

## Supplementary files

### SIPPOM-WOSR: a Simulator for Integrated Pathogen POPulation Management of phoma stem canker on Winter OilSeed Rape. II. Sensitivity analysis

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## Appendix 1. Sensitivity analysis of the inoculum production sub-model

### Introduction

The inoculum production sub-model of SIPPOM, a simulator for integrated pathogen population management, simulates between harvest of the crop in the previous season and the beginning of winter in the current season i) the impact of tillage on vertical displacement of stubble in soil, ii) the subsequent potential density of pseudothecia on stubble present at soil surface, and iii) the effect of climate on pseudothecial maturation and the release of ascospores. The quantity of released ascospores also depends on the severity of the disease in the previous year (Lô-Pelzer *et al.*, 2009b; 2009c). Sensitivity analysis of this sub-model was divided into four parts: variation in parameters involved in the simulation of vertical stubble displacement in soil layers, of potential quantity of inoculum due to disease severity the previous year, of potential ascospores production, and of pseudothecial maturation.

This appendix presents the results for the vertical stubble displacement in soil layers, and the potential quantity of inoculum due to disease severity the previous year.

### Material and methods

#### *Vertical stubble displacement*

The vertical displacement of stubble was distributed over four layers of soil in SIPPOM (soil surface, 0-10 cm, 10-20 cm, 20-30 cm). Each tillage operation (sowing, rotary harrowing, stubble disking, chisel plough, mouldboard ploughing) was represented by a 4\*4 matrix that describes the displacement of stubble from a soil layer to another (Schneider, 2005). Successive tillage operations in a given field are simulated by multiplying matrices. The values of the elements of each matrix describing stubble displacement within soil have been estimated by Schneider (2005). The inputs date of chisel plough, weather and G2 values were tested in this part. The number of tested input situation was therefore 24 (Table 1). Instead of varying each parameter of the matrices with restriction, two contrasting boundary matrices were defined from the nominal matrix for each tillage tool according to the following decision rule: the proportion of stubble arriving in the surface layer from other layers was increased or decreased by 50% and the difference was allocated uniformly to the other layers (the sum of the parameter of each column of the considered matrix is 1). If the proportion of stubble arriving in surface layer exceeded 1, it was fixed at 1 and the others are fixed at 0. Stubble disking (that could be simulated in SIPPOM) was not considered in the sensitivity analysis as its matrix value was quite similar to that of chisel plough.

**Table 1. Numbers of simulations carried out for each sub-model for various input variables and parameters, and the analysed output variables**

Sub-model	Process	Input variables (number)	Number of parameters	Number of simulations	Output variables tested
Primary inoculum production	Vertical stubble displacement in soil layers	4 crop management systems (2*2 dates of chisel plough) 2 years 3 G <sub>2</sub> values	3 matrices per tillage tool 4 tillage tools	24*3 <sup>4</sup> = 1944	Cumulative number of pseudothecia Cumulative number of released ascospores
	Potential quantity of inoculum due to disease severity in previous years	4 crop management systems (2*2 dates of chisel plough) 2 years 9 G <sub>2</sub> values	3 <sup>4</sup> values of a <sub>i</sub> (Table 2) 3 values of prop <sub>i</sub> (Table 2) per G <sub>2</sub> value	8*9*3 <sup>4</sup> *3 = 17496	

