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The effects of sowing date and nitrogen availability during vegetative stages on *Leptosphaeria maculans* development on winter oilseed rape

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Abstract

Phoma stem canker, caused by *Leptosphaeria maculans* (anamorph *Phoma lingam*), is one of the most serious diseases of oilseed rape world-wide. However, little is known about the effects of cultural practices on phoma stem canker development. We carried out a field experiment, in 2000/2001 and 2001/2002, at Grignon Experimental Unit (Paris Basin, France) to assess the effects of sowing date and nitrogen availability during vegetative stages on phoma stem canker development on two winter oilseed rape cultivars. We studied eight treatments corresponding to the combination of two sowing dates — early (beginning of August) and typical (beginning of September) — two levels of nitrogen availability during vegetative stages —application of 0 or 250 kg $N.ha^{-1}$ before the end of autumn, subsequent spring nitrogen fertilizer application being adjusted according to the needs of the crop — and two cultivars — Bristol (susceptible to phoma stem canker) and Pollen (slightly susceptible). Early sowing resulted in smaller crown cankers, whereas high nitrogen availability during the vegetative stage favoured crown canker development. Significant interactions between cultivar susceptibility and cultural practices were observed in the second year of the experiment. Crown canker development was more strongly limited by early sowing for Pollen than for Bristol. Similarly, high nitrogen availability during the vegetative stage increased crown canker development more strongly for Pollen than for Bristol. The results presented here should facilitate integration of the risk of phoma stem canker development into the choice of the sowing date and nitrogen management within the cropping system.

1. Introduction

Phoma stem canker, or blackleg (caused by *Leptosphaeria maculans* (Desm.) Ces. & de Not.), is a major disease of oilseed rape world-wide. In France, this disease has been estimated to be responsible for mean yield losses of 5 to 20% (Aubertot et al., 2002). Chemical, genetic, biological and cultural control methods may be used to contain the disease. Fungicide treatments of seeds, soil or foliage are the principal means of controlling the disease in various regions of the world (West et al., 2001). However, fungicides are effective only if applied during the phoma leaf spot stage (West et al., 1999). As fungicides are effective for only limited periods of time — generally two to three weeks — total control of the disease using fungicides is not possible. The use of resistant cultivars efficiently controls the pathogen, but such resistance may break down due to the selection pressure exerted on the pathogen population (Brun et al., 2002; Rouxel et al., 2003). Several biological control agents that might contain phoma stem canker development have been studied (Chakraborty et al., 1994; Tewari et al., 1997; Kharbanda et al., 1999; Maksymiak and Hall, 2000; Beatty and Jensen, 2002; Hysek et al., 2002). However, none of these agents are currently widely used by farmers as a means of biological control for *L. maculans*. Due to the limited efficiency of chemical, genetic and biological control methods and durability problems, cultural control strategies are needed for controlling the disease, in conjunction with the methods currently used.

Appropriate residue management (*e.g.*, burying infected debris by tillage before autumn) and sufficiently long crop rotations (a 4-year interval between oilseed rape crops is usually recommended) reduce the risk of primary inoculum production from

infected stubble (Alabouvette and Brunin, 1970; Kharbanda and Tewari, 1996; Turkington et al., 2000; West et al., 2001). Other cultural practices may also affect disease development, and are thus potentially useful for pathogen control. Sowing date is used to restrict pest development in many crops (Flint and Gouveia, 2001). In western Europe, concentrations of air-borne ascospores of *L. maculans* generally peak between September and December (Alabouvette and Brunin, 1970; Gladders and Musa, 1980; Thürwachter et al., 1999; West et al., 2002a; West et al., 2002b). The severity of the symptoms at harvest is highest if infection occurs soon after emergence (Brunin and Lacoste, 1970; Alabouvette et al., 1974; MacGee and Petrie, 1979; Hammond and Lewis, 1987; Sun et al., 2000). Hence, sowing date should directly influence the primary infection stage of the epidemic cycle of the disease. However, the relationship between sowing date and severity of phoma stem canker at the end of the crop cycle is unclear. Late sowing dates have been reported to be associated with lower levels of stem canker development in Australia (MacGee and Emmet, 1977) and Europe (Scheibert-Bohm, 1979, in Germany and the Netherlands; Lepage and Penaud, 1995, in France). However, early sowing has been reported to limit stem canker development in England (Gladders and Musa, 1980) and in western Australia (Khangura and Barbetti, 2001). Several studies of spring canola in Canada concluded that sowing date did not affect phoma stem canker development because ascospores are released throughout the growing season (Kharbanda and Tewari, 1996). Furthermore, very early sowing (about one month earlier than the usual date) was reported to have no overall effect on phoma stem canker development in 36 trials carried out over a three-year period in France (Dejoux et al., 2003). However, crops sown very early generally displayed higher levels

of phoma stem canker than crops with a typical sowing date in situations in which a significant difference was identified (10 of 13 cases).

The application of fertilizers, especially nitrogen, may also affect the activity of many pest species, to the benefit or detriment of the crop (Flint and Gouveia, 2001). Few studies to date have analysed the effects of nitrogen status on phoma stem canker development on oilseed rape crops (Kharbanda and Tewari, 1996). A Canadian study reported that the amount of nitrogen fertilizer applied did not affect the severity of crown canker on winter oilseed rape (Hall et al., 1993). Two other experiments showed that differences in the level of nitrogen fertilization did not affect the development of phoma stem canker (Sadowski et al., 1998; Söchting and Verret, 2003). However, high nitrogen concentrations often increase the susceptibility of plants to disease (Agrios, 1997). In these previous experiments, nitrogen fertilizer was typically applied in spring, after the fungus had infected the plants. It is therefore of interest to analyse the effects of nitrogen availability on phoma stem canker development, not during the spring, but at the beginning of the vegetative stage of the crop cycle.

We report here the results of field experiments investigating the effects of sowing date and nitrogen management within the cropping system and their interactions on phoma stem canker development. We used two oilseed rape cultivars to analyse possible interactions between known cultivar susceptibility and sowing date, and between cultivar susceptibility and nitrogen availability during vegetative stages. The aim of this study was not to compare representative agricultural practices, but to investigate the

potential effects of cultural practices on phoma stem canker. We deliberately introduced extreme cultural practices to generate broad differences in crop development.

