# Methods for Sampling and Assessment in Relation to the Spatial Pattern of Phoma Stem Canker (Leptosphaeria maculans) in Oilseed Rape

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Aubertot J.N., Schott J.J., Penaud A., Brun H., Doré T., 2004 How can crown canker caused by *Leptosphaeria maculans* be assessed in an oilseed rape field? *Eur. J. Plant Path, 110 (2), 183-192.*

Doi: 10.1023/B:EJPP.0000015359.61910.3b

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25 text pages; 1 table page; 3 figure pages.

**Additional keywords**: blackleg, *Brassica napus*, disease assessment, disease spatial pattern, sampling.

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## Abstract

Sound assessment of phoma stem canker symptoms is needed to develop epidemiological, agronomical and physiological studies on the pathosystem. A specific analysis was therefore carried out to: (i) compare 4 methods of crown canker assessment; (ii) test the among and within assessor repeatability of one of the methods compared; (iii) characterise the spatial pattern of the disease, and (iv) define the sample size to achieve a given level of disease assessment precision. The methods compared examined the symptoms with different procedures and graded the plants observed into 6 severity classes. A disease index summarised the severity distribution observed. Examination of crown cross-sections was the most precise method for assessing crown cankers. The method was repeatable, though an "assessor effect" was apparent. The disease generally had a random pattern although significant spatial correlations were detected for 4 out of the 15 plots studied at the scales examined. A relationship between the coefficient of variation of the disease index and the sample size was established, evaluated with experimental field data, and exemplified for typical severity distributions.

## Introduction

Phoma stem canker (blackleg), caused by *Leptosphaeria maculans* (Desmaz.) Ces. & De Not. [anamorph *Phoma lingam* (Tode: Fr.) Desm.], is an oilseed rape (*Brassica napus* L.) disease with major economic consequences in Australia, Canada and Europe (West et al., 2001). Most research programs dealing with the pathogen require reliable estimation of the disease's symptoms. There are several reasons for assessing the disease: to compare the effects of one or several factors on stem canker development and/or yield loss in field experiments (*e.g.* to compare cultivars for resistance); and to perform a diagnosis, such as defined by Doré et al. (1997), of agronomic problems in cropping systems. Several studies have been carried out on direct and indirect methods for disease assessment, and related sampling methods for different crops (Cooke, 1998). However, few methodological studies on the assessment of stem canker disease severity have been carried out so far.

Rimmer and van den Berg (1992) listed methods used to evaluate phoma stem canker resistance in *B. napus* and *B. rapa*. Several rating scales applied to cotyledon, leaf and stem, in association with different screening methods, were reported. However, the disease is usually rated according to the observation of crown or stem cankers, since the incidence and severity of leaf lesions are unrelated to yield losses (McGee, 1973; Pierre and Regnault, 1982). Van den Berg et al. (1993) compared 4 rating scales to measure the severity of phoma stem canker based on (i) the length of the externally visible stem lesion; (ii) girdling at the hypocotyl and tap root by necrosis in the periderm; (iii) stem penetration of infected (discolored) tissue in the cortex and pith; and (iv) the area of infected (discolored) tissue in the cross-section of the hypocotyl and tap root. The authors recommended using the area of diseased tissue in a cross-section,

since the corresponding rating scale revealed the most symptoms. It is important to note that the amount of discolored tissue is not necessarily equivalent to the area infected. Infection hyphae can proceed into symptomless tissue some time before symptoms appear. The observation of the area of discolored tissue should therefore be considered more as an indicator than as a true measurement of disease development.

Disease incidence, disease severity, the area under the disease incidence curve, the area under the disease severity progress curve, and minimum and maximum incidence and severity were compared in order to rank genotypes of oilseed rape in terms of resistance to phoma stem canker (Rempel and Hall, 1996). Disease severity (measured as a percentage discoloration of a cross section of the stem base) and disease incidence (measured as a percentage of sampled plants with basal stem canker) in mature plants appeared to be the most appropriate measurement of resistance.

Gilligan (1980) analysed the size and shape of sampling units to estimate the incidence of stem canker in oilseed rape stubble in a field plot after swathing. Four sampling units of different sizes and shapes were compared. As expected, larger sampling units resulted in greater precision.

Although these methodological studies have improved phoma stem canker assessment, some questions remain unanswered. In France, field workers sometimes use different methods to assess *L. maculans* crown cankers. Irrespective of the assessment method used, plants are classified into 6 severity classes according to their crown canker severity. The severity distribution observed is summarised by a disease index. Does the method of crown canker assessment significantly modify the overall disease index? If so, what would be the most reliable method for observing crown canker? Are the methods of assessment repeatable among and within assessors? In addition to these

questions, field sampling requires clarification. Is the disease structured spatially within a field? If so, what would be the best strategy for sampling plants? How many observations are needed to remain below a given coefficient of variation of the disease index? Consequently, a methodological study was carried out to answer these questions.

## Materials and methods

Four methods of crown canker assessment commonly used in France were compared in several trials performed in different French regions. For one of the methods tested, the ratings of the same plants by 2 independent assessors were compared to test the among assessor repeatability of the method. Then, the rating was repeated by each assessor and compared in order to assess the within assessor repeatability of the method. The spatial pattern of the disease within several plots was characterised for 2 years in 2 regions. Lastly, a numerical analysis was performed to assess the number of observations needed to obtain a given level of precision in the disease index and field observations were used to evaluate the mathematical formula proposed.

