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Identifying epidemiological predictors for quantitative host plant resistance: application to the sunflower-phoma pathosystem

André Schwanck

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Université
de Toulouse

THÈSE

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RESISTANCE QUANTITATIVE: APPLICATION AU PATHOSYSTEME
TOURNESOL-PHOMA

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INTRODUCTION

Host plant resistance

Host plant resistance (HPR) is an environment-friendly and low-cost technology for disease management (e.g., McDonald and Linde, 2002; Poland et al 2009; Brown 2015). HPR can be classified according to two general categories: (i) *complete* or *qualitative resistance* and (ii) *incomplete* or *quantitative resistance* (e.g., Poland et al. 2009). Qualitative resistance is expressed as the absence of disease, and is generally governed by single major genes for resistance (R genes; Poland et al. 2009). Qualitative resistance is prone to the "boom and bust" cycle that occurs when resistance is overcome by pathogen populations that progressively adapt to the deployed HPR (e.g., McDonald and Linde 2002). Quantitative (or partial) HPR, on the other hand, is expressed as a reduction in disease, and is often conditioned by multiple minor genes (Poland et al. 2009). Quantitative resistance is conditioned by quantitative trait loci (QTLs), which are portions of chromosomes that include a gene that contributes significantly to the expression of a quantitative trait (Lannou et al. 2012). Quantitative HPR has been associated to a higher durability as compared to qualitative HPR (Parlevliet 2002; Mundt 2014; Zhan et al. 2015). Quantitative HPR therefore represents a critical component when aiming at developing sustainable agricultural production systems (e.g., Poland et al. 2009; Savary and Willocquet, 2014).

The particular easiness in selection of qualitative resistance, due to the phenotypic contrast it involves (absence or not of symptoms), makes this type of resistance the basis of most breeding programs worldwide (Vale et al. 2001; Stuthman et al. 2007). However, attention on quantitative resistance has increased as a result of its higher durability (Zhan et al. 2015; Mundt et al. 2014), and of its genetic identification and tracking which is now technically possible (e.g., StClair 2010). The need to develop high quality phenotyping analysis and screening methodologies for quantitative resistance has been recently advocated (e.g. St Clair 2010; Mundt et al. 2014; Brown 2015).

From an epidemiological perspective, quantitative resistance reduces the rate of speed of epidemics by affecting one or several components of the disease cycle (Parlevliet 1979). These *components of resistance* (Zadoks 1972; Parlevliet 1979) can be measured, through monocyclic experiments (Zadoks and Schein 1979) involving host genotypes expressing a range of quantitative resistance. Low infection efficiency, long latency period, small lesions, low number of propagules per lesion, short infectious period, for example, are considered components of

quantitative resistance and the slow development of an epidemic is a consequence of these components acting alone or in combination (Parlevliet 1979; Mundt 2014).

QTLs driving other quantitative traits [e.g. plant morphological traits] can also affect disease through disease escape (or avoidance) processes (Ando et al. 2007; Poland et al. 2009; Andrivon, et al. 2013; Srinivasachary et al. 2013). Thus, identifying morphological traits that are associated with reduced disease intensity, through plant phenotyping, can represent a useful basis for plant breeding for resistance.

Components of resistance and plant morphological traits can be considered as potential *predictors of quantitative resistance* [e.g. desirable host plant traits, phenotyping targets] for plant breeding programs.

Monocyclic epidemics

Epidemics have been classified into two broad categories based on disease progress processes: *simple interest* and *compound interest* epidemics (e.g., Van der Plank 1965; Zadoks and Schein 1979). In simple interest epidemics, only one cycle of infection occurs per growing season. These have been referred to as *monocyclic* epidemics (Zadoks and Schein 1979). By contrast, a polycyclic epidemic is characterized by the occurrence of secondary infections during the course of the growing seasons, which originate from lesions produced from primary or secondary infections (Zadoks and Schein 1979; Campbell and Madden 1990). Knowledge on disease cycle constitutes fundamental information in plant pathology. Literature in plant epidemiology brings a range of spatiotemporal experimental and analytical methods that allow inferring on the occurrence of secondary cycles in crop diseases (e.g. Campbell and Madden 1990; Madden et al. 2007).

When considering monocyclic epidemics, primary inoculum is crucial for field epidemics. Disease cycles of fungi associated to monocyclic epidemics mainly involve the production of structures resilient for overwintering/oversummering, i.e., source of inoculum for the next crop cycle (Bergamin Filho and Amorim 1996; Vidhyasekaran 2004). The cotton wilt (*Fusarium oxysporum*), is an example provided by Vanderplank (1963) of a typical monocyclic disease (Campbell and Madden 1990). *F. oxysporum* dissemination relies on infected seeds, contaminated water or soil, and wind (Cia and Salgado 2005), and the inoculum present in the soil at the beginning of the season remains the main source of inoculum for cotton wilt (Campbell and Madden 1990). Nevertheless, defining whether a disease is monocyclic or polycyclic is not always simple. For some diseases, the pathogen cycle is known to produce spores for potential

secondary infections, but the role of secondary infections in epidemics can be highly dependent on a range of factors (e.g. synchrony between spore production and crop susceptibility period; environmental effects; pathogen population). This is illustrated by two examples. Stem canker of oil seed rape (*Leptosphaeria maculans* Sowerby) can be associated to monocyclic epidemics (West et al. 2001). *L. maculans* secondary cycles, from pycnidiospores (Travadon et al. 2007), can be more frequently observed in Australia rather than in Canada and Europe, possibly due to pathogen populations aggressiveness differences (West et al. 2001). Fusarium head blight of wheat (*Gibberella zeae* Schwein.), in which primary infections occur on wheat spikes between flowering and soft dough, has been associated to monocyclic epidemics as well (e.g., Fernando et al. 1997; Del Ponte et al. 2004). Although *G. zeae* produces macroconidia on infected spikelets within the season (Trail 2009), gradient and spatiotemporal studies have suggested that no secondary cycles occur within the crop cycle (Ferdando et al. 1997; Spolti et al. 2015).

Quantitative resistance against pathogens associated with monocyclic epidemics

Assessing quantitative resistance through the concept of components of resistance was developed for air-borne fungi associated with polycyclic epidemics (Parlevliet 1979). In the case of monocyclic epidemics, such an approach is limited, since only a few components such as infection efficiency and lesion expansion can be measured. The concept of component of resistance is however still relevant in the case of pathogens associated with monocyclic epidemics, and can be mobilized in order to develop phenotyping methods for quantitative host plant resistance. The study presented in this Ph.D. dissertation exemplifies the use of the concept of components of resistance in a disease associated to monocyclic epidemics.

Phoma black stem

Phoma black stem, caused by the fungus *Leptosphaeria lindquistii* Frezzi (synonym *Phoma macdonaldii* Boerema; MacDonald 1964), has become an important sunflower disease in France (Debaeke and Pérès 2003). Short rotations (mainly sunflower-wheat) and reduced tillage, which were introduced in the 90s in France, may have contributed to an increase in inoculum density, leading to more severe epidemics (Debaeke and Pérès 2003; Bordat et al. 2011). The disease currently occurs in all sunflower production zones in France (Bordat et al. 2011).

Epidemic onset usually occurs after flowering, with lesions on the stem progressing from the bottom to upper leaf nodes. Typical symptoms are black lesions on the stem, localized at the leaf nodes, and expanding around and along the stem. Although phoma black stem symptoms

have been described, a diagram to assess disease severity on the stem has not been developed. Lesions on the stem originate from infections which take place at the trough located at the basis of the leaf petioles, or along the petioles. Lesions on the petiole or on the stem accelerate leaf senescence (Quiroz et al. 2014). Lesions on the leaf veins can be observed at the end of the crop cycle when epidemics are severe. Lesions can also develop on collars, triggering the senescence and death of the plant a few weeks before normal maturity. This is referred to as premature death or premature ripening, and can be associated with high yield losses (Donald et al. 1987; Debaeke and Pérès 2003; Seassau et al. 2010).

It is generally considered that *Leptosphaeria lindquistii* overwinters as pseudothecia, pycnidia, and mycelium produced on infected sunflower stubble residues (Gulya et al. 1997). In France, the main source of primary inoculum appears to consist in ascospores released from infected sunflower residues (Délos et al. 1997; Seassau et al. 2010; Bordat et al. 2011). The occurrence of pycnidia on lesions developed on the stems during the growing season were observed in the USA (McDonald 1964), and in the former Yugoslavia (Maric et al. 1988). In South West of France, however, the presence of pycnidia on black stem lesions has not been observed during epidemics (Délos et al. 1997; Bordat et al. 2011). In this area, the disease is therefore considered to be associated to monocyclic epidemics (*sensu* Zadoks and Schein 1979), e.g., to not involve secondary infections over the course of the crop cycle. Nevertheless, this appears to be an untested working hypothesis, since no study to our knowledge has formally explored the occurrence of secondary cycles in phoma black stem epidemics.

Sources of quantitative resistance to phoma black stem have been identified (e.g., Roustae et al. 2000), and several quantitative traits loci (QTLs) for resistance to phoma have been characterised (Al-Chaarani et al. 2002; Bert et al. 2004; Darvishzadeh et al. 2007a), using phenotyping methods involving inoculation under controlled conditions on 10-day-old seedlings. Phenotypic variation explained by individual QTLs was moderate, ranging between 6 and 20%. However, no varieties resistant to phoma black stem have been deployed until now, and no specific disease management tools are currently deployed to control the disease.

Objectives of the Ph.D. thesis and outline of the Ph.D. dissertation

The main objective of the Ph.D. dissertation presented here was to develop and implement methods to identify predictors for quantitative resistance of host plants, using phoma black stem of sunflower as a study case. This work involved a range of complementary **experimental** and **analytical** approaches which mobilize epidemiological concepts and methods. The objective can

be divided into three studies, each corresponding to a **chapter** in this Ph.D. dissertation, as presented in Figure 1.

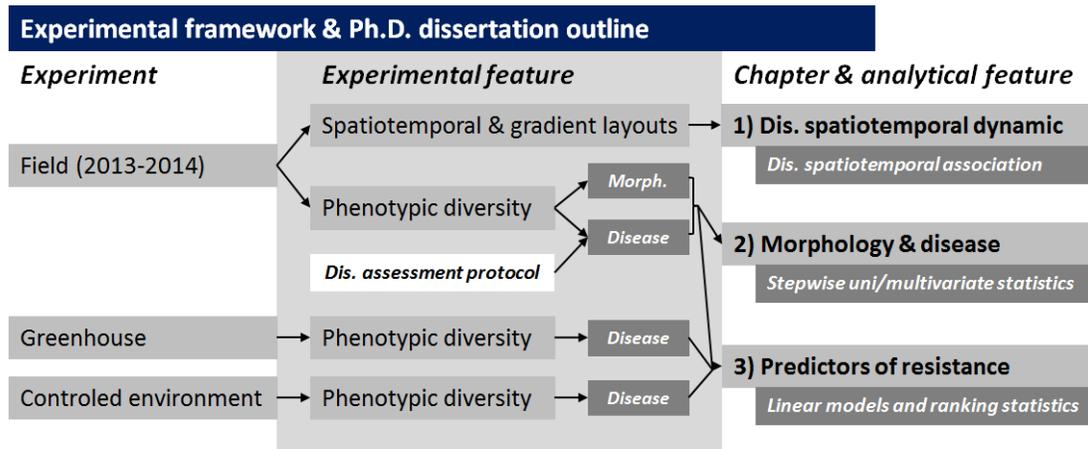


Figure 1. Flowchart of the experimental framework and Ph.D. dissertation outline.

The first specific objective was to explore the occurrence of secondary cycles in phoma black stem epidemics. Findings may have important implications for the management of the disease in general, and for the for host plant resistance in particular: the development of relevant phenotyping methods for HPR will depend on whether the disease is associated to monocyclic or polycyclic epidemics. Experiments were conducted in the field over two years in order to describe the (i) spatiotemporal attributes and (ii) disease gradients of phoma black stem, as well as (iii) the association between phoma black stem and defoliation. This work is presented in **Chapter 1**.

The second specific objective was to (i) develop a protocol for assessment of phoma black stem in sunflower (including a sampling design and a scale for severity on stem), and (ii) to apply it to address relationships between sunflower morphological traits and phoma black stem intensity. Experiments were conducted over two years with a range of sunflower genotypes, on which both morphological traits and disease intensity features were measured, in order to assess relationships between plant morphology and disease intensity. This work is presented in **Chapter 2**.

The third specific objective was to identify predictors of phoma black stem resistance in the field through (i) measuring components of physiological resistance under different experimental conditions and plant ages, (ii) identifying morphological traits associated with reduced disease, and (iii) conducting complementary analyses to identify predictors best

associated to disease intensity in the field. This work involved replicated experiments under growth chamber, greenhouse, and field conditions involving a set of sunflower genotypes for which predictors for resistance were measured, together with disease intensity in the field. This work is presented in **Chapter 3**.

A last section presents a general **discussion** with conclusions and perspectives drawn from this work.

1. Exploring spatiotemporal patterns of phoma black stem in sunflower

André Aguiar Schwanck and Laetitia Willocquet

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1.1. Abstract

Field experiments were conducted over two growing seasons with three sunflower cultivars to explore the spatio-temporal dynamics of *Phoma* black stem epidemics and to test hypotheses pertaining to (i) disease spread from a known inoculum source; (ii) spatial patterns of the disease; (iii) disease spatiotemporal association; and (iv) association between disease intensity and sunflower defoliation. The spatial patterns of disease were random in most of epidemics, and disease gradients were not detected. Our results suggest absence of secondary infections, that is, that the studied *phoma* black stem epidemics were monocyclic under the experimental conditions reported here. Significant associations between the number of dead leaves per plant and the number of *phoma* black stem lesions per plant were detected towards the end of epidemics.

1.2. Introduction

Phoma black stem is a sunflower disease caused by the fungus *Leptosphaeria lindquistii* Frezzi (syn. *Phoma macdonaldii* Boerema). The importance of this disease has increased in France at the end of the last century (Debaeke and Pérès 2003). Short rotations (sunflower-wheat) and reduced tillage, which were introduced in 90's in France, may have contributed to amplify disease inoculum in fields and landscape, leading to more frequent and stronger epidemics (Debaeke and Pérès 2003; Bordat et al. 2011). Infections appear along the leaf petioles, or at the insertion of the petiole on the stem (stem node), and then expand around and along the stem. Typical symptoms on the stem are black lesions that are well delimited by a slight brownish halo. Lesions on the leaf veins can be observed at the end of the crop cycle under favourable conditions (Bordat et al. 2011). Lesions can also develop on collars, causing plant senescence and death a few weeks before normal maturity. This is referred to as premature death or premature ripening, and can be associated to high yield losses (Donald et al. 1987; Debaeke and Pérès 2003; Seassau et al. 2010). Although the disease has gained importance in France, no disease control methods are currently deployed against this disease.

It is generally considered that *L. lindquistii* overwinters as pseudothecia, pycnidia and mycelium produced on infected sunflower stubble residues (Gulya et al. 1997). Phoma black stem epidemic onset typically occurs at flowering, with lesions on the stem progressing from bottom to upper stem nodes (Schwanck et al. 2016). Lesions on the petiole or on the stem are associated to leaf senescence (Quiroz et al. 2014). In France, the main source of primary inoculum appears to consist in ascospores released from infected sunflower residues (Délos et al. 1997; Seassau et al. 2010; Bordat et al. 2011). The occurrence of pycnidia on lesions developed on the stems during the growing season were observed in the USA (McDonald 1964), and in the former Yugoslavia (Maric et al. 1988). In South West of France, however, the presence of pycnidia on black stem lesions has not been observed during epidemics (Délos et al. 1997; Bordat et al. 2011). In this area, the disease is therefore considered to be associated to monocyclic epidemics (*sensu* Van der Plank 1965; Zadoks and Schein 1979), i.e., to not involve secondary infections over the course of the crop cycle. Nevertheless, this appears to be an untested working hypothesis, since no study to our knowledge has formally explored the occurrence of secondary cycles in phoma black stem epidemics.

The characterization of spatiotemporal disease patterns and the measurement of disease or spore dispersal gradient are two complementary approaches that allow deriving hypotheses on the physical and biological mechanisms that determine disease development in time and space (e.g., Fitt et al. 1987; Madden et al. 2007). Knowledge on the spatiotemporal dynamics of plant diseases has considerable implications for disease management and for further epidemiological research, including the improvement of disease sampling strategy (e.g., Alexander et al. 2005), short or long term inoculum influence on epidemics (e.g. Nita et al. 2012), or environmental influence on epidemics (e.g. Shah et al. 2005). Statistical approaches such as Spatial Analysis by Distance IndicEs (SADIE; Perry 1995; Perry 1998) allow considering other processes that may be associated with the progress of plant disease epidemics, such as defoliation (Pethybridge et al. 2005), or the occurrence of other diseases (Pethybridge and Turechek 2003; Spolti et al. 2012). The measurement of disease gradients allows describing the occurrence and spatial range of secondary infections from a known source of inoculum artificially created (e.g., Fitt et al 1987; Campbell and Madden 1990; Madden et al. 2007). This approach further allows addressing questions on the origin of infections that build epidemics, as was done to assess the relative role of sexual or asexual spores in wheat Fusarium head blight progress (Fernando et al. 1997).

The objectives of this study were to describe (i) the spatiotemporal attributes and (ii) disease gradients of phoma black stem, as well as (iii) the association between phoma black stem and defoliation.

1.3. Materials and Methods

All experiments were conducted in Auzeville, near Toulouse (South-West of France), at the INRA experimental unit, in 2013 and 2014. The experiments were performed with three commercial hybrids (cultivars) currently grown in France (Schwanck et al. 2016): Kerbel, ES Paulina and NK Ferti. The experimental plots were sown at a rate of 7 seeds.m⁻², on May 6 and April 15, in 2013 and 2014, respectively. Nitrogen was applied once in both years at 50 days after sowing, at a rate of 35 and 70 kg.ha⁻¹ in 2013 and 2014, respectively. Each experimental unit consisted in a plot of six rows with a length of six meters and an inter-row space of 0.5 m, corresponding to an area of 18 m² (6 × 3 m).

Experiments were grown under rainfed conditions and weather data were collected with a weather station (CE-180, Cimel, France) located less than 400 m from the experimental site. Mean monthly temperature and accumulated rainfall were computed each year from April to September.

1.3.1. Spatiotemporal experiments

The spatiotemporal experiments involved one plot of each cultivar (Kerbel, ES Paulina and NK Ferti) in both years. All plants of the four central rows in the plots were assessed every seven days during five weeks, starting when the first phoma black stem symptoms were observed. At each assessment, the number of phoma black stem lesions and the number of dead leaves per plant were counted in all assessed plants. Furthermore, the total number of leaves per plant was counted at the first assessment. A leaf was considered dead when more than 50% of its area was not green. Coordinates (x; y) of each plant within the plot were recorded and used to construct bubble maps of the lesion density per plant with the ggplot2 package (Wickham 2009) of R software (R Core Team 2014).

Complementary analyses were performed in order to characterize the spatio-temporal patterns of phoma black stem epidemics: no single method is expected to identify all of the spatial characteristics of a given population (Madden et al. 2007). Combining different methods to analyse spatial patterns is therefore recommended (Perry et al. 2002; Madden et al. 2007). First, the Morisita's index (I_{δ}) (Morisita 1962; Madden and Hughes 1995), a variance-mean

statistics which provides a measure of the degree of spatial aggregation for counting data, was computed as:

$$I_{\delta} = \frac{n[\sum(x^2) - \sum x]}{(\sum x)^2 - \sum x}$$

Where n is the number of plants and x is the number of lesions (or dead leaves) per plant. $I_{\delta} < 1$ indicates a uniform distribution; $I_{\delta} = 1$ indicates a random distribution, and $I_{\delta} > 1$ indicates an aggregated distribution (Morisita 1962; Madden et al. 2007). The indexes and the chi-squared based probability test (H_0 : randomness) were calculated using the “dispindmorisita” function of the R vegan package (Oksanen et al. 2015).

Second, the spatial arrangement of lesions and dead leaves counts was evaluated using spatial analysis by distances (SADIE) indices (SADIE software version 1.22; Perry 1995). Similar to a correlation-based analysis, results from SADIE reflect the spatial arrangement of counting data at the sampling unit level and above (x ; y coordinates) and depends on the level of heterogeneity in the dataset (Perry 1995). In SADIE, the distance to regularity, D_r , is the minimum total distance that units (= number of lesions or dead leaves) would need to be moved to achieve the same number m in each plant, i.e., a uniform (regular) distribution. The degree of no randomness within a set of data is quantified by comparing the observed spatial pattern with rearrangements obtained after random permutations of the units among the plants. An overall index of aggregation is given by $I_a = D_r / E_a$, where E_a is the mean distance to regularity of the randomized samples. An aggregation index (I_a) > 1 indicates an aggregated pattern, while indices equal to and below 1 indicate random and regular patterns, respectively (Perry 1995; Perry 1998). Third, the Taylor’s power law (Taylor 1961; Madden et al. 2007) was used to examine the overall relationship between variance (s^2) and mean (μ) of the number of lesions per plant across all assessments and cultivars, as follows:

$$\ln(s^2) = \ln(A) + b \ln(\mu)$$

where s^2 is the variance, μ is the mean and $\ln(A)$ and b are the intercept and slope of the linear model, respectively. A random pattern is expected when $\ln(A) = 0$ and $b = 1$, while $b > 1$ indicates heterogeneity among plants (aggregation; Madden and Hughes 1995; Madden et al. 2007). t-tests were performed to test the null hypothesis H_0 where: $\ln(A) = 0$ and $H_0: b = 1$ using the “pt” function of R. Model fitness was evaluated based on the coefficient of determination (R^2) with the “lm” function of R software (multiple R^2 provided by the “summary” function).

Fourth, the association of the number of lesions and the number of dead leaves per plant between assessment dates was assessed through the association test of SADIE X (1: first and

third; 2: third and fifth; 3: first and fifth assessment; Perry et al. 1999). The association test (X) is equivalent to the correlation coefficient between cluster indices of each assessment (Perry 1999; Madden et al. 2007). Finally, the association between the number of dead leaves and the number of phoma black stem lesions was characterized for each assessment and cultivar, using the association test of SADIE (X).

1.3.2. Disease gradient experiments

The disease gradient experiments were arranged as a complete randomized block design with four replications (blocks), whereby individual plots (a cultivar within a block) represented the elementary units. Plants located at the centre of the four central rows (3 m apart from the edge of the plot, one plant per row) were inoculated 66 days after sowing (DAS) in 2013 (July 11) and 77 DAS in 2014 (July 1), corresponding to the development stage “internode between last leaf and inflorescence between 0.5 and 2 cm long” (Schneider and Miller 1981; Merrien and Milan 1992). This pattern of source plant inoculation corresponds to a line source of inoculum (Campbell and Madden 1990). The inoculation was performed before the time where epidemic onsets generally occur. No lesions were observed at the time of inoculation, indicating that the experiment was established in absence of a background noise (Gregory 1968; Savary and van Santen 1992) of spontaneous infections.

An aggressive strain of *L. lindquistii* (MPH2), isolated from infected sunflower stem residues collected near Auzeville in 2006 (Seassau et al. 2010), was used for inoculations. Inoculum consisted in 5 mm-diameter mycelial plugs of a two-week-old colony grown on potato-dextrose-agar medium at 25° C under darkness. The inoculation method was adapted from that used to inoculate sunflower collars (Seassau et al. 2010). A mycelial plug was applied against the stem of each plant, just above the first, second and third petiole insertion point (node). Each plug was then covered by a layer of humid cotton, and the inoculated nod was wrapped with aluminium foil in order to keep the cotton humid. The cotton and aluminium foil were removed 7 days after inoculation.

Plants located at 50, 100, 150 and 200 cm away from the inoculated plants (two plants per distance and per plot) were assessed for disease intensity. The plants were chosen so that two plants per row, located each on both sides of the inoculated plants, were assessed. Assessments were conducted every seven days during three weeks, starting when the first phoma black stem symptoms were observed. At each assessment, the number of phoma black stem lesions and the number of dead leaves (green area less than 50%) per plant were counted. Furthermore, the total

number of nodes per plant (cumulated numbers of dead and green leaves, i.e., the total number of leaves) was counted at the first assessment. The means of the number of lesions and of the number of dead leaves per plant were computed for each distance in each plot for further analyses.