2. Materials and methods

2.1 Field experiments

Field experiments were carried out in 2000/2001 and 2001/2002 at Grignon (Paris Basin, France), on an orthic luvisol (0.67 $g.g^{-1}$ silt, 0.25 $g.g^{-1}$ clay and 0.08 $g.g^{-1}$ sand). The plots used for the experiment had not been sown with oilseed rape for at least four years and crops were infected by the natural inoculum present in the area. The experimental treatments consisted of combinations of sowing dates (early sowing, $\mathbf{D_1}$; and a typical sowing date, $\mathbf{D_2}$), nitrogen availability during vegetative stages (low, $\mathbf{N_{Low}}$; and high, $\mathbf{N_{High}}$) and cultivar (Bristol, susceptible to phoma stem canker, **B;** and Pollen, slightly susceptible, **P**). The experimental design was a split-split-plot arranged in a randomized complete block design with three replicates. Sowing date was the main plot factor, cultivar the subplot factor, and nitrogen availability, the sub-subplot factor. For early sowing treatments, crops were sown on August 3rd 2000 and July 31st 2001. For typical sowing date treatments, crops were sown on August 31st 2000 and September 4th 2001. We generated different levels of nitrogen availability by not supplying nitrogen during the autumn or by supplying 250 kg ammonium $nitrate.ha^{-1}$. For the fertilized plots, nitrogen was applied in three installments: 50 $kg.ha^{-1}$ after sowing, 100 $kg.ha^{-1}$ one month after sowing and 100 $kg.ha^{-1}$ two months after sowing. Bristol, the cultivar susceptible to phoma stem canker displays early flowering and maturity, whereas

Pollen, which is slightly susceptible to this disease, displays semi-late flowering and maturity (CETIOM, 2000). Each sub-sub plot was 12 m x 15 m in size, including a 1.5 m wide separation border to limit interactions between adjacent plots.

2.2 Crop management

The sowing rate was 117 seeds.m^{-2} for all treatments and both years. Each sub-sub plot was drip-irrigated to ensure that seedling emergence was not delayed due to lack of moisture. From August 1st to September 30th of both years, 25 mm of water was applied as soon as the balance between rainfall plus irrigation minus potential evapotranspiration was negative over a period of ten consecutive days. For each treatment, spring nitrogen fertilizer application was adjusted according to the needs of the crop, determined with the balance sheet method adapted for oilseed rape crops (Reau et al., 1997). Nitrogen was applied as ammonium nitrate. High levels of nitrogen availability during the vegetative stages were achieved by applying 50 kg.ha^{-1} of N after sowing (August 7th 2000 and August 1st 2001 for early sowing dates, September 4th 2000 and September 7th 2001 for usual sowing dates), 100 kg.ha^{-1} one month later (September 4th 2000 and September 7th 2001 for early sowing dates, October 13th 2000 and October 3rd 2001 for typical sowing dates) and 100 kg.ha^{-1} two months after sowing (October 13th 2000 and October 3rd 2001 for early sowing dates, October 30th 2000 and October 30th 2001 for typical sowing dates). These plots required no spring fertilization in either of the two study years. Plots that received no nitrogen fertilizer during the vegetative stages received two applications of 80 kg.ha^{-1} N, at intervals of about one month, in late winter 2000/2001 (February 20th 2001, March 27th 2001). In 2002, $\mathbf{N_{Low}}$ plots received two applications of nitrogen fertilizer: 80 kg.ha^{-1} on each $\mathbf{N_{Low}}$ plot

(March 8th 2002) plus 30 kg.ha^{-1} for early sowing dates (March 25th 2002) or 80 kg.ha^{-1} for typical sowing dates (March 25th 2002). For crop protection, we applied a molluscicide (mercaptodimethur at 1.5 kg a.i.ha^{-1}), herbicide (tebutam+clomazone at 3 kg a.i. ha^{-1} and 0.1 kg a.i. ha^{-1} respectively), insecticide (deltametrine at 6.5 g a.i. ha^{-1}), and fungicide treatments (carbendazim at 0.5 kg a.i. ha^{-1}) controlling sclerotinia (*Sclerotinia sclerotiorum*) and light leaf spot (*Pyrenopeziza brassicae*) uniformly on all plots, according to regional CETIOM recommendations (CETIOM, 2000). We applied no fungicides active against phoma stem canker development or plant growth regulators.

2.3 Climate and soil measurements

Mean daily temperature and daily rainfall were recorded with an automatic meteorological station located less than 300 m from the field experiments. A regional climatic data set for 1971 to 1999 was used for comparison with the climate during the two years of the experiment.

A few days before or after sowing, the amount of residual mineral N in the top 90 cm was measured for three soil layers: 0-30 cm, 30-60 cm, and 60-90 cm. Samples were taken only from $\mathbf{N_{Low}}$ plots if it was not possible to take samples before nitrogen fertilizer application. Soil samples were obtained by mixing three separate 3x377 cm^3 cores taken from each of the three blocks, for each soil layer. Soil inorganic N content was determined in a KCl extract (300 ml of 1.0 mol.l^{-1} KCl per 150 g of fresh soil, shaken for half an hour, carried out in duplicate) with an autoanalyser (Skalar Analytical, Breda, The Netherlands), using cadmium reduction and the Griess Ilosvay

reaction for nitrate (Henriksen and Selmer-Olsen, 1970) and the indophenol method for ammonium (Verdow, 1977).

2.4 Plant measurements

Throughout the crop cycle, seven samples were taken from each sub-sub plot at different periods or growth stages (GS; Sylvester-Bradley, 1985): GS 1.2, second true leaf exposed; GS 1.6, sixth true leaf exposed; GS 1.10, tenth true leaf exposed; early winter (mid-December); late winter (beginning of February); GS 4.5, flowering; GS 6.3, most seeds green. For each set, we sampled an area of 0.85 m x 1 m in each sub-sub plot to determine green leaf area index (GLAI) and nitrogen nutrition index, calculated as the ratio of the measured mineral N concentration in the aerial parts of the plant to the critical nitrogen concentration (Lemaire and Gastal, 1997). The critical nitrogen concentration, for a given plant biomass, is the lowest nitrogen concentration in the aerial parts of the plant at which growth is not limited by nitrogen. This value was obtained from the critical curve described by Colnenne et al. (1998), except after flowering, when an alternative critical curve described by Jeuffroy et al. (2003) was used. Total nitrogen concentration was determined by a procedure adapted from the Dumas method (Dumas, 1831). This procedure involved the combustion of dehydrated and ground plant tissue at about 1800°C, the reduction of nitrogen oxides by reduced Cu at 600°C and N_2 determination by catharometry (NA 1500 analyser, Fisons Instruments, Rodano, Italia). For each sample, growth stage was determined on a subsample of 10 representative plants. We did not analyse the relationship between crown canker severity and yield loss because yield formation also depends on the cultural practices analysed.