### *Experiment details*

An experimental network (Exp. 1) was set up in 6 locations spread over several regions of France to compare 4 methods of crown canker assessment and the experiments took place at these locations in 1999/2000 and 2000/2001. The locations were: Grignon (Yvelines, Ile-de-France, 1999/2000), Guyancourt (Yvelines, Ile-de-France, 1999/2000), Le Rheu (Ille-et-Vilaine, Bretagne, 2000/2001), Mondonville (Haute-Garonne, Midi-Pyrénées, 1999/2000), Oucques (Loir-et-Cher, Centre, 1999/2000), and Toury (Eure-et-Loir, Centre, 2000/2001). Six cultivars of different

susceptibilities to phoma stem canker were sown at each location in a complete block design with 4 replications: Eurol, Falcon, Vivol, Jet Neuf, Columbus, Goéland. The sowing dates ranged from August 30$^{th}$ to September 24$^{th}$ in the different locations and years. After sowing, infected stubble was arranged (about 1 per m$^2$) on each plot (4 m x 1.5 m) in order to increase inoculum levels of *L. maculans*.

An experiment (Exp. 2) was carried out in 1999/2000 at the INRA Experimental Centre of Grignon (Yvelines, Ile-de-France, France) to test the within and among assessor repeatability of *L. maculans* crown canker assessments and to characterise the spatial pattern of the disease. Nine plots (51 m x 30 m) were sown on August 26$^{th}$ with cultivar Capitol. A third experiment (Exp. 3) was carried out in 2001/2002 at the INRA Experimental Centre of Grignon and at Le Louroux (Indre et Loire, Centre) to supplement the disease spatial pattern analysis. At Grignon, 3 plots (51 m x 30 m) were sown with cultivar Capitol on August 29$^{th}$. At Le Louroux, 3 plots (51 m x 30 m) were sown on August 20$^{th}$ with cultivar Pollen. In each site of Exp. 2 and 3, oilseed rape crops were naturally infected by the inoculum of the experiment area.

*Disease assessment*

Four methods of crown canker assessment were compared in Exp. 1: scraping (**Scr**), section (**Sec**), scraping and section (**Scr+Sec**) and visual assessment of the crown circumference (**Vis**). At crop maturity (growth stage 5.3-5.5: seeds green-brown to brown in lower pods; Harper and Berkenkamp, 1975), 2 samples of 60 plants were collected in each elementary plot (2 adjacent rows x 30 consecutive plants or 3 adjacent rows x 20 consecutive plants) for all the sites except at Oucques, where only 1 sample of 60 plants was collected per plot. All the assessments of a given site were performed

by the same assessor. For the first sample, the plants were assessed after scraping the circumference of the crowns with a knife (**Scr**). A second assessment was performed after sectioning the plants at crown level (**Scr+Sec**). For the second sample, plants were graded according to the visual assessment of the crown circumference (**Vis**). A second grading was performed after sectioning the plants at crown level (**Sec**). At Oucques, only the **Scr** and **Scr+Sec** methods were performed. At Toury, the **Vis** method was not performed. For each method, the plants were graded according to 6 severity classes. For **Scr** and **Vis**, the classes were: 1, no canker observed; 2, canker weakly developed; 3, canker developed on less than half of the crown circumference; 4, canker developed on more than half of the crown circumference; 5, canker almost developed on the whole crown circumference; 6, plant lodged or broken during sampling. For **Scr+Sec** and **Sec**, the classes were: 1, healthy plant, no visible lesion; 2, canker weakly developed; 3, canker developed on less than half of the crown section; 4, canker developed on more than half of the crown circumference; 5, canker almost developed on the whole crown section; 6, section without any living tissue, plant lodged or broken at the crown level during sampling.

For Exp. 2 and Exp. 3, 120 plants were collected from positions corresponding to the 8 x 15 points of intersection of a 3.3 m x 3.3 m grid in each plot, at crop maturity (growth stage 5.3-5.5: seeds in lower pods green-brown to brown; Harper and Berkenkamp, 1975)". For Exp. 2 and Exp. 3, crown cankers were rated according to the **Sec** method. One of the 2 assessors of Exp. 2 graded the plants of Exp. 3.

In order to summarise the observed severity class distribution, a disease index (*DI*, derived from Pierre and Regnault, 1982) widely used in France, was calculated as follows:

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

where $n_i$ is the number of plants in category $i$. $DI$ increases with crown canker severity, starting from 0 for healthy plants to 9 for completely lodged plants.

*Among and within assessor repeatability of crown canker assessment*

Two assessors were trained by experts on real plants, using sketches and pictures. Each sampled plant from the 9 plots at Grignon (Exp. 2) was labeled individually. The 2 assessors rated the plants from 7 plots simultaneously and independently according to the **Sec** method. Some of the plots were rated twice by each assessor (6 plots for assessor 1 and 5 plots for assessor 2) or by one only at intervals ranging from a half day to 2 days. The plants were cut at cotyledon scar level in order to prevent any variability due to the localisation of the section. Between 2 assessments, the plants were stored at 4°C in order to prevent the symptoms from progressing. All the available assessments were gathered to test the among and within assessor repeatability. The variable analysed was the difference between 2 crown canker classes of the same plant (pair wise comparison).

