

EFFECT OF SUNFLOWER CROP MANAGEMENT ON STEM CANKER (*DIAPORTHE HELIANTHI*) ATTACKS FOR SUSCEPTIBLE AND TOLERANT GENOTYPES

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Abstract

The effects of crop management (plant density, N fertilization) on sunflower stem canker (*Diaporthe helianthi*) were analysed for a range of genotypes (susceptible, tolerant) in Toulouse (SW France) under conditions of high inoculum (2000). The evolution of leaf and stem symptoms was related to canopy development, microclimatic conditions and the timing of leaf infection. The number of girdling lesions per plant was maximum with high N fertilization but more leaves were infected without N fertilization. Increasing plant density resulted in a greater proportion of girdling stem lesions which are detrimental to yield.

Introduction

Phomopsis stem canker, whose causal agent is *Diaporthe helianthi* Munt.-Cvet. et al. was reported in many regions of sunflower production in Argentina, France, Hungary, Rumania, USA and Yugoslavia (Gulya et al., 1997). It spread over the entire French sunflower area from 1992-93 onwards (Delos and Moinard, 1996). This pathogen causes drastic reductions in yield (up to 1.5 t/ha) and oil content (up to 25 %), as a result of premature leaf and plant wilting and lodging (Gulya et al., 1997). In France, the use of disease-tolerant genotypes and fungicides is the basis of current disease control (Cetiom, 1995). Both for economic and environmental reasons, integrated disease management is required, exploiting the ability of sunflower to self-regulate disease occurrence, given appropriate canopy management.

In 1998-2000, a cooperative program was conducted by INRA and CETIOM to analyze and predict the effects of sunflower crop management on the frequency and severity of *Phomopsis* and the first results were recently published (Debaeke and Estragnat, 2003; Debaeke et al., 2003). It was shown that reduction of plant density and N supply was not in itself sufficient to lower the infection rate to the level given by chemical protection, but in conjunction with delayed sowing, infection levels were comparable to early and protected sowings.

As plant density and nitrogen rate significantly affect the development of sunflower leaf area, it was suggested that leaf area index (LAI) and the fraction of photosynthetically active radiation intercepted by the canopy ($fPAR_i$), which are closely related and may constitute synthetic variables for representing the effect of canopy management and crop intensification level (Debaeke and Estragnat, 2003). Increasing plant density and N amount generally resulted in higher values of relative humidity within the canopy (Debaeke et al., 2000), enhancing ascospore germination and the resulting leaf infection processes. Epidemiological

models such as Asphodel (Delos and Moinard, 1996) can represent satisfactorily the influence of genotype, sowing date and weather conditions on potential crop damage.

The objective of this research was to investigate in more detail the stem canker infection process in several sunflower canopies differing by genotypic susceptibility, plant density, nitrogen status, and water availability under contrasted conditions of inoculum pressure.

Materials and Methods

In 2000, a field experiment was carried out at INRA-Toulouse (SW France, 43°36 N, 1°26 E) on a deep silty-clay soil. Conditions of infection, either natural (Ni) or semi-natural (SNi), were applied to 2 adjacent large plots. In the latter case, sunflower stalks from infected fields were placed between the crop rows according to the method described by Viguié et al. (2000). Stalks were introduced on 5 June and removed on 15 June. Sprinkler irrigation (20 mm) was applied on 8 June on the SNi plot for promoting leaf infection and fungicide protection against *Phoma* black stem (*Phoma macdonaldii* Boerema) was applied on 26 May and 20 June with Dithane DG at 2.5 l/ha.

Each plot (Ni, SNi) was composed of 48 unreplicated subplots of 90 m sq. each. Four cultivars either susceptible (Proleic 204, DK3790) or tolerant (Inedi, Santiago) were sown on 10 April at two plant densities (d1= 5 pl/m sq., d2= 8 pl/m sq.) with fertilization (N2= 120 kg N/ha: 50 N at sowing + 70 N on 22 May) or without (N1= 0). Three regimes of irrigation were applied (IRR1= no irrigation except for promoting leaf infection; IRR 2: 35 mm on 25/06; IRR3: 35 mm on 25/06 + 35 mm on 6/07).

The appearance of visible leaf (and stem) symptoms and their vertical distribution were scored on 20 tagged plants at eight dates between 21 June to 26 July. At each date, new infected leaves and new stem lesions (shallow or girdling) were scored. When a leaf symptom failed to develop a stem lesion, the causes were identified: either senescence or *Phoma* attack. Only spots girdling the stem and wilted or broken stems were considered to be detrimental to yield (CETIOM, 1995).