2.5 Disease measurements

The percentage of plants with at least one phoma leaf spot (incidence) was determined from GS 1.2 to GS 6.3, on the samples used for plant measurements. Crown canker severity was also determined on the samples used for plant measurements from late winter to GS 6.3 and on an additional sample taken at crop maturity (GS 6.6: most seeds dark brown). Six severity classes were used to grade crown cankers: 1, healthy plant, no visible lesions; 2, weakly developed canker; 3, canker developed on less than half of the crown section; 4, canker developed on more than half of the crown circumference; 5, canker developed over almost all the crown section; 6, section with no living tissue, plant lodged or broken at the crown during sampling. The minimum sample size was 80 plants per sub-sub plot. A disease index (DI) with values from 0 for healthy plants to 9 for plants totally lodged because of the disease was then used to summarize the observations (Aubertot et al., in press):

$$DI = \frac{\sum_{i=2}^{6} [2(i-2)+1]n_i}{\sum_{i=1}^{6} n_i} \qquad [1]$$

where n_i is the number of plants in class i.

We determined the concentration of *L. maculans* air-borne ascospores daily, using a spore trap (7-day recording volumetric spore trap, Burkard Manufacturing Company, Rickmansworth, UK) placed in the middle of the experimental plot. This device is similar to the trap described by Hirst (1952). The ascospores trapped within a 24-hour period were counted under a microscope and the ascospore concentration was calculated

as a function of throughput at the orifice (10 l.min^{-1}). This device was used from August 28th 2000 until June 25th 2001 and from August 2nd 2001 until July 17th 2002.

2.6 Statistical analysis

We used SAS Release 6.12 for Windows (SAS Institute Inc., 1989) for statistical analysis. We performed split-split-plot analyses of variance (Little and Hills, 1978), using the GLM procedure. Separate analyses were performed individually for each of the sample sets taken at a given phenological stage or in a given period. Probability values are presented directly in the text to support our interpretations. For each analysis of variance, we checked homoscedasticity, by Levene's test (confidence level of 0.95), and the normality of the residuals by the Shapiro-Wilks test (confidence level of 0.95). Logarithmic transformations were carried out before analysis if Levene's test revealed heteroscedasticity. Arcsine transformations were applied to percentage data containing both values smaller than 30% and greater than 70% (Gomez and Gomez 1984).

3. Results

The autumn was mild and wet in the first year of the experiment (2000/2001, Figure 1). Total cumulative rainfall was 274 mm from October to December 2000, whereas the 29-year mean for these months was 159 mm. In 2001, there was a wet period from March to April, followed by a dry period from May to June. The autumn was mild, with typical levels of rainfall in the second year of experiment. However, temperatures in December 2001 were below the 29-year mean. For that month, mean daily temperature remained below 0°C for four consecutive days (data not shown). In 2002, there was a wet period from February to March, followed by a dry period in April.

Soil nitrogen content at sowing was between 20 and 50 kg.ha^{-1} at depths of 0-90 cm for early-sown crops in both years (Table 1). Soil nitrogen content was higher in plots sown at the usual date (45-72 kg.ha^{-1}) because of summer soil N mineralization. In late winter, differences were observed in soil nitrogen content, due to differences in nitrogen fertilization during the autumn. As expected, soil mineral nitrogen content was higher in plots to which fertilizer was applied in autumn than in $\mathbf{N_{Low}}$ plots in late winter. Early sowing improved the nitrogen absorption of the oilseed rape crop (data not shown), as previously reported by Dejoux et al. (2003).

Differences in nitrogen availability during the vegetative stages led to differences in leaf development (Figure 2). Green leaf area index was significantly higher for $\mathbf{N_{High}}$ plots from sowing to spring re-growth than for $\mathbf{N_{Low}}$ plots, for both cultivars and sowing dates in both years ($P < 0.01$ in 2000/2001 and in 2001/2002 for the maximum GLAI from sowing to spring re-growth). Differences between $\mathbf{N_{High}}$ and $\mathbf{N_{Low}}$ plots were greatly reduced by late winter 2001/2002, because of leaf fall, whereas the differences remained large during the winter of 2000/2001. The lower temperatures recorded during the winter of 2001/2002 were associated with the observed differences in leaf fall between the two years of the experiment. Spring fertilization of the $\mathbf{N_{Low}}$ plots decreased the differences in GLAI caused by differences in nitrogen availability during the vegetative stage, although the difference between $\mathbf{N_{High}}$ and $\mathbf{N_{Low}}$ plots was still significant (at flowering; $P < 0.01$ for both years of experiment). On the $\mathbf{N_{Low}}$ plots, for both years and both cultivars, sowing at the usual date led to a greater GLAI at the third sampling than early sowing ($P < 0.01$ in 2000 and in 2001, for the sowing date x nitrogen availability

interaction). In both years, differences in GLAI due to sowing date were reduced in early winter. The differences between the GLAI values of the two cultivars were limited and, in most cases, were not significant for the seven sampling sets.

Differences in nitrogen nutrition index (NNI) were also observed between $\mathbf{N_{High}}$ and $\mathbf{N_{Low}}$ plots, from the two-leaf stage until late winter ($P < 0.01$, Figure 3). In $\mathbf{N_{High}}$ plots, NNI was generally 1 or more during the entire crop cycle, indicating that nitrogen was not limiting. In contrast, NNI was smaller than 1 for all $\mathbf{N_{Low}}$ plots before flowering, and was generally between 0.5 and 0.8 in both years. On $\mathbf{N_{Low}}$ plots, sowing at the usual time generally led to slightly greater NNI ($P < 0.054$ for the first three stages sampled in 2000 and 2001). These differences disappeared in early winter ($P = 0.10$ in 2000 and $P = 0.15$ in 2001). No difference in NNI was observed between the two cultivars analysed ($P = 0.15$ - 0.71 for the entire sample set in both years, except for flowering in 2002, when $P < 0.01$). As for GLAI, differences in NNI during the vegetative stage were reduced by spring fertilization of the $\mathbf{N_{Low}}$ plots in both years (for the effect of the nitrogen availability during the vegetative stages, $P = 0.55$ in 2001 and $P = 0.61$ in 2002, at flowering).