*Spatial analysis*

Data from Exp. 2 and 3 were analysed by mapping and by distance class analyses. For mapping, the plants were represented individually by quadrats with shading increasing with disease intensity, ranging from white for class 1 to black for class 6. Distance class analyses were performed on each of the 15 plots of Exp. 2 and 3

using the 2DCORR method (Ferrandino, 1998). The data were first split into two classes: healthy to moderately infected plants (severity classes ≤ 3), and severely infected plants (severity classes > 3). The analysis consisted in: i) testing probabilities of deviation from a random arrangement for all possible distance classes using conservative Bonferroni tests (at a level of confidence $\alpha = 1-(1-0.05)^{1/224}$); and ii) in performing radial correlation analyses. The radial correlation analysis is based on the comparison of observed and predicted (under the assumption of randomness) cumulative probability density functions for the total number of severely infected plant pairs within a given distance. The maximum value of the difference between observed and predicted cumulative probability distribution functions was used as a Kolmogorov-Smirnov statistic at a level of confidence $\alpha = 0.05$. The distance at which maximum deviation occurs provided a measurement of the spatial range of correlation.

*Sample size determination*

Disease scores are often summarised by an index. Regarding the general case of a disease rated according to k categories, disease indexes are usually calculated as follows:

$$DI = \frac{\sum_{i=1}^{k} c_i n_i}{n} \qquad [3]$$

where $n_i$ is the number of plants in category $i$; $c_i$ denotes arbitrary coefficients and $n = \sum_{i=1}^{k} n_i$ is the total number of plants observed. If we consider the assessments as a sequence of independent, identically distributed random variables, plant rating corresponds to a process of multinomial trials. Let $p_i$ be the probability that an

assessment is in category $i$, for $i = 1, 2, \ldots, k$, with $\sum_{i=1}^{k} p_i = 1$. Under these assumptions, it can be shown (Appendix, equations A2 and A3) that *DI* has the following expected value and variance:

$$E(DI) = \sum_{i=1}^{k} c_i p_i \qquad [4]$$

$$VAR(DI) = \frac{1}{n}\left( \sum_{i=1}^{k} c_i^2 p_i (1 - p_i) - 2 \sum_{i=1}^{k-1} \left( c_i p_i \sum_{j=i+1}^{k} c_j p_j \right) \right). \qquad [5]$$

The coefficient of variation of *DI* is defined by:

$$CV = \frac{\sqrt{VAR(DI)}}{E(DI)} \qquad [6]$$

It can be therefore written as:

$$CV = \sqrt{\frac{\sum_{i=1}^{k} c_i^2 p_i (1 - p_i) - 2 \sum_{i=1}^{k-1} \left( c_i p_i \sum_{j=i+1}^{k} c_j p_j \right)}{n \left( \sum_{i=1}^{k} c_i p_i \right)^2}} \qquad [7]$$

and sample size can be calculated as a function of coefficients $c_i$, $p_i$, and the coefficient of variation of *DI*:

$$n = \frac{\sum_{i=1}^{k} c_i^2 p_i (1 - p_i) - 2 \sum_{i=1}^{k-1} \left( c_i p_i \sum_{j=i+1}^{k} c_j p_j \right)}{CV^2 \left( \sum_{i=1}^{k} c_i p_i \right)^2}. \qquad [8]$$

Equation 7 was evaluated with field data for two sample sizes. The evaluation consisted in comparing an observed *DI* coefficient of variation of each plot of Exp. 2 and 3 to the value predicted by equation 7, assuming that the real $p_i$ distribution of the plot was equal to the observed distribution. A sampling strategy was chosen arbitrarily. It consisted in observing consecutive plants chosen at random within the 8 x 15 square grid according to a North-South or East-West transect. Because only 8 x 15

observations were available on each plot, the evaluation was performed on small sample sizes to obtain a sufficient number of estimations of *DI* to evaluate its coefficient of variation. The observed *DI* coefficient of variation of a given plot $CV^{Obs}$ was calculated as follows:

$$CV^{Obs}=\frac{\sqrt{\sum_{i=1}^{n_s}\left(DI_i-\overline{DI}\right)^2}}{\overline{DI}\sqrt{n_s-1}} \quad [9]$$

where $n_s$ represents the number of possible ways to sample the plants within the square grid according to the chosen sampling strategy, $DI_i$, the i$^{th}$ estimation of *DI* and

$$\overline{DI}=\frac{1}{n_s}\sum_{i=1}^{n_s}DI_i \ , \quad [10]$$

is the mean observed value of *DI*.

Lastly, a sensitivity analysis of equation 8 was performed as a function of the distribution of $p_i$, with $k = 6$, $c_1=0$ and $c_i = 2(i-2)+1$ for $i = 2$ to 6. Three contrasted severity distributions observed in Exp. 1 were taken as 3 real populations: weakly infected plants (0.24, 0.42, 0.31, 0.03, 0, 0; *DI* = 1.5); moderately infected plants (0.02, 0.27, 0.13, 0.28, 0.15, 0.15; *DI* = 4.5); and severely infected plants (0, 0, 0, 0, 0.75, 0.25; *DI* = 7.5). The first 6 numbers in the parenthesis are the probabilities associated with each crown canker severity class: $p_1$, $p_2$, $p_3$, $p_4$, $p_5$, $p_6$. Another moderately infected plant distribution, observed in Exp. 1, was added to the analysis to exemplify the effect of the severity distribution on *CV* for a given *DI*: (0.03, 0.07, 0.15, 0.67, 0.05, 0.03; *DI* = 4.5).