The fraction of radiation intercepted by the sunflower canopy ($fPAR_i$) was measured regularly from star bud to early anthesis stages using a hand-held Picqhelios apparatus (Debaeke and Estragnat, 2003). Relative humidity (RH) and temperature within the canopy were recorded using thermohygrometers (Rotronic) placed in the middle of the interrow (row width= 0.50 m) at 0.4 m above the soil for one susceptible cultivar (cv. Proleic) and 4 treatments: d1-N1, d1-N2, d2-N1, d2-N2.

Results

Effects of Genotype and Management on the Proportion of Infected Stems. The importance of the primary inoculum was demonstrated by the comparison of plots SNi and Ni which did not differ except for the presence of infected stalks in the crop interrow: 75 % of the stems were infected with SN infection versus 58 % under natural conditions (Table 1). The difference would have been greater with less natural inoculum in June. The presence of inoculum did not change the genotypic ranking, but the difference in infected stems between SNi and Ni was greater for tolerant than for susceptible cultivars. With SNi, the proportion of infected stems did not differ with plant density while a higher infection rate was observed at high plant density under Ni conditions. Irrigation around flowering increased the proportion

of stems bearing lesions, especially with semi-natural conditions. The effect of nitrogen was unusual: a higher proportion of stems was attacked with no nitrogen than with 120 kg N/ha, in both infection conditions.

Table 1. Effects of crop density, nitrogen fertilization and irrigation on the proportion of sunflower plants with at least one stem lesion of *Phomopsis* on 28/07: natural (Ni) and semi-natural (SNi).

Plot	Nitrogen		Crop density		Cultivar (T = Tolerant; S = Susceptible)				Irrigation		
	N1 (0)	N2 (120)	d1 (5)	d2 (8)	Inedi (T)	Santiago (T)	DK3790 (S)	Proleic204 (S)	IRR 1	IRR 2	IRR 3
SNi	79 a	71 b	81	82	31 c	72 b	98 a	99 a	68 b	80 a	78 a
Ni	64	53	31 b	56 a	16 d	46 c	78 b	93 a	53	59	63

Within each line, comparing N rates, crop densities, cultivars or irrigation amounts, values followed by the same letter are not significantly different at $p < 0.05$. The absence of letters indicates nonsignificant effects at $p < 0.05$.

Simulation of Infection Conditions. According to Asphodel which was run using hourly climatic data at 2 m, asci were mature on 17 May and numerous episodes of spore deposition were observed in May (17, 26), June (4-5, 11, 30) and July (10, 14-15). In 2000, conditions were optimal for successful infection from the end of May to mid-June (sunflower stage, rainfall: 128 mm between 5 and 13 June, air saturation, infected stalks in the interrows) and again in mid-July. Symptoms appeared early on leaves (14 June), 10 days before anthesis, and the first lesions were observed on stems on 26 June for the most susceptible cultivars. The Asphodel model identified three major periods where leaf infection was significantly possible (5-6 June, 11-12 June, 15-16 July). As leaf symptoms are visible about 20 days after initial infection, we expected a bursting of symptoms from 20 to 35 days after stalk placement (5 June). This was effectively observed irrespective of the cultivars and the management options (Figure 1). An additional peak was observed (on the dense canopies) corresponding to natural spore rain on 26 May before the stalk deposition.

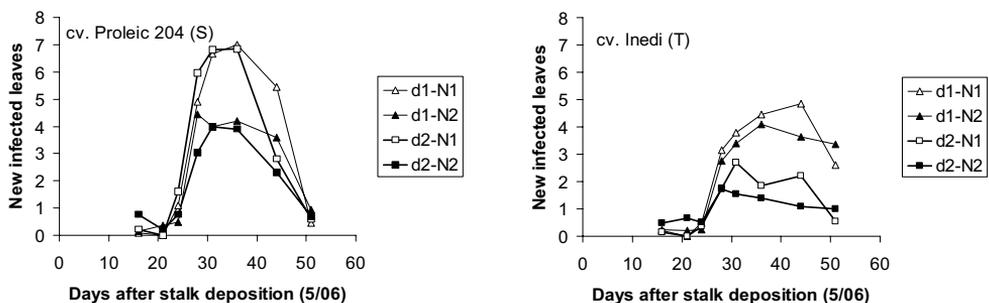


Figure 1. Evolution of the number of new infected leaves (major infection events: 5-6 June, 11-12 June) for 2 cultivars (Proleic 204, susceptible; Inedi, tolerant) and four management options: d1 (5 pl/m sq.); d2 (8 pl/m sq.); N1 (N = 0); N2 (N = 120 kg/ha).

