ for the three variables). Highest values of leaves percentage showing secondary infections and location of these leaves in the plants (58% affected leaves and 2.88 value of location) were obtained for 80 and 60 kg N/ha respectively. Lowest values (27% affected leaves and 1.54) were associated to 0 kg N/ha. A clear response to dose was obtained for the LSI, that ranged from 0.87 to 2.06 for 0 and 140 kg N /ha respectively with no significant differences for doses between 40 and 120 kg N/ha (average 1.85 LSI) (Table 1). Similarly, significant differences of LAI were associated to nitrogen fertilization. Values of LAI varied from 1.37 for 0 to 2.94 for 140 kg N/ha, and no statistical differences were recorded for LAI at doses ranging from 40 to 120 kg N/ha (average 2.24) (Table 1). Our results show that the incidence of EPS caused by *P. halstedii* is not affected by the nitrogen dose. On the contrary, the incidence, location and severity of ESS are favored by moderate to high doses of nitrogen, which are associated to a dense crop canopy. The influence of nitrogen fertilization on some diseases caused by airborne pathogens has been earlier reported, but the effect that nitrogen inputs may have on crop canopy development and hence canopy microclimate conducive to pathogen infections depend on the crop, the pathogen, and the range of doses of nitrogen fertilizer that are considered (Walters, 2009). The correlation between leaf area (LAI) and severity of secondary symptoms (LSI) of downy mildew in sunflower should be confirmed in future experiments.

Table 1. Early (primary and secondary) symptoms of downy mildew and leaf area index of sunflower in a field naturally infested by *Plasmopara halstedii* in Córdoba (Spain), in full bloom and fertilized with different doses of nitrogen

Dose of nitrogen (kg / ha)	**Early symptoms**				**LAI**
	Incidence of EPS (%)	ESS			
		Affected leaves (%)	Location in the plant (0-6)	LSI	
0	11.7	27.2 D	1.54 D	0.87 D	1.37 D
20	23.3	41.0 C	2.16 C	1.39 C	1.46 CD
40	17.5	51.1 B	2.32 BC	1.65 B	1.99 BC
60	14.2	51.2 B	2.88 A	2.03 AB	2.17 B
80	20.0	57.8 A	2.35 BC	1.89 AB	2.21 B
100	23.3	53.1 AB	2.59 ABC	1.94 AB	2.33 B
120	27.5	50.2 B	2.53 ABC	1.72 AB	2.52 AB
140	15.0	55.3 AB	2.66 AB	2.06 A	2.94 A

EPS, early primary symptoms; ESS, early secondary symptoms; LSI, leaf severity index; LAI, leaf area index.
Percentage values were transformed (transformed variable=log[variable]) prior to statistical analyses.
Values followed by the same letter within a column did not differ significantly (P = 0.001) as determined by ANOVA and the Fisher's protected LSD test.

At the end of flowering significant differences of LPS were associated to fertilization in block I, with values ranging from 17 for 0 kg N/ha to 50% for 120 kg N/ha (Table 2). The same trend of response to dose of N was obtained for the early symptoms of SDM in this block as compared to the response of the randomized complete block experiment: no significant effects of dose on the incidence of EPS were recorded, but LSI was significant dependent of the fertilizer, ranging from 0.46 for 0 kg N/ha to an average of 2.15 for 60 and 140 kg N/ha (Table 2). Higher incidences of LPS as compared to incidences of EPS were observed in the block I analyses. The most feasible explanation is that some plants initially displaying secondary infections become systemically infected, thus increasing the final incidence of plants showing primary symptoms. This could be also the reason for the significant effect of N fertilizer on the incidence of LPS. Transitions from local to systemic infections of *P. halstedii* have been previously observed (Spring, 2009), and they are known as well from other agronomically relevant downy mildew pathogens such as *P. tabacina* (Johnson, 1989). Aerial infections by *P. halstedii* have been described to give rise to local lesions but rarely to result in systemic infections (Zimmer, 1975; Gulya et al., 1997), but our results show that these secondary infections can evolve systemic and cause typical dwarfing and extended chlorosis of the leaves, indistinguishable from those due to early infection of seedlings. The effect of moderate increases of nitrogen fertilization under different water supplies on the development of secondary symptoms as well as on late primary symptoms of SDM as compared to their impact on crop yield will be further studied in order to consider the advisability of the combined management of water supply and N fertilization for the control of infections by *P. halstedii*.

Table 2. Incidences of early and late primary symptoms and leaf severity index associated to natural infection by *Plasmopara halstedii* in a field experiment conducted in Córdoba (Spain) under high water supply and fertilized with different doses of nitrogen

Dose of nitrogen (kg / ha)	Incidence of EPS (%)	LSI	Incidence of LPS (%)
0	13.3	0.46 C	16.7 C
20	36.7	1.66 AB	40.0 AB
40	16.7	1.34 B	40.0 AB
60	13.3	2.15 A	36.7 AB
80	16.7	1.67 AB	36.7 AB
100	13.3	1.85 AB	33.3 B
120	43.3	1.58 AB	50.0 A
140	13.3	2.16 A	23.7 BC

EPS, early primary symptoms; LSI, leaf severity index; LPS, late primary symptoms.
Data were analyzed following a completely randomised statistical design (block I in the randomised complete block experiment).
Percentage values were transformed (transformed variable=log[variable]) prior to statistical analyses.
Values followed by the same letter within a column did not differ significantly (P = 0.001) as determined by ANOVA and the Fisher's protected LSD test.

ACKNOWLEDGMENTS

This work was supported by the Spanish Ministry for Science and Innovation (MICINN) and the European Regional Development Fund (ERDF) of the EU within the framework of the grant AGL2010-17909.

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