***Statistical analysis***

Statistical analysis was carried out using procedures from SAS Release 6.12 for Windows (SAS Institute Inc., 1989). Analyses of variance were performed with the GLM procedure. Data collected in Exp. 1 were examined by an analysis of variance. The Student-Newman-Keuls test was used to compare *DI* obtained by the different methods. Furthermore, an analysis of variance was performed for each site and for each method of crown canker assessment. For each method of assessment, coefficients of variation were averaged over the sites to provide a general comparison of their accuracy. The Wilcoxon non-parametric signed rank test of the Univariate procedure was used to analyse whether the mean difference between the crown canker severity classes given by 2 assessors was significantly different from zero (Exp. 2, pairwise comparison). The mean difference between 2 crown canker severity classes given by an assessor to identical plants was analysed in the same way. An analysis of variance was performed on the data collected in Exp. 2 on *DI* obtained on several plots by 2 assessors at 2 different times. The prediction performance of equation 7 was evaluated by calculating the root mean square error (RMSE), the mean relative absolute error (MRAE), and mean bias error (MBE) across all the 15 plots of Exp. 2 and 3. These measurements of prediction deviation were calculated as follows:

$$RMSE=\sqrt{\frac{1}{n}\sum_{i=1}^{n}\left(CV_i^{Obs}-CV_i^{Pred}\right)^2} \quad [11]$$

$$MRAE=\frac{1}{n}\sum_{i=1}^{n}\frac{\left|CV_i^{Obs}-CV_i^{Pred}\right|}{CV_i^{Obs}} \quad [12]$$

$$MBE=\frac{1}{n}\sum_{i=1}^{n}\left(CV_i^{Pred}-CV_i^{Obs}\right) \quad [13]$$

where $CV_i^{Obs}$ are the observed *DI* coefficients of variation, *n* is the total number of $CV_i^{Obs}$, $CV_i^{Pred}$ are the predicted *DI* coefficient of variation, and $\overline{CV}$ is the mean of the observed $CV_i^{Obs}$.

## Results

*Comparison of 4 methods of crown canker assessment*

The analysis of variance performed on the results of Exp. 1 revealed significant effects of assessment method ($df = 3$, $P < 10^{-4}$), cultivar ($df = 5$, $P < 10^{-4}$) and site ($df = 5$, $P < 10^{-4}$, confounded with the effects of season and assessor) on *DI*, as well as interactions between site and cultivar ($df = 25$, $P < 10^{-4}$), and site and method ($df = 12$, $P < 10^{-4}$). The effects of site, cultivar and their interaction were expected. The interaction between site and method most likely resulted from discrepancies in the way plants were classified amongst the assessors of each site. Interaction between cultivar and method of assessment was not significant ($P = 0.91$). This indicates that each method could be used successfully to rank cultivars consistently. However, the 4 methods tested led to significant discrepancies in *DI*. *DI* obtained by **Scr+Sec** and **Sec** were not significantly different from each other but they were significantly higher than **Scr** and **Vis** (Table 1). **Scr** and **Vis** were also significantly different from each other (Table 1). The 4 methods of assessment compared led to 3 distinct groups of Disease Index values. However, the mean values observed were within a relatively narrow interval ($2.3 \leq DI \leq 2.6$) and did not modify cultivar susceptibility ranking (data not shown). **Sec** was the most precise method of crown canker assessment. The mean coefficient of variation for the 6 sites was: 0.176 for **Sec**, 0.193 for **Scr+Sec**, 0.204 for **Scr** and 0.218 for **Vis**.

*Among and within assessor repeatability of crown canker assessment*

The difference between the classes given by 2 assessors for the same plants was statistically different from 0 (mean of the difference = 0.12; $p < 0.001$; n = 2400). However, the classes given by the 2 assessors were the same or differed only by one category in 96% of cases (plants were graded with exactly the same score in 54% of cases).

The differences between the classes given by an assessor for the same plants at different times were not statistically different from 0 for either assessor (mean of the difference = $-4.7 \times 10^{-2}$; p = 0.09; n = 720 for assessor 1; mean = $-4.2 \times 10^{-3}$; p = 0.81; n = 480 for assessor 2). The classes were the same or differed only by one category in 95% of cases (plants were graded with the same score in 57% of cases). The crown canker assessment appeared to be repeatable for each assessor, even though minor discrepancies were observed. The analysis of variance performed on *DI* revealed that neither the "assessor factor" (df = 1, P = 0.57), nor the "repetition factor" (df = 1, P = 0.79) was significant. In addition, plot * assessor interaction was not significant (df = 1, P = 0.23).

*Spatial analysis*

The visual examination of spatial data maps of Exp. 2 and 3 plots did not provide strong evidence for departure from randomness (Figure 1). None of the Bonferroni tests performed on all the distance classes was significant. However, the Kolmogorov-Smirnov tests revealed a significant spatial correlation of like pairs of plants for 4 plots out of the 15 analysed. For these cases, the maximum value of the

difference between observed and predicted cumulative probability functions ranged from 5 to 6 distance units (17 to 20 m).

*Sample size determination*

Equation 7 correctly predicted the Disease Index coefficient of variation for both sample sizes tested on the 15 plots of Exp. 2 and 3 (Figure 2), even if slight biases were observed. The error measurements were: for sample size = 2, RMSE = 0.020, MRAE = 0.036, MBE = -0.012; for sample size = 8, RMSE = 0.020, MRAE = 0.089, MBE = $5.5 \times 10^{-3}$. RMSE was respectively lower than 5% and 10% of the mean observed *DI* coefficient of variation for a sample size of 2 and 8.

Figure 3 illustrates equation 8. It allows the determination of sample size as a function of the level of precision required defined by *CV* for four severity distributions. Disease severity distribution greatly influences the sample size required. For a given level of *CV*, the required sample size for the weakly infected severity distribution is 5.3 times greater than that required for the first moderately infected distribution and 57 times greater than that required for the severely infected severity distribution. The second moderately infected distribution ($DI = 4.5$) requires 1.9 times the number of plants needed for the first moderately infected distribution ($DI = 4.5$) for a given level of *CV*. For a sample of 60 plants, *CV* ranges from 0.015 to 0.11 for the 4 severity distributions studied.