We observed differences between years in terms of changes in air-borne ascospore concentration over time (Figure 4). Two peaks were observed in 2000, on September 20th and October 20th. In autumn 2001, a major peak was observed on November 7th. The number of days with captured ascospores and the total number of ascospores detected were higher during autumn 2000 than during autumn 2001. The first days with a mean daily ascospore concentration greater than 1 spore per m^3 (*i.e.*, with more than 2

ascospores actually observed) were September 7th 2000 and September 20th 2001. On these dates, plots sown at **D_2** were at the cotyledon growth stage (GS 1.0) and the one-leaf growth stage (GS 1.1), whereas plots sown at **D_1** were at the six-leaf (GS 1.6) and eight-leaf growth stages (GS 1.8), in 2000 and 2001, respectively.

In both years, the maximum phoma leaf spot incidence was high for the **N_{High}** plots, regardless of sowing date, and for the **N_{Low}** plots sown at **D_2** (73-97%, Table 2), regardless of cultivar. Maximum incidence was lower on **N_{Low}** plots sown at **D_1** than on the other plots (29-50%). In 2001, the incidence was very low until late winter on **N_{Low}** plots sown at **D_1** (7% for cultivar **B**, 9% for cultivar **P**). For **N_{Low}** plots sown at **D_1**, disease incidence increased in late winter, due to the increased concentrations of air-borne ascospores (Figure 4). In both years, phoma leaf spot incidence decreased significantly at flowering (incidence range: 0 to 35%) and no phoma leaf spot was observed at crop maturity. Cultivar susceptibility to phoma stem canker had no significant effect on the incidence of phoma leaf spot. In 2000, the first phoma leaf spots were observed on September 3rd. Thus, infection must have occurred before the spore trap was set up on August 28th 2000, because leaf lesions do not develop until several days after infection (West et al., 2001).

The cultural practices analysed in this experiment led to a wide range of phoma crown canker severity in both years (Figure 5). Low levels of canker development were already visible in late winter in 2002. However, crown cankers really started to develop after flowering in both years. The year 2001/2002 was more favourable for disease development (DI of 2.2-8.8, at crop maturity) than 2000/2001 (DI of 1.4-5.9, at crop

maturity). In both years, DI was higher for $\mathbf{N_{High}}$ plots than for $\mathbf{N_{Low}}$ plots ($P < 0.01$ in 2001 and 2002, at crop maturity). Disease indices were higher for the usual sowing date than for early sowing ($P < 0.01$ in 2001 and $P = 2.9 \times 10^{-2}$ in 2002, at crop maturity). The expected difference in disease susceptibility between the cultivars was observed in 2002 ($P < 0.01$ at crop maturity) but not in 2001 ($P = 0.79$, at crop maturity). The only significant interaction observed in 2000/2001 was that between sowing date and nitrogen availability before late winter ($P < 0.01$); nitrogen had a greater effect on crops sown at the usual date than for crops sown early. In 2001/2002, there was a significant interaction between cultivar and sowing date ($P = 1.2 \times 10^{-2}$, at crop maturity), and between cultivar and nitrogen availability ($P < 0.01$, at crop maturity). The cultivar Pollen was more sensitive to cultural practices than the cultivar Bristol in the second year of the experiment.

4. Discussion

The different cultural practices led to differences in crop development, which in turn resulted in a wide range of phoma crown canker development in the two years. The sooner the infection occurs after emergence, the more severe are the symptoms at harvest. Thus, for both years of the experiment, early sowing shifted the period of maximum susceptibility to infection and the time at which the first significant release of ascospores was observed. This clearly reduced crown canker severity in crops sown earlier than usual. This finding is consistent with the results of some other studies (Gladders and Musa, 1980; Khangura and Barbetti, 2001), but conflicts with other studies in which sowing date was found to have no effect on phoma stem canker development (Kharbanda and Tewari, 1996; Dejoux et al., 2003) or late sowing was

found to restrict phoma development (MacGee and Emmet, 1977; Scheibert-Bohm, 1979; Lepage and Penaud, 1995). Other elements must be responsible for the reported variability of the effect of sowing date on phoma development.

The pattern of ascospore release clearly varies between regions and years, and contributes considerably to the variability observed. Factors affecting crop development may also influence the effect of sowing date on disease development. For instance, for a given pattern of ascospore concentration, the effect of early sowing depends on the physical conditions controlling emergence (drought, presence of a crust, sowing depth, etc). Thus, published results might not have been as contradictory as they initially appear if information other than sowing date and symptom severity had been taken into account (*e.g.*, ascospore release pattern, date of emergence). The results presented here are consistent with the notion that the risk of severe crown canker is increased if infection occurs soon after emergence. A frequency analysis of first ascospore release pattern is required to elucidate the effect of sowing date on phoma epidemics. In such an analysis, the effect of sowing date could be represented in a probabilistic manner, and could be used to take into account the risk of severe phoma epidemics when choosing the sowing date.

Nitrogen availability during vegetative stages greatly influenced phoma stem canker development. This finding conflicts with published results suggesting that nitrogen fertilization does not affect the percentage of stems of winter oilseed rape infected by *L. maculans* (Hall et al., 1993; Sadowski et al., 1998; Söchting and Verret, 2003). However, in the reported experiments, nitrogen fertilizer was applied in spring. Thus, our results may not conflict with those of previous studies if the timing of nitrogen

fertilization is taken into account. Several mechanisms may account for the effect of crop nitrogen status on phoma stem canker development. Higher nitrogen availability during the early growth stages results in larger leaves, which are more likely to come into contact with spores. When such leaves fall, they leave larger scars, which may act as an important point of entry for secondary inoculum (Pérès et al., 1996). Oilseed rape crops with high nitrogen contents are also more sensitive to frost than those with low nitrogen contents (Pellet et al., 2002). They may therefore present a larger number of frost wounds, and wounding is known to increase the incidence and severity of phoma stem canker (Hall, 1992). Furthermore, Snoeijers et al. (2000) speculated that a lack of nitrogen within the plant might result in the induction of several pathogenicity, avirulence/virulence genes. Thus, high nitrogen availability during vegetative stages may limit the transcription of induced pathogen genes by modifying the nutritional status of the plant. Finally, more extensive foliage development may modify the microclimate, increasing the rate of pathogen spore germination. Our experimental design precluded analysis of the respective contributions of these mechanisms.