### ***Potential quantity of inoculum due to disease severity***

In SIPPOM, the estimated disease index is called G<sub>2</sub> disease index. In the model, it is directly estimated from the number of leaf spots (Lô-Pelzer *et al.*, 2009a), whereas in fields, it results from the assessment of canker severity on several plants (often more than 40) in a 6-class scale, from 1 (healthy plants) to 6 (stem crown totally cankered) (Aubertot *et al.*, 2004, Lô-Pelzer *et al.*, 2009b). We call ‘distribution’ the proportion of plants in each canker severity classes, leading to a given value of G<sub>2</sub>. As G<sub>2</sub> can vary from 0 to 9, 9 mean distributions have been defined in SIPPOM, corresponding to intervals of values of G<sub>2</sub>: [0; 1], ]1; 2], ]2; 3], etc., called G<sub>2</sub>-interval (Lô-Pelzer *et al.*, 2009b). In the model, the relationship between the disease severity and the subsequent production of inoculum is calculated from the G<sub>2</sub> severity disease index in two steps: the distribution of plants in each class of canker severity is deduced from G<sub>2</sub>, and the potential production of inoculum per stubble is deduced from the distribution in each class of canker severity (Lô-Pelzer *et al.*, 2009a; 2009b; 2009c). Parameters associated to these two steps were i) the mean distributions associated to the 9 G<sub>2</sub>-interval, and ii) the 6 coefficient of production of ascospores related to the 6 canker severity classes (Lô-Pelzer *et al.*, 2009c).

In addition to the effect of crop management, date of chisel plough and weather, all the non-zero integer G<sub>2</sub> severity disease index values were tested (from 1 to 9, instead of only three values of G<sub>2</sub> tested before). The number of tested input situation was therefore 72 (Table 1). The nominal value of distributions of each G<sub>2</sub>-interval as well as two boundary values corresponding to the previous and next intervals (Lô-Pelzer *et al.*, 2009b) were tested. For G<sub>2</sub>-intervals 1 and 9, lower (interval 1) and higher (interval 9) boundary values were fixed as follows: the highest proportion of plants in canker severity classes (corresponding to class 1 for G<sub>2</sub>-interval 1 and to class 6 for G<sub>2</sub>-interval 9) were increased by 20 % of their value and other proportions of plants (for the 5 other canker severity classes) were uniformly decreased.

Concerning the production of inoculum per canker severity class, coefficient of production relative to the most severe canker class (class 6) has been estimated for class 1 to 5 (Lô-Pelzer *et al.*, 2009c). Nominal values of relative coefficients were used, and two boundary values were fixed as follows: all canker severity classes produce as much as the class 6 (all the coefficients are fixed at 1); the relationship between canker severity class and production of inoculum is linear and corresponds to the average necrotic area: coefficients are 0 for class 1, 0.125 for class 2, 0.5 for class 3 and 4 (that are not significantly different, Lô-Pelzer *et al.*, 2009c), 0.875 for class 5 and 1 for class 6 (Table 2).

**Table 2 Description of parameters of SIPPOM-WOSR subjected to sensitivity analysis and their values. The base for thermal times is 0°C.**

Sub-Model	Process	Parameter	Description	Nominal value	Boundary values	Reference
Primary inoculum production	Vertical stubble displacement in soil layers	Matrix 4*4	One matrix per tillage tool, describing vertical movement of stubble in 4 layers			Schneider, 2005
	Potential quantity of inoculum due to disease severity the previous year	$(prop)_i)_{G_2=j}$ $a_i$	Proportion of plants in each canker severity class $i$ (one distribution per integer $G_2$ value $j$ ) Production of inoculum depending on canker severity class $i$ relative to the more severe class (6)	$(prop)_i)_{G_2=j}$ $a_1 = 0.18$ $a_2 = 0.54$ $a_{34} = 0.76$ $a_5 = 0.91$ $a_6 = 1$	$(prop)_i)_{G_2=j-1}$ $(prop)_i)_{G_2=j+1}$ $a_1 = 0; 1$ $a_2 = 0.125; 1$ $a_{34} = 0.5; 1$ $a_5 = 0.875; 1$ $a_6 = 1$	Lô-Pelzer <i>et al.</i> , 2009b Lô-Pelzer <i>et al.</i> , 2009c