## Discussion

There are several reasons why the observation of crown cross-sections should be preferred when assessing phoma stem canker symptoms. Firstly, sectioning the plant at

crown level and assessing the proportion of diseased tissues appeared to be the most precise method of assessing crown canker. Secondly, the assessment of a cross-section is more closely linked with disturbance of plant physiology than external symptoms, which are only rough indicators of the disease. In addition, sectioning the plant appeared to be an easy and fast way of proceeding. This recommendation is consistent with those of van den Berg et al. (1993), who advised the use of a scale based on the area of diseased tissue in a cross-section of the plant. They pointed out that the rating for the area of discolored tissue in the cross-section of the hypocotyl and tap root was higher than the rating for stem penetration. They concluded that preference should be given to the rating scale that reveals the most symptoms, *i.e.* the area of diseased tissues in the cross-section of hypocotyl and tap root. However, even at crown level, the symptoms observed may differ according to the location of the cross-section. Three procedures can be considered to address this problem. Firstly, plants could be cut at cotyledon scar level, in order to standardise disease assessment. Second, several successive sections located at crown level could be assessed. This would provide more detailed information but would be time-consuming. Lastly, plants could be cut at crown level where the external symptom is most serious, if visible externally. A specific study is needed to assess the effects of cross-section location at crown level in disease assessment.

The classes assigned by different assessors differed slightly but significantly. This drawback must be taken into consideration when assessing the disease, especially when numerous observations requiring several assessors are needed. Thus the “assessor factor” can be integrated in the blocking factor. Whenever several assessors are required to rate an experiment, each block should be rated by one and only one assessor to

account for the "assessor factor". To increase the repeatability of the assessment and prevent any drift, several procedures can be considered. The simplest way to proceed is to frequently refer to sketches and pictures during observation. We propose more precise definition of the severity classes to be used as a function of the percentage of the discolored section: 1, healthy plant, no visible lesion; 2, 0-25% of the discolored cross-section; 3, 25-50% of the discolored cross-section; 4, 50-75% of the discolored cross-section; 5, 75-100% of the discolored section; 6, section without any living tissue, plant lodged or broken at the crown level during sampling. This proposition is consistent with the recommendation to avoid arbitrary categories in order to maintain standardisation of assessment keys (Cooke, 1998) and is compatible with an existing rating scale (Gugel et al., 1990). Furthermore, a computer-aided training program could be developed in order to train assessors better. This method has been developed successfully for other crops and diseases (Nutter and Schultz, 1995; Cooke, 1998). Lastly, image analysis could also be used to enhance the accuracy and precision of assessment. The drawback of this technique is that it may need substantial development before it is operational and, moreover, it may be much more time-consuming than visual assessment.

No particular disease spatial structure was observed on a general level. For each plot analysed, oilseed rape had not been cultivated for at least 4 years on the experimental plots. Local inoculum within the plots can therefore be considered as negligible (West et al., 2001). Plants were infected by the natural inoculum of the surroundings of the experiment area. The typical distances analysed ranged from 3 to 52 m. These distances are relatively small compared with typical distances of ascospore dispersion, which can reach several kilometers (Hall, 1992; West et al., 2001). Thus, the general lack of disease spatial pattern observed for the 9 plots of Exp. 2 and for 2 plots

of Exp. 3 is coherent with the typical distance of ascospore dispersion. The spatial correlations observed for 4 plots of Exp.3 may have resulted from spatial heterogeneity within the plots. Spatial heterogeneity of environmental factors (such as soil nitrogen availability) or crop characteristics (such as plant density) may have influenced the development of the disease. However, the experiment set up did not permit investigating these hypotheses. Further studies are still needed to investigate disease spatial patterns at smaller and larger scales. Because the study revealed spatial patterns in some situations, caution is required when defining the sampling design within a plot. It is prudent to consider using stratified sampling which has the advantage of ensuring that samples are taken from each section of a field (Campbell and Madden, 1990).

The sample size depends on the severity distribution, not only because severity distribution directly affects the expected *DI,* which is inversely proportional to *CV*, but also because it affects the standard deviation of *DI*. The 2 moderately infected distributions in Figure 3 had similar values of *DI* (4.5), but their *CV* differed by a factor of 1.9. Since the severity distribution within a plot is not known *a priori*, it is first necessary to estimate it to determine the sample size required to remain below a given *CV*. The following procedure can be proposed: (i) choose a maximum value for *CV*; (ii) assess the disease distribution (say, with n = 30); (ii) use equation 8 with the observed distribution to define the sample size; (iii) complete assessments to obtain the sample size calculated; (iv) estimate *CV* using equation 7; (v) if *CV* is greater than the chosen threshold, repeat steps (ii), (iii), (iv) and (v). In some cases, the first sample size required to assess the disease distribution (n = 30) may be greater than the sample size required for *DI* at a given level of precision. Although these cases require additional work, precision on *DI* is increased. Equation 7 is valid only if the assessments can be

considered as a sequence of identically distributed random and independent variables. This implies that the size of the population analysed, *i.e.* the plants in the field studied, must be quasi-infinite compared with the size of the sample. This constraint is generally fulfilled for plots such as commercial fields.

The use of a Disease Index appeared to suffer from several drawbacks. Firstly, the information contained in one number is inevitably poorer than that contained in several numbers describing a distribution. For instance, different distributions can lead to the same Disease Index. Consequently, it may be prudent to look at complete distributions in addition to Disease Indexes when disease assessment is performed according to categories. Moreover, the coefficients involved in the calculation of the Disease Index used are arbitrary and are not directly related to yield loss. This difficulty should be overcome by a study aimed at assessing yield losses associated with each disease severity class. Thus, a less arbitrary weighting could be used to define a new Disease Index.