Mixed model analyses of variance were performed to test the effect of distance from the inoculum source on the number of phoma black stem lesions (Savary and van Santen 1992; Willocquet et al. 2008). Analyses were performed for each [cultivar x assessment] combination, considering distance from the source as a fixed effect and block as a random effect. The mixed model analyses of variance were performed with the “lme” function from the “nlme” R package (Pinheiro et al. 2015).

1.4. Results

The climatic conditions under which experiments were conducted were very different in 2013 and 2014 (Figure I. 1). Monthly temperature from April to June was lower ($\approx 2\text{ }^{\circ}\text{C}$) in 2013 than in 2014. The opposite trend was observed in July and August, when temperature was greater in 2013 than in 2014. Temperature in September was similar in both years. Major differences in monthly accumulated rainfall were observed in May (2013 = 104 mm vs. 2014 = 63 mm) which was drier in 2014 than in 2013. In July and August, however, the accumulated rainfall was much lower in 2013 than in 2014.

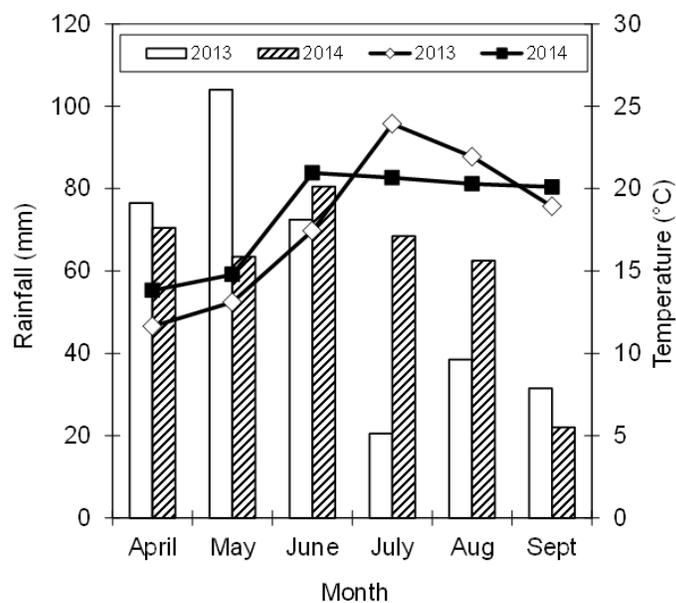


Figure I. 1. Monthly accumulated rainfall and mean temperature in 2013 and 2014.

1.4.1. Spatiotemporal experiments

The total number of emerged plants in the four central rows of the plots varied according to cultivar and year: in 2013, the total number of emerged plants were 42, 46, and 44 for Kerbel, ES Paulina and NK Ferti, respectively; while in 2014, the total number of emerged plants were 44, 47, and 43 plants Kerbel, ES Paulina and NK Ferti, respectively. The first assessments were performed at 93 and 92 DAS in 2013 and 2014, respectively. In 2013, the mean total number of leaves per plant was 29, 29, and 27 for Kerbel, ES Paulina and NK Ferti, respectively; while in 2014, it was 26, 28, and 25 for Kerbel, ES Paulina and NK Ferti, respectively. The disease progress curves indicated that epidemics were much more severe in 2014 than in 2013, with a terminal number of lesions per plant ranging from 2 to 3.5 in 2013, and from 8 to 12 in 2014 (Figure I. 2A, B). The disease progress curves had a linear shape in 2013, and an exponential shape in 2014. NK Ferti showed the highest number of lesions in all assessments in both years. Disease intensity was higher on Kerbel than on ES Paulina in 2013. In 2014, disease levels were similar in Kerbel and ES Paulina during the first four assessments, but were larger in ES Paulina than in Kerbel at the last assessment.

The number of dead leaves per plant increased linearly over the four first assessments for all cultivars in both years (Figure I. 2C; D). Compared to the increase over the four first assessments, the increase in dead leaves between the fourth and fifth assessment was much larger in 2013, but only slightly larger in 2014. The number of dead leaves per plant was larger in 2013 than in 2014 in all cultivars, and the difference between years was the largest at the last assessment, with numbers ranging from 25 to 28 dead leaves in 2013, vs. 14 to 17 dead leaves in 2014. The number of dead leaves was in general lowest in NK Ferti, intermediate in Kerbel, and highest in ES Paulina. This ranking between cultivars was in general opposite to the ranking observed in terms of disease level (Figure I. 2A, B).

The spatial distribution of disease over assessments for each cultivar is displayed for 2013 (Figure I. 3) and 2014 (Figure I. 4). Patterns in 2013 indicate that disease intensity could vary greatly between plants at each assessment date, but not specific spatial pattern could be detected from visual observation. Similar features were observed in 2014, except that the overall level of disease intensity was larger than in 2013.

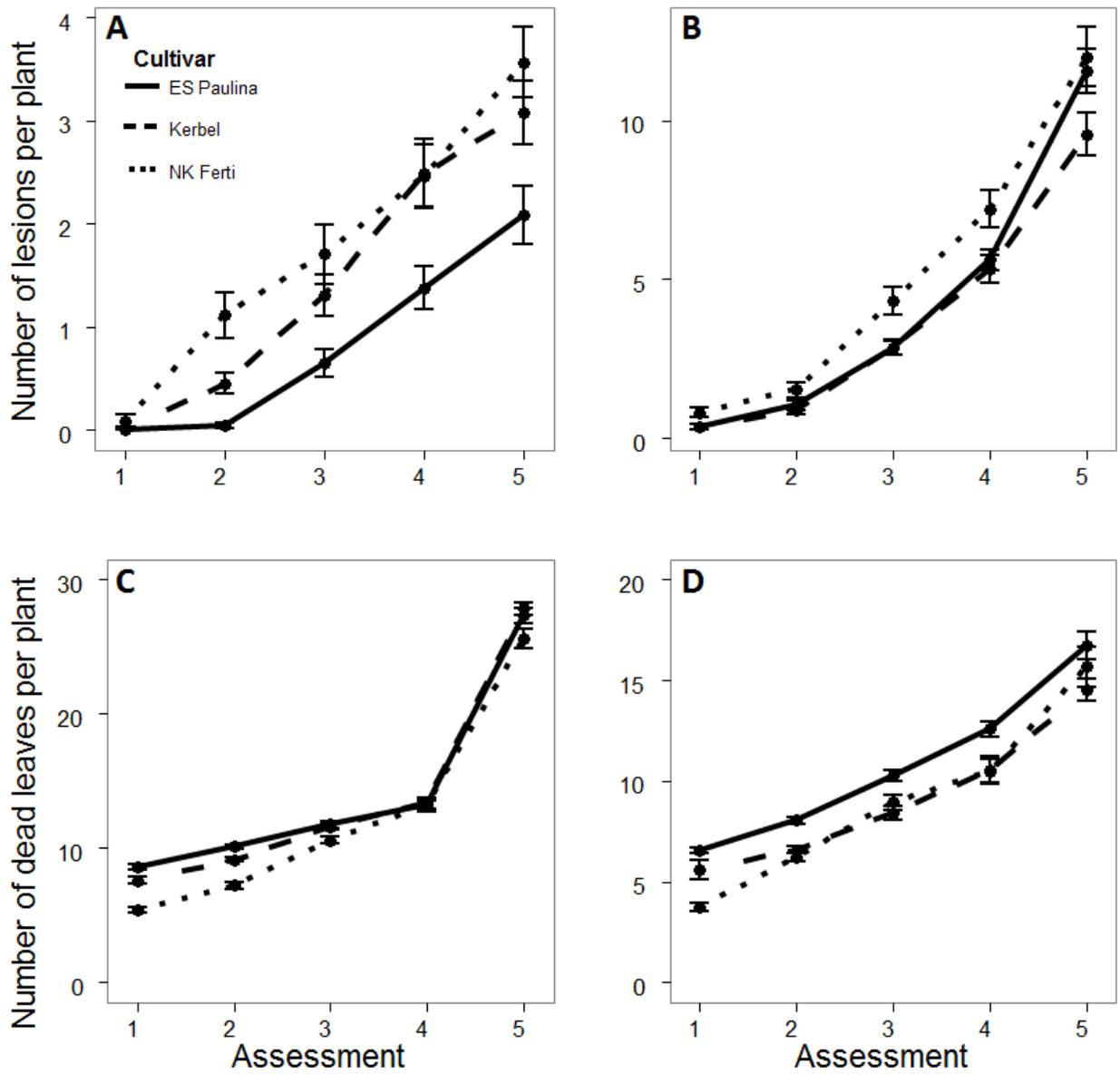


Figure I. 2. Temporal progress of the mean number of Phoma black stem lesions (A: 2013; B: 2014) and dead leaves (C: 2013; D: 2014) per plant in spatiotemporal experiments with cultivars ES Paulina, Kerbel and NK Ferti. Dots and error bars represent the mean and standard error of the mean, respectively, from all assessed plants.

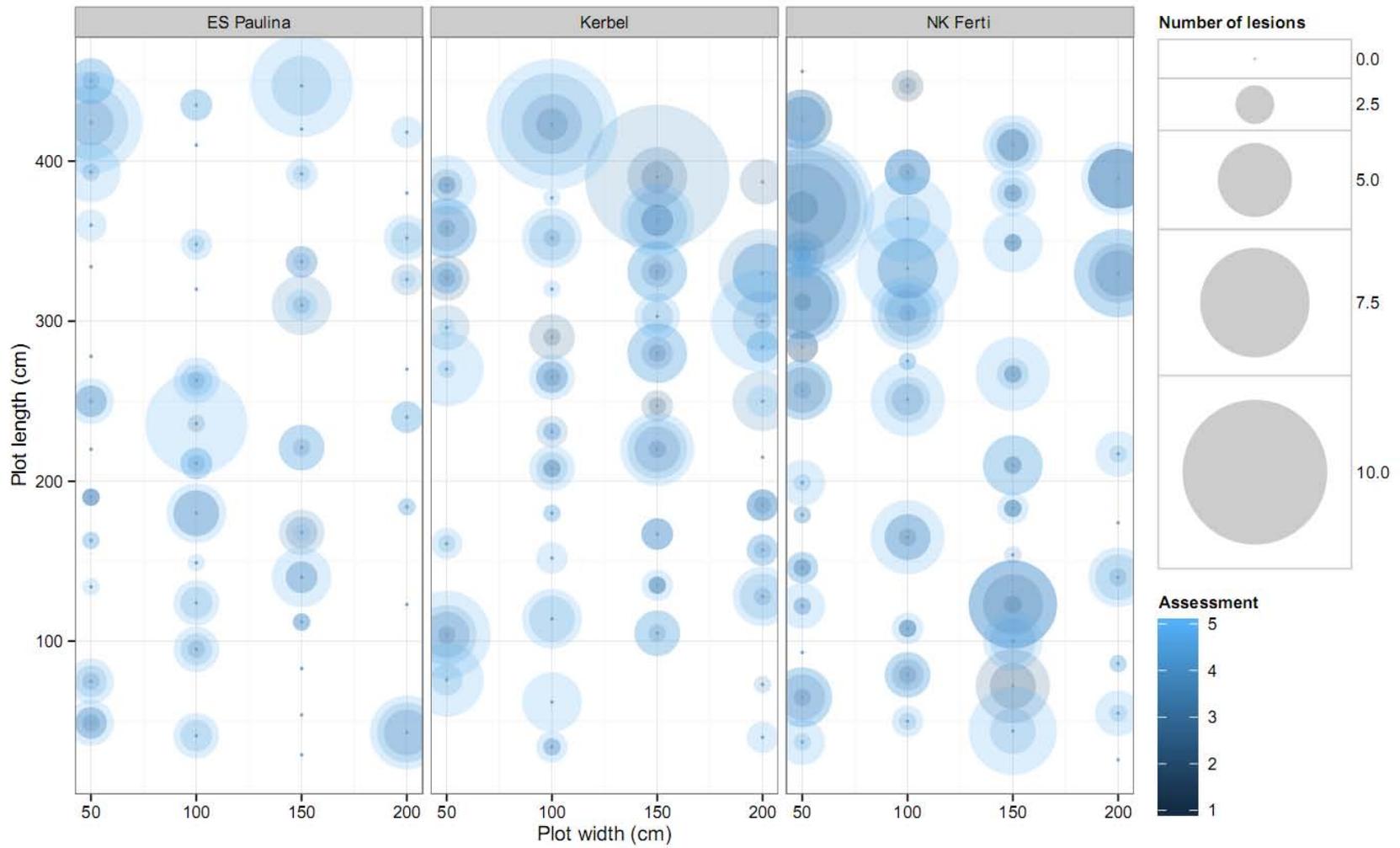


Figure I. 3. Bubble plots representing field plots from the spatiotemporal experiment conducted in 2013. Circles represent the number of Phoma black stem lesions per plant colored according to assessment from dark (earlier) to light (later) shade.

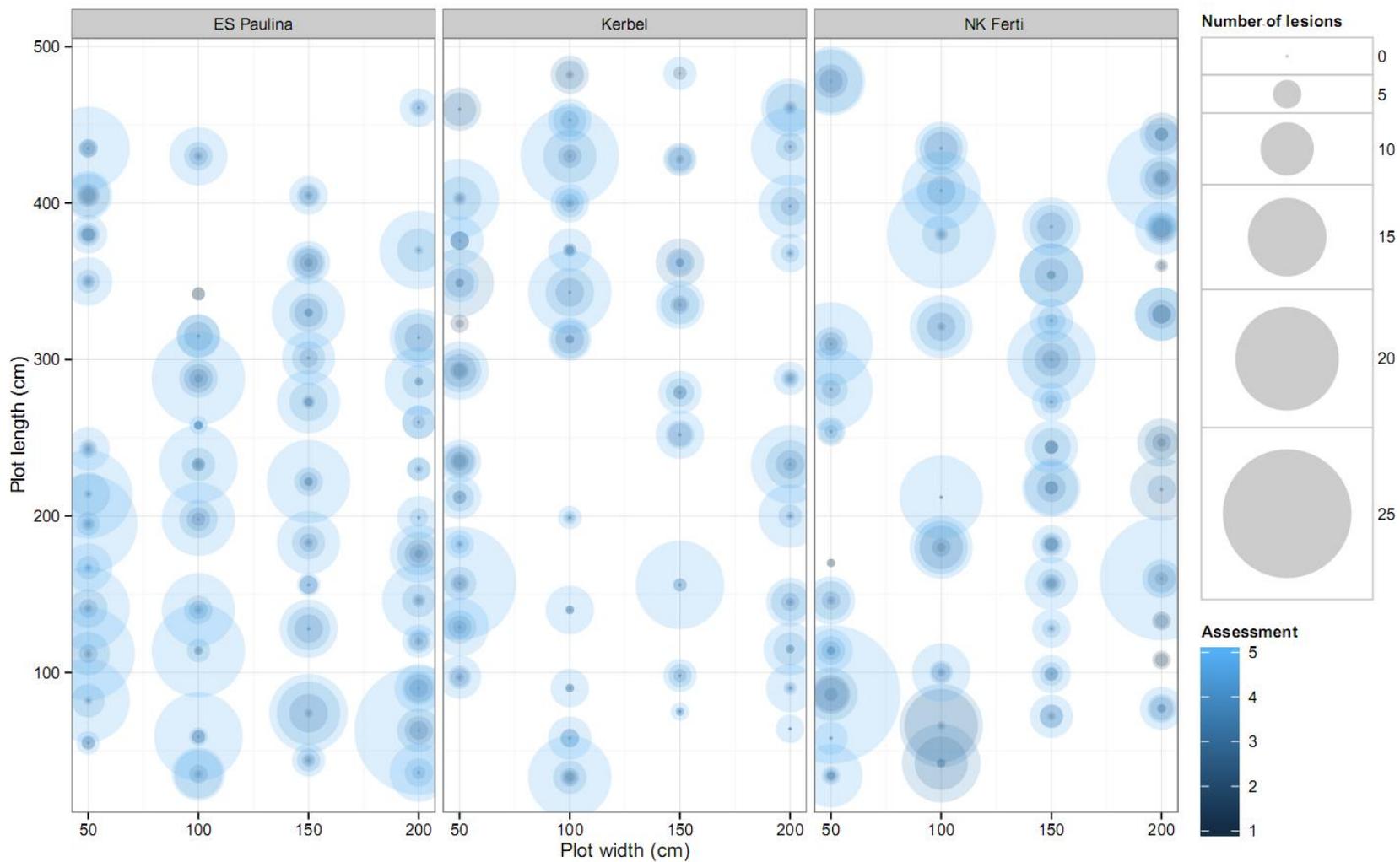


Figure I. 4. Bubble plots representing field plots from the spatiotemporal experiment conducted in 2014. Circles represent the number of Phoma black stem lesions per plant colored according to assessment from dark (earlier) to light (later) shade.

Morisita and SADIE aggregation indices for each assessment and cultivar in both years with respect to the number of lesions and number of dead leaves per plant are displayed in Table I. 1. Morisita's index was associated to significant ($P < 0.05$) aggregation of the number of lesions per plant in one or two assessments for each cultivar by year combination, except for Kerbel in 2013, where no aggregation was detected, and for NK Ferti in 2013, where aggregation was detected in four assessments. SADIE index of aggregation indicated a significant ($P < 0.05$) aggregation in the number of lesions per plant for Kerbel (3 assessments) and NK Ferti (4 assessments) in 2013, and for Kerbel (1 assessment) and ES Paulina (1 assessment) in 2014. Both indices (Morisita and SADIE) significantly ($P < 0.05$) detected aggregation in the number of lesions per plant in only four instances: assessments 2, 3 and 4 in NK Ferti in 2013 and assessment 5 in 2014 for Kerbel. Morisita's index indicated a significant ($P < 0.05$) aggregation in the number of dead leaves per plant for Kerbel in 2014 (2 assessments) and for NK Ferti in 2014 (1 assessment). Significant ($P < 0.05$) aggregation in the number of dead leaves was detected from the SADIE index for Kerbel in 2013 (4 assessments) and in 2014 (2 assessments), and for NK Ferti in 2013 (1 assessment). Both indexes (Morisita and SADIE) significantly ($P < 0.05$) detected aggregation in the number of dead leaves only in one instance (Kerbel, 2014, assessment 4).

Taylor's power law provided a very good description of variation in observed variance of the number of lesions per plant when all plots across cultivars and years were combined (Figure I. 5). The regression was associated with an R^2 value of 0.97. The slope ($b = 1.09$) was not significantly greater than 1 (t-test; $P = 0.123$) and the intercept ($a = 0.11$) was significantly greater than zero (t-test; $P = 0.008$). These results indicate an overall random distribution of the number of phoma black stem lesions within plots across assessments, cultivars and years.

The spatiotemporal dynamics of phoma black stem epidemics was analysed with the association test of SADIE (X) between assessments dates on the three cultivars in both years (Table I. 2). Significant ($P < 0.05$) spatial associations were detected in five [cultivar x year] combinations out of six when associations between two assessments dates were considered (assessment dates 2 and 4, 3 and 5 for 2013 and assessments 1 and 3 and 3 and 5 for 2014). A significant ($P < 0.05$) association between assessments 1 and 5 was detected only once (NK Ferti, 2013).

Table I. 1. Morisita's index and Index of aggregation (SADIE) of the number of black stem lesions and number of sunflower dead leaves per plant in five assessments performed at the spatiotemporal experiments.

Year	Cultivar	Assessment ^a	Lesions ^b		Dead leaves ^b	
			I_{δ}^c	I_a^d	I_{δ}^c	I_a^d
2013	Kerbel	1	- ^e	- ^e	0.91	1.51*
		2	0.72	1.76*	0.91	2.43*
		3	1.30	1.87*	0.93	2.40*
		4	1.11	1.85*	0.95	1.66*
		5	1.06	1.31	0.98	1.04
	ES Paulina	1	- ^e	- ^e	0.91	1.25
		2	- ^e	- ^e	0.91	0.82
		3	1.45	1.16	0.93	1.04
		4	1.26	0.77	0.95	1.11
		5	1.34**	0.93	0.98	0.97
	NK Ferti	1	6.50*	1.03	0.89	1.06
		2	1.95**	1.77*	0.92	0.84
		3	1.68**	1.60*	0.94	1.57*
		4	1.17*	1.64*	0.97	1.16
		5	1.07	1.64*	0.99	0.93
2014	Kerbel	1	1.54	0.93	1.16**	1.16
		2	1.08	1.36	0.88	1.14
		3	0.93	1.36	0.96	1.36
		4	1.11*	1.43	1.08**	1.36*
		5	1.09**	2.00*	1.00	1.77*
	ES Paulina	1	1.75	1.96*	0.87	1.12
		2	0.94	0.98	0.9	1.07
		3	0.94	1.01	0.94	1.19
		4	0.99	1.00	0.96	1.26
		5	1.09**	0.79	1.01	1.21
	NK Ferti	1	1.43	1.01	0.86	0.91
		2	1.46*	0.82	0.88	1.19
		3	1.01	0.78	0.92	0.74
		4	1.07	0.76	0.95	0.74
		5	1.11**	0.82	1.06**	0.82

^a Assessments were performed every seven days. See text for details.

^b * and ** = $P < 0.05$ and $P < 0.01$, respectively.

^c Morisita's index (I_{δ}).

^d Index of aggregation (I_a) provided by the spatial analysis by distances indices (SADIE).

^e No lesions observed.

Table I. 2. Analysis of association between assessments of the number of black stem lesions per plant in spatiotemporal experiments.

Year	Cultivar	Assessments^b	X^c
2013	Kerbel	2 and 4	0.05
		3 and 5	0.46**
		2 and 5	0.23
	ES Paulina	2 and 4	0.36*
		3 and 5	0.32*
		2 and 5	0.28
	NK Ferti	1 and 3	0.52**
		3 and 5	0.65**
		1 and 5	0.40**
2014	Kerbel	1 and 3	0.38*
		3 and 5	0.64**
		1 and 5	0.05
	ES Paulina	1 and 3	0.41**
		3 and 5	0.01
		1 and 5	-0.11
	NK Ferti	1 and 3	0.39*
		3 and 5	0.58**
		1 and 5	0.16

^a * and ** = $P < 0.05$ and $P < 0.01$, respectively.

^b Assessments used in the association analysis. Assessments were performed every seven days. See text for details.

^c X : SADIE association function of the number of lesions per plant between two assessments.

Significant ($P < 0.05$) spatial associations between the number of dead leaves and the number of phoma black stem lesions were detected in most of the last assessments (assessments 3 to 5; 13 significant associations out of 18; Table I. 3), whereas such an association was significant ($P < 0.05$) only in one instance when considering the two first assessments. No clear difference between cultivars, or between years, was detected.

Table I. 3. Analysis of association between the number of sunflower dead leaves and the number of black stem lesions per plant performed in five assessments in the spatiotemporal experiment.

Cultivar	Assessment ^a	X^b	
		2013 ^c	2014 ^c
Kerbel	1	-	0.09
	2	0.51**	0.03
	3	0.46**	0.94**
	4	0.28*	0.88**
	5	0.22	0.80**
ES Paulina	1	-	0.55**
	2	0.13	-0.08
	3	-0.01	0.41*
	4	0.31*	0.34*
	5	0.33*	-0.20
NK Ferti	1	0.13	-0.05
	2	-0.13	-0.09
	3	0.45**	0.97**
	4	0.21	0.81**
	5	-0.07	0.99**

^a Assessments were performed every seven days. See text for details.

^b X : SADIE association function of the number of lesions and number of dead leaves per plant at each assessment.

^c * and ** = $P < 0.05$ and $P < 0.01$, respectively.

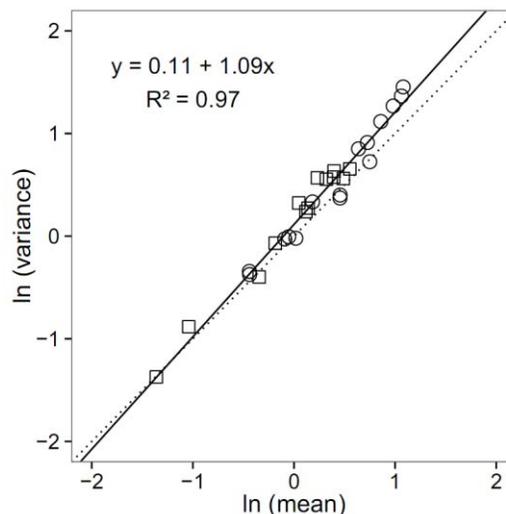


Figure I. 5. Relationship between the logarithm of the observed variance and the logarithm of mean number of Phoma black stem lesions per plant collected on three cultivars (Kerbel, ES Paulina and NK Ferti) over five assessments in spatiotemporal experiments. Squares and circles display data collected in 2013 and 2014, respectively. The dotted line represents the 1:1 line. The plain line represents the linear regression of the variance over the mean of the logarithm of the number of Phoma black stem lesions per plant.