## Results

### *Vertical stubble displacement*

Matrices were considered as qualitative variables in the variance analysis model. Effects of parameters were introduced and the coefficient of determination ranged from 91 to 97 % depending on the output variable and the crop management \* weather \*  $G_2$  severity disease index situation considered. The “cumulative number of pseudothecia” and “cumulative number of released ascospores” were calculated every 30 days from harvest of WOSR to the end of winter (around 180 days after harvest). But for this part, only the cumulative number over 90, 120, 150 and 180 days were analysed, as all tillage operations were carried out within 90 days after harvest. Whatever the  $G_2$  severity disease index and the date of chisel plough, results were similar (Table 3). The impact of different tillage tools depended on the considered output variable. The chisel had more effect than other tools on the “cumulative number of pseudothecia” and “cumulative number of released ascospores” over 90 days, as it was used early in the season, whereas rotary harrowing and sowing had more effect than other tools on the “cumulative number of pseudothecia” and “cumulative number of released ascospores” over 120 days and more (Table 3). Mouldboard ploughing had a small effect on output variables for the integrated crop management, but the number of released ascospores with this crop management was significantly smaller than the number of released ascospores with the intensive crop management because of this tillage operation. Differences between years, revealed by the interaction between weather conditions and tillage operations, appeared for the “cumulative number of released ascospores” (Table 3), as the rain is very important for ascospore release.

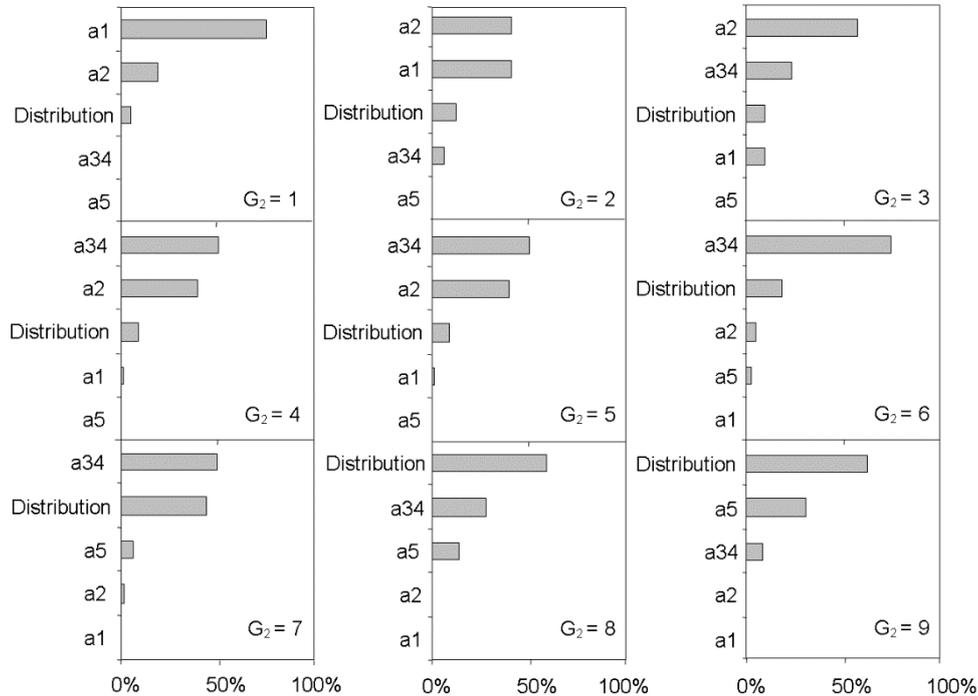
**Table 3. Effect of the variations in the tillage operation matrices on the cumulative number of pseudothecia and ascospores over 90 to 180 days after harvest depending on the crop management \* weather input variables. The tillage operation with the biggest effect is shown, as well as the proportion of the coefficient of determination explained (between brackets). Results were similar whatever the G<sub>2</sub> value and the date of chisel plough.**