## Appendix

Consider the general case of a disease characterised by $n$ sampled units graded according to $k$ severity classes. Grading corresponds to a process of multinomial trials if the observations can be viewed as a set of independent and identically distributed realisations of a discrete random variable. Let $p_i$ be the probability that an assessment is in category $i$ ($\sum_{i=1}^{k} p_i = 1$). A disease index can be used to summarise the severity distribution observed. It is commonly written as:

$$DI = \frac{\sum_{i=1}^{k} c_i n_i}{n} \qquad [A1]$$

where $n = \sum_{i=1}^{k} n_i$ if the number of sampled units, $k$, is the number of severity classes, $n_i$ is the number of observations in class $i$, and $c_i$ are coefficients. The expected value and variance of the Disease Index can therefore be calculated as follows.

*Calculus of the expected value of the Disease Index E(DI)*

According to equation A1, the expected value of $DI$ is:

$$E(DI) = E\left(\frac{1}{n}\sum_{i=1}^{k} c_i n_i\right),$$

which can be also be written as:

$$E(DI) = \frac{1}{n}\left(\sum_{i=1}^{k} c_i E(n_i)\right).$$

Since $E(n_i) = np_i$ (Wackerly et al., 1996), the expected value of the Disease Index is:

$$E(DI) = \sum_{i=1}^{k} c_i p_i \qquad [A2]$$

*Calculus of the variance of the Disease Index VAR(DI)*

According to equation A1, the variance of DI is:

$$VAR(DI)=\frac{1}{n^2}VAR\left(\sum_{i=1}^{k}c_i n_i\right).$$

This expression can be developed as:

$$VAR(DI)=\frac{1}{n^2}\left(\sum_{i=1}^{k}c_i^2 VAR(n_i)+2\begin{pmatrix}c_1c_2COV(n_1,n_2)+...+c_1c_kCOV(n_1,n_k)+c_2c_3COV(n_2,n_3)+...\\+c_2c_kCOV(n_2,n_k)+...+c_{k-1}c_kCOV(n_{k-1},n_k)\end{pmatrix}\right),$$

where $COV(n_i,n_j)$ is the covariance of variables $n_i$ and $n_j$. Since $VAR(n_i)=np_i(1-p_i)$ and $COV(n_i,n_j)=-np_ip_j$ (Wackerly et al., 1996). The expression can be simplified to yield:

$$VAR(DI)=\frac{1}{n}\left(\sum_{i=1}^{k}c_i^2 p_i(1-p_i)-2\sum_{i=1}^{k-1}\left(c_i p_i\sum_{j=i+1}^{k}c_j p_j\right)\right) \qquad [A3]$$

**Acknowledgements**

The authors thank D Le Floch and R Gosse for their technical assistance, J Troizier and his team for field trial management (Exp. 2 and 3) and all those in charge of the experimental network (Exp. 1). The authors would like also to acknowledge F Ferrandino for his help in providing the software for performing 2DCORR analysis and giving useful advice for the spatial analysis. In addition they are grateful to the anonymous referees for their very helpful comments. A Champeil and H Dupré de Boulois analysed some of the data. The English text was checked by Keith Hodson.

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**Table 1. Comparison of mean phoma stem canker disease indexes (*DI*[1]) obtained with four methods of assessment for six sites and six winter oilseed rape cultivars (Exp. 1).**

| Method of assessment[2] | Mean *DI* | N[3] | SNK grouping[4] |
|---|---|---|---|
| **Scr+Sec** | 2.64 | 144 | **A** |
| **Sec** | 2.59 | 120 | **A** |
| **Scr** | 2.41 | 144 | **B** |
| **Vis** | 2.26 | 96 | **C** |

[1] $DI = \sum_{i=2}^{6}[2(i-2)+1]n_i / \sum_{i=1}^{6} n_i$, where $n_i$ is the number of plants in category *i*.

[2] **Scr**: scraping; **Sec**: section; **Vis**: visual assessment of the crown circumference.

[3] Number of *DI* assessments.

[4] Student-Newman-Keuls test for *DI*. Means with the same letter are not significantly different ($P \leq 0.05$).

**Figure captions**

**Figure 1.** Maps of spatial pattern data observed for Exp. 2 and 3. Plants were sampled on a square grid (3.3 m x 3.3 m, 120 plants per plot). Each quadrat represents the symptom severity observed on a plant. Shading increases as a function of disease severity class:

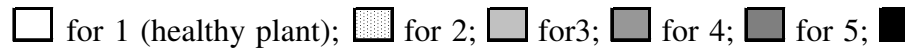 for 1 (healthy plant); for 2; for3; for 4; for 5; 6 (lodged plant). For convenience, the relative position of the plots was not represented. a: Exp. 2, Ile-de-France 1999/2000; b: Exp. 3, Ile-de-France 2001/2002; c: Exp. 3, Centre 2001/2002.

**Figure 2.** Comparison of predicted and observed Disease Index coefficient of variation for the 15 plots of Exp. 2 and 3. For each plot, the Disease Index coefficient of variation was predicted using equation 7, assuming that the real $p_i$ distribution was equal to the observed distribution. The observed Disease Index coefficient of variation was calculated using all the possible values that the Disease Index could take if n consecutive plants were sampled at random within the 8 x 15 square grid according to a North-South or East-West transect. ●: n = 2 sampled plants, ○: n = 8 sampled plants. The straight line is the 1:1 line.