1.4.2 Disease gradient experiments

The first assessments in gradient experiments were performed at 112 and 104 DAS in 2013 and 2014, respectively, when first lesions were detected in the plots. All inoculations led to lesions on stems with appearance similar to lesions that are observed in natural infections. Lesions on inoculated plants had lengths ranging between 12 and 24 mm seven days after inoculation, when the aluminium foils and cotton were removed. The number of lesions per plant according to the distance from the source of inoculum over assessments, cultivars and years is displayed in Figure I. 6. No pattern according to the distance to the source could be visually detected. Phoma lack stem intensity was higher in 2014 than in 2013. The mean numbers of lesions in 2013 at the last assessment were 2.6, 2.8 and 3.2 for Kerbel, ES Paulina and NK Ferti, respectively. These means were 6.8, 8.2 and 8.9 for Kerbel, ES Paulina and NK Ferti, respectively, in 2014.

Table I. 4. Results from mixed model analyses of variance of the effects of distance from the inoculum source on the number of lesions per plant of sunflower black stem in the disease gradient experiments.

Year	Cultivar	Assessment ^a	F ^b	P ^c
2013	Kerbel	1	0.11	0.75
		2	0.09	0.77
		3	1.65	0.22
	ES Paulina	1	0.01	0.93
		2	0.79	0.39
		3	0.07	0.80
	NK Ferti	1	3.37	0.09
		2	0.04	0.84
		3	0.40	0.54
2014	Kerbel	1	<0.01	0.98
		2	0.02	0.90
		3	0.11	0.74
	ES Paulina	1	3.78	0.06
		2	3.67	0.07
		3	2.34	0.15
	NK Ferti	1	1.55	0.23
		2	<0.01	0.99
		3	1.26	0.28

^a Assessments were conducted every seven days. See text for details.

^b Fisher associated to the effect of distance on number of lesions per plant.

^c P-value associated to the effect of distance on number of lesions per plant.

The mixed model analysis of variance indicated no significant ($P < 0.05$) effect of the distance from inoculum source on the number of phoma black stem lesions in any of the [cultivar x assessment x year] combinations analysed (Table I. 4). Therefore, no further analysis pertaining to characterizing disease gradients was considered.

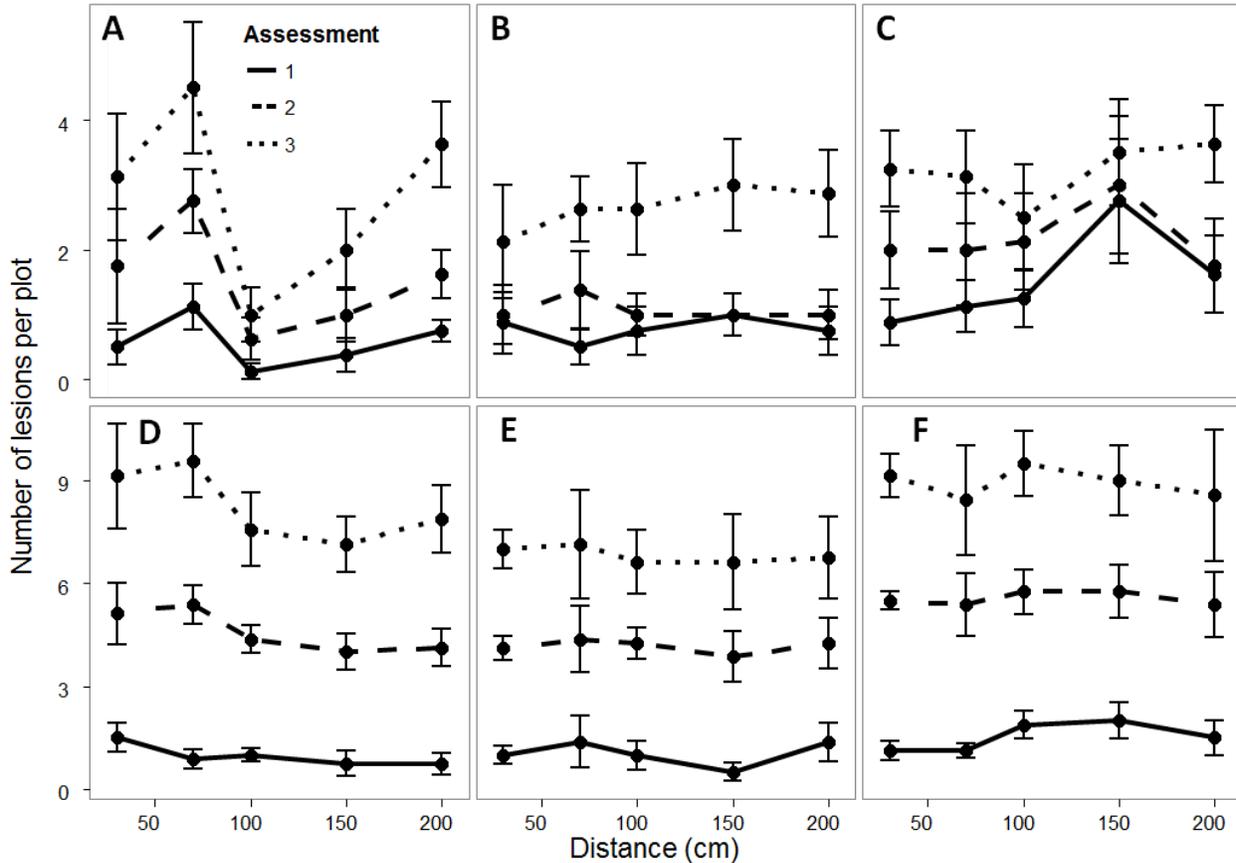


Figure I. 6. Disease gradient of sunflower Phoma black stem per plant in ES Paulina (A: 2013; D: 2014), Kerbel (B: 2013; E: 2014) and NK Ferti (C: 2013; F: 2014). Dots and error bars represent the means and standard error of the mean derived from four replications (blocks), respectively.

1.5. Discussion

Analyses of the spatiotemporal experiments indicated that both Morisita's index and SADIE index of aggregation detected aggregation of the number of lesions in four epidemics out of 28 (Table I. 1). The Taylor power law analysis further indicated a random distribution of phoma black stem lesions. These results indicate that lesions of phoma black stem were in general randomly distributed within the plots studied. Aggregated patterns are expected when secondary disease cycles are occurring in pathogens with rain-splash spores being produced in field epidemics (e.g., Pethybridge et al. 2005; Nita et al. 2012).

The results obtained in both years from the disease gradient experiments did not allow detecting any gradient along the total of 24 plots where line sources of inoculum had been established. Disease was observed on the plants assessed at any distance from the line source, indicating that absence of gradient was not due to host or environmental conditions unfavourable to infection. The random patterns of disease under natural epidemics, on the one hand (spatio-temporal experiments), and the non-detection of disease gradients (gradient experiment) over two years of experiment, on the other hand, would suggest that under the experimental conditions reported here no secondary infections took place.

Disease association between assessments separated by two weeks was detected in 10 instances (experimental plots) out of 12, whereas disease association between assessments separated by four weeks was detected only in one experimental plot out of six (Table I. 2). The lack of association between 4-week-apart assessments may be explained by the fact that lesions that appeared during this period originated from primary infections randomly distributed (originating from exogenous, airborne inoculum - ascospores). These lesions therefore were not associated to the lesions observed at the beginning of this period. The closer association found between assessments separated by two weeks may reflect the fact that lesions detected at the beginning of this period were also observed at the end of this period. The number of new lesions which appeared during this period (and which were randomly distributed) was small enough, as compared to the number of lesions detected at the beginning of the period (and still present and observed at the end of the period), to detect the association between both assessment dates.

Within-year analyses of the spatiotemporal experiments indicated that phoma black stem intensity and the number of dead leaves were associated over the last three assessments (Table I. 3).

These results conform with recent findings from field experiments where phoma black stem was associated to premature leaf senescence (Quiroz et al. 2014). Our experimental approach did not allow identifying if leaves senescing on plants were the cause, or the consequence, of phoma black stem infections. Specific experiments with inoculation of troughs corresponding to leaves with varying levels of physiological senescence would be necessary to quantify the relative effects of (1) physiological senescence on susceptibility to infection and (2) infection on disease-induced leaf senescence.

Between-year comparison indicates that 2013 was characterized by low rainfall in July and August (i.e., during flowering and grain filling), lower phoma black stem disease, and lower number of dead leaves per plant, compared to 2014. The difference in disease intensity between

years may be related to the difference in rainfall patterns: *L. lindquistii* ascospores release is favoured by rainfall (Délos et al. 1998). Under the assumption that phoma disease is associated to defoliation (this article, Table I. 3; Quiroz et al. 2014), the patterns detected in the spatiotemporal experiments between years (lower disease and higher defoliation in 2013 than in 2014) may seem contradictory. This difference may however be explained by a more important effect of water stress (higher in 2013) on defoliation, than of the association between phoma black stem (higher in 2014) and defoliation.

Our results are suggestive of black stem epidemics being driven by widespread inoculum sources located beyond any given field's limits. Other diseases, such as Fusarium head blight (FHB) of cereals, exhibit similar epidemiological features. As exemplified in the case of FHB (Savary 2014), adequate disease management strategies may be aiming at (1) inoculum reduction at the landscape scale through a reduction of sunflower in the rotation and cultivation practices; and (2) a reduction of infections through the use of host plant resistance, and fungicide applications when necessary.

1.6. Acknowledgements

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2. Effects of plant morphological traits on phoma black stem in sunflower

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2.1. Abstract

Despite the importance of Phoma black stem of sunflower in France, no specific management tools are currently deployed to control this disease. The deployment of host plant resistance could be a cost-effective and sustainable way to manage the disease. Relationships between plant morphological traits and disease intensity may provide guidance towards the identification of sunflower morphological ideotypes associated with reduced disease intensity and therefore partial resistance. Such relationships were quantified in field experiments conducted over 2 years with a set of 21 sunflower genotypes, where several morphological attributes and several disease intensity variables were measured. Plant morphology was assessed prior to epidemic onset. Disease intensity was assessed at different scales of crop and plant hierarchy, using a nested sampling design, and implementing the concept of conditional disease intensity. The various analyses performed indicated that experimental plots grouped according to morphological attributes of sunflower at the flowering stage were associated with experimental plots grouped according to disease intensity variables, therefore indicating an association between morphological traits and disease intensity. Low disease intensity was associated with a morphological ideotype with large number of green leaves and tall stature. A sunflower plant morphological ideotype with more leaves and taller stature may represent an operational target in sunflower breeding when considering resistance to Phoma black stem.

Keywords: *Helianthus annuus*, *Leptosphaeria lindquistii*, *Phoma macdonaldii*, Plant morphology, Sunflower, Botanical epidemiology.

2.2. Introduction

Phoma black stem, caused by the fungus *Leptosphaeria lindquistii* Frezzi (synonym *Phoma macdonaldii* Boerema), is an important disease of sunflower in France (Debaeke and Pérès 2003). The pathogen overwinters as pseudothecia, pycnidia and mycelium produced on infected sunflower stubble residues (Gulya et al. 1997). In France, lesions observed on stems appear to be mainly due to infections from exogenous inoculum consisting of ascospores released from infected sunflower residues (Seassau et al. 2010; Bordat et al. 2011). Furthermore, it is hypothesized that epidemics are monocyclic, because no pycnidia (which may lead to secondary infections) can be observed on lesions on the host during the growing season (Bordat et al. 2011). Symptoms on the stem correspond to black, round to oval lesions enlarging from leaf nodes. Epidemics generally start after flowering and the appearance of symptoms on the stem usually follows acropetal progress (from the lower to upper leaf nodes). *L. lindquistii* infections can also occur on the collar, where lesions develop and may lead to senescence and death of the plant a few weeks before normal maturity. This is referred to premature death or premature ripening, and can lead to high yield losses (Donald et al. 1987; Debaeke and Pérès 2003; Seassau et al. 2010).

Phoma black stem is influenced by several components of cropping practice. The disease is favoured by increased nitrogen input and irrigation, which are in turn associated with a larger leaf area index (LAI) in the canopy (Debaeke and Pérès 2003). It is also possible that short rotations (sunflower–wheat) and reduced tillage, which were introduced in the 90s in France, may have triggered an increase in inoculum density, leading to the recent increase in importance of the disease in France (Debaeke and Pérès 2003). No complete resistance has been identified for Phoma black stem. Although several QTLs (Quantitative Trait Loci) for resistance to *L. lindquistii* have been identified (e.g., Roustae et al. 2000), no varieties with partial resistance to Phoma black stem as yet have been deployed to manage the disease.

Measuring disease in plants is a key methodological component in most research, development, and applied activities involving phytopathological aspects (Large 1966; Zadoks and Schein 1979). Applications include plant breeding, where various germplasm, varieties and/or breeding materials are rated through visual assessment (e.g., Poland and Nelson 2011; Xie et al. 2012). Disease assessment involves several components, including keys and diagrams describing the host development and morphology, standard area diagrams for disease severity (e.g., James 1971), and sampling design (Zadoks and Schein 1979). Recent years marked a renewed interest in the development and evaluation of diagrams for disease severity (e.g., Bock et al. 2010; Schwanck and Del Ponte 2014). Disease assessment aims at collecting data pertaining

to disease intensity, which can be expressed as incidence, severity, or lesion density (Nutter et al. 1991).

Plant morphology can affect disease intensity, as shown in many pathosystems (e.g., Zadoks and Schein 1979; Savary et al. 1995a; Ando et al. 2007). Plant morphology can in turn vary in response to several factors, including numerous cropping practices (e.g., level of nutrients, pruning), and plant genotype (Poland et al. 2009; Andrivon et al. 2013). The identification of morphological traits associated with reduced disease intensity may therefore represent a useful basis to improve disease management, through breeding of varieties with morphological attributes associated with less disease. In the case of Phoma black stem in sunflower, relationships between morphological traits and disease intensity have not been quantitatively documented, and may provide useful information to improve management of the disease.

The objectives of the work presented here were: (1) to develop a protocol for assessment of Phoma black stem in sunflower (including a sampling design and a scale for severity on the stem), and (2) to apply it to address relationships between sunflower morphological traits and Phoma black stem intensity.

2.3. Materials and methods

2.3.1. Experimental design and general features

Two field experiments were conducted in Auzeville, near Toulouse (Haute-Garonne, South-West France), in 2013 and 2014. Both experiments were planted according to a randomized complete block design with four replications, and 21 sunflower genotypes randomly established in each block. Both experiments were conducted under natural conditions for epidemic development (no inoculation was performed).

The experimental plots were sown at a rate of 7 seeds/m², on 6 May and 15 April in 2013 and 2014, respectively. Both experiments were grown under rainfed conditions. Nitrogen was applied once in both years at 50 days after sowing, at a rate of 35 and 70 kg/ha in 2013 and 2014, respectively. Each experimental unit (one genotype in a block) consisted of a plot of six rows with a row length of 6 m and an inter-row space of 0.5 m, corresponding to an area of 18 m² (6 × 3 m). Due to the limited amount of seeds available for several genotypes tested, the two border rows of each plot were sown with a commercial hybrid in both years (NK Kondi). Weather data was collected with a weather station (CE-180, Cimel, France) located less than 400

m from the experimental site. 10-day mean temperature and accumulated rainfall were computed from April to September in each experimental year.

2.3.2. Plant material

Plant material consisted of 21 sunflower genotypes (Table II. 1). The inbred lines FU (INRA core collection ID: SF056) and PAZ2 (INRA core collection ID: SF306) were used in this study. FU had shown higher Phoma black stem intensity than PAZ2 in a previous study performed under controlled conditions on seedlings, using inoculation of cotyledon petioles (Bert et al. 2004).

Table II. 1. List and description of sunflower genotypes used in the experiments.

Codes^a	Male lines^b	Genetic material type	
550	(Tub-1709-1)-1-6A	Hybrids: FU x line from INRA core collection	
558	SF306		
574	97B7		
586	SF336		
592	SF247		
598	SF332		
610	FP109	Hybrids: FU x RIL ^c	
616	FP111		
628	FP146		
634	FP176		
640	FP043		
652	FP056		
658	FP072		
664	FP102		
SF056 ^d			Lines
SF306 ^e			
FP055			
FP066			
Kerbel		Commercial hybrids	
ES Paulina			
NK Ferty			

^a Genotype codes used in this study.

^b Male parental lines used to produce hybrids.

^c RIL = Recombinant inbred lines, see text for details.

^d other name: FU.

^e other name: PAZ2.

Six hybrids were produced from crosses involving the susceptible inbred line FU as the female tester, and six lines from the INRA *Helianthus annuus* core collection as the males. One male parental line was PAZ2, while the five other male parental lines were characterized as

having a range of levels of field resistance to premature death (Bordat et al. 2012). Eight additional hybrids were produced from crosses between FU and recombinant inbred lines (RILs; F7-F10) obtained by single seed descent from a crossing between FU and PAZ2. The eight RILs had a range of levels of quantitative resistance to premature death (610, 616, 628 and 634 with two alleles of quantitative resistance, and 640, 652, 658, and 664 with two alleles of susceptibility; Bordat et al. 2012). Furthermore, two lines, FP055, characterized as having two alleles for susceptibility, and FP066, with two alleles of quantitative resistance against premature death, were included. Three commercial hybrids currently grown in France, Kerbel (RAGT Semences), ES Paulina (Euralis Semences) and NK Ferti (Syngenta Seeds), were used in order to include genetic material grown in farmers' fields. Hybrids were used in this study because this represents the main genetic make-up currently used in commercial sunflower fields.

The genotypes tested therefore included: (1) parental lines with varying characteristics of resistance to *L. lindquistii*, (2) hybrids produced from crosses between a susceptible line (FU) and lines or RILs with a range of levels of resistance to *L. lindquistii*, and (3) commercial hybrids.

2.3.3. Disease assessments

One disease assessment was conducted in 2013, at 122 days after sowing (DAS; September 5). Two disease assessments were performed in 2014, at 112 DAS (August 5) and 119 DAS (August 12). On each assessment date, 15 plants per plot were randomly selected and classified according to four categories: (A) no disease; (B) symptoms on the stem only; (C) symptoms on the collar only and (D) symptoms on both the stem and collar. Disease incidence on the stem (proportion of plants with at least one Phoma black stem lesion; INCS) and disease incidence on collar (proportion of plants with a lesion on the collar; INCC) were derived from these assessments (Table II. 2).

Table II. 2. List of measured or computed variables for Phoma black stem disease intensity or sunflower morphological traits

Variable meaning	Variable acronym	Calculation	Unit
<i>Disease variables</i>			
Phoma black stem disease incidence (fraction of plants with at least one black stem lesion)	INCS	-	-
Collar disease incidence (fraction of plants with a collar lesion)	INCC	-	-
Disease height (distance from plant base to highest lesion on the stem)	DISH	-	cm
Relative disease height (proportion of disease height over plant height)	RDISH	DISH/PH	-
Disease severity on stem at <i>i</i> internode level ^a , measured on diseased plants)	CSEV _{<i>i</i>}	-	%
Mean of disease severity over three internode levels, measured on diseased plants	CMSEV	$\Sigma CSEV_i/3$	%
Conditional disease severity on stem (disease severity on diseased plants)	CSEV	CMSEV x RDISH	%
[absolute] Disease severity on stem	SEV	INCS x CSEV	%
Conditional number of lesions on stem per plant (number of lesions on stem – number of diseased nodes - per plant on diseased plants)	CNUML	-	Nb lesions/ plant
Number of lesions per plant (number of diseased nodes per plant)	NUML	CNUML x INCS	Nb lesions/ plant
Lesion incidence on stem (fraction of diseased nodes)	INCN	NUML/TL	-
Proxy for lesion size	LESI	CSEV / CNUML	%/Nb lesions/ plant
<i>Morphological variables</i>			
Number of dead leaves per plant	DL	-	Nb leaves
Number of green leaves per plant	GL	-	Nb leaves
Total number of leaves per plant	TL	DL + GL	Nb leaves
Proportion of dead leaves on total nb leaves	DLFR	$100 \times DL / TL$	%
Plant height	PH	-	cm
Stem diameter	SD	-	mm
Width of insertion point at leaf nodes 3 and 6 (3 rd and 6 th node from plant base)	N3, N6	-	mm
Leaf area index	LAI	-	-

^a *i* = low, medium and high internode level (see text for details).

Among the stem-diseased plants, three plants per plot were randomly selected for additional disease measurements of disease intensity (severity, density, height). The distance between the stem base and the highest lesion on the stem was measured (disease height; DISH). Relative disease height (RDISH) was further computed as the ratio of DISH to plant height. The number of lesions on the stem was counted (CNUML). This number corresponds to a conditional disease measurement (Shaw 1995, 1996; McRoberts et al. 2003), because it is the number of lesions on diseased plants only; that is, conditional on the plant being diseased. CNUML was multiplied by INCS to derive the [absolute] number of lesions on a stem per plant (NUML). Furthermore, disease incidence at the node scale (fraction of diseased nodes per plant, INCN) was derived by dividing NUML by TL, the total number of leaves (nodes) per plant (see below, assessment of morphology). Disease severity on the stem was assessed using a standard area diagram with six grades, ranging from 1 to 70 % (Figure II. 1). The diagram was first designed using digital photos of symptoms on stems displaying a range of severity. The software ASSESS© 2.0 (APS Press, St. Paul, MN, USA) was used to fine tune symptom densities so that they correspond to the six grades with pre-set levels of disease severity.

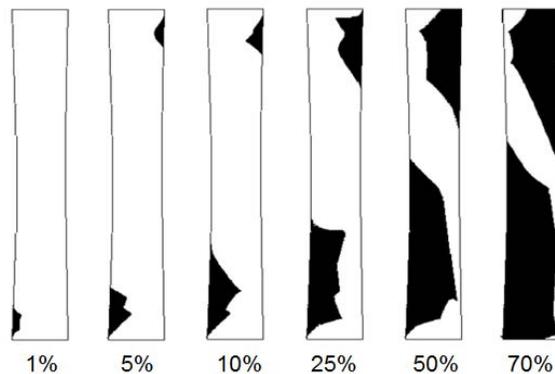


Figure II. 1. Standard area disease diagram used to assess sunflower Phoma black stem severity. Percentage values represent the percentage of stem area covered by lesions between two nodes.

Disease severity assessments were performed on three internodes located on the lowest part of the stem (where disease develops), i.e.,: closest to soil surface (CSEV1), on the highest diseased internode (CSEV3), and on the internode located at an intermediate height (CSEV2). When the number of diseased internodes was not large enough to fit three measurements, only one or two measurements were taken. Mean severity (CMSEV) over the three internodes was computed. Conditional disease severity on the stem (CSEV) was derived from CMSEV and RDISH, and the [absolute] disease severity on the stem was calculated from CSEV and INCS

(see computations in Table II. 2). A proxy for lesion size (LESI) was derived as the fraction of conditional disease severity over the conditional number of lesions per plant.

In 2014, disease incidence on stems (INCS) was variable between plots in assessment 1, whereas INCS was consistently close to 1 at the time of the second assessment, and thus had much less variation. Furthermore, conditional disease intensity (e.g., CNUML) was very small at the first assessment, and displayed less variation as compared to assessment 2. Therefore, INCS corresponding to the first assessment was retained for data analysis, whereas the other disease intensity variables (e.g., NUML) collected at the second assessment were used for the analyses.

2.3.4. Morphological assessments

Morphological variables were measured in both years at the ‘star-bud’ (Schneider and Miller 1981; Merrien and Milan 1992) stage (July 3, 2013, 58 DAS; and June 18, 2014, 65 DAS) and at the flowering stage (July 30, 2013 and July 9, 2014). Three representative plants per plot were chosen and assessed for morphological variables (Table II. 2).

Variables collected were plant height (PH), stem diameter at the base of the plant (SD), width of the petiole trough at the stem node (N3: measurement made at ‘star-bud’ on the third node from plant base; and N6: measurement made at flowering on the sixth node), number of green leaves (GL), and number of dead leaves (DL). A leaf was considered dead when more than 50 % of its area was not green. Furthermore, when dead leaves were detached from the stem (defoliated), DL could be derived by observing the petiole insertion point at stem nodes. The total number of leaves ($TL = DL + GL$) and the fraction of dead leaves ($DLFR = DL/TL$) were computed from GL and DL. TL also corresponds to the total number of nodes on the stem. Leaf area index (LAI) was determined using the Pouzet and Bugat (1985) method based on the measurement of length and width on the largest and lowest leaves of the plants and on the number of leaves per plant.