The expected difference in disease susceptibility between cultivars was observed in 2001/2002, but not in 2000/2001. The reasons responsible for this difference in the results obtained in the two years are unknown. However, one key finding of this study was the interaction between cultivar susceptibility and cultural practices. In 2001/2002, the slightly susceptible cultivar Pollen was more sensitive to cultural practices (sowing date and nitrogen availability during vegetative stages) than the susceptible cultivar Bristol. Thus, the level of cultivar tolerance should perhaps be seen as only a rough indicator of phoma stem canker risk. Instead, the combination of cultural practices and

cultivar should be taken into account when assessing the risk of severe phoma stem canker epidemics. The existence of such an interaction may make it more difficult for breeders to assess the susceptibility of cultivars to phoma stem canker. However, the interaction observed in the second year of experiment still requires confirmation from the analysis of additional data.

Plots with severe crown cankers had high incidences of phoma leaf spot during autumn, but high phoma leaf spot incidence resulted in a wide range of severity of crown cankers. There was therefore no evidence of a consistent relationship between phoma leaf spot incidence during autumn and severity of crown canker at crop maturity. This is consistent with previous reports of a poor correlation between the incidences of phoma leaf spots and basal stem cankers (West et al., 2001). Further studies are required to analyse the pathway between primary infection and stem canker development.

In this study, some of the cultural practices were deliberately taken to extremes that are rarely applied in practice. In the Centre region of France, the 20th and 80th percentiles for sowing date were August 22nd and August 29th, respectively, in 2000 (CETIOM, 2002). We chose to study very early sowing dates to maximize the separation between the period of highest crop susceptibility and the initial flush of ascospores. Furthermore, only 5% of commercial fields are fertilized with mineral nitrogen in autumn (CETIOM, 2002). However, it has been reported that oilseed rape can absorb more than 250 kg N.ha^{-1} in early winter (Dejoux et al., 2003), a quantity consistent with the levels of nitrogen applied to the N_{High} plots in this study. High levels of mineral nitrogen fertilization in autumn were used to simulate levels of nitrogen availability that might

result from the application of organic manure in summer or over-fertilization of the preceding crop.

The use of different cultural practices made it possible to identify significant interactions between crop development and phoma stem canker development. The results presented provide insight into the effects of cultural practices on phoma stem canker development, but the interaction between crop management and cultivar in phoma stem canker development has to be substantiated with additional testing. Our results should make it possible to define cultural control strategies for containing the pathogenic agent to integrate the risk of phoma epidemics into proposals for innovative cultural practices for oilseed rape. However, although phoma stem canker is one of the major diseases of oilseed rape, effective recommendations for cultural control must also take into account other constraints, such as the control of other important pests, economic and environmental objectives (*e.g.*, optimization of gross margin, reduction of pesticide use). The integration of these multiple constraints will require the development of an integrated model representing the effects of cultural practices on yield formation, and the yield loss caused by the main pests of oilseed rape. The results presented should provide some of the information required to develop such a model for the formulation of integrated pest management strategies for oilseed rape, combining cultural, genetic and, as a last resort, chemical control methods.

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Table 1. Total soil nitrogen content (kg.ha^{-1}, at 0-90 cm depth) at sowing and in late winter as a function of sowing date and nitrogen availability during vegetative stages. 95% confidence intervals are indicated in brackets.

	At sowing[1]			In late winter[2]	
Sowing date[3]	2000/2001	2001/2002	**N availability**[4]	2000/2001	2001/2002
D1	19.7 (17.3)	50.4 (33.2)	$\mathbf{N_{Low}}$	12.1 (0.9)	16.8 (1.2)
			$\mathbf{N_{High}}$	16.4 (3.1)	107.8 (17.4)
D2	44.6 (16.6)	72.0 (33.1)	$\mathbf{N_{Low}}$	10.3 (1.4)	19.6 (10.0)
			$\mathbf{N_{High}}$	86.8 (38.4)	181.1 (70.8)

[1] Soil was sampled on August 2nd 2000 and August 1st 2001 for $\mathbf{D_1}$; and on September 19th 2000 and September 5th 2001 for $\mathbf{D_2}$.

[2] Soil was sampled on January 31st 2001 and February 4th 2002.

[3] $\mathbf{D_1}$: early sowing dates (August 3rd 2000 and July 31st 2001); $\mathbf{D_2}$: usual sowing dates (August 31st 2000 and September 4th 2001).

[4] Nitrogen availability during vegetative stages. $\mathbf{N_{Low}}$: no fertiliser applied during autumn; $\mathbf{N_{High}}$: 250 kg.ha^{-1} N was applied in three installments, within two months of sowing.

Table 2. Changes over time in the observed percentage of plants with at least one phoma leaf spot (incidence) in 2000/2001 and in 2001/2002. D_1: early sowing date; D_2: usual sowing date; B: cultivar Bristol, susceptible to phoma stem canker; P: cultivar Pollen, slightly susceptible; N_{High}: high nitrogen availability during vegetative stages; N_{Low}: low nitrogen availability during vegetative stages. Numbers in brackets are the lower and upper limits of the 95% confidence intervals (Agresti-Coull, 1998).