**Figure 3.** Sample size as a function of coefficient of variation of the disease index $DI = \sum_{i=2}^{6}[2(i-2)+1]n_i / \sum_{i=1}^{6} n_i$ for 4 severity distributions: ····· weakly infected plants (0.24, 0.42, 0.31, 0.03, 0, 0; $DI$ = 1.5); ——— moderately infected plants 1 (0.03, 0.07, 0.15, 0.67, 0.05, 0.03; $DI$ = 4.5); — — moderately infected plants 2, same $DI$ but with another distribution (0.02, 0.27, 0.13, 0.28, 0.15, 0.15; $DI$ = 4.5); and ——— severely infected plants (0, 0, 0, 0, 0.75, 0.25; $DI$ = 7.5). The first 6 numbers in parentheses are

1 the probabilities of occurrence of each crown canker severity class. The sample size was
2 calculated using equation 8.
3

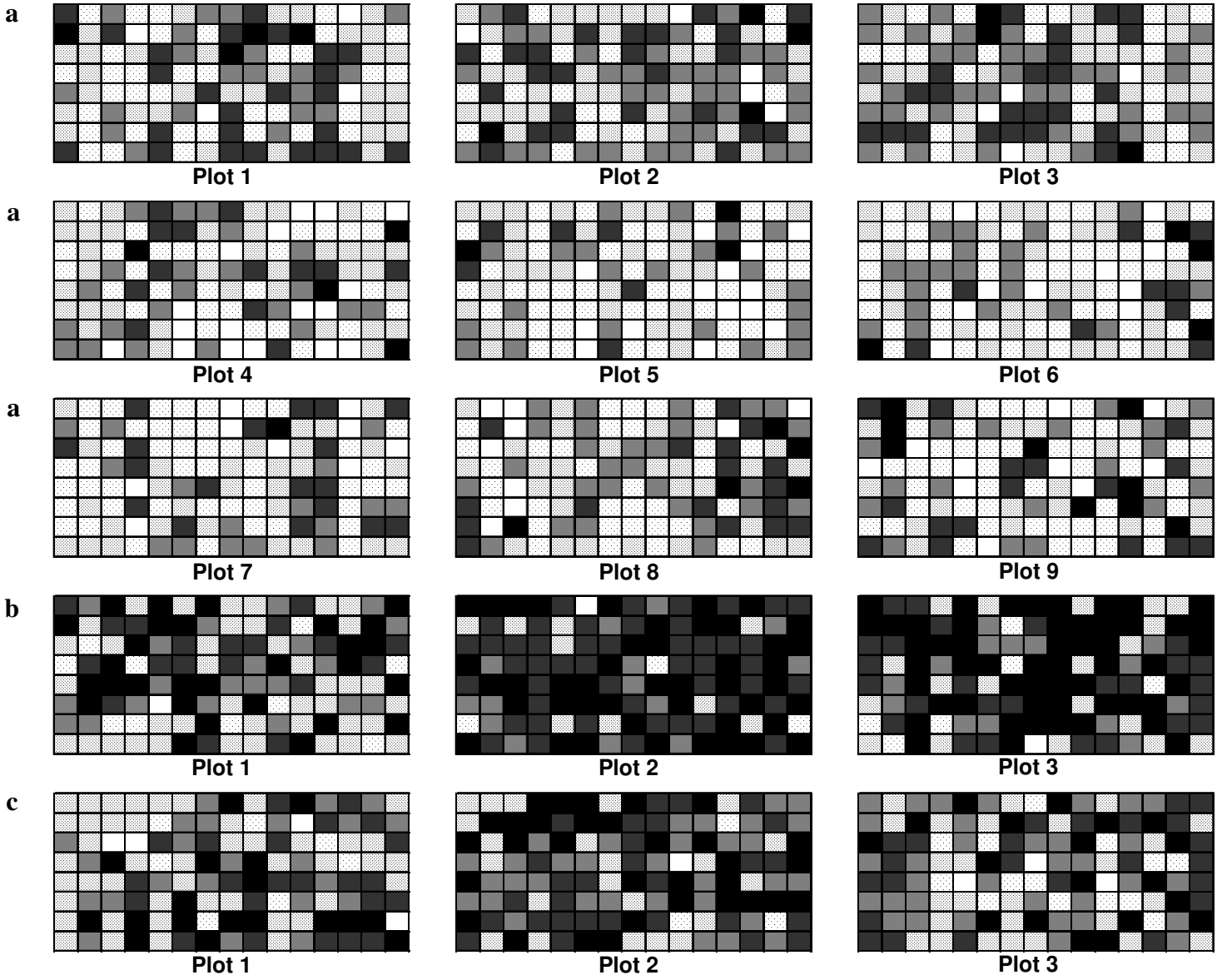
a
Plot 1
Plot 2
Plot 3
a
Plot 4
Plot 5
Plot 6
a
Plot 7
Plot 8
Plot 9
b
Plot 1
Plot 2
Plot 3
c
Plot 1
Plot 2
Plot 3