2.3.5. Data analysis

A series of analyses were performed on the data from each year in order to assess the effect of plant morphology on disease intensity, considering each individual plot as a statistical unit. Complementary multivariate analyses were implemented, in order to consider disease intensity and morphological traits as combinations of variables. Statistical analyses were performed with R (R Core Team: Rv3.2: A Language and Environment for Statistical Computing. Vienna, Austria: R Foundation for Statistical Computing), SAS v.13 (SAS Institute Inc., Cary, NC) and SYSTAT v.13 (SYSTAT, San Jose, CA). Adapting from Willocquet et al. (2012) and Srinivasachary et al.

(2013), the analyses were conducted in six main steps (Figure II. 2): (1) analyses of variance of disease and morphological variables according to genotype and block, based on the statistical design of field experiments, in order to provide an overview of the datasets and of any patterns in the variables; (2) multivariate analyses of disease variables to characterize associations between disease variables, and to group experimental plots according to disease variables; (3) similar to step (2), grouping of experimental plots according to morphological variables; (4) contingency table analyses between disease-based and morphology-based groups; (5) correspondence analyses between disease- and morphology-based groupings; and (6) logistic regressions in order to assess the relationships between disease intensity and morphological characteristics. These steps are described below in more detail.

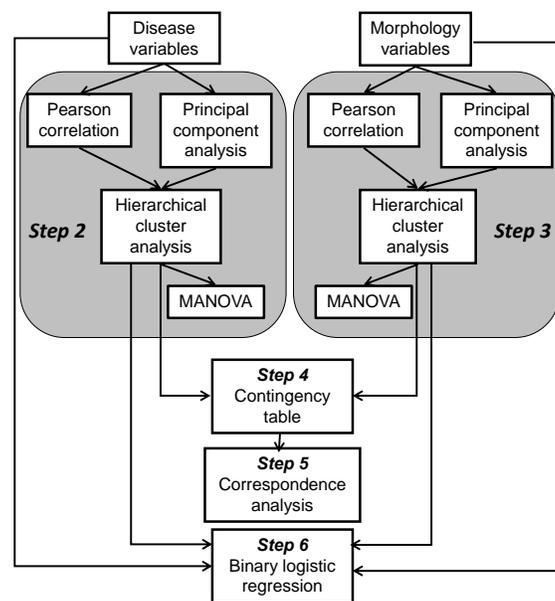


Figure II. 2. Main steps implemented to characterize the association between sunflower Phoma black stem disease intensity and morphological traits. See text for details.

Transformation (arcsine for disease incidence variables and square root for other variables) was applied to variables for which homoscedasticity was not satisfied (using Levene's test) so as to stabilize the variance (Snedecor and Cochran 1989). The 2013 INCC and INCN data were arcsine-transformed and DISH, SEV, NUML and LESI were square-root transformed; in 2014, INCC, INCS and INCN data were arcsine-transformed, while SEV and LESI were square-root transformed. In the first step, mixed model analyses of variance (ANOVA) were performed for each (disease and morphological) variable and each assessment, considering genotypes as

fixed effects and blocks as random effects. The MIXED procedure of SAS was used to perform the analyses of variance.

In the second and third steps (groupings according to disease or morphology variables), Pearson correlations and principal component analyses were first performed separately for disease and morphological variables in order to assess associations between variables, using the function “corr” in R for correlation analyses, and SYSTAT for the principal component analysis. All analyses described from this point were conducted with SYSTAT. Experimental plots were grouped according to (1) disease intensity and (2) morphological variables, as a result of hierarchical cluster analyses using the Ward criterion and the Mahalanobis distance (Saporta 1990). The latter distance was chosen because disease- and morphology-variables were expressed in very different metrics and had very different ranges (Table II. 2), and because the Mahalanobis distance is scale invariant (Wilkinson et al. 2007). Disease clusters (D groups) were derived according to INCN, NUML, SEV, LESI and INCS. Variables used to form morphological clusters (M groups) at star-bud and flowering were DL, GL, LAI, N3 (star-bud) or N6 (flowering assessment), SD and PH. Boxplots were then built to characterize the groups formed for both D and M clusters using un-transformed variables, and multivariate analyses of variance (MANOVA) were conducted to assess the effects of the different groups formed on the variables from which they were derived. The resulting D groups and M groups may be seen as disease- and morphology-meta variables, which synthesize experimental plot information.

In the fourth step, contingency tables between morphological and disease groups derived from cluster analyses were performed to assess the association between these two meta-variables. Contingency tables were built in both experimental years, using M clusters from star-bud or flowering data on the one hand, and D clusters on the other hand, totalling four contingency tables.

In the fifth step, correspondence analyses (Benzécri 1973; Greenacre 1984; Savary et al. 1995b) were performed in both years using morphological groups and disease intensity groups as active variables. Binary logistic regressions (Steinberg and Colla 2009) were performed in the sixth step to further assess the association between morphological variables or formed M clusters and disease intensity. For this, binary variables defining high or low disease intensity were generated from D groups based on disease clusters characterization. Four binary logistic regression models were tested involving morphological groups and morphological variables as predictors for low or high disease intensity clusters:

$$\ln(P(Di)/(1-P(Di)))=a+bx$$

Where $P(D_i)$ is the probability of a plot belonging to the disease cluster D_i , where disease intensity is low (Models 1 and 3) or high (Models 2 and 4) and x is the predictor variable to be tested: M clusters (Models 1 and 2) or morphological variables (Models 3 and 4).

2.4. Results

2.4.1. Overview of disease intensity and morphological traits

Several plots for which crop establishment was poor were excluded from the analyses: four plots in 2013 and eight plots in 2014. As a result, the total number of plots included in the analyses was 80 and 76 in 2013 and 2014, respectively. Experiments were subject to different weather patterns (Figure II. 3). In general the mean 10-day temperature from April to June was 2–3 °C lower in 2013 compared with 2014, and in July and August was 2–3 °C higher in 2013 compared with 2014. Major differences in 10-day accumulated rainfall were observed in May (41 mm more rainfall in 2013 compared with 2014) and July and August, when rainfall was much lower in 2013 than in 2014. Rainfall around flowering was much larger in 2014 than in 2013.

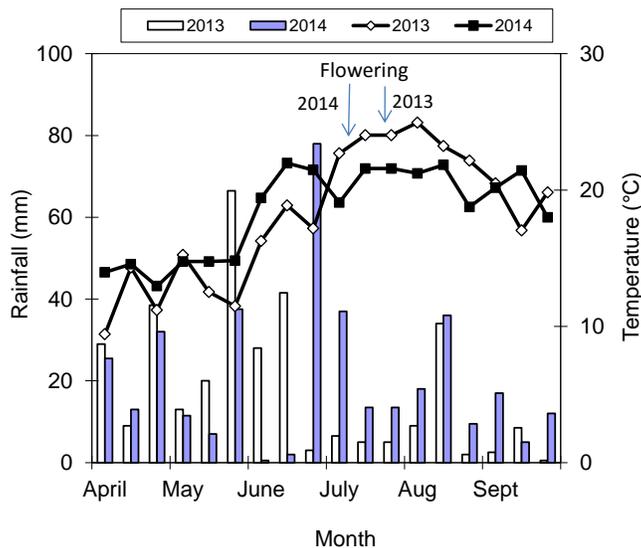


Figure II. 3. 10-day rainfall and mean temperature in 2013 and 2014.

The mixed models ANOVAs indicated that, except for DL and DLFR, all disease intensity and morphological variables were significantly ($P < 0.05$) affected by sunflower genotype in both years (Table II. 3). Variables for disease incidence at the plant scale (e.g., INCS) and LESI were similar in both years, whereas other disease variables had higher values in 2014 compared to 2013. Disease severity was 1.4 and 4.9 % in 2013 and 2014, respectively, and

the number of lesions per plant (NUML) was much lower in 2013 (1.5) compared to 2014 (6.9). Variation among plots (standard error) was higher in 2014 for all variables except INCC (Table II. 3). Means of morphological variables were much lower at the ‘star-bud’ development stage than at flowering, except for GL and SD, for which a small increase was observed between ‘star-bud’ and flowering (Table II. 3). When comparing years, morphological variables were lower in 2013 compared to 2014, except DL, DLFR and TL at flowering which were lower in 2014 compared to 2013 (Table II. 3).

Table II. 3. Summary of statistics and results from mixed model analyses of variance of the effects of sunflower genotypes on sunflower Phoma black stem disease and morphological variables

Variable type	Variable ^a	2013			2014		
		Mean (SE) ^b	F	P	Mean (SE) ^b	F	P
Disease	INCS	0.55 (0.03)	2.59	<0.01	0.56 (0.06)	5.47	<0.01
	INCC	0.17 (0.02)	3.75	<0.01	0.18 (0.02)	4.78	<0.01
	DISH	20.47 (0.86)	1.96	0.02	35.92 (4.12)	4.99	<0.01
	RDISH	0.21 (0.01)	2.70	<0.01	0.34 (0.04)	4.82	<0.01
	CSEV	2.05 (0.21)	2.52	<0.01	5.05 (0.58)	7.04	<0.01
	SEV	1.41 (0.20)	2.20	0.01	4.90 (0.56)	6.99	<0.01
	CNUML	2.38 (0.17)	2.32	0.01	7.17 (0.82)	3.07	<0.01
	NUML	1.52 (0.15)	2.20	0.01	6.94 (0.80)	3.09	<0.01
	LESI	0.82 (0.06)	3.72	<0.01	0.67 (0.08)	6.39	<0.01
	INCN	0.10 (0.01)	2.49	<0.01	0.32 (0.04)	3.40	<0.01
Morphology (Star bud)	DL	0.54 (0.08)	2.77	<0.01	1.13 (0.13)	1.89	0.03
	GL	15.47 (0.23)	2.64	<0.01	17.01 (1.95)	2.90	<0.01
	TL	16.01 (0.25)	2.46	<0.01	18.14 (2.08)	3.54	<0.01
	DLFR	3.15 (0.44)	2.54	<0.01	6.10 (0.70)	1.86	0.04
	PH	53.33 (1.51)	9.73	<0.01	62.68 (7.19)	5.70	<0.01
	SD	14.51 (0.27)	4.00	<0.01	14.99 (1.72)	2.46	<0.01
	N3	6.50 (0.14)	3.20	<0.01	6.98 (0.80)	2.41	<0.01
	LAI	1.19 (0.05)	3.05	<0.01	1.24 (0.14)	3.11	<0.01
Morphology (Flowering)	DL	5.74 (0.18)	1.34	0.19	2.85 (0.32)	1.79	0.05
	GL	17.89 (0.35)	9.46	<0.01	19.49 (2.23)	13.02	<0.01
	TL	24.61 (0.80)	13.54	<0.01	22.33 (2.56)	12.41	<0.01
	DLFR	23.62 (0.35)	2.10	0.02	12.60 (1.44)	1.59	0.09
	PH	107.78 (2.54)	16.54	<0.01	111.72 (12.81)	20.93	<0.01
	SD	16.46 (0.26)	2.94	<0.01	19.67 (2.25)	2.46	<0.01
	N6	8.77 (0.15)	2.23	0.01	8.76 (1.00)	4.91	<0.01
LAI	1.49 (0.07)	4.22	<0.01	2.42 (0.27)	3.08	<0.01	

^a Variables acronyms and units are shown in Table II. 1.

^b Mean is followed by standard error of the mean (SE).

2.4.2. Association between disease variables and definition of disease groups

Most disease variables were positively and significantly ($P < 0.05$) correlated among themselves in both years (Figure II. 4). NUML was highly correlated ($r > 0.70$) to DISH and RDISH in both years (Figure II. 4). NUML was also highly correlated to SEV (2013: $r = 0.84$; 2014: $r = 0.60$). INCC was the variable least correlated with other disease variables, and was significantly ($P < 0.05$) correlated only with DISH in 2014 (Figure II. 4b). LESI was also poorly correlated to most other disease variables, except with SEV (2013: $r = 0.60$; 2014: $r = 0.88$).

Principal component analyses generated two axes which captured most of the variance for disease variables in 2013 (factor 1 = 58 % and factor 2 = 15 %) and 2014 (factor 1 = 54 % and factor 2 = 19 %). All disease variables were positively associated with factor one of the principal component analyses in both years (Figure II. 5a and b). In both years, RDISH, DISH, INCN and NUML were clustered with positive values close to 1 on axis 1 and values close to -0.25 on axis 2, LESI being near-independent from these clusters. INCC and LESI were closely projected in 2013; both being more linked to factor 2, with positive coordinates higher than 0.5. In 2014, LESI was closely projected to INCS and both variables were highly linked to factor 2 (coordinate > 0.5), while INCC was only weakly linked to either of the two factors, being projected close to the axes origin.

Hierarchical cluster analysis using INCS, SEV, NUML, LESI and INCN disease variables generated four disease intensity clusters in 2013 (D1 to D4) and three clusters in 2014 (D5 to D7). Clusters D1, D2, D3 and D4 included 37, 22, 10 and 11 plots, respectively; while clusters D5, D6 and D7 included 25, 20 and 31 plots respectively. MANOVA results confirmed that these clusters differed significantly with respect to the disease intensity variables used to define them. In 2013, Wilk's lambda value associated with MANOVA was 0.02 (F -ratio = 39.6; with 3 and 76 degrees of freedom; $P < 0.001$), while in 2014 it was 0.13 (F -ratio = 25.0; with 3 and 72 degrees of freedom; $P < 0.001$).

Cluster D4 corresponded to high disease intensity whereas D2 and D3 were associated with low disease intensity in 2013 (Figure II. 6, upper graphs). Cluster D3 was however associated with high LESI values. Cluster D1 had high (INCS, NUML, INCN) to intermediate (SEV, LESI) disease intensity values. Clusters D5 and D6 corresponded to low and high disease intensity, respectively, in 2014 (Figure II. 6, lower graphs). Cluster D5 was ranked differently amongst the other disease groups, depending on the disease variable considered (Figure II. 6).

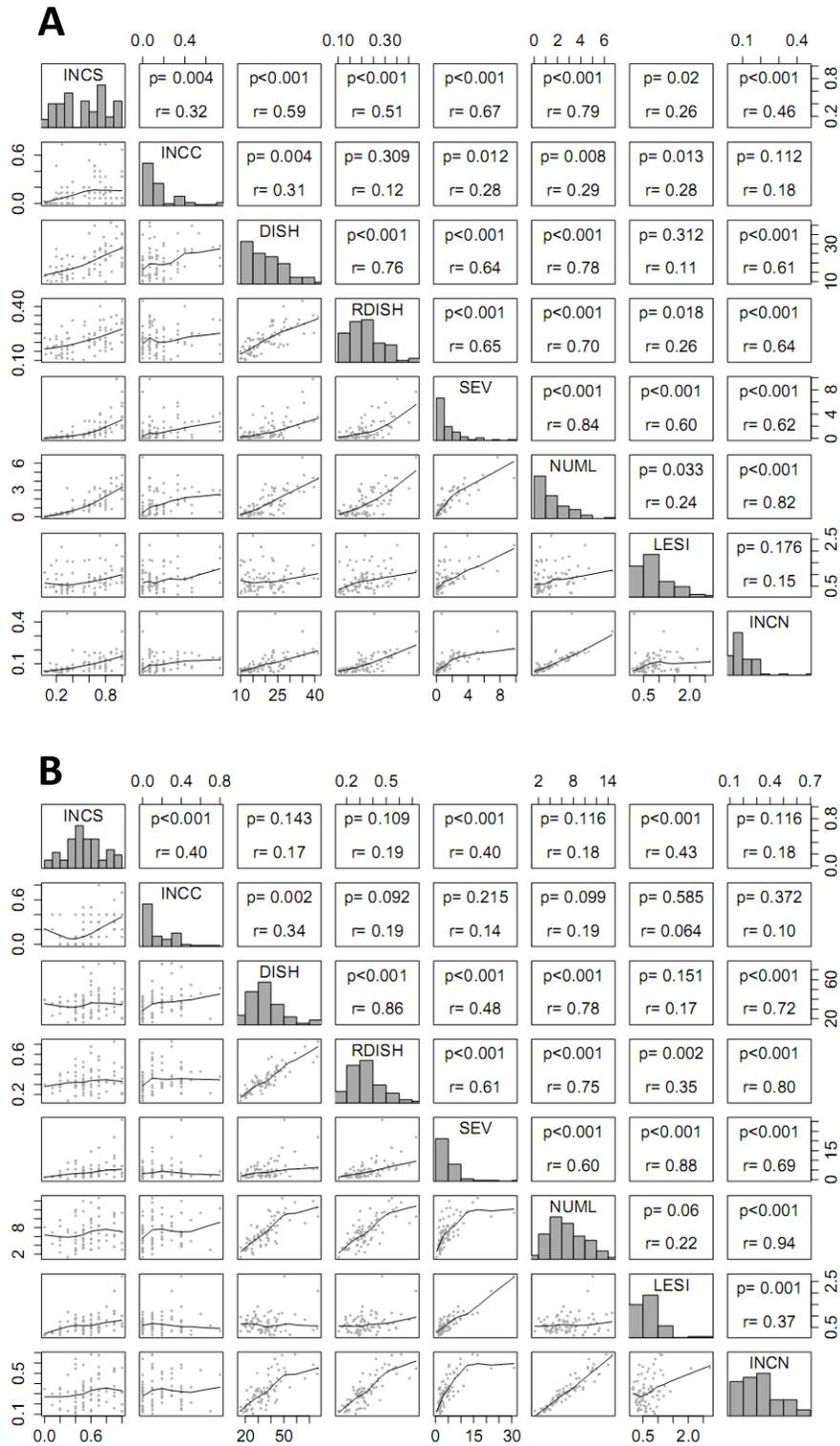


Figure II. 4. Phoma black stem disease variables frequency distribution (*diagonal*), pairwise variable scatterplots (*lower left triangle*), and Pearson correlations with associated *P*-values (*upper right triangle*). A: 2013; B: 2014. Symbols for variables and their units are listed in Table II. 2.

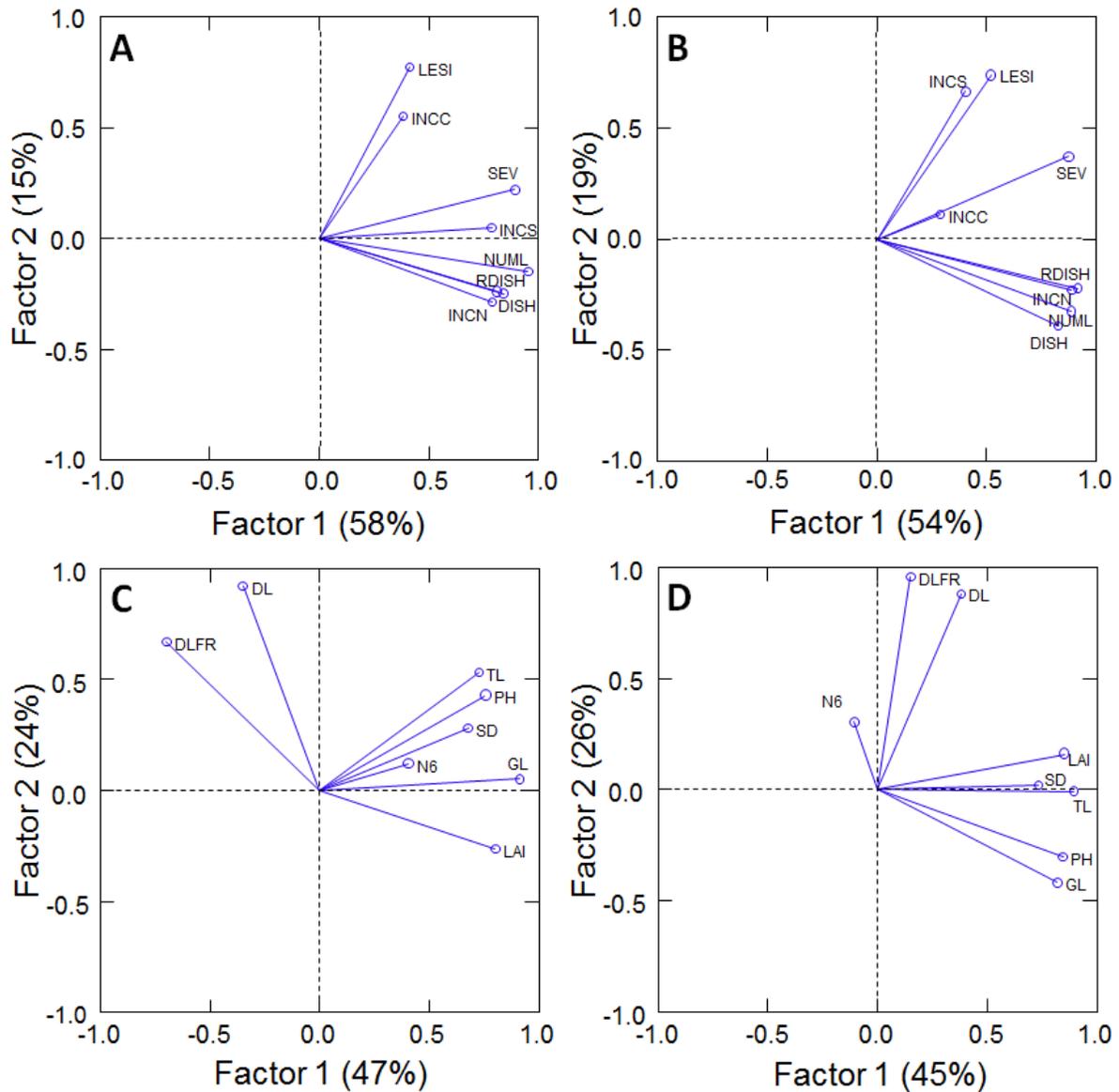


Figure II. 5. Principal component analyses of sunflower Phoma black stem and morphological variables measured in field experiments. A, C: 2013; B, D: 2014. A, B: disease variables. C, D: morphological variables measured at flowering. Symbols for variables and their units are listed in Table II. 2.

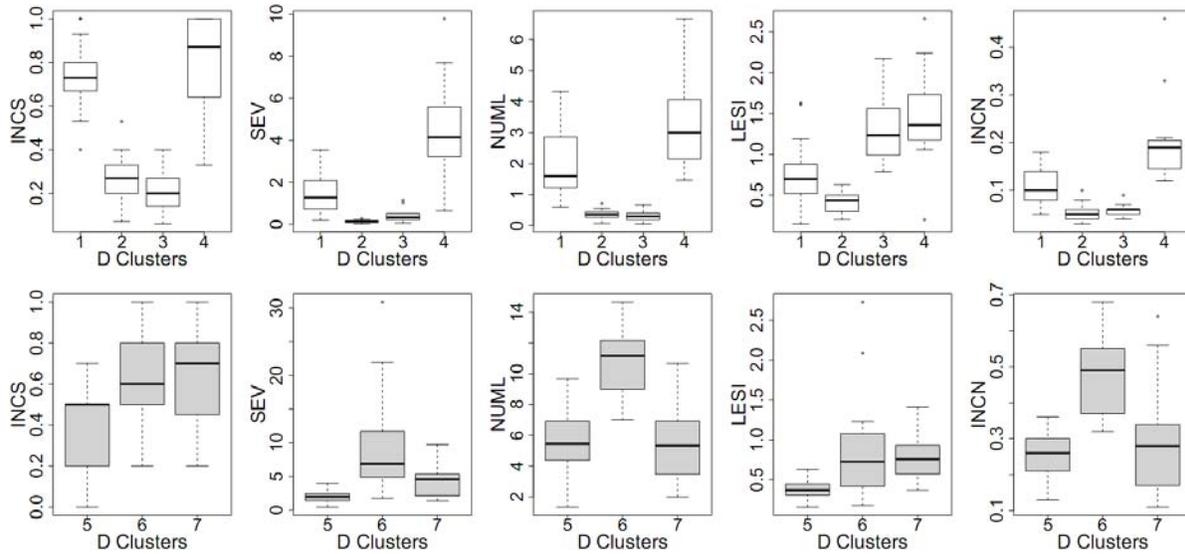


Figure II. 6. Boxplots of sunflower Phoma black stem disease variables according to groups generated from hierarchical cluster analysis. Top graphs, white: 2013; bottom graphs, gray: 2014. The boxes represent the interquartile range, the whiskers indicate the 5- and 95-percentiles, and the line within each box represents the median. Dots refer to outlying data points. Symbols for variables and their units are listed in Table II. 2.