		Growth stage[1] or period						
Season	**Treatment**	GS 1.2[2]	GS 1.6[3]	GS 1.10[4]	Early winter[5]	Late winter[6]	GS 4.5[7]	GS 6.3[8]
2000/2001	$D1BN_{High}$	0 (0, 13)	37 (22, 55)	60 (42, 75)	83 (66, 93)	57 (39, 73)	17 (7, 34)	0 (0, 13)
	$D1PN_{High}$	0 (0, 13)	40 (25, 58)	63 (45, 78)	80 (62, 91)	40 (25, 58)	0 (0, 13)	0 (0, 13)
	$D2BN_{High}$	0 (0, 13)	90 (74, 97)	97 (82, 100)	87 (70, 95)	50 (33, 67)	0 (0, 13)	0 (0, 13)
	$D2PN_{High}$	3 (0, 18)	83 (66, 93)	93 (78, 99)	70 (52, 83)	40 (25, 58)	0 (0, 13)	0 (0, 13)
	$D1BN_{Low}$	0 (0, 13)	37 (22, 55)	7 (1, 22)	0 (0, 13)	7 (1, 22)	0 (0, 13)	0 (0, 13)
	$D1PN_{Low}$	0 (0, 13)	17 (7, 34)	7 (1, 22)	37 (22, 55)	43 (27, 61)	0 (0, 13)	0 (0, 13)
	$D2BN_{Low}$	10 (3, 26)	93 (78, 99)	40 (25, 58)	13 (5, 30)	10 (3, 26)	3 (0, 18)	0 (0, 13)
	$D2PN_{Low}$	10 (3, 26)	80 (62, 91)	43 (27, 61)	13 (5, 30)	13 (5, 30)	0 (0, 13)	0 (0, 13)
2001/2002	$D1BN_{High}$	0 (0, 2)	0 (0, 2)	81 (74, 86)	85 (79, 90)	80 (71, 86)	35 (25, 46)	0 (0, 13)
	$D1PN_{High}$	0 (0, 2)	0 (0, 2)	51 (44, 58)	73 (66, 79)	31 (25, 39)	25 (19, 33)	0 (0, 9)
	$D2BN_{High}$	0 (0, 2)	64 (58, 70)	91 (86, 94)	75 (69, 81)	54 (47, 62)	30 (23, 39)	0 (0, 13)
	$D2PN_{High}$	0 (0, 2)	82 (77, 86)	80 (75, 85)	81 (75, 85)	22 (17, 28)	24 (17, 31)	0 (0, 13)
	$D1BN_{Low}$	0 (0, 2)	0 (0, 2)	0 (0, 2)	7 (5, 11)	29 (24, 35)	17 (13, 22)	0 (0, 13)
	$D1PN_{Low}$	0 (0, 2)	0 (0, 2)	6 (4, 10)	9 (6, 13)	50 (44, 56)	4 (2, 7)	0 (0, 13)
	$D2BN_{Low}$	0 (0, 2)	55 (49, 61)	95 (91, 97)	65 (59, 71)	32 (27, 38)	8 (5, 13)	0 (0, 13)
	$D2PN_{Low}$	0 (0, 2)	82 (78, 86)	92 (88, 95)	69 (63, 74)	15 (11, 19)	3 (1, 6)	0 (0, 13)

[1] Sylvester-Bradley (1985): GS 1.2, second true leaf exposed; GS 1.6, sixth true leaf exposed; GS 1.10, tenth true leaf exposed; GS 4.5, flowering; GS 6.3, most seeds green.

[2] August 15st 2000 and August 15st 2001 for **D1**; September 18th 2000 and September 27th 2001 for **D2**.

[3] September 3rd 2000 and August 4th 2001 for **D1**; October 2nd 2000 and October 18th 2001 for **D2**.

[4] October 24th 2000 and November 6th 2001 for **D1**; November 6th 2000 and November 19th 2001 for **D2**.

[5] December 11th 2000 and December 13th 2001.

[6] February 12th 2000 and February 4th 2001.

[7] April 2nd 2000 and April 2nd 2001 for cultivar Bristol; April 9th 2000 and April 11th 2001 for cultivar Pollen.

[8] May 14th 2000 and May 13th 2001 for cultivar Bristol; May 21st 2000 and May 17th 2001 for cultivar Pollen.

Figure 1. Mean monthly temperature (**a**) and cumulated rainfall (**b**) for the months of the year. ■ 29-year average (1971/1999); □ 2000/2001; ▩ 2001/2002.

Figure 2. Changes in green leaf area index (GLAI) over time in 2000/2001 (**a**) and 2001/2002 (**b**). Error bars indicate 95% confidence intervals for the means of three replicates. ● **D_1BN_{High}**; ■ **D_1PN_{High}**; ◆ **D_2BN_{High}**; ▲ **D_2PN_{High}**; ○ **D_1BN_{Low}**; □ **D_1PN_{Low}**; ◇ **D_2BN_{Low}**; △ **D_2PN_{Low}**.

D_1: early sowing date; **D_2**: usual sowing date; **B**: cultivar Bristol, susceptible to phoma stem canker; **P**: cultivar Pollen, slightly susceptible; **N_{High}**: high nitrogen availability during vegetative stages; **N_{Low}**: low nitrogen availability during vegetative stages.

Figure 3. Changes in nitrogen nutrition index (NNI) over time in 2000/2001 (**a**) and 2001/2002 (**b**). Error bars indicate 95% confidence intervals for the means of three replicates. ● **D_1BN_{High}**; ■ **D_1PN_{High}**; ◆ **D_2BN_{High}**; ▲ **D_2PN_{High}**; ○ **D_1BN_{Low}**; □ **D_1PN_{Low}**; ◇ **D_2BN_{Low}**; △ **D_2PN_{Low}**.

D_1: early sowing date; **D_2**: usual sowing date; **B**: cultivar Bristol, susceptible to phoma stem canker; **P**: cultivar Pollen, slightly susceptible; **N_{High}**: high nitrogen availability during vegetative stages; **N_{Low}**: low nitrogen availability during vegetative stages.

Figure 4. Changes in mean daily concentration of air-borne ascospores of *Leptosphaeria maculans* over time in 2000/2001 (**a**) and 2001/2002 (**b**).

Figure 5. Changes over time in the disease index for phoma crown canker in 2000/2001 (**a**) and 2001/2002 (**b**). Error bars indicate 95% confidence intervals for the means of three replicates. ● **D_1BN_{High}**; ■ **D_1PN_{High}**; ◆ **D_2BN_{High}**; ▲ **D_2PN_{High}**; ○ **D_1BN_{Low}**; □ **D_1PN_{Low}**; ◇

D_2BN_{Low};

△ **D_2PN_{Low}**. **D_1**: early sowing date; **D_2**: usual sowing date; **B**: cultivar Bristol, susceptible to phoma stem canker; **P**: cultivar Pollen, slightly susceptible; **N_{High}**: high nitrogen availability during vegetative stages; **N_{Low}**: low nitrogen availability during vegetative stages.