Dynamics of Leaf and Stem Infection. By the observation of vertical distribution of leaf symptoms with time, it was possible to associate a leaf spot to a time of spore deposition as simulated by the Asphodel model. Early infection (26 May) was detected on leaves 1-9 (position 1), major infection from 5-6 June on leaves 10-15 (position 2), and infection from 11-12 June on leaves 16-22 (position 3). Using this method, the fate of a given leaf attack was related to the time of infection. For instance, the proportion of girdling lesions was 33 % for stem lesions in position 1, 16 % for position 2, and 0 for position 3 (cv. Proleic 204) (Table 2). On 25 July, for cv. Proleic 204 (S), 34 % of the leaf symptoms had resulted in a stem lesion, 33 % were still progressing, 30 % had been stopped by senescence and only 5 % by Phoma. On cv. Inedi (tolerant), the corresponding values were 2 % (stem lesion), 38 % (in progress), 52 % (senescence) and 7 % (Phoma).

Table 2. Evolution of leaf infection under four crop management options (SNi, cv. Proleic 204, full irrigation).

	d1 – N1	d1 – N2	d2 – N1	d2 – N2
Number of leaves per plant with one or more <i>Phomopsis</i> spot (a)	14.8	13.4	13.8	12.6
Stem spots / Infected leaves (b)	0.34	0.37	0.22	0.21
Girdling lesions/ Total stem lesions (c)	0.13	0.22	0.22	0.36
Number of girdling lesions per plant a * b *c	0.65	1.09	0.67	0.95
Vertical distribution of stem lesions (% girdling)				
Bottom (leaf 1-9)	44 (10)	27 (15)	48 (52)	37 (53)
Middle (leaf 10-16)	56 (12)	47 (20)	48 (12)	62 (21)
Top (leaf >16)	0	26 (0)	4 (0)	1 (0)
Phoma black stem (spots/plant)	2.3	3.2	1.4	3.2

The effect of crop management on the number of girdling lesions per plant was studied step by step. The number of girdling lesions was decomposed as the number of infected leaves x the proportion of leaf symptoms resulting in a stem lesion x the proportion of girdling stem lesions (Table 2). When compounding all the periods of infection, in the context of the high inoculum pressure of 2000, the less covering canopies (low plant density and low nitrogen, d1-N1) were the most favourable for leaf infection. The higher proportion of infected leaves producing a stem lesion was observed for low stand densities (d1), especially d1-N2: this was attributed to a lower rate of senescence in these plots which favoured the progress of the mycelium towards the stem. Generally, Phoma black stem is frequent in these situations (vigorous stems), but here, the chemical control maintained Phoma at a low level. The most harmful lesions were observed in dense canopies (eight plants/m sq.), especially with high nitrogen. This was explained by the earliness of the infection process in dense canopies and to the lower stem diameter which resulted in rapid pith damage. The situations with high plant density and nonlimiting N fertilization were responsible for a rapid canopy development which was more receptive to early spore deposition, which has a greater potential of damage (position 1). Using the vertical distribution of symptoms, we observed that the early leaf attacks had a lower chance to reach the stem as they were more affected by

Phoma and senescence, but once they had reached the stem they resulted more frequently in severe lesions as they had more time to develop. The final number of harmful stem lesions resulted from successive processes, each of them diversely affected by crop management and its effects on microclimate and canopy structure.

Simulating Crop Management Effect on RH with Asphodel. Relative humidity (RH) within the canopy measured in the 1-30/06 period was positively related to light interception (and leaf area index), indicating variations of microclimate with crop management and a potential influence on stem canker epidemics (Table 3). Values of RH measured in situ were used as input variables for the Asphodel model and the number of days where leaf infection was successful were simulated. During the first period of infection (1-15 June), increasing plant density and N fertilization (d2-N2) resulted in a higher value of RH and more plants (and leaves) infected which confirmed the observations of Figure 1 and Table 2. The effect of the sunflower canopy was no more discriminating during the second period of infection (10-20 July).