2.4.3. Association between morphological variables and definition of morphological groups

Morphological variables at flowering were positively associated, resulting in 18 significant ($P < 0.05$) pairwise correlations out of 28, and displayed a wide range of r values (from 0.01 to 0.95; Figure II. 7). GL, TL and PH variables were highly correlated ($r > 0.6$; Figure II. 7) in both years. DL was not significantly correlated with PH, SD and N6, but was highly correlated with DLFR in both years. Correlation coefficients involving N6 were below 0.4 in all cases, except with SD in 2013 ($r = 0.53$).

Principal component analyses generated two axes which captured most of the variance of morphological variables measured at flowering in 2013 (factor 1 = 47 % and factor 2 = 24 %) and 2014 (factor 1 = 45 % and factor 2 = 26 %). In both years, most morphological variables were linked to the first factor except DLFR and DL, which were more linked to the second axis, and N6, which was not well represented by either of the two first axes (Figure II. 5c and d). PH was closely linked with TL in 2013 and with GL in 2014. DL and DLFR were well represented by axis 2, indicating a weak association with variables well represented by axis 1 (TL, PH, SD, GL and LAI).

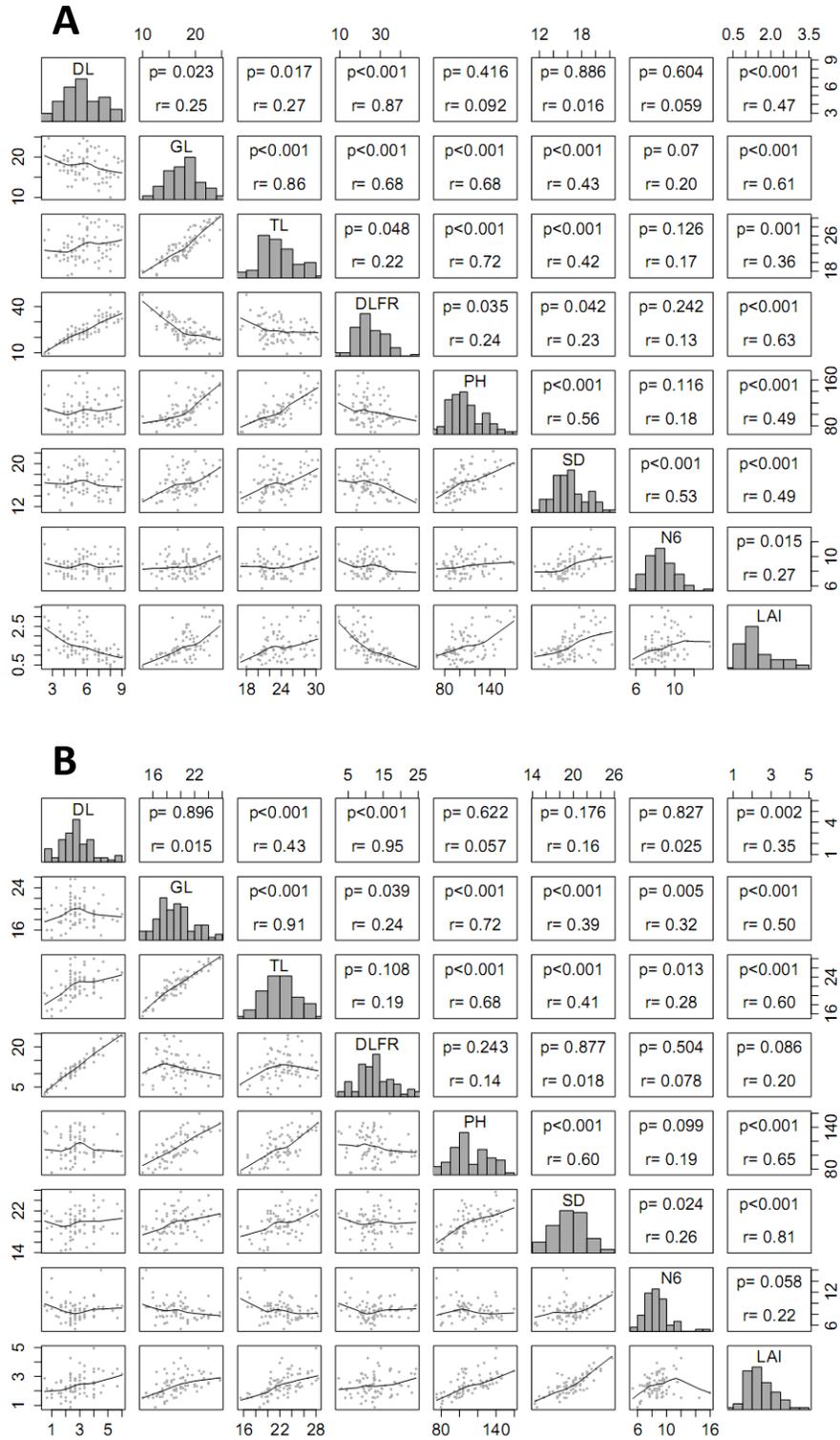


Figure II. 7. Sunflower morphological variables frequency distribution (*diagonal*), pairwise variable scatterplots (*lower triangle*), and Pearson correlations with associated *P*-values (*upper right triangle*). A: 2013; B: 2014. Symbols for variables and their units are listed in Table II. 2.

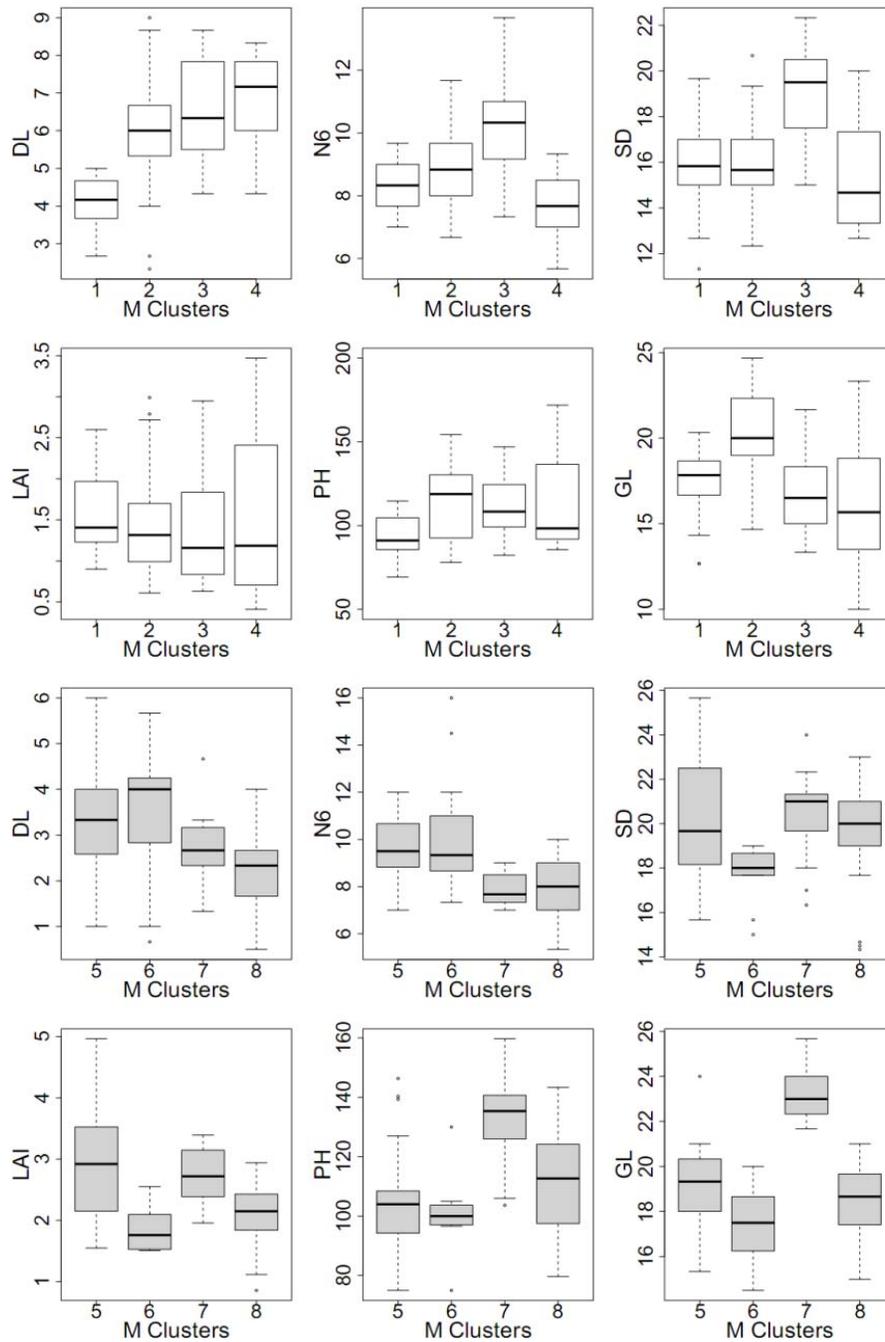


Figure II. 8. Boxplots of sunflower morphological variables collected at flowering stage according to groups generated from hierarchical cluster analysis. Upper plots, white: 2013; bottom plots, gray: 2014. The boxes represent the interquartile range, the whiskers indicate the 5- and 95-percentiles, and the line within each box represents the median. Dots refer to outlying data points. Symbols for variables and their units are listed in Table II. 2.

Cluster analyses using DL, GL, N6, SD, LAI and PH at flowering generated four morphology clusters in both years (2013: morphology clusters M1 to M4; and 2014: M5 to M8). Clusters M1, M2, M3 and M4 included 22, 26, 16 and 16 plots respectively, while clusters M5, M6, M7, and M8 included 23, 11, 15 and 27 plots respectively. MANOVA confirmed that morphology clusters differed with respect to the variables used to define them. Wilk's lambda value associated with MANOVA was 0.07 (F -ratio = 17.8; with 3 and 76 degrees of freedom; $P < 0.001$) in 2013, while it was 0.06 (F -ratio = 16.8; with 3 and 72 degrees of freedom; $P < 0.001$) in 2014.

In 2013, morphology cluster M1 corresponded to plots with low DL and PH, while cluster M2 was characterized with highest GL values (Figure II. 8, 2 top rows). Plots with the highest values of SD and N6 were grouped in cluster M3, while cluster M4 had the highest values of DL and the lowest N6 values. In 2014, plots with the highest values of DL, N6, and PH were included in clusters M5 and M6, whereas plots with the lowest values of DL and N6 were grouped in clusters M7 and M8. Cluster M6 also grouped plots with the lowest values of SD, LAI and GL, while M7 grouped with the highest values of PH and GL (Figure II. 8, 2 bottom rows).

2.4.4. Associations between disease intensity and morphological traits

Contingency table analyses showed that clusters involving morphological variables at star-bud were not significantly (2013: Chi-square = 3.62; $P = 0.93$; and 2014: Chi-square = 3.24; $P = 0.77$) associated with disease clusters in either year. However, Chi-squares associated with the contingency tables of morphology clusters at flowering and disease clusters were 17.02 and 18.24 in 2013 and 2014, respectively, indicating a significant ($P < 0.05$) association between these two meta-variables (M and D clusters) in both years. Morphological variables at flowering were therefore retained in subsequent analyses.

Correspondence analyses yielded a good representation of disease intensity and morphology clusters along the first two generated axes in both years (Figure II. 9). The fraction of total inertia accounted for by the first axis was very large: 89 and 97 % in 2013 and 2014, respectively. Projections of clusters D2 and D3 (low disease intensity) were close to that of cluster M2 (highest GL and PH values; Figure II. 9a) in 2013. Furthermore, cluster M3 (high SD and N6) was closely projected to D4 (high disease cluster). Clusters M1, M4 and D1 were projected close to the origin of axis 1. In 2014, cluster M7 (high PH and GL) was closely projected to cluster D5 (low disease; Figure II. 9b), while cluster D7 was close to clusters M6 and M8 (low GL and LAI).

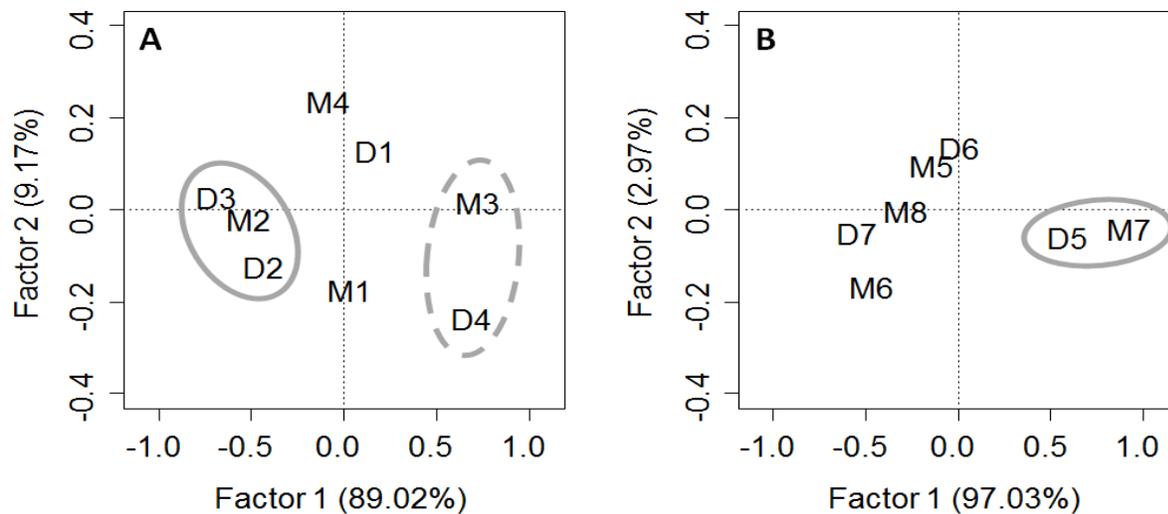


Figure II. 9. Coordinates representation of sunflower Phoma black stem disease intensity and sunflower morphology groups generated by correspondence analysis. A: 2013; B: 2014. Di (D1 to D4; 2013; D5 to D7, 2014) and Mj (M1 to M4, 2013; M5 to M8, 2014) groups are derived from hierarchical cluster analyses. Grey circles present close projections of M groups to high (*dashed*) and low (*continuous*) disease intensity groups. Inertia is represented between parentheses on axes.

Results from cluster analyses and the characterization of disease clusters (Figure II. 6) led to the definition of binary variables representing low and high disease intensity in each year: plots with low disease intensity in year 2013 corresponded to plots belonging to disease clusters D2 and D3, while high disease intensity plots corresponded to plots belonging to disease cluster D4. In the same way, in 2014, low and high disease intensities were attached to disease clusters D5 and D6, respectively. Three of the binary logistic regressions of the effects of morphological variables, or of clusters of morphological traits, on binarized disease intensity yielded significant ($P < 0.05$) results, and were associated to areas under the receiver operating characteristic curve (Harrell 2001) ranging between 0.64 and 0.74 (Table II. 4). The first model indicated that M7 (high PH and GL) significantly predicted low disease intensity (estimate = 2.26; $P = 0.002$). The third model indicated a significant ($P < 0.05$), negative association of SD (2013) and positive associations of PH, LAI and GL (2014) with low disease intensity. Finally, GL had a significant ($P < 0.05$) and negative association to high disease intensity in 2014.

Table II. 4. Binary logistic regressions of the effects of morphological traits and morphology clusters on low and high Phoma black stem disease intensity in sunflower

Model tested ^a	Year	Predicted disease level	AUROC ^b	Predictor ^c	Estimate ^d	SE ^e	P ^f
Model 1	2014	low (cluster D5)	0.734	M7	2.264	0.745	0.002
Model 3	2013	low (clusters D2 and D3)	0.641	SD	-0.254	0.122	0.038
			0.751	PH	0.052	0.015	0.001
	2014	low (cluster D5)	0.712	LAI	1.129	0.388	0.004
			0.736	GL	0.360	0.114	0.002
Model 4	2013	high (cluster D4)	0.698	GL	-0.238	0.116	0.041

^a Models involved morphological groups (Models 1 and 2) or morphological variables (Models 3 and 4) as predictors for low (Models 1 and 3) and high (Models 2 and 4) disease levels. Low disease levels correspond to disease clusters D2 and D3 in 2013, and to disease cluster D5 in 2014. High disease levels correspond to disease cluster D4 in 2013, and to disease cluster D6 in 2014.

^b Area under receiver operating characteristic curve.

^c Acronym meanings are indicated in Table II. 1.

^d Only estimates with corresponding $P < 0.05$ are displayed in the Table.

^e Standard error of the estimate.

^f Probability associated to the estimate.

2.5. Discussion

2.5.1. Experimental framework, protocols of assessment and features of variables measured

The experimental framework applied in this study generated variation in both disease intensity and plant morphological traits, which enabled hypothesis testing. A first factor of variation was the set of sunflower genotypes used (commercial hybrids, experimental hybrids, lines), which were a significant ($P < 0.05$) source of variance both in terms of morphological traits and disease intensity (Table II. 3). A second factor of variation was the experimental year. Both experimental years had different weather (Figure II. 3): in 2013 the spring was cooler and wetter compared to 2014, whereas in 2013 the summer was warmer and drier compared to 2014. These differences were associated with weather conditions which were more favourable to the epidemic in 2014 compared to 2013: rainfall during flowering was higher in 2014 compared to 2013, and rain events are positively associated with ascospores release (Délos et al. 1998; Bordat et al. 2011).

The protocols applied to assess Phoma black stem intensity and sunflower morphology in experimental plots provided a detailed description of the variables characterizing these features,

their variation within and between years, and their associations (Table II. 3; Figures 4, 7). Variation of disease intensity variables within year was similar across variables and years, with a coefficient of variation in the range of 10 %. Disease incidence was similar in both years, whereas disease intensity within plants (e.g., disease severity) was higher in 2014 compared to 2013. Correlation matrixes and principal component analyses (Figure II. 4, 5) highlighted the strong associations between disease variables DISH, RDISH, NUML and INCN in both years, while the other variables had weaker or less robust (i.e., not appearing in both years) associations. Morphological variables varied between years, generally being larger in 2014 compared to 2013. Strong associations between TL and GL, between DLFR and DL, and to a lesser extent between PH and (GL and TL) were detected in both years (Figure II. 5, 7). Other associations between morphological variables were weaker, or were not observed in both years.

The assessment of Phoma black stem intensity at different scales of hierarchy was conducted according to a nested sampling design, whereby disease was first assessed at a high level of hierarchy (population of plants assessed for presence of black stem), on 15 plants per plot. A sample of diseased plants was subsequently selected to conduct more detailed assessments of conditional disease intensity (number of lesions, disease height). Finally, the diseased portion of the stem (aggregated on the lower part of the stem) was again sampled to assess disease severity between nodes. This nested sampling strategy, making use of the concept of conditional severity (Shaw 1995, 1996; McRoberts et al. 2003), provides an efficient way to derive accurate estimates of disease intensity at different levels of hierarchy. Such a sampling design could be applied to other plant diseases.

2.5.2. Relationships between phoma black stem intensity and morphological attributes of sunflower

The results indicate an association between morphological traits measured at flowering and disease intensity measured towards crop maturity. In both years, no disease was observed at flowering: the measurement of morphological traits at flowering took place before epidemic onset. The relationships found here therefore do not reflect the effect of disease on morphology, and can be hypothesized as reflecting the effects of morphology on disease.

The statistical steps taken in this work involved mainly multivariate analyses, and provided a description of the relationships between Phoma black stem intensity and sunflower morphological traits (correspondence analyses), as well as related hypotheses testing (chi-square test on contingency tables, logistic regressions). Similar approaches have been used by

Willoquet et al. (2012) and Srinivasachary et al. (2013), and are powerful tools to assess relationships between meta-variables such as groups of variables that characterize a given feature (e.g., morphological traits and disease intensity). In view of the large differences in disease intensity between years, and of comparatively smaller differences observed with respect to morphological traits, it was decided to perform the analyses separately for each year, and to identify relationships between morphological traits and disease intensity patterns which could be detected in both years. Such relationships may be considered as robust, because they could be observed under contrasted environmental (weather) and disease conditions.

The various analyses made on Phoma black stem intensity and morphological traits of sunflower measured over two field experiments indicate that groups defined according to morphological attributes of sunflower were associated with groups defined according to disease intensity. To our knowledge, such a relationship has not previously been documented in the case of sunflower Phoma black stem. Considering plants according to multivariate attributes can lead to the notion of plant ideotype or plant type (Donald 1968), which refers to a holistic point of view, as compared to considering one specific morphological attribute, or several attributes separately. Such a notion corresponds to operational entry points for plant breeding, and was critical in producing high yielding varieties that were deployed during the green revolution (Hedden 2003). Plant [morphological] ideotypes associated with reduced disease have been considered as a potential entry point for disease management (e.g., Ando et al. 2007; Poland et al. 2009; Andrivon et al. 2013). On the other hand, characterizing disease intensity according to several disease variables may represent a first step towards considering epidemiological processes occurring at different spatial or temporal scales. Grouping according to different disease variables therefore enables a holistic characterization of diseases epidemics, in the same way as grouping according to multiple morphological traits. Association between multiple morphological and disease attributes has been shown in other pathosystems. Lower disease intensity was associated with taller plants, lower number of tillers per plant, and lower number of leaves per tiller in rice sheath blight (Willoquet et al. 2012; Srinivasachary et al. 2013). Among traditional rice varieties, varieties with taller plants and larger leaf area were associated with lower leaf blast lesion intensity (Schlösser et al. 2000). In the case of *Fusarium* head blight in barley, taller plants with more seeds per inflorescence were associated with lower disease severity (Zhu et al. 1999).

Contingency tables and correspondence analyses indicated that in both years low Phoma black stem was associated with sunflower morphological groups characterized by a large number

of green leaves and tall plants. These relationships were further detected in logistic regressions in both years with respect to the number of green leaves, and with plant height in 2014. Taller plants, for a given LAI, may be associated with a microclimatic environment where wetness duration is decreased, therefore leading to decreased infection efficiency, and to reduced epidemic development, as shown in other pathosystems where wetness is required for infection (Scott et al. 1985). However, trade-off with other agronomic traits (harvest index, lodging tolerance) needs to be accounted for when considering plant height as a target in breeding programmes. A larger number of green leaves may also correspond to inoculum dilution, when considering ascospores deposited on the top of the canopy and flowing with rainfall from node to node towards the base of the plant. The greater the number of green leaves, the more spore dilution occurs in node troughs, and the lower spore concentration at the lowest nodes, where infection occurs.

2.5.3. Implications for morphological and disease assessment and for breeding

The associations found between disease intensity variables within and across years can be used in the future to optimize assessments of Phoma black stem. For example, the number of lesions per plant was highly correlated to disease height in both years ($r = 0.78$; Figure III. 4). Disease height could therefore be assessed in studies where time for data collection is a limiting factor: disease height takes much less time to measure compared with the number of lesions. Sunflower morphological assessments indicate that measurements made at flowering were associated with Phoma black stem intensity, whereas measurements made at star bud were not. Morphological assessments may therefore be taken in priority at flowering in experiments addressing morphology and Phoma black stem intensity.

Low disease intensity was associated with a morphological ideotype with a large number of green leaves and tall stature. To our knowledge such a relationship has not previously been identified. A sunflower morphological plant ideotype with larger number of leaves and taller stature may represent an operational target in sunflower breeding.

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3. Predicting quantitative host plant resistance against phoma black stem in sunflower

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3.1. Abstract

Phoma black stem is an important disease in sunflower, against which no specific management method is currently deployed in France. Relevant phenotyping methods for quantitative resistance are critical for integration of this trait into breeding programmes. Components of resistance associated with physiological resistance, and morphological traits associated with disease escape were measured on 21 sunflower genotypes under growth chamber (on seedlings), greenhouse (on adult plants), and field conditions, together with disease intensity in the field. Potential predictors were first selected for sensitivity and robustness from mixed model anovas. Analyses involving ranking tests and logistic regressions were then performed to identify predictors for field resistance. The identification of predictors for resistance involved analyses conducted in two broad steps: process-oriented experiments, and epidemic-oriented experiments. This stepwise approach departs from many studies aimed at identifying predictors for field resistance, which rely mainly on the computation of correlation coefficients between predictors and measured field disease variables. Predictors for quantitative resistance were identified: (i) lesion length and lesion expansion on stems of plants before flowering stage, and (ii) lesion length on first leaf petioles of seedlings. A high number of leaves and tall plants were associated with disease escape. Control genotypes for susceptibility and quantitative resistance were identified, and implications for breeding and improvements were derived from this work.

Keywords: components of resistance, *Helianthus annuus*, *Leptosphaeria lindquistii*, phenotyping method, *Phoma macdonaldii*, plant morphology

3.2. Introduction

Phoma black stem, caused by the fungus *Leptosphaeria lindquistii* (synonym *Phoma macdonaldii*), has become an important disease of sunflower in France (Debaeke & Pérès, 2003). The most visible symptoms are black lesions on the stem, localized at the leaf nodes, and expanding around and along the stem. These lesions originate from infections that take place at the trough located at the base of leaf petioles, or along the petioles. Epidemic onset generally occurs after flowering, with lesions on the stem progressing from the bottom to upper leaf nodes. Lesions on the petiole or on the stem accelerate leaf senescence (Quiroz et al., 2014). Lesions on the leaf veins can be observed at the end of the crop cycle when epidemics are severe. Lesions can also develop on collars, and cause the senescence and death of the plant a few weeks before normal maturity. This is referred to as premature death or premature ripening, and can be associated with high yield losses (Donald et al., 1987; Debaeke & Pérès, 2003; Seassau et al., 2010). The pathogen overwinters as pseudothecia, pycnidia, and mycelium produced on infected sunflower stubble residues (Gulya et al., 1997). In France, lesions observed on stems appear to be mainly due to infections from ascospores released from infected sunflower residues (Seassau et al., 2010; Bordat et al., 2011). Sources of partial resistance to phoma black stem have been identified (e.g. Roustaee et al., 2000), and several quantitative trait loci (QTLs) for resistance to phoma have been characterized (Rachid Al-Chaarani et al., 2002; Bert et al., 2004; Darvishzadeh et al., 2007a) using phenotyping methods involving inoculation under controlled conditions on 10-day-old seedlings. Phenotypic variation explained by individual QTLs was moderate, ranging between 6 and 20%. No varieties resistant to phoma black stem have been deployed until now, and no specific disease management tools are currently deployed to control the disease. Furthermore, phoma black stem resistance is not currently included as a criterion for variety registration in France.

Relevant phenotyping methods are critical in breeding programmes aiming to incorporate quantitative resistance. The relevance of phenotyping methods depends on the generation of predictors that represent the expression of genotype resistance in field conditions (Zadoks, 1972; Mundt, 2014): reducing epidemics in the field is the true practical target associated with breeding for host plant resistance. Phenotyping methods for quantitative resistance may be developed by considering two main types of resistance: physiological resistance and disease escape (Parlevliet, 1979; Poland et al., 2009; Srinivasachary et al., 2011, 2013). Physiological resistance corresponds to plant processes, constitutive or induced, that are associated with a decrease in efficiency of one or several of the infection or reproductive stages of the pathogen (Russell, 1978;

Poland et al., 2009; Srinivasachary et al., 2011). Physiological resistance can be achieved by mobilizing the concept of components of resistance (Parlevliet & Zadoks, 1977; Parlevliet, 1979). These components of resistance affect processes involved in the disease cycle when physical host–pathogen interactions take place (Butt & Royle, 1980). In the case of aerial fungi causing polycyclic epidemics, components of resistance that affect infection efficiency, latency period, and sporulation intensity are classically considered.

Measurement of the reduction in efficiency of these processes (i.e. measurement of components of resistance), together with the measurement of the reduction in epidemic severity for a range of genotypes, allows the identification of predictors of field resistance. This has been achieved for several pathosystems, e.g. in barley leaf rust (Parlevliet & van Ommeren, 1975), groundnut rust (Savary et al., 1988; Savary & Zadoks, 1989) and leaf rust on durum wheat (Herrera-Foessel et al., 2007). Such an approach allows relevant components of resistance to be targeted in breeding programmes, i.e. components of resistance that are the best predictors of, or that are highly correlated with, disease epidemics in the field.

Disease escape, or disease avoidance, can also reduce epidemics, but is unrelated to molecular and biochemical plant–pathogen interactions (Parlevliet, 1979; Srinivasachary et al., 2011). Disease escape can be associated with morphological traits that hamper processes involved in the disease cycle, therefore reducing epidemic severity (Poland et al., 2009; Srinivasachary et al., 2013). These morphological traits can in turn be genetically driven (Poland et al., 2009; Willocquet et al., 2012; Andrivon et al., 2013). The effects of disease escape traits on disease epidemics have been quantified in some pathosystems, including septoria tritici blotch in wheat (Arraiano et al., 2009) and rice sheath blight (Willocquet et al., 2012; Srinivasachary et al., 2013).

Improving resistance against phoma black stem in sunflower represents a relevant way to manage the disease: varietal resistance is efficient, environment-friendly, and of no cost for the growers. Qualitative, complete resistance is very often prone to the boom and bust cycle, which renders major resistance genes short-lived (e.g. McDonald & Linde, 2002). Partial resistance, by contrast, only reduces epidemic severity, but is more durable (Mundt, 2014). Developing methods that enable improved partial resistance to phoma black stem may therefore improve the sustainability of sunflower farming. Such methods should, as stated above, involve predictors for quantitative resistance that are associated with field resistance, i.e. resistance expressed at its operational deployment scale.

The goal of this work was to identify predictors of phoma black stem resistance in the field, using a set of sunflower genotypes for which predictors for resistance were measured, together with disease intensity in the field, in a range of complementary experiments. The specific objectives were to (i) measure components of physiological resistance under different experimental conditions and plant ages, and (ii) identify morphological traits associated with reduced disease in field experiments, in order to (iii) identify predictors for field resistance.

3.3. Materials and Methods

3.3.1. Overview of the experiments

Experiments were conducted at different scales (leaf petiole on seedlings, stem node on adult plants, and field plot) with the same set of sunflower genotypes in order to identify predictors for field resistance. Components of physiological resistance to phoma black stem disease were measured under growth chamber conditions on inoculated seedlings, and in a greenhouse on inoculated adult plants, yielding two initial groups of potential predictors for field resistance. Morphological characteristics at flowering were measured on uninoculated plants (naturally infected) in field experiments, representing a third group of potential predictors for field resistance. Disease intensity was also assessed in the same field experiments, in order to estimate field resistance levels for the genotypes tested. Sunflower genotype was the only factor involved in the experiments, and thus predictors for field disease intensity are considered as predictors for quantitative resistance against the disease.

3.3.2. Sunflower genotypes

Plant material consisted of 21 sunflower genotypes (Table III. 1). The inbred line FU (INRA core collection ID: SF056; https://www.heliagene.org/Web/public/core/Core_collections_list.html), an unbranched maintainer genotype, and the inbred line PAZ2 (INRA core collection ID: SF306), a branched restorer genotype, were used in this study. PAZ2 had shown lower phoma disease levels than FU in a previous study performed under controlled conditions on sunflower seedlings, using inoculation of cotyledons (Bert et al., 2004). Furthermore, PAZ2 and FU were classified as resistant and susceptible, respectively, to premature death of sunflower (Bordat et al., 2012).

Table III. 1. List and description of sunflower genotypes used in the experiments.

Codes^a	Resistance^b	Male lines^c	Genetic material type	
550	R	(Tub-1709-1)-1-6A	Hybrids: FU x line from INRA core collection	
558	R	SF306		
574	R	97B7		
586	S	SF336		
592	S	SF247		
598	S	SF332		
610	R	FP109	Hybrids: FU x RIL ^d	
616	R	FP111		
628	R	FP146		
634	R	FP176		
640	S	FP043		
652	S	FP056		
658	S	FP072		
664	S	FP102		
SF056 ^e	S			Lines
SF306 ^f	R			
FP055	S			
FP066	R			
Kerbel			Commercial hybrids	
ES Paulina				
NK Ferti				

^a Genotype codes used in this study.

^b Resistance level to Phoma black stem (SF056 and SF306) and premature death (all genotypes) determined for parental male lines (hybrids), and for lines in previous studies (Bert et al, 2004; Bordat et al, 2012); R: resistant; S: susceptible.

^c Male parental lines used to produce hybrids.

^d RIL: Recombinant inbred lines, see text for details.

^e other name: FU.

^f other name: PAZ2.

Six hybrids were produced from crosses between the susceptible inbred line FU as female tester, and six lines from the INRA *Helianthus annuus* core collection as males. One male parental line was PAZ2, two lines [(Tub-1709-1)-1-6A, and 97B7] were characterized as resistant to premature death from previous field experiments, and the three other lines (SF336, SF247, and SF332) as susceptible (Bordat et al., 2012). Eight additional hybrids were produced from crosses between FU and recombinant inbred lines (RILs; F7-F10) obtained by single-seed descent from a crossing between FU and PAZ2. Among the RILs, four (FP043, FP056, FP072, and FP102) were characterized as having two alleles for susceptibility to premature death (on two different QTLs), and four RILs as having two alleles for quantitative resistance to premature death (FP109, FP111, FP146, and FP176; Bordat et al., 2012). Two RILs characterized as having two alleles for

susceptibility (FP055), and two alleles for quantitative resistance (FP066), were included in the study. Three commercial hybrids currently grown in France, Kerbel, ES Paulina and NK Ferti, were used in order to incorporate genetic material commonly grown by farmers into the study.

In summary, 21 genotypes were tested and they included: (i) parental lines with varying levels of resistance to *L. lindquistii*, (ii) hybrids produced from crossings between a susceptible line (FU) and lines or RILs with varying levels of resistance, and (iii) commercial hybrids (Table III. 1). This study involved hybrids because they represent the main genetic make-up currently used in commercial sunflower fields better than inbred lines. The 21 above genotypes were used in both field experiments. However, due to limitations in seed supply, only 19 genotypes were used in greenhouse experiments (genotypes 558 and 664 could not be included), and 18 genotypes were used in growth chamber experiments (genotypes 558, 664 and FP066 could not be included).

3.3.3. Growth chamber experiments on seedlings

Growth chamber experiments were conducted in order to measure potential predictors for quantitative resistance on seedlings in 18 genotypes described above. Two independent experiments were conducted in a growth chamber, both according to a randomized complete block design, with four blocks within which the 18 genotypes were randomly arranged. Each experimental unit consisted of a set of eight seedlings of a given genotype, grown in eight adjacent (2×4) plastic pots of 0.1 L (soil composition: blond sphagnum peat and clay; Proveen® A16894). Groups of three such experimental units were placed in transparent plastic boxes. Plants were grown in a growth chamber under a photoperiod of 14 h (25 °C; light intensity of 200 $\mu\text{mol m}^{-2} \text{s}^{-1}$) and 10 h dark period (20 °C).

An aggressive strain of *L. lindquistii* (MPH2), isolated from infected sunflower stem residues collected near Auzeville in 2006 (Seassau et al., 2010), was used for inoculations. A suspension of pycnidiospores (105 spores mL^{-1} ; 0.01% Tween 20) was prepared from a 2-week-old colony grown at 25 °C under a 12 h photoperiod (Roustae et al., 2000). The inoculation was performed on 20-day-old seedlings at the petiole bases of the first pair of leaves (two petioles per plant, i.e. 16 inoculation sites per experimental unit) by applying 20 μL of the pycnidiospore suspension (i.e. 2000 pycnidiospores/site of inoculation) at the insertion point of the petiole on the seedling stem. Seedlings were sprayed with distilled water after inoculation and the plastic boxes were then hermetically covered with a transparent plastic lid until the first assessment to provide a humid environment.

The number of diseased petioles was counted on all seedlings 8 days after inoculation (DAI). A petiole was considered as a site (*sensu* Zadoks, 1971), and therefore a diseased petiole was considered as a lesion. Petiole lesion length (PLL) was measured on all diseased petioles at 4 and 8 DAI, and the mean petiole lesion length per experimental unit was calculated. Infection efficiency (IE) was calculated for each experimental unit as: $IE = \text{total number of lesions} / \text{total number of spores deposited}$ (Table III. 2; Schein, 1964; Zadoks & Schein, 1979).

Table III. 2. List of measured or computed variables used as predictors Phoma black stem disease resistance in sunflower.

Experiment type	Predictor	Variable acronym	Unit
Growth chamber	Infection efficiency	IE	Nbpetioles.Nbspores ⁻¹
	Petiole lesion length	PLL	mm
Greenhouse	Stem lesion length at node 1	SLL1	mm
	Stem lesion length at node 2	SLL2	mm
	Stem lesion length at node 3	SLL3	mm
	Stem lesion expansion at node 1	SLE1	mm ² .day ⁻¹
	Stem lesion expansion at node 2	SLE2	mm ² .day ⁻¹
	Stem lesion expansion at node 3	SLE3	mm ² .day ⁻¹
Field	Number of dead leaves per plant	DL	Nb leaves
	Number of green leaves per plant	GL	Nb leaves
	Total number of leaves per plant	TL	Nb leaves
	Percentage of dead leaves per plant	DLFR	%
	Plant height	PH	cm
	Stem diameter	SD	mm
	Width of petiole trough at 6 th leaf node from plant base	N6	mm
	Leaf area index	LAI	-

3.3.4. Greenhouse experiments on adult plants

Experiments were conducted in a greenhouse in order to measure the components of resistance on sunflower adult plants in 19 genotypes described above. Two independent experiments were conducted, both according to a randomized complete block design with two blocks. Sowing was carried out on 9 and 30 January 2014 in the first and second experiment, respectively. Two seeds of the same genotype were sown per pot, into 5.3 L plastic pots filled with organic soil (soil composition: blond sphagnum peat and clay; Proveen® A16894), in which 0.6 g of nitrogen was incorporated at sowing. A 12 h photoperiod was ensured by cool-white fluorescent lamps (providing a light intensity of 60 $\mu\text{mol m}^{-2} \text{s}^{-1}$). The minimum and maximum temperatures registered during the experiments were 18 and 37 °C, respectively.

Isolate MPH2 of *L. lindquistii* was used for these experiments. Inoculum consisted of 5 mm-diameter mycelial plugs of a 2-week-old colony grown on potato dextrose agar medium at 25 °C under darkness. The inoculation method was adapted from Seassau et al. (2010) for collar infection. Inoculation was performed at 47 days after sowing (DAS) – at the development stage ‘immature bud elongated about 1 cm above the nearest leaf’ – by applying a mycelial plug against the stem of each plant, just above the first, second and third petiole insertion points (node). In order to maintain wetness, each plug was then covered by a layer of wet cotton, surrounded by aluminium foil. Cotton and aluminium foil were removed 7 DAI.

Disease assessments were made at 7, 10, 13 and 16 DAI on each inoculated node. Phoma black stem lesion length (SLL1, 2 or 3) and width (SLW1, 2 or 3) were measured using a flexible ruler. Lesion expansion (SLE1, 2 or 3) was computed as the growth in lesion size between 7 and 16 DAI, where lesion size is $SLL \times SLW$.

3.3.5. Field experiments

Two field experiments were conducted (one in 2013 and one in 2014) in order to measure morphological traits and disease intensity in the 21 genotypes described above. Both experiments were conducted under natural conditions of infection (no inoculation was performed). Both experiments were established according to a randomized complete block design with four blocks. These experiments were conducted at the Auzeville INRA Experimental Unit, near Toulouse, France.

The experimental plots were sown at a density of 7 seeds m^{-2} on 6 May 2013 and 15 April 2014. Plants were grown under rainfed conditions in both years. Nitrogen was applied, according to local recommendation, once in both years at 50 DAS, at a rate of 35 and 70 $kg\ ha^{-1}$ in 2013 and 2014, respectively. Experimental units (one genotype in a block) were plots with an inter-row space of 0.5 m and six rows with a length of 6 m. Due to the limited amount of seeds available for several genotypes tested, the two border rows of each plot were sown with a commercial hybrid in both years (NK Kondi).

Morphological variables were assessed at flowering on 30 July 2013 and 9 July 2014. Three representative plants per plot were selected at random and were carefully assessed for morphological characteristics. Variables collected for morphological characteristics of each genotype were: plant height (PH), stem diameter at the base of the plant (SD), width of the petiole trough at sixth node (N6), number of green leaves (GL), and number of dead leaves (DL).

A leaf was considered dead when more than 50% of its area was discoloured. Where dead leaves had detached from the stem (defoliated), DL could be derived by observing the petiole insertion point at stem nodes. The total number of leaves ($TL = DL + GL$) and the percentage of dead leaves ($DLFR = 100 \times (DL/TL)$) were calculated from GL and DL. TL corresponds also to the total number of nodes on the stem. Leaf area index (LAI) was determined using the Pouzet & Bugat (1985) method based on the measurement of length and width on the largest and lowest leaves of the plants, and on the number of leaves per plant (Table III. 2).

Disease assessments were recorded once in 2013, at 122 DAS (5 September), and twice in 2014, at 112 DAS (5 August) and 119 DAS (12 August). At each assessment date, 15 plants per plot (i.e. per genotype in each block) were randomly selected and categorized according to four classes: (i) no disease, (ii) symptoms on the stem only, (iii) symptoms on plant collar only, and (iv) symptoms on both stem and collar. Phoma black stem incidence (INCS) and collar disease incidence (INCC) were calculated from these assessments (Table III. 3). Three diseased plants per plot were then randomly selected for further disease assessments. The number of lesions on the stem was counted (CNUML). Disease severity on the stem (CSEV) was determined from (i) the distance between the base of the stem and the highest lesion along the stem, (ii) plant height, and (iii) disease severity estimated at the lowest, highest and intermediate diseased internode, according to a 6-grade diagram scale, with disease severities of 1, 5, 10, 25, 50 and 70% (Schwanck et al., 2016). CNUML and CSEV correspond to conditional disease measurements, i.e. conditional on a plant being diseased (McRoberts et al., 2003; Willocquet & Savary, 2004). CNUML and CSEV were each multiplied by INCS to give the (absolute) number of lesions on stem per plant (NUML) and severity (SEV), respectively. In 2014, INCS showed high variation between plots in assessment 1, whereas INCS was close to 1 at the second assessment, and, therefore, had a much smaller variation. Furthermore, conditional disease intensities (CNUML and CSEV) were very small at the first assessment, and displayed less variation compared to assessment 2. Therefore, INCS corresponding to the first assessment was retained for data analysis, whereas NUML and SEV corresponding to the second assessment were retained for the analyses.

Table III. 3. List of Phoma black stem variables measured in the field.

Disease variable	Variable acronym	Unit
Black stem disease incidence (fraction of plants with at least one black stem lesion)	INCS	-
Collar disease incidence (fraction of plants with a collar lesion)	INCC	-
Disease severity on stem	SEV	% ^a
Number of lesions per plant (number of diseased nodes per plant)	NUML	Nlesions.plant ⁻¹

^a Disease severity estimated at the lowest, highest and intermediate diseased internode, according to a 6-grade diagram scale, with disease severities of 1, 5, 10, 25, 50 and 70% (Schwanck et al., 2016).

3.3.6. Data analysis

The identification of predictors for quantitative resistance to phoma black stem in sunflower involved three main steps. First, mixed model analyses of variance involving the effects of genotype, experiment, and their interaction, were performed on predictors and on disease intensity variables, in order to (i) preselect predictors of disease resistance according to sensitivity and robustness, and (ii) identify field disease intensity variables to use for further analyses. Secondly, rank tests (Kendall's correlation coefficient and Kendall's coefficient of concordance) were applied to assess associations among predictors, and between predictors and field disease intensity variables. Thirdly, binary logistic regressions were used to identify predictors that could predict the belonging of a genotype to a group characterized by low disease intensity in the field, i.e. by partial resistance. All statistical analyses were conducted using R (R Core Team, 2014).

Selection of predictors measured in the different experiments involved two criteria: (i) predictors for which values varied according to genotype (sensitivity of the predictor), and (ii) predictors for which the ranking between genotypes would not vary over independent experiments (robustness of the predictor). These criteria were assessed by conducting mixed model analyses of variance considering the effects of genotype, experiment, and their interaction. Genotype and experiment were considered as fixed effects, while block was considered as a random effect. Predictor variables were selected when (i) a significant ($P < 0.05$) genotype effect was found, and (ii) a nonsignificant ($P > 0.05$) effect of the interaction between genotype and experiment was observed, to fulfil the first and second criteria, respectively. When variables were measured at different times, the assessment time corresponding to the lowest P value associated with genotype effect was retained, representing the assessment with highest genotype effect. Means of selected predictors per genotype were then computed over blocks and experiments for

further analyses. The mixed model analyses of variance were performed with the *lme* function from the *nlme* R package. The adequacy of model assumptions was assessed by graphical analysis of residuals.

Mixed model analyses of variance were conducted for disease intensity variables measured in field experiments, using the same statistical procedures as above. These analyses considered experiment (i.e. year) and genotype as fixed effects, and blocks as random effects. Genotype means over blocks and experiments were computed when the genotype \times experiment interaction was not significant ($P > 0.05$). Means for each genotype were computed individually for each experimental year when a significant ($P < 0.05$) genotype \times experiment interaction was found. In that case, numerical indices were added before variable acronyms to identify the first and second experimental years (2013 = 1 and 2014 = 2; for example: 1SEV and 2SEV).

Kendall's rank correlation (τ) was used to assess pairwise correlations (i) among the different predictors measured, within and between experiment types, and (ii) between predictors and field disease variables, in order to assess the predictive power of the predictors. Kendall's τ was calculated using the *cor* function and coefficient significances in the *cor.test* function (method = Kendall).

Kendall's coefficient of concordance (W) was calculated to assess the overall rank concordance of predictors and field disease variables (Legendre, 2005). Kendall's W was calculated to assess (i) the rank order among all field disease variables, and (ii) the rank order among predictors (according to experiment type) and field disease intensity (altogether, or per type of variable). Specifically for this analysis, predictors showing negative Kendall's τ correlation were multiplied by -1 because perfect inverted ranks would correspond to a complete rank disagreement ($W = 0$; Legendre, 2005). Kendall's coefficient of concordance (W) was calculated using the *irr* package (Gammer et al., 2012).

Binary logistic regressions (Steinberg & Colla, 2009) were performed to test the prediction power of predictors for field disease intensity variables. Binary variables were defined as above (0, susceptible) and below (1, resistant) the median of the genotypes. Binary logistic regressions were performed according to the general model:

$$\ln[P(r)/(1 - P(r))] = a + bx$$

where $P(r)$ is the probability of a genotype to belong to the group with a disease intensity below the median (1, resistant); x is the predictor to be tested; a and b are model parameters. The effect of each predictor was tested for each field disease binary variable. The binary logistic regressions were performed using the *glm* function in R. Conditional density plots, which display the

probability of each binary data occurring at a given level of each predictor variable, were used to illustrate the predictive power of selected predictors. Conditional density plots were built from the *cdplot* function of R software, and scatterplots were built with the *ggplot2* package.

3.4. Results

3.4.1. Overview of the data set and variable selection

For the experiments on seedlings in the growth chamber, the overall mean of infection efficiency (IE) was 3.19×10^{-4} , and petiole lesion length increased from 3.9 mm at 4 DAI to 5.7 mm at 8 DAI (Table III. 4). All three variables derived from these experiments were significantly ($P < 0.05$) affected by genotype, whereas the interaction between experiment and genotype was not significant. The *P* value associated with the effect of genotype was lower for petiole lesion length (PLL) measured at 8 DAI than PLL measured at 4 DAI, indicating a higher level of significance. Therefore, PLL at 8 DAI was used for further analyses, together with IE, which represents processes different from those represented by PLL.

In the greenhouse experiments on adult plants, stem lesion length (SLL) increased with plant node height (Table III. 4). SLL increased from 7 to 16 DAI, but the rate at which the length extended decreased with time. Lesion expansion (SLE) was greater at node 3 than node 1, but there was a larger difference between node 1 and node 2 than between node 2 and node 3. All variables derived from greenhouse experiments were significantly ($P < 0.05$) affected by genotype, except stem lesion length at nodes 1 and 2 measured at 7 DAI (Table III. 4). The effect of interaction between genotype and experiment was not significant ($P > 0.10$). When comparing SLL measured on the three nodes at different assessment dates, *P* values showed the effect of genotype to be most significant at 13 DAI. Measurements of SLL made at this time were therefore retained for further analyses.

Morphological variables measured at flowering in field experiments indicated an average total number of leaves of 23, among which 4 were dead (DL), and an average plant height of 110 cm (Table III. 4). Except DL, all morphological variables were significantly ($P < 0.05$) affected by genotypes. Interaction between genotypes and experiments was not significant ($P > 0.05$), except for the size of the petiole at the sixth node (N6). DL and N6 were therefore excluded from further analyses.

Table III. 4. Results from mixed model analyses of variance of the effects of genotypes and experiments on predictors of Phoma black stem disease resistance in sunflower.

Experiment	Predictor ^a	Assessment time (DAI) ^{bc}	Mean ^d	P-value ^e		
				Gen	Exp ^f	GenxExp
Growth chamber	IE	8	3.19×10 ⁻⁴	<0.001	0.130	0.357
	PLL	4	3.88	0.023	0.238	0.695
	PLL	4	5.70	0.018	0.003	0.567
Greenhouse	SLL1	7	34.78	0.058	<0.001	0.322
	SLL1	10	44.04	0.020	<0.001	0.739
	SLL1	13	46.83	<0.001	<0.001	0.764
	SLL1	16	49.41	<0.001	<0.001	0.817
	SLL2	7	44.17	0.142	<0.001	0.673
	SLL2	10	52.52	0.001	<0.001	0.593
	SLL2	13	56.04	<0.001	<0.001	0.253
	SLL2	16	56.88	0.001	<0.001	0.803
	SLL3	7	51.29	<0.001	<0.001	0.771
	SLL3	10	61.66	0.001	<0.001	0.837
	SLL3	13	64.69	<0.001	<0.001	0.649
	SLL3	16	65.86	0.001	<0.001	0.787
	SLE1	-	74.10	0.003	<0.001	0.369
	SLE2	-	84.17	0.001	<0.001	0.234
	SLE3	-	88.83	0.017	<0.001	0.150
Field	DL	-	4.33	0.085	<0.001	0.170
	GL	-	18.67	<0.001	<0.001	0.309
	TL	-	23.00	<0.001	<0.001	0.110
	DLFR	-	18.82	0.031	<0.001	0.050
	PH	-	109.71	<0.001	0.092	0.772
	SD	-	18.03	<0.001	<0.001	0.506
	N6	-	8.77	<0.001	0.918	0.003
	LAI	-	1.95	<0.001	<0.001	0.775

^a IE: infection efficiency; PLL: petiole lesion length; SLL1–3: stem lesion length at nodes 1–3; SLE1–3: stem lesion expansion at nodes 1–3; DL: number of dead leaves per plant; GL: number of green leaves per plant; TL: total number of leaves per plant; DLFR: percentage of dead leaves per plant; PH: plant height; SD: stem diameter; N6: width of petiole trough at 6th leaf node from plant base; LAI: leaf area index.

^b DAI: days after inoculation.

^c Variables measured in the field were assessed at flowering.

^d Means of predictors over genotypes, replications and experiments

^e Associated *P*-values from mixed model analyses of variance using block as random effect and genotype (Gen.) and experiment (Exp.) as fixed effects.

^f Experiment refers to two independent experiments conducted in growth chamber and greenhouse environments and to field experimental years of 2013 and 2014.

Means over both field experiments of collar incidence (INCC), phoma black stem incidence (INCS) and severity (SEV), and number of lesions per plant (NUML) were 0.18, 0.57, 3.11 and 4.16%, respectively (Table III. 5). All disease variables were significantly ($P < 0.001$) affected by genotypes. Neither incidence variable was significantly ($P < 0.05$) affected by the interaction between genotype and experiment, whereas the two other disease variables, NUML and SEV, were significantly affected by this interaction. INCS and INCC means over blocks and years were therefore used for correlation analyses with predictors, whereas SEV and NUML means over blocks were considered separately for each year (2013: 1SEV and 1NUML; 2014: 2SEV and 2NUML) in correlation analyses.

Table III. 5. Results from mixed model analyses of variance of the effects of sunflower genotypes and experiments conducted in 2013 and 2014 years on Phoma black stem variables.

Disease variable ^a	Mean ^b	P-value ^c		
		Gen	Exp	Gen*Exp
INCC	0.18	<0.001	0.463	0.200
INCS	0.57	<0.001	0.893	0.192
SEV	3.11	<0.001	<0.001	<0.001
NUML	4.16	<0.001	<0.001	0.032

^a INCC: collar disease incidence; INCS: black stem disease incidence; SEV: disease severity on stem; NUML: number of lesions per plant.

^b Means of predictors over genotypes, replications and experiments.

^c Associated P -values for mixed model analyses of variance using block as random effect and genotype (Gen) and experiment (Exp) as fixed effects.

3.4.2. Pairwise correlations among predictors

Most Kendall's rank correlations (τ) between variables derived from a given experiment type (growth chamber, greenhouse or field) were significant ($P < 0.05$; Table III. 6). IE and PLL, measured on seedlings in growth chamber experiments, were significantly correlated ($\tau = 0.55$). Among the 15 pairwise correlations performed on predictors measured in greenhouse experiments, 11 were significant ($P < 0.05$) and positive, with τ values ranging between 0.34 and 0.63 (between SLL1 and SLL2). Among the 15 pairwise correlations performed on field morphological variables, 13 were significant ($P < 0.05$), among which 10 were positive (mean $\tau = 0.60$) and 3 were negative (mean $\tau = -0.41$). The percentage of dead leaves (DLFR) was not significantly ($P > 0.05$) correlated with the total number of leaves (TL) or with the stem diameter (SD). Only three pairwise correlations between predictors from different types of experiments were significant: PLL and SLL3, SLE2 and GL, and SLE2 and TL ($P < 0.05$; Table III. 6).

Table III. 6. Kendall's tau (τ) rank correlation coefficient matrix among predictors for Phoma black stem resistance in sunflower.^a

Growth chamber	Greenhouse									Field					
	IE	PLL	SLL1	SLL2	SLL3	SLE1	SLE2	SLE3	GL	TL	DLFR	PH	SD	LAI	
IE	1														
PLL	0.55	1													
SLL1	0.19	0.31	1												
SLL2	0.04	0.18	0.63	1											
SLL3	0.11	0.45	0.35	0.29	1										
SLE1	0.32	0.33	0.54	0.43	0.34	1									
SLE2	0.09	0.12	0.45	0.48	0.31	0.48	1								
SLE3	0.08	0.28	0.22	0.19	0.60	0.35	0.45	1							
GL	0.00	-0.19	-0.03	-0.26	-0.22	-0.10	-0.39	-0.28	1						
TL	-0.01	-0.19	-0.05	-0.28	-0.22	-0.15	-0.46	-0.31	0.92	1					
DLFR	-0.04	0.20	-0.09	-0.07	0.13	-0.15	-0.02	0.11	-0.41	-0.33	1				
PH	-0.05	-0.11	0.05	-0.07	-0.04	0.03	-0.28	-0.18	0.63	0.66	-0.44	1			
SD	0.01	-0.08	0.13	-0.02	0.00	0.06	0.01	-0.07	0.40	0.37	-0.28	0.42	1		
LAI	0.05	-0.03	0.05	-0.07	-0.01	0.03	-0.25	-0.12	0.66	0.63	-0.39	0.71	0.58	1	

^aIE: infection efficiency; PLL: petiole lesion length; SLL1–3: stem lesion length at nodes 1–3; SLE1–3: stem lesion expansion at nodes 1–3; GL: number of green leaves per plant; TL: total number of leaves per plant; DLFR: percentage of dead leaves per plant; PH: plant height; SD: stem diameter; LAI: leaf area index. Colours are set according to correlation coefficients, varying from -1 (red), 0 (white) to 1 (blue). Black lines in the matrix highlight pairs within each type of experiments. Numbers associated to P values < 0.05 are in bold.

Note that rank correlation coefficients ≥ 0.45 or ≤ -0.45 correspond to $P < 0.001$.

3.4.3. Prediction of field disease intensity

Four correlation patterns were identified between predictors and field disease intensity. First, INCS was positively and significantly ($P < 0.05$) correlated to all predictors derived from growth chamber and greenhouse experiments, except IE (Table III. 7). Significant τ values ranged from 0.34 to 0.41, for SLL3 and SLL1, respectively. Secondly, the stem lesion length at second node (SLL2) measured in greenhouse experiments was significantly ($P < 0.05$) associated with SEV measured in both field experiments (1SEV: $\tau = 0.38$; 2SEV: $\tau = 0.34$) and to NUML in 2013 (1NUML: $\tau = 0.42$). Thirdly, several morphological traits measured at flowering in field experiments were significantly ($P < 0.001$) and negatively associated with SEV measured in 2014 (GL, TL, PH, and LAI; Table III. 7), with τ values ranging from -0.44 to -0.56 . Fourthly, Kendall's τ between predictors and INCC were not significant (with associated P values ranging from 0.12 to 0.90), and ranged between -0.14 (DLFR) and 0.25 (PH; data not shown).

Table III. 7. Kendall's tau (τ) rank correlation coefficients between predictors for sunflower Phoma black stem resistance and field disease variables.

Experiment	Predictor ^a	Disease variable ^b				
		INCS ^c	1SEV ^d	1NUML ^e	2SEV ^d	2NUML ^e
Growth chamber	IE	0.26	-0.06	-0.13	0.04	-0.16
	PLL	0.35	0.08	-0.03	0.14	-0.19
Greenhouse	SLL1	0.41	0.25	0.29	0.07	0.15
	SLL2	0.37	0.38	0.42	0.34	0.29
	SLL3	0.34	0.16	0.18	0.21	-0.04
	SLE1	0.36	0.16	0.1	0.11	0.08
	SLE2	0.38	0.18	0.29	0.37	0.15
	SLE3	0.38	0.15	0.15	0.27	0.03
Field	GL	-0.27	-0.29	-0.15	-0.56	-0.08
	TL	-0.25	-0.25	-0.13	-0.56	-0.06
	DLFR	0.14	0.03	-0.01	0.21	-0.16
	PH	-0.16	-0.11	0.01	-0.44	-0.01
	SD	-0.07	-0.04	0.12	-0.29	-0.01
	LAI	-0.22	-0.24	-0.14	-0.5	-0.18

^aIE: infection efficiency; PLL: petiole lesion length; SLL1–3: stem lesion length at nodes 1–3; SLE1–3: stem lesion expansion at nodes 1–3; GL: number of green leaves per plant; TL: total number of leaves per plant; DLFR: percentage of dead leaves per plant; PH: plant height; SD: stem diameter; LAI: leaf area index.

^bColours are set according to correlation coefficients, varying from -1 (red), 0 (white) to 1 (blue). Numbers are in bold when associated to P values < 0.05 .

^cPhoma black stem incidence, mean over 2013 and 2014 experimental years.

^dPhoma black stem severity measured in experimental years of 2013 (1SEV) and 2014 (2SEV).

^eNumber of sunflower black stem lesions in experimental years of 2013 (1NUML) and 2014 (2NUML).

Note that rank correlation coefficients ≤ -0.43 correspond to $P < 0.001$.

The P values associated with Kendall's coefficient of concordance (W) for rank order of sunflower genotypes according to field disease intensity and to predictor variables were significant ($P < 0.01$) in most cases, indicating an overall agreement in ranking among genotypes according to predictors and to field disease intensity variables (Table III. 8). Although a

significant interaction between genotype and experiments was detected in mixed model analyses of variance for SEV and NUML, Kendall's W associated with the five disease intensity variables (INCS, 1SEV, 2SEV, 1NUML, and 2NUML) was significant ($W = 0.64$; $P < 0.001$), indicating an overall agreement in disease scores attributed to genotypes between the two experimental years.

The predictors derived from growth chamber experiments were associated with significant ($P < 0.05$) rank concordances with respect to INCS, 1SEV, and 2SEV; however, such was not the case with 1NUML and 2NUML. All comparisons in rank concordance performed on greenhouse predictors and disease intensity variables were significant ($P < 0.01$), and INCS was associated with the highest rank concordance ($W = 0.61$), in comparison with the other field disease intensity variables. The ranking of genotypes using the SEV variable had a higher concordance ($W = 0.42$), compared to when genotypes were ranked using either INCS ($W = 0.38$) or NUML ($W = 0.32$).

Binary logistic regressions based on NUML and INCC, and variables measured in the growth chamber, in the greenhouse, and in the field, always yielded nonsignificant effects of predictors, with P values attached to predictors ranging from 0.10 to 0.94 (data not shown). By contrast, about half of the binary logistic regressions considering INCS and SEV were attached to significant ($P < 0.05$) likelihood ratios, and about a quarter of the regressions involved significant ($P < 0.05$) parameters attached to the predictors (Table III. 9). The likelihood of a genotype having low disease intensity could be significantly ($P < 0.05$) predicted from variables measured in the growth chamber only when INCS (binarized) was predicted from PLL. For predictors measured in greenhouse experiments (lesion length or lesion expansion on adult plants), the likelihood of low disease intensity was significantly ($P < 0.05$) associated with SLL3 (predicting disease incidence, INCS) and SLE3 (predicting disease severity, SEV). Among the morphological variables measured in the field, plant height significantly ($P < 0.05$) predicted the likelihood of a genotype to have a low disease incidence in the field. Furthermore, the likelihood of a genotype to have low disease severity in the field was significantly ($P < 0.05$) associated with several morphological variables: number of green leaves, total number of leaves, plant height, and LAI.

Table III. 8. Kendall's coefficients of concordance (W) for rank order of sunflower genotypes according to field disease intensity and to predictors for resistance to Phoma black stem.

Experiment	Dis. var. ^a	Node ^b	Nb of variables ^c	W	χ^2	P -value	
-	All	-	5	0.645	58.1	<0.001	
Growth chamber ^d	All	-	7	0.405	48.2	0.000	
	INCS	-	3	0.674	34.4	0.008	
	1SEV, 2SEV	-	4	0.440	29.9	0.027	
	1NUML, 2NUML	-	4	0.318	21.6	0.200	
Greenhouse ^e	All	all	11	0.479	94.9	<0.001	
	INCS	all	7	0.619	78.0	<0.001	
	1SEV, 2SEV	all	8	0.527	76.0	<0.001	
	1NUML, 2NUML	all	8	0.500	72.0	<0.001	
	All	1	1	7	0.508	64.0	<0.001
			2	7	0.570	71.9	<0.001
			3	7	0.499	62.9	<0.001
	INCS	1	1	3	0.726	39.2	0.003
			2	3	0.701	37.8	0.004
			3	3	0.724	39.1	0.003
	1SEV, 2SEV	1	1	4	0.512	36.9	0.005
			2	4	0.634	45.7	0.000
			3	4	0.551	39.6	0.002
	1NUML, 2NUML	1	1	4	0.525	37.8	0.004
			2	4	0.600	43.2	0.001
			3	4	0.475	34.2	0.012
Field ^f	All	-	11	0.367	80.8	<0.001	
	INCS	-	7	0.383	53.7	<0.001	
	1SEV, 2SEV	-	8	0.422	67.5	<0.001	
	1NUML, 2NUML	-	8	0.328	52.5	<0.001	

^a Dis. var.: disease variable; INCS: black stem disease incidence; SEV: disease severity on stem; NUML: number of lesions per plant; each line indicates the variables included in the analysis; INCS: genotypes mean over blocks and years; 1SEV and 1NUML: genotype means over blocks in 2013; 2SEV and 2NUML: genotype means over blocks in 2014; All: all five variables previously described.

^b all: variables from all nodes were included in the analysis; 1, 2, 3: variables measured on the first, second, and third nodes were used for the analysis.

^c Number of variables included in the analysis.

^d Predictors from growth chamber experiment used in the analysis were infections efficiency and petiole lesion length assessed at eight days after inoculation (DAI).

^e Predictors from greenhouse experiment used in the analysis were stem lesion length and stem lesion expansion assessed on the first, second, and third stem node at 13 DAI.

^f Predictors from field experiment were multiplied by -1 prior to analysis (six variables; see text for details).

Table III. 9. Binary logistic regressions of the effects of predictors measured in growth chamber, greenhouse and field experiments on low disease intensity of sunflower Phoma black stem.

Disease variable ^a	Experiment type	Likelihood ratio		AUROCC ^c	Predictor ^d	Predictor statistics		
		LR	<i>P</i> -value ^b			Estimate	SE ^e	<i>P</i> -value ^b
INCS	Growth chamber	3.79	0.052	0.72	IE	-8.651	5.473	0.114
		6.08	0.014	0.82	PLL	-1.307	0.686	0.037
	Greenhouse	6.20	0.013	0.81	SLL1	-0.139	0.073	0.056
		3.70	0.055	0.71	SLL2	-0.079	0.048	0.101
		5.92	0.015	0.79	SLL3	-0.080	0.039	0.040
		1.83	0.177	0.66	SLE1	-0.023	0.018	0.206
		5.09	0.024	0.72	SLE2	-0.040	0.022	0.075
		3.74	0.053	0.74	SLE3	-0.040	0.023	0.079
	Field	4.91	0.027	0.75	GL	0.471	0.248	0.057
		4.06	0.044	0.72	TL	0.386	0.217	0.075
		5.33	0.021	0.77	DLFR	-0.598	0.324	0.065
		4.89	0.027	0.74	PH	0.058	0.029	0.048
		1.38	0.239	0.62	SD	0.366	0.325	0.260
		4.35	0.037	0.77	LAI	2.439	1.333	0.067
SEV	Growth chamber	0.33	0.567	0.59	IE	2.116	3.801	0.578
		0.08	0.781	0.54	PLL	-0.113	0.408	0.781
	Greenhouse	2.90	0.088	0.68	SLL1	-0.077	0.054	0.151
		5.91	0.015	0.77	SLL2	-0.112	0.060	0.060
		3.48	0.062	0.73	SLL3	-0.056	0.033	0.092
		0.43	0.512	0.54	SLE1	-0.011	0.016	0.518
		8.41	0.004	0.82	SLE2	-0.065	0.035	0.061
		5.25	0.022	0.76	SLE3	-0.049	0.025	0.046
	Field	9.19	0.002	0.84	GL	0.768	0.346	0.026
		10.0	0.002	0.84	TL	0.771	0.338	0.023
		1.58	0.208	0.63	DLFR	-0.270	0.230	0.241
		6.79	0.009	0.79	PH	0.071	0.032	0.027
		2.19	0.139	0.65	SD	0.473	0.342	0.167
		9.92	0.002	0.87	LAI	4.559	1.970	0.021

^a INCS: black stem disease incidence; SEV: disease severity on stem.

^b *P* values are displayed in bold when *P* < 0.05.

^c Area under the Receiver Operating Characteristic Curve.

^d IE: infection efficiency; PLL: petiole lesion length; SLL1–3: stem lesion length at nodes 1–3; SLE1–3: stem lesion expansion at nodes 1–3; GL, number of green leaves per plant; TL: total number of leaves per plant; DLFR: percentage of dead leaves per plant; PH: plant height; SD: stem diameter; LAI: leaf area index.

^e Standard error of the estimate.

The conditional density plots displayed in Figure III. 1 illustrate the relationship between binarized variables and predictors derived from the different experiment types. Plots indicate that the presence of genotypes belonging to the low disease intensity group decreased as predictor

variables derived from growth chamber and greenhouse experiments increased (Figure III. 1a–d). Genotypes with an SLL3 lower than 50 mm had a likelihood of 80% to belong to the low phoma black stem incidence (INCS) group (Figure III. 1d), while this likelihood decreased to less than 10% when SLL3 was higher than 90 mm. An opposite pattern of likelihood levels was observed with field predictors, for which likelihood levels of a genotype belonging to the low severity group increased with predictor values (Figure III. 1e,f). The high association between SEV and the total number of leaves (TL) is highlighted in Figure III. 1e. The likelihood level of a genotype with 20 leaves belonging to the low severity group was close to 5%, and increased near-linearly up to 98%, which corresponds to a genotype with 28 leaves (Figure III. 1e).

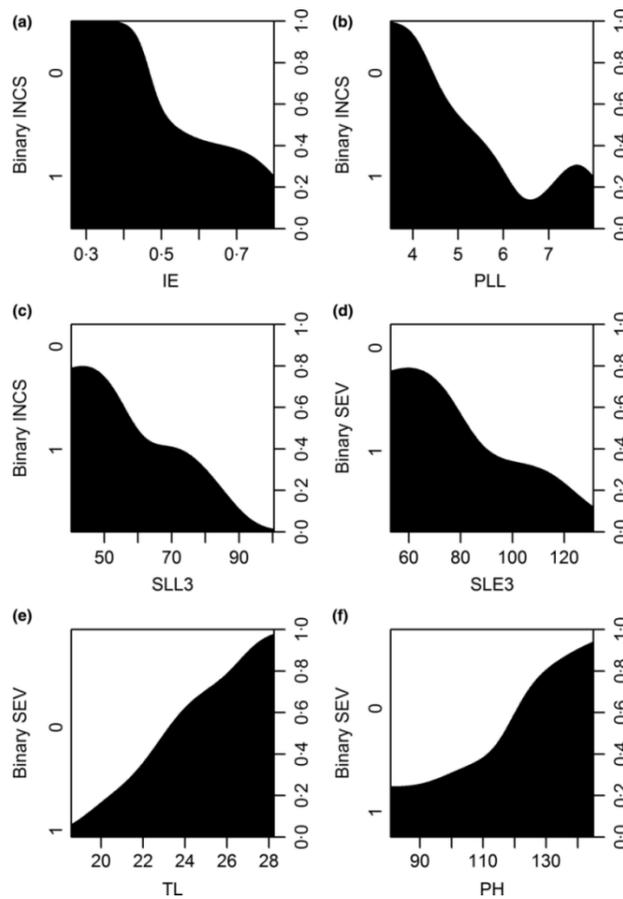


Figure III. 1. Conditional density plots for growth chamber (a, b), greenhouse (c, d) and field (e, f) predictors of sunflower phoma black stem in the field. The dark grey region represents the binary class of resistant (low incidence, (a–c) or low disease severity, (d–f)), while light grey background shows the absence of belonging to this class. The probability of belonging to these classes is displayed on the right-hand y-axes. Binary variables were created according to the median of genotypes for phoma black stem intensity variables (resistant = 1; susceptible = 0). Thus, the resistant class was attributed to genotypes with values below the median of phoma black stem severity (binary SEV: median = 2.57) and incidence on stem (binary INCS: median = 0.57). IE: infection efficiency; PLL: petiole lesion length; SLL: stem lesion length; SLE: stem lesion expansion; TL: total number of leaves; PH: plant height. Values for infection efficiency were multiplied by 10^4 .

Scatterplots of genotypes according to the pairwise variables displayed in Figure III. 1 are presented in Figure III. 2. In this figure, the dashed line represents the median value used as a threshold to binarize the field disease intensity (INCS and SEV) variables. Although genotypes with IE larger than 2.5×10^{-4} were above and below the threshold in similar frequency, the two genotypes with IE lower than 2.5×10^{-4} were below the median (Figure III. 2a). A similar pattern was displayed when considering PLL, with all three genotypes with PLL lower than 4.5 mm being below the threshold, and other genotypes partitioned below and above the threshold with similar frequencies (Figure III. 2b). INCS generally increased as SLL3 increased (Figure III. 2c). SLE3 below $90 \text{ mm}^2 \cdot \text{day}^{-1}$ was associated with six and two genotypes below and above the threshold, respectively, while SLE3 above $90 \text{ mm}^2 \cdot \text{day}^{-1}$ was associated with three and seven genotypes below and above the threshold, respectively. Disease severity in the field generally declined as predictors derived from field experiments increased (Figure III. 2e,f).

3.5. Discussion

Components of resistance measured in the greenhouse had, in general, a higher (although moderate) predictive value than those measured in the growth chamber. However, depending on the analyses, there were differences in the components of resistance measured in the greenhouse that best predicted disease intensity in the field. Ranking coefficient analysis highlighted SLL2 as the best predictor, whereas logistic regressions identified SLL3 and SLE3 as the best predictors. It therefore appears that measurements made on nodes 2 or 3 should be retained. Furthermore, as there was little difference in performance between lesion expansion and lesion length, lesion length should be used as it takes less time to measure.

There have been several studies aimed at identifying quantitative resistance and mapping QTLs in sunflower for resistance to *L. lindquistii*; these involved experiments under controlled conditions, where petioles of cotyledons of young seedlings were inoculated, and the fraction of petiole length diseased was measured (Roustae et al., 2000; Rachid Al-Chaarani et al., 2002; Bert et al., 2004; Darvishzadeh et al., 2007a). These studies enabled the identification of QTLs, but no detailed studies were conducted to assess the expression of these QTLs in the field. Genotypes inoculated on petioles (young seedlings) or stem nodes (fifth leaf pair and flowering development stages) had similar ranking when comparing disease intensity on genotypes at different ages grown under controlled conditions (Roustae et al., 2000). Furthermore, the comparison of disease level measured on plants from five genotypes inoculated at different ages in the greenhouse and in the field (one experimental year) indicated a good agreement in

genotype ranking according to disease levels measured in the greenhouse at different ages and in the field (Larfeil et al., 2010). The results of the present study confirm the agreement in genotype ranking according to disease intensities measured at different ages and under different experimental environments, and provides further quantitative analyses, on a larger range of sunflower genotypes.

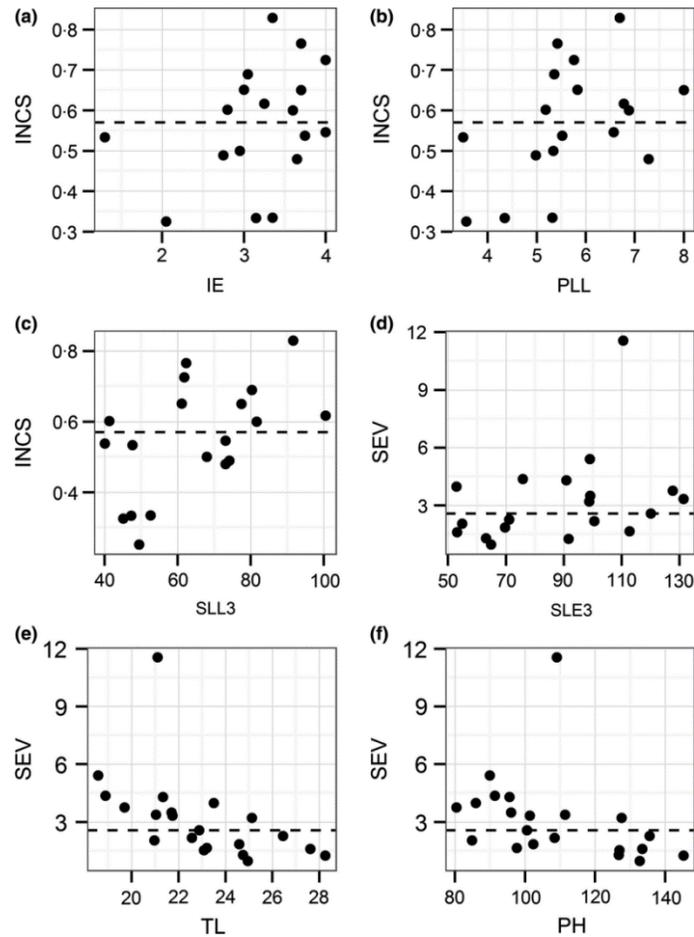


Figure III. 2. Scatterplot diagrams of sunflower phoma black stem intensity (y-axis) against disease predictors (x-axis) measured in growth chamber (a, b), greenhouse (c, d) and field (e, f) experiments. The dashed line indicates the phoma black stem intensity median, used as a threshold to create binary variables used in the logistic regressions. INCS, black stem disease incidence; SEV: disease severity on stem; IE: infection efficiency; PLL: petiole lesion length; SLL3: stem lesion length at node 3; SLE3: stem lesion expansion at node 3; TL: total number of leaves; PH: plant height. Values for infection efficiency (x-axis in (a)) were multiplied by 10^4 .

In the present investigation, inoculations were made using one isolate of *L. lindquistii*, and involved suspensions of (asexual) pycnidiospores. Natural infections in the field experiments occurred from a pathogen population, and were mainly caused by ascospores. However, a good association was observed between disease level intensities from growth chamber and greenhouse

experiments, on the one hand, and from field experiments on the other (Tables 7–9). This suggests that (i) inoculation with pycnidiospores related well with infections from ascospores; and (ii) if interactions between the isolate and the sunflower genotypes used exist, they were small enough not to be detected from these analyses: the genotype ranking according to disease intensity from inoculation by one isolate was similar to that from natural infection by a local pathogen population (Table III. 8). Interactions between isolates and sunflower genotypes have been detected from experiments conducted on young seedlings, where cotyledons were inoculated (Darvishzadeh et al., 2007b). Nevertheless, such interactions were not detected when inoculations were made on seedling collars in the greenhouse (Bordat et al., 2011). It is possible that interactions between pathogen isolate and host genotype detected under controlled conditions do not occur, or are expressed with a smaller magnitude, under field conditions, because of interactions with the environment (Mundt, 2014).

Logistic regressions showed that plant height was associated with a decreased probability for a genotype to belong to a susceptible group, in terms of disease incidence and severity. The total number of leaves, number of green leaves, and LAI were also associated with a decreased probability of a genotype to belong to the genotype group with high severity in the field. In previous field experiments, when LAI varied as a response to a range of crop management factors (nitrogen and water supply, and plant density), phoma black stem intensity increased with increasing LAI (Debaeke & Pérès, 2003). In contrast, in the present study, genotypes with larger LAI were associated with a decrease in disease intensity. Based on these results, it would be interesting to quantify the relative effects of genotype and crop management factors on LAI and on phoma in the future. Plant height, total number of leaves, number of green leaves, and LAI were highly correlated among each other (Table III. 6). The experimental design of this study does not allow the separation of specific effects of the morphological traits on disease intensity, but some hypotheses can be put forward to explain the associations found between some of these morphological traits and disease intensity (Schwanck et al., 2016): first, a larger number of green leaves may lead to inoculum dilution if ascospores are deposited on the top of the canopy and flow with rainfall from node to node towards the base of the plant. Secondly, taller plants may have a microclimate where wetness duration is decreased, therefore leading to decreased infection efficiency, and so reduced epidemics, as shown in other pathosystems where wetness is required for infection (Scott et al., 1985). Among the four morphological traits associated with disease intensity, plant height and the total number of leaves represent traits with high heritability, and may be retained when considering breeding for resistance to phoma.

Although not the focus of this study, the different genotypes were assessed in the field for incidence of disease on the collar (INCC). The results are in very good agreement with previous studies using the same genotypes [Figure III. S1 (*supplementary figure*)], therefore cross-validating results obtained in both studies. None of the predictors for phoma black stem derived in the present study were significantly associated with collar disease incidence and subsequent premature death. This suggests that resistance to phoma black stem might be governed by genes (or QTLs) that differ from those involved in resistance to premature death, and/or that pathogen specialization processes have occurred according to the type of tissue infected (collar or stem). If so, breeding for resistance to phoma black stem and breeding for resistance to premature death need to be distinguished.

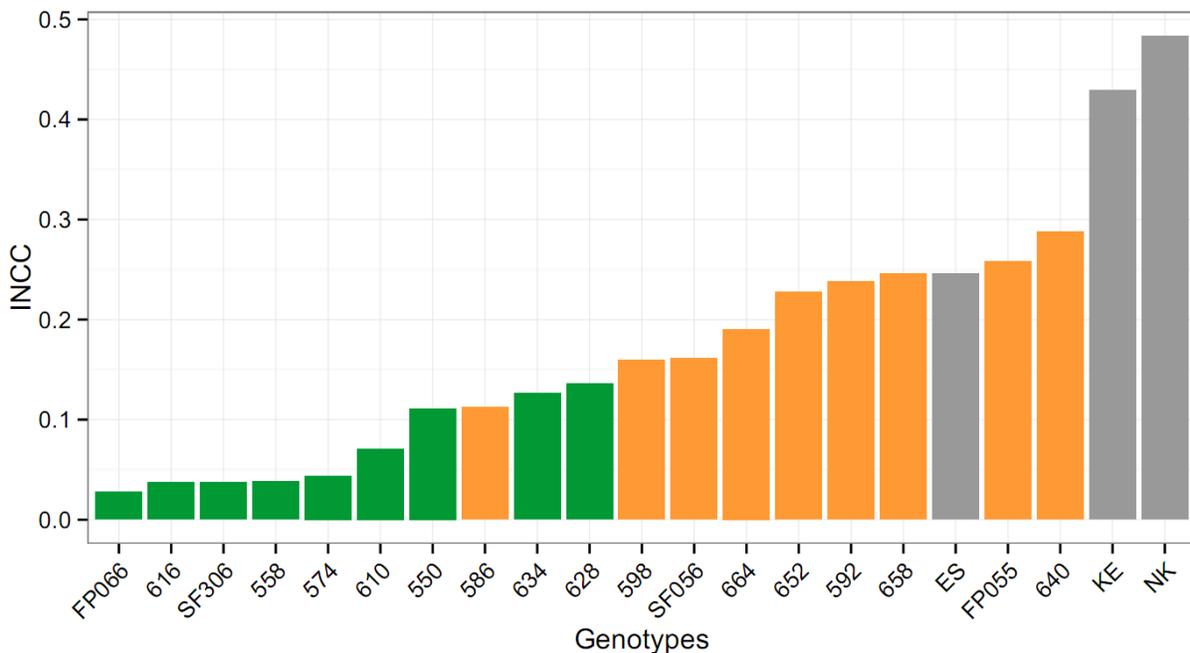


Figure III. S1. Bar chart displaying collar disease incidence (INCC) measured in the field for 21 sunflower genotypes. Each bar represents the collar disease incidence mean per genotype over two years and four replications per year. Green and orange bars represent genotypes classified as resistant and susceptible to premature death, respectively, according to previous studies (Bordat et al., 2012). Gray bars display INCC for genotypes for which no information is available from previous studies.

The concept of components of resistance was mainly developed for airborne fungi associated to polycyclic disease epidemics. Several components of resistance have been considered for these diseases: relative resistance affecting infection efficiency, latency period, infectious period duration, sporulation intensity, and lesion expansion (Parlevliet & Zadoks,

1977; Parlevliet, 1979). These relative resistances may be assembled in one overall relative resistance that expresses partial resistance in a genotype (Savary & Zadoks, 1989). However, in the case of diseases associated with monocyclic epidemics, such as phoma on sunflower, only a few components of resistance can be considered, and, therefore, the implementation of the concept of components of resistance appears limited (Savary, 2014). In the present study, infection efficiency and lesion expansion were addressed and this yielded predictors for quantitative resistance associated to these two components of resistance.

The identification of predictors for resistance involved analyses conducted in two broad steps: process-oriented experiments, and epidemic-oriented experiments. This stepwise use of complementary process- and epidemic-oriented analyses follows earlier analyses (Savary & Zadoks, 1989; Willocquet et al., 2012; Srinivasachary et al., 2013), but is different from many studies aiming at identifying predictors for field resistance, which rely mainly on the computation of correlation coefficients between predictors and measured field disease variables (e.g. Herrera-Foessel et al., 2007). In this work, rank assessment methods were used, which are relevant when considering the practical aim attached to this study, i.e. to identify predictors to be used for ranking genotypes in selection programmes. Kendall's W has also been used, for example, to assess rank agreement between genotypes according to disease levels across experimental years or locations (e.g. Shah et al., 2000). Logistic regression was also used, which is well suited to the objective of decision making, in this case decisions pertaining to genotype selection in breeding programmes. Logistic regressions have previously been applied, e.g. for the development and evaluation of forecasting systems (e.g. Yuen et al., 1996), and for the prediction of risk factors for crop health (Savary et al., 2011).

The results generated from this work provide several elements that can be used in sunflower plant breeding programmes. First is the identification of control genotypes for susceptibility and for quantitative resistance. Genotype 640 had the highest levels of disease in the field in both years and for all disease variables. It was ranked between 11 and 14, and 14 and 17 (1 being rank of genotype with lowest value of disease variable) for predictors from growth chamber and greenhouse experiments, respectively. For total number of leaves and plant height, it was ranked 4 and 12, respectively. Therefore, this genotype is suitable for use as a susceptible control in future studies on resistance to phoma. Genotypes 550 and 574 had the lowest disease incidence in the field, and ranked, on average, 3 for the other field disease variables. Genotype 550 ranked 2 for infection efficiency and lesion length in growth chamber experiments, while genotype 574 ranked 10 and 6 for the corresponding components of resistance. Average rankings

for components of resistance measured in the greenhouse were 3.8 and 8.8 for genotypes 550 and 574, respectively; both genotypes ranked between 13 and 15 for total number of leaves and plant height. Therefore, genotypes 550 and 574 could be used as controls for quantitative resistance.

A second element of information useful for breeding programmes concerns the predictors for physiological resistance. Components of resistance measured in the greenhouse were, in general, more strongly associated with field disease intensity than components of resistance measured in the growth chamber, although lesion length on seedling petioles in the growth chamber was also associated with disease in the field (Table III. 8). This measurement on seedlings may be preferred in future studies, because it requires much less time and space compared to measurements made in the greenhouse. Infection efficiency is expected to be an important component of resistance, particularly as epidemics of phoma are monocyclic (Lannou, 2012) under the experimental conditions of the current work. Infection efficiency is also expected to be associated with field variables representing the level of infection, i.e. disease incidence (INCS), and the number of lesions per plant (NUML). It would therefore be useful to improve the methodology in order to measure this component of resistance.

A third element of information pertains to predictors from morphology. The results from the present study are in agreement with a complementary analysis performed on results from the same field experiment, which indicated that low disease intensity was associated with high number of leaves and tall plants (Schwanck et al., 2016). The two morphological traits are correlated and so plant height may be used in future work, because it is faster to measure than counting the number of leaves.

The results presented here provide a first series of measurements of components of resistance and morphological traits associated with quantitative resistance to phoma black stem, on a limited number of sunflower genotypes. Although several predictors with significant effects were identified, their correlation or predictive ability with respect to disease in the field was moderate. Further improvement of phenotyping methods for this disease may be obtained by improving the methods to measure infection efficiency, and by assessing a larger number of sunflower genotypes, which would be representative of the genetic diversity of this species.

3.6. Acknowledgements

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DISCUSSION

Main findings

Results from spatiotemporal and disease gradient experiments have suggested that phoma black stem is randomly distributed and that there was no evidence of disease gradient from a source of inoculum (*chapter 1: “Exploring spatiotemporal patterns of phoma black stem in sunflower”*). These results suggest the absence of plant-to-plant dispersion of *Leptosphaeria lindquistii* in sunflower, and that phoma black stem is associated to monocyclic epidemics in southwest of France

The protocol developed to assess phoma black stem, including a diagram scale for disease severity on the stem, enabled to assess the relationships between disease intensity and plant morphology. The association with disease was identified for morphological traits measured at flowering, but not at the star-bud sunflower development stage. Low phoma black stem levels were associated with sunflower plant morphological traits characterized by a large number of green leaves and large plant height, which can constitute targets for sunflower breeding for phoma black stem resistance. A larger number of green leaves may correspond to inoculum dilution at the plant scale, while taller plants may be associated with a microclimatic environment where wetness duration is decreased. These associations were detected from results obtained in two independent field experiments, from analyses conducted using plot-scale data (*chapter 2: “Effects of plant morphological traits on phoma black stem in sunflower”*) and plant genotype-scale data (*chapter 3: “Predicting quantitative host plant resistance against phoma black stem in sunflower”*), indicating that these associations could be detected from the phenotypic and genetic perspectives, respectively.

A *predictor of resistance* may be defined as a variable derived from phenotyping methods applied to a group of plant genotypes, and which is associated [has predictive power] with field disease intensity variable(s). Predictors of quantitative resistance to phoma black stem on sunflower could be derived from field (plant morphology), greenhouse (disease intensity on adult plants) and growth chamber experiments (disease intensity on inoculated seedlings; *chapter 3*). Components of resistance measured on adult plants (lesion length and lesion expansion on stem) had in general a higher predictive value than those measured on seedlings (infection efficiency and lesion length on petiole). Plant morphological traits measured in the field (plant height, number of leaves) had higher predictive power of disease intensity in the field than components of resistance. These results are in agreement with the concordance in genotype ranking found

according to disease intensities measured at different ages and under different experimental environments (Roustae et al. 2000; Larfeil et al. 2010). Additionally, the results presented in *chapter 3* constitute useful information for the use of phenotyping methods in breeding programmes for quantitative resistance on sunflower against *L. lindquistii*.

Broad applications of this work: methodological aspects

Chapter 2 used data collected in a well-known experimental design [randomized complete block design with four replications (21 treatments = genotypes)] to test hypotheses on the association between plant morphology and disease intensity.

The analyses used data at the plot scale, and the analytical steps (an overview is provided in Figure 2 of chapter 2) involved: (1) analyses of variance of disease and morphological variables according to genotype and block, based on the statistical design of field experiments, in order to provide an overview of the datasets and of patterns of variables; (2) multivariate analyses of disease variables to characterize associations between disease variables, and to group experimental plots according to disease variables; (3) similar to step (2), grouping of experimental plots according to morphological variables; (4) contingency table analyses between disease-based and morphology-based groups, (5) correspondence analyses between disease- and morphology-based groupings, and (6) logistic regressions in order to assess the relationships between disease intensity and morphological characteristics. I highlight that the presented analytical framework (1) provided data correlation overview and (2) enabled testing hypotheses on data collected using a well-defined assessment protocol.

Thus, I encourage other researchers to consider applying the presented analytical framework to other pathosystems, in order to test equal, similar or even other hypotheses (e.g. associations among multiple diseases in the field). Additionally, I feel useful to highlight that such an analytical framework could be applied on datasets from experiments which are conducted to test other hypotheses rather than disease escape processes. For example, data collected from experiments under complete randomized block design, when a relatively large number of variables derived through plant and disease phenotyping are involved, or plant and disease phenotypic large datasets collected within a given regional area under the same environmental conditions.

The *components of resistance* concept could be applied to a disease associated with monocyclic epidemics in a set of complementary experiments (*chapter 3*). Even though the number of components of resistance assessed was limited (infection efficiency and lesion length

on seedlings and lesion length and expansion on adult plants), the components of resistance could be compared to disease intensity in the field in order to identify predictors for quantitative resistance.

Results presented in *chapter 3* involved experiments conducted on the same set of genotypes, on which (1) components of physiological resistance were measured on inoculated seedlings and adult plants, (2) disease escape components (morphological traits) were measured on adult plants in the field, and (3) disease intensity variables were measured. The two first sets of measurements correspond to predictors of resistance, while the third set corresponds to response variables. The analyses were conducted to assess the relationships between predictors and the response variables, and involved (1) linear mixed models to select predictors according to their robustness and sensitivity, (2) Kendall's ranking uni- and and (3) binary logistic regressions. The experimental framework and analytical approach presented in *chapter 3* exemplify a useful research strategy for identifying predictors of quantitative resistance against pathogens associated with monocyclic epidemics. It allowed inferring and hypothesizing on physiological resistance and disease escape processes occurring in phoma black stem of sunflower.

Additionally, I utilized *conditional density plots* in *chapter 3* to illustrate probability levels of a particular binary variable class (resistant genotype) in condition to predictors of resistance. It is the first time this sort of plot is applied in plant pathology to my knowledge, therefore I feel appropriate providing further details and addressing potentialities of its use. Conditional density plots are similar to *spinograms* ("spineplot" function of "grDevices" package in R) and are derived by a smoothing technique via density (R Core Team 2014). In fact, a conditional density plot is related to a spinogram in the way a density plot relates to a frequency histogram (Glynn and Robinson 2014). Although conditional density plots are less reliable in regions with only few observations in the abscissa axis (R Core Team 2014), displaying conditional density plots to 21 cases (genotypes) showed a clear visual congruence to logistic regression results (*chapter 3*), which also deal with probability levels of a binary variable according to a quantitative variable. I identify potential in using conditional density plots in other studies with binary variables, e.g. (1) selection of binary or quantitative variables through graphical analysis, (2) studies involving dichotomy [e.g. spatial pattern studies: *aggregated* and *random*; as recently applied by Schwanck and Del Ponte (20--)] for visualizing the sensitiveness of different statistical tests in detecting aggregation of brown spot lesions on rice leaves according to disease severity]. The interested reader may also see articles I used to interpret

conditional density plots in the fields of remote sensing (e.g. Falkowski et al. 2009) and medicine (e.g. Coassin et al. 2010; Thelin et al. 2013).

Knowledge gaps on phoma black stem and perspectives

This PhD work provided information on phoma black stem epidemiology and sunflower quantitative resistance. I now present some questions that remain on this pathosystem, i.e., hypotheses that are still to be tested pertaining to (1) the epidemiology of the disease and to (2) sunflower breeding for durable resistance against phoma black stem. As I address these knowledge gaps, I propose research priorities (*in italic*) to some of them.

Several questions with respect to infection processes of *L. lindquistii* involved in phoma black stem epidemics still remain: (1) Why do epidemics generally start after flowering? (2) Why do lesions follow an acropetal progress on stem nodes: is it related to leaf senescence influencing the susceptibility to infections? (3) Why do lesions occur predominantly at the node of the plant, specifically starting by the insertion point of the petiole on the plant stem? Although the knowledge currently available provides indications of answers, these questions have not been formally addressed yet.

An investigation on the infection processes occurring with respect to genetic, histological, and morphological aspects, and on the effects of the environment on these processes, would contribute to a better understanding of phoma black stem cycle and epidemics.

Some hypotheses on *L. lindquistii* population biology could be derived from *chapter 3*. The results suggest the absence, or a weak effect, of interactions between *L. lindquistii* isolates and sunflower genotypes. Interaction between isolates and sunflower genotypes have been detected from experiments conducted on young seedlings, where cotyledons were inoculated (Darvishzadeh et al. 2007b). Nevertheless, such interactions were not detected when inoculations were made on seedling collars in greenhouse experiments (Bordat et al. 2011). Furthermore, none of the predictors for phoma black stem derived in our study were significantly associated with collar disease incidence. This suggests that resistance to phoma black stem might be governed by genes (or QTLs) which differ from those involved in resistance to premature death, and/or that pathogen specialization processes have occurred according to the type of tissue infected (collar or stem). If so, breeding for resistance to phoma black stem and breeding for resistance to premature death need to be distinguished. It can be hypothesized that *L. lindquistii* population may have

evolved as two phylogenic groups, as a consequence of the selection imposed by the plant on the pathogen fitness (Zhan et al. 2015). Knowledge on the genetic diversity of a pathogen population constitutes important information for plant breeding, since it is related to the effectiveness and durability of host resistance deployed in crops (Peever et al. 2000).

Population biology and diversity of L. lindquistii causing phoma black stem is still to be studied.

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TITRE : *Identifying epidemiological predictors for quantitative host plant resistance: application to the sunflower-phoma pathosystem*

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RÉSUMÉ

La maladie de taches noires (TN ; champignon *Leptosphaeria lindquistii*) est une maladie importante en France. L'étude présentée dans cette thèse fournit des informations utiles sur l'épidémiologie de TN et sur la résistance quantitative du tournesol contre TN. Des expérimentations ont été menées sur petites parcelles au champ, plantes adultes (serre), et plantules (phytotron) pour (1) caractériser la dynamique spatio-temporelle de TN, (2) identifier les traits morphologiques affectant TN via des processus d'esquive de la maladie et en utilisant une procédure standardisée d'évaluation de la maladie, et (3) identifier des prédicteurs de résistance quantitative à TN. Cette étude suggère que (1) TN est associée à des épidémies monocycliques dans le sud ouest de la France, (2) les niveaux faibles de TN sont associés à des plantes ayant un grand nombre de feuilles vertes et de grande taille, et (3) des prédicteurs de résistance quantitative à BS peuvent être identifiés expérimentalement.

MOTS-CLES : *Leptosphaeria lindquistii*, tournesol, résistance quantitative, monocyclique

ABSTRACT

Phoma black stem (BS) is caused by the fungus *Leptosphaeria lindquistii*, and is an important disease in France. The study presented in this dissertation provides useful information on BS epidemiology and sunflower quantitative resistance against the disease. Experiments were conducted on plants grown in small plots (field), adult plants (greenhouse), and seedlings (growth chamber) in order to (1) characterize the spatiotemporal dynamics of BS, (2) identify morphological traits affecting BS through disease escape processes and utilizing a standardised disease assessment procedure, and (3) identify predictors of quantitative resistance to BS. This study suggests that (1) BS is primarily associated to monocyclic epidemics in south west France, (2) low BS levels are associated with sunflower plants characterized by a large number of green leaves and large height, and (3) predictors of quantitative resistance to BS can be experimentally identified.

KEYWORDS: *Leptosphaeria lindquistii*, sunflower, quantitative resistance, monocyclique