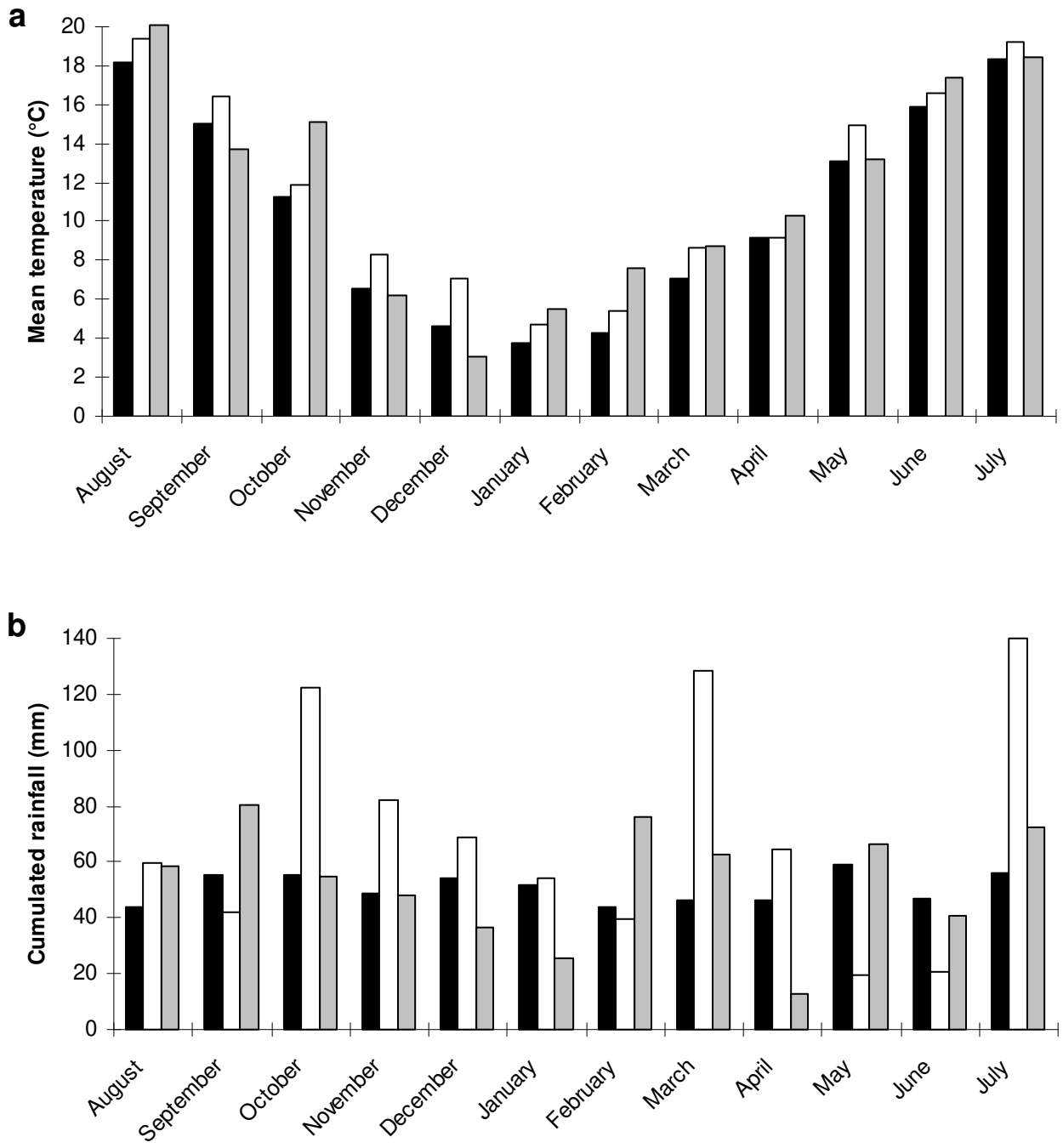
a
Mean temperature (°C)
0
2
4
6
8
10
12
14
16
18
20
August
September
October
November
December
January
February
March
April
May
June
July
b
Cumulated rainfall (mm)
0
20
40
60
80
100
120
140
August
September
October
November
December
January
February
March
April
May
June
July

Figure 1.

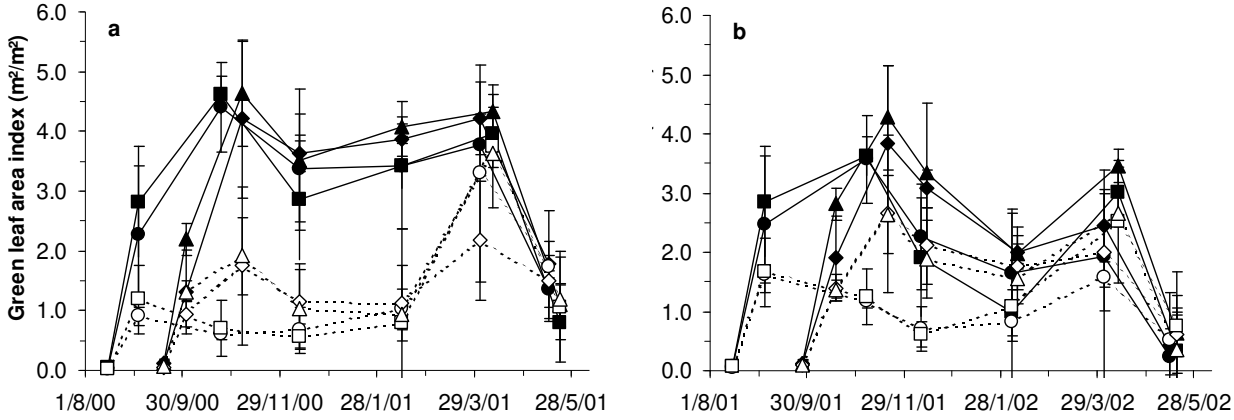
a
b
Green leaf area index (m²/m²)
6.0
5.0
4.0
3.0
2.0
1.0
0.0
1/8/00
30/9/00
29/11/00
28/1/01
29/3/01
28/5/01
1/8/01
30/9/01
29/11/01
28/1/02
29/3/02
28/5/02

Figure 2.