Table 3. Simulation of infection events with the Asphodel model using relative humidity at 2 m in the air and within the canopy (at 0.4 m from the soil) for different crop management options varying by the fraction of PAR intercepted (SNI, cv. Proleic 204, full irrigation).

	Period or date of measurement	RH from sensors above the canopy (2 m from the soil)	RH from sensors within the canopy (0.4 m from the soil)			
			d1 – N1	d1 – N2	d2 – N1	d2 – N2
Mean RH (%)	1-30/06	76.9	78.6	80.1	80.9	81.4
jPARi (%)	7/06	-	82	85	86	90
	15/06	-	89	92	91	94
	5/07	-	90	92	89	93
Number of days of leaf infection	1-15/06	4	6	7	7	9
	10-20/07	2	7	6	6	7

Discussion

Effect of Crop Density. The proportion of plants infected by *Phomopsis* increased at higher plant densities. This effect has been mentioned in the literature but never thoroughly explored or discussed. This effect may have two causes. Firstly, canopy closure is more rapid in dense stands, creating conditions of high relative humidity necessary to make leaf infection more likely; this should result in early infection and a higher proportion of stems with girdling lesions or total senescence 3-4 weeks before physiological maturity. Secondly, dense crops are characterized by small leaves and thin stems which could be more rapidly destroyed by *Phomopsis*, causing plant wilting and stem breakage. The effect of crop density is especially important in growing seasons where early infection and isolated rainy events occurred. In such conditions, the degree of canopy closure during the early stages of flower bud is the

main factor limiting successful leaf infection. Crop density has less influence during later attacks because the differences in leaf area index and microclimatic conditions between high and low plant densities decrease with time. In addition, as natural leaf senescence is accelerated in dense stands because of the low PAR penetration deep into the canopy and because of early water depletion under rainfed management, the progression of the mycelium towards the stem could be hindered by leaf senescence more than in a more open stand. The influence of crop density is also limited in frequently irrigated canopies where inoculum was continuously available and where the relative humidity was high enough for infection even within the most open stands (for instance, SNI).

Effect of Nitrogen Fertilization. The effect of nitrogen fertilization (and of N status generally) on the proportion of stems attacked by *Phomopsis* was not clear-cut. As for the effect of crop density, rapid canopy closure occurs with the highest amounts of N applied at sowing time through an increase in individual leaf area for a given plant density. It results, in most cases, in an increase of the proportion of stems infected by *Phomopsis*, although the effect is less pronounced than with crop density (Jinga et al., 1992; Debaeke et al., 2003). Conversely, in several independent experiments (as in 2000), more symptoms were observed on leaves and stems without fertilizer input. We suggest that with natural infection, which occurs mainly in May and June, highly fertilized crops are more likely to be successfully infected on leaves than low-fertilized crops, because N deficiency during this period may be critical in terms of leaf area index and resulting microclimate within the canopy. Under semi-natural infection, the presence of infected stalks in the interrow is a source of permanent inoculum for numerous infection events throughout the growing season. As the ascospores are splashed by frequent irrigation (or rain), we may assume that the distribution of the spores is more efficient in open canopies resulting from N deficiency. The shorter laminae and petioles in such canopies could hasten the spread of the mycelium towards the axil. A similar observation was reported by Estragnat (pers. com.) in 1992 when a sunflower crop was exposed to excessive rainfall in June (17 rainy days) which resulted in a severe N stress: 30 % of the stems were infected on no-nitrogen plots compared with 5 % on plots receiving 120 kg N/ha. The hypothesis of a greater receptivity of N-deficient crops to stem canker attack cannot be discussed without an appraisal of N deficiency (for instance Nitrogen Nutrition Index or leaf nitrogen). Only studies on isolated plants could help to separate the influence of nitrogen on microclimate from the intrinsic susceptibility of plant tissue.

Conclusions

A strong interaction exists between the inoculum level and the effects of crop management and genotypes on leaf and stem symptoms. In conditions of high pressure (as in 2000), a fungicide application was justified irrespective of genotypic tolerance and management. But, under moderate conditions of inoculum (3 years out of 5 in SW France), the spraying decision should be modulated by crop canopy development at the flower bud stage. The detailed analysis of infection process provided a key for understanding the final infection rate.

Acknowledgements

This programme was supported by INRA (AIP Ecopath). The authors are grateful to D. Chesneau for his helpful assistance in field scoring.

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