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Effect of crop management on epidemics of phomopsis stem canker (*Diaporthe helianthi*) for susceptible and tolerant sunflower cultivars

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ABSTRACT

Phomopsis stem canker (*Diaporthe helianthi*) is a worldwide fungal disease which is responsible for high yield losses in sunflower crop in the main regions of production. Field data on the influence of crop management on the incidence and severity of stem canker were reported by Debaeke et al. (2003) but a more thorough study was required to analyse step-by-step the spread of the fungus within the canopy from spore deposition on leaves to stem injury.

In a 2-year study (2000–2001), the effects of crop management (plant density, N fertilization, and irrigation) and genotypic tolerance (susceptible vs tolerant cultivars) on the epidemics of *Diaporthe helianthi* were monitored under conditions of reinforced inoculum. The incidence and severity of leaf and stem symptoms were closely related to canopy development (leaf area index) and microclimatic conditions (relative humidity) resulting from different crop management options. Increasing plant density resulted in a greater proportion of girdling stem lesions, detrimental to yield, because of earlier infection under dense canopies. The number of girdling lesions per plant was maximal with high N fertilization but more leaves were infected without N fertilization.

The field data were used to evaluate satisfactorily the epidemiological Asphodel model regarding the main periods of leaf infection. The relative humidity within the canopy (observed), the number of leaf infection events (simulated by Asphodel) and the final proportion of stem lesions (observed) were positively related which clearly demonstrated the key role of crop management in the development of stem canker in sunflower.

1. Introduction

Stem canker of sunflower caused by the Ascomycete *Diaporthe helianthi* (anamorph: *Phomopsis helianthi*) was first described in 1981 (Muntanola-Cvetkovic et al., 1981). Since then it has been reported in many sunflower production regions such as Argentina, USA and southern and eastern Europe (Gulya et al., 1997). It spread over the entire French sunflower area from 1992 to 1993 onwards and is still endemic in spite of recent drought events and the repeated use of tolerant cultivars (Moinard and Lecomte, 2004).

The main epidemiological aspects have been thoroughly described in the literature (Acimovic and Straser, 1981; Pérès and Regnault, 1988; Penaud et al., 1995; Delos and Moinard, 1997; Gulya et al., 1997). Ascospores are actively released from perithecia maturing on the sunflower debris and are spread by wind and rain splash throughout the growing season. Leaves are infected at the margin via guttation drops and the mycelium progresses along the veins and down the petiole until it reaches the stem where an elongated grey-brown necrotic lesion is formed, always centred on axils. A relative humidity of 90% must be maintained for 10–12 h for leaf infection to be successful. On average, leaf spots are visible 20–25 days after spore deposition for air temperatures ranging from 20 to 24 °C and yield loss is related to the severity of attacks on stems (girdling lesions) (Pinochet, 1995). Frequent or abundant rainfall from budding to flowering appears to be more important for successful infection than temperature, but values exceeding 32 °C after leaf infection can stop growth or even kill the mycelium in its progression towards the stem (Pérès and Regnault, 1988).

Yield losses up to 30% have been commonly observed, resulting from early senescence, plant wilting and stem breakage in unprotected conditions or after very early infections (Masirevic and Gulya, 1992; Delos and Moinard, 1995). The oil content may also be reduced by 15–25% (Acimovic, 1986; Pérès and Regnault, 1988; Diaz Franco and Ortegon Morales, 1997).

In France, a predictive model of disease spread (Asphodel) was developed by the Plant Protection Service of the French Ministry of Agriculture to estimate the potential development of *D. helianthi* epidemics at a micro-regional level and improve farmer's spraying decisions (Delos and Moinard, 1996, 1997; Debaeke et al., 2001; Battilani et al., 2003). This model was developed on the basis of

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data collected in southwestern France but no field evaluation study has been published till now.

In France, the use of disease-tolerant genotypes and fungicides is the basis of current disease control (Cetiom, 1995; Jouffret, 2005). 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. N fertilization and plant density generally increase the proportion of stems with necrotic lesions (Debaeke and Estragnat, 2003; Debaeke et al., 2003; Encheva et al., 2003) although the magnitude of the response depends on the inoculum amount and the timing of spore release.

Most of the conclusions from previous studies on crop management effects have been drawn on the basis of final scorings of crop injury. Moreover, disease symptoms have not been related to measurements of canopy microclimate resulting from crop management.

Therefore, a detailed study was conducted to analyse the stem canker infection process in several sunflower canopies differing in genotypic susceptibility, plant density, nitrogen status, and water availability. The main objective was to determine step-by-step whether and how the sunflower canopy development and the resulting microclimatic conditions affect leaf infection and stem attack. In addition, the field data on phomopsis epidemics were used to evaluate the Asphodel model in a range of crop canopies.

2. Materials and methods

2.1. Experimental design

In 2000 and 2001, sunflower (*Helianthus annuus* L.) crop was grown under natural (Ni) and semi-natural infection (SNi) at INRA in Auzeville, near Toulouse (Haute-Garonne, SW France, 43°36N, 1°26E) on a deep silty–clay soil.

In the semi-natural protocol of infection, sunflower stalks from infected fields were placed between the crop rows according to the method described by Viguié et al. (2000a) in order to reinforce the natural inoculum.