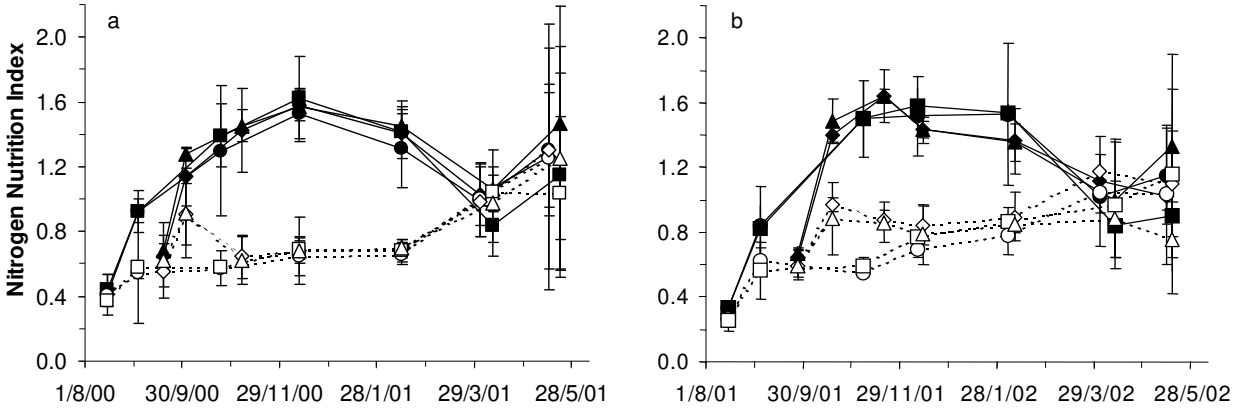
a
b
Nitrogen Nutrition Index
2.0
1.6
1.2
0.8
0.4
0.0
1/8/00
30/9/00
29/11/00
28/1/01
29/3/01
28/5/01
1/8/01
30/9/01
29/11/01
28/1/02
29/3/02
28/5/02

Figure 3.

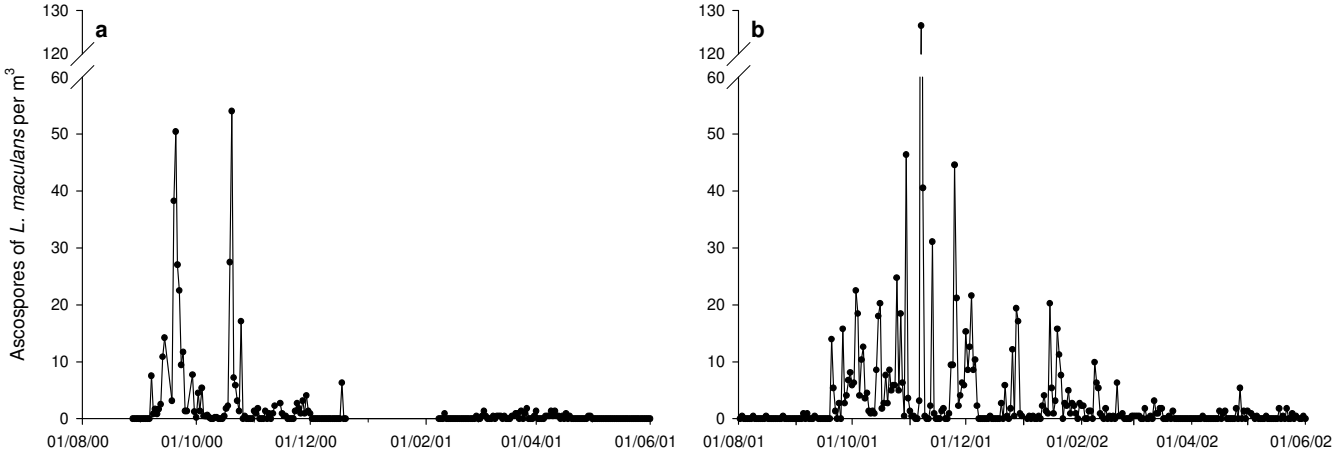
a
b
Ascospores of L. maculans per m3
130
120
60
50
40
30
20
10
0
01/08/00
01/10/00
01/12/00
01/02/01
01/04/01
01/06/01
01/08/01
01/10/01
01/12/01
01/02/02
01/04/02
01/06/02

Figure 4.

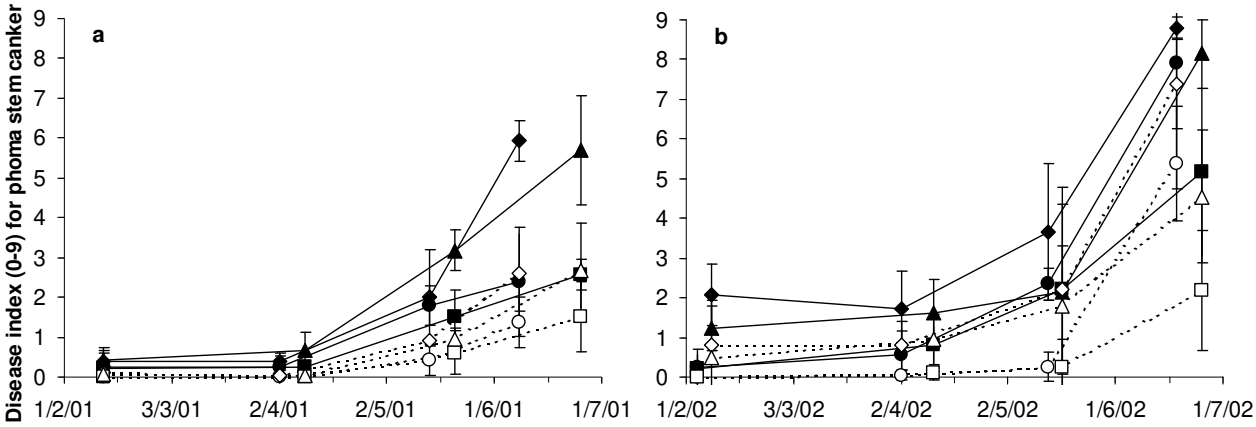
a
b
Disease index (0-9) for phoma stem canker
0
1
2
3
4
5
6
7
8
9
1/2/01
3/3/01
2/4/01
2/5/01
1/6/01
1/7/01
1/2/02
3/3/02
2/4/02
2/5/02
1/6/02
1/7/02

Figure 5.