**Figure 1.**

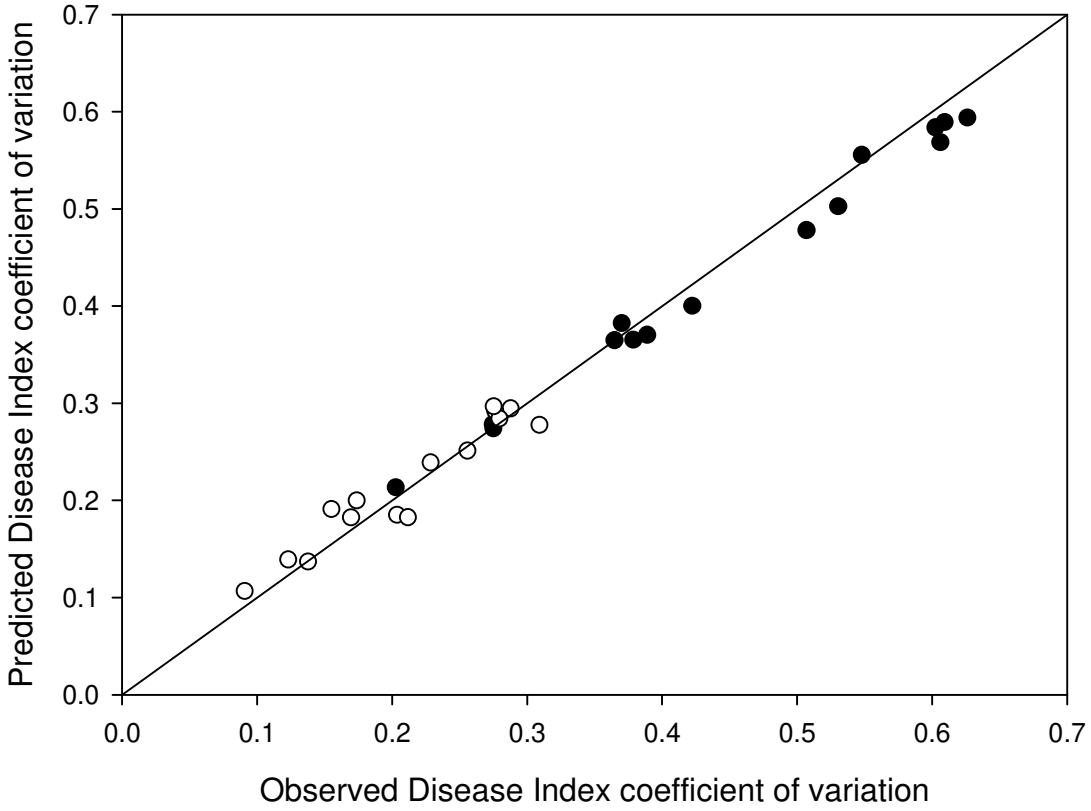
Predicted Disease Index coefficient of variation
Observed Disease Index coefficient of variation
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7

**Figure 2.**

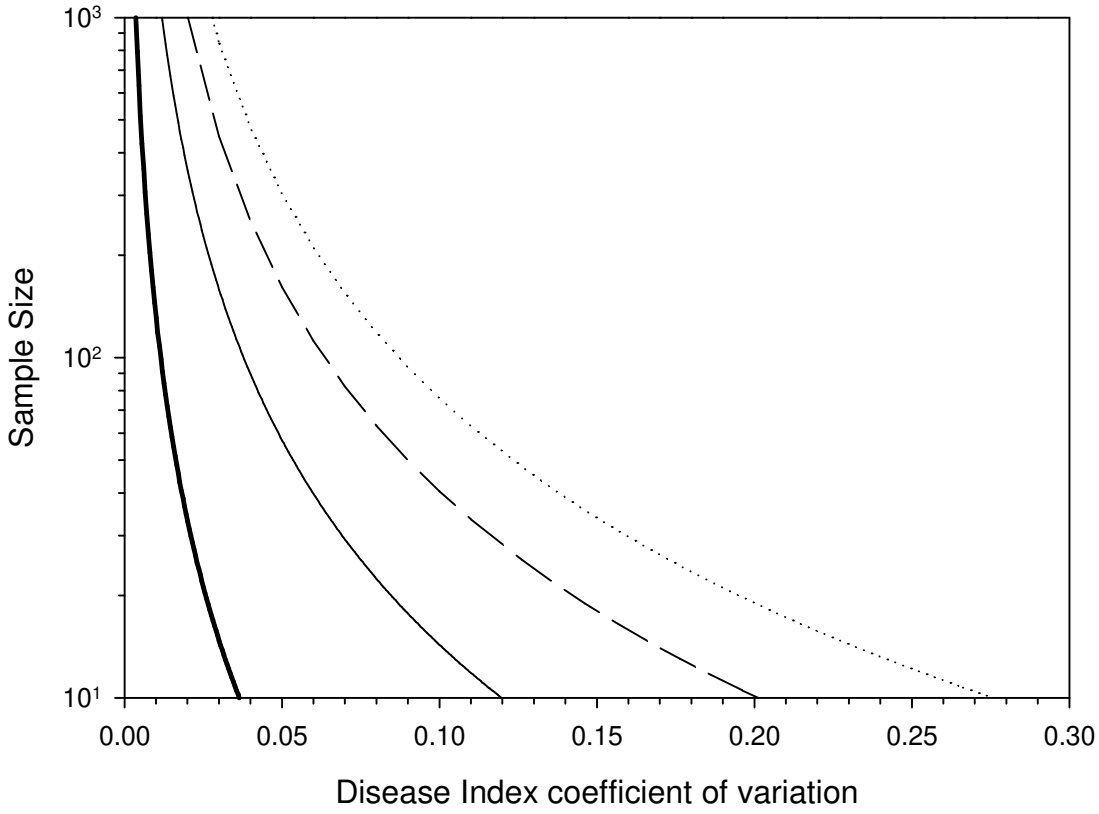
10³
10²
10¹
Sample Size
0.00
0.05
0.10
0.15
0.20
0.25
0.30
Disease Index coefficient of variation

**Figure 3.**