In 2000, the stalks were introduced on 5 June and removed on 15 June. Sprinkler irrigation (20 mm) was applied on 8 June to promote leaf infection. Fungicide protection against phoma black stem (*Phoma macdonaldii* Boerema) was applied on 26 May and 20 June with Dithane DG (mancozeb) at 2.5 l ha^{-1}. In 2001, stalks were spread between the rows on 15 June and removed on 2 July. Irrigation was applied on 20 June (25 mm), 22 June (25 mm) and 25 June (20 mm) to promote spore release in drier conditions than in 2000. Mancozeb was sprayed twice on 28 May and 19 June to control phoma selectively. In 2000, a parallel experiment was conducted under natural conditions of infection (Ni), *i.e.* without the depositing of infected stalks.

In 2000, each of the two experiments (SNi, Ni) was composed of 48 treatments applied to unreplicated plots of 90 m^2 each, irrigation being the main plot treatment (3 levels) and nitrogen (2), cultivar (4) and plant density (2) being the sub-plot treatments. Four cultivars, either susceptible (cv. Proleic 204, cv. DK3790), or tolerant (cv. Inedi, cv. Santiago) were sown on 10 April at two plant densities (d1 = 5 plants m^{-2}, d2 = 8 plants m^{-2}) with nitrogen fertilization (N2 = 120 kg N ha^{-1}:50 N at sowing + 70 N on 22 May) or without (N1 = 0). The four cultivars differed in their earliness at anthesis (from 22 June to 5 July): cv. Santiago and cv. DK3790, being the earliest cultivars, cv. Inedi intermediate, and cv. Proleic 204 the latest. Three irrigation regimes were applied (IRR1: no irrigation except for promoting leaf infection; IRR2: 35 mm on 25 June; IRR3: 35 mm on 25 June + 35 mm on 6 July).

In 2001, the experimental design was restricted to 16 unreplicated treatments. As in 2000, irrigation was the main plot treatment (2 levels), while nitrogen (2), cultivar (2) and plant density (2) being applied to sub-plots. Two cultivars (cv. Isun, susceptible; cv. Butisol, tolerant) were sown on 4 April at two plant densities (d1 = 4.5 plants m^{-2}; d2 = 7.5 plants m^{-2}) with N-fertilization (N2 = 165 kg N ha^{-1}:120 N at sowing + 45 N on 28 May) or not (N1 = 0). Two irrigation regimes were applied after initial leaf infection (IRR1 = no irrigation except for promoting leaf infection; IRR2 = 30 mm on 25 July).

2.2. Disease assessment

In 2000, on two sunflower cultivars (cv. Proleic 204, cv. Inedi) fully irrigated (IRR3), the appearance of visible leaf and stem symptoms and their vertical distribution were scored on 20 tagged plants at 8 dates between 21 June to 26 July. At each date, new infected leaves and new stem lesions (shallow or girdling) were tagged and scored. When a leaf symptom failed to develop into a stem lesion, the causes were identified: either physiological senescence or phoma attack. Only spots girdling the stem and wilted or broken stems were considered to be detrimental to yield (Cetiom, 1995). The final vertical distribution of the type of symptoms (healthy, leaf spot, stem lesion, girdling lesion, and wilted plant) by leaf layer was determined more extensively on 28 July by sampling 30 plants; in this paper, only the results obtained for cv. Proleic 204 on six crop management systems (IRR3: d1−N1, d1−N2, d2−N1, d2−N2; IRR2: d2N2; IRR1: d2N2) will be exposed.

A similar method was applied in 2001 on the two cultivars cv. Isun (S) and cv. Butisol (T). Twenty plants per plot were tagged and symptoms of *D. helianthi* were regularly scored on leaves and stems at 8 dates between 3 July and 7 August. The final determination of *D. helianthi* attacks was made on 40 plants per plot.

2.3. Canopy and microclimate measurements

In 2000 and 2001, the fraction of radiation intercepted by the sunflower canopy (*f*PARi), which is closely related to leaf area index (LAI), was measured regularly from star bud to early anthesis stages using a hand-held Picqhelios (Aeric, Balma, France) apparatus as described in Debaeke and Estragnat (2003). Leaf area index was determined on 20 plants at star bud and anthesis stages using the Pouzet and Bugat (1985) method based on the measurement of lamina length and width on two characteristic leaves per plant. At early anthesis, chlorophyll content was measured on 20 plants per treatment on the 7th leaf beneath the head with a Minolta SPAD 502 (Spectrum Technologies, Inc., Plainfield, IL, USA) to provide an indication on plant nitrogen status according to Debaeke and Raffaillac (2006). Relative humidity (RH) and temperature within the canopy were recorded from mid-June to early August using thermohygrometers Rotronic MP100A (Campbell Scientific Ltd., Les Ulis, France) placed in the middle of the inter-row (row width = 0.50 m) at 0.4 m above the soil. The sensor accuracy was 1.5% over the RH range 5–95%. Microclimatic variables were recorded every 15 min and compiled hourly, and measurements were stored on Campbell CA10 data loggers. Only selected management treatments (4 in 2000; 6 in 2001) representing the range of sunflower canopies and two susceptible cultivars (cv. Proleic 204 in 2000; cv. Isun in 2001) were equipped with the sensors: d1−N1 (IRR3), d1−N2 (IRR3), d2−N1 (IRR3), d2−N2 (IRR3) in 2000; d1−N1 (IRR1 and IRR2), d1−N2 (IRR2), d2−N2 (IRR1 and IRR2) and d2−N1 (IRR2) in 2001.

2.4. Simulation with Asphodel

Asphodel is a deterministic model able to describe the effect of weather (temperature, precipitation, and relative humidity) on fungal dynamics in relation to plant susceptibility (Delos and