Output variable		Intensive crop management		Integrated crop management	
		Year 1	Year 2	Year 1	Year 2
Cumulative number of pseudothecia	90 days	Chisel plough (39-51 %)		Rotary harrowing (40-46 %)	
	120-180 days	Rotary harrowing (41-44 %) Sowing (40-44 %)		Sowing (40-43 %)	
Cumulative number of ascospores	90 days	Chisel plough (51-76 %)		Chisel plough (78-81 %)	Rotary harrowing (59-60 %)
	120 days	Chisel plough (37-40 %)	Harrow (47-48 %)	Rotary harrowing (35-47 %)	
	150 days	Rotary harrowing (37-48 %)			
	180 days			Sowing (46 %)	Rotary harrowing (45-46 %)

***Potential quantity of inoculum due to disease severity***

Effects of parameters were introduced into the variance analysis model, and the coefficient of determination ranged from 80 to 94 % depending on the G<sub>2</sub> severity disease index situation considered. The results were similar whatever the crop-management \* weather situation. Variations in coefficients a<sub>i</sub> representing the production of inoculum depending on canker severity class was more important for lower G<sub>2</sub> severity disease index (1 to 6, Figure 1) whereas the variation in distribution of plants in each canker severity class (considered as a qualitative variable) had more impact on the output variables for higher G<sub>2</sub> severity disease index (7 to 9, Figure 1).

**Fig 1. Sensitivity analysis of the calculation of the potential quantity of inoculum due to disease severity the previous year in the primary inoculum production sub-model. Sensitivity of the output variable “cumulative number of released ascospores over 180 days” to variations in parameters as a function of the  $G_2$  disease index. Results were the same whatever the simulated situation (crop management systems and weather conditions). Parameters are described in Table 2. The parameter ‘distribution’ in the figure correspond to  $(prop_i)_{G_2=j}$  (Table 2).**



## Discussion

The effect of tillage operations on the number of released ascospores depended on the tilling date and the weather conditions. Matrix parameters were accurately estimated by Schneider (2005). The sensitivity analysis showed that although mouldboard ploughing significantly reduced the number of pseudothecia and ascospores (in agreement with experts' expectation), the variation in the corresponding matrix had little effect on the output variables. This may be due to the fact that chisel ploughing before mouldboard ploughing buries most of the stubble in layer 2 or leaves it on the surface, whereas very little stubble was lifted onto the surface layer from these layers using a mouldboard ploughing (most was displaced onto the surface layer from layer 3; Schneider, 2005). Variation in the ploughing matrix therefore had little effect on the output variables. The sensitivity analysis itself is questionable rather than experts' expectation or the model. A proper sensitivity analysis of this part, varying all the matrix parameters and taking into account interaction with various weather conditions, could be interesting but difficult to carry out, there being 16 parameters per matrix.

Parameters for the part that links disease severity to the quantity of primary inoculum were estimated accurately (Lô-Pelzer *et al.*, 2009a; 2009b). It is not surprising that distributions had more effect than the coefficients of production of inoculum (depending on canker severity class) for higher  $G_2$  severity disease index, as the sixth class coefficient was more involved for these  $G_2$  severity disease index values, and it did not vary. The results of the sensitivity analysis strengthen the legitimacy of this sub-model that is one of the first attempt to simulate the link between the disease severity and the subsequent amount of inoculum produced.

## Appendix 2. Sensitivity analysis of the dispersal and genetic sub-model

## Introduction

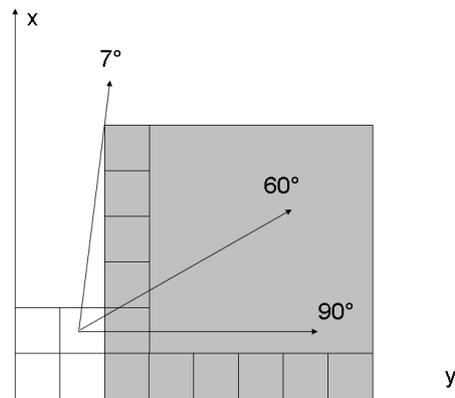
In addition to the sensitivity analysis of sub-models presented in the main paper, sensitivity analysis of the dispersal and genetic sub-models was also carried out. Spores flow between a source and a target field as well as the sensitivity of the pathogen population dynamics (*i.e.*, evolution of frequencies of pathotypes) to the initial structure (initial frequencies of pathotypes and initial pathogen population size) and to the recombination were tested. It necessitated testing the sensitivity of sub-model outputs to both variations in parameters and input variables.

## Material and methods

### *Dispersal sub-model*

Daily ascospore flow was investigated with a source field and a target field. Four values of distance between the fields were chosen: 0 km (adjacent), 1 km, 5 km and 10 km. This study also included three sizes of source and target fields: 1, 4 and 9 ha. For analysing sensitivity of weather input variables, three contrasting wind speeds (low:  $1 \text{ m.s}^{-1}$ , medium:  $5 \text{ m.s}^{-1}$  and high:  $10 \text{ m.s}^{-1}$ ) and three wind directions ( $7^\circ$ ,  $60^\circ$  and  $90^\circ$  from north; Figure 2) were chosen. Wind directions were chosen according to the layout of the fields: “direct dispersal” ( $90^\circ$ ) and “biased” dispersal ( $7^\circ$  and  $60^\circ$ ).  $7^\circ$  has been chosen as the minimum angle allowing ascospores to reach the target field when source and target fields are adjacent, source field having the smallest size and target field having the biggest size (Figure 2).

**Fig 2. Distribution of source (white) and target (grey) fields, and wind directions tested chosen according to the adjacent fields in the smallest source field-biggest target field situation ( $50 \text{ m} * 50 \text{ m}$  pixels). The angle  $60^\circ$  was chosen as intermediate.**



The effect of the pathogen population size in source pixels was not tested because the number of ascospores landing on a target pixel and coming from a given source pixel was proportional to the number of ascospores released in this source pixel. The total number of tested input variables in this sub-model was 324 (Table 4).

**Table 4. Numbers of simulations carried out for each sub-model for various input variables and parameters, and the analysed output variables**

Sub-model	Input variables (number)	Number of parameters	Number of simulations	Output variables tested
Dispersal	4 distances 3 source field sizes 3 target field sizes 3 wind directions 3 wind intensities	2	324*3 <sup>2</sup> = 2916	Number of spores landing in target field
Genetic	40 initial frequencies 3 population sizes	1	120*3 = 360	Double virulent pathotype frequency

Three values of the parameter associated with the dispersal function ( $\mu_{\text{Cauchy}}$ ) were tested: the nominal value corresponding to spores of *L. maculans* was 10 s. The value reported by Diggle *et al.* (2002) for spores of *Colletotrichum* (50 s) was also tested as well as a smaller value (5 s; chosen as VN – 50 % VN). In addition, the pixel size of the raster was tested. It is fixed at 50 m \* 50 m in SIPPOM-WOSR, 10 m \* 10 m and 100 m \* 100 m values were also tested. Because of the excessive computation time, the test of the 10 km distance between source and target field was not possible with a 10 m \* 10 m pixel.

The output variables analysed was the number of ascospores per m<sup>2</sup> on the target field. The effect of variations in parameter values, as well as the effect of variations in input variable (and interactions) were entered in the variance analysis.

#### **Genetic sub-model**

The sensitivity analysis of the genetic sub-model was carried out for 2 avirulence genes (4 possible pathotypes: a double virulent, two simple virulent and a double avirulent pathotype). Input variables tested were: initial size of pathogen population (1,000, 10,000 and 100,000 ascospores per square meter per day) and initial frequencies of pathotypes: 0 (for double virulent only), very low: 10<sup>-4</sup> (Lannou and Mundt, 1997), low: 1 %, medium: 10 % and equally probable, the frequency of the avirulent pathotype being fixed as the difference between 1 and the sum of the frequencies of simple and double virulent pathotypes. As both simple virulences are symmetric, all the combinations of frequencies were not tested. Moreover, the cases where the initial frequency of the double virulent pathotype was higher than the frequencies of the simple virulent pathotypes were not considered. In total 40 values of initial frequencies were tested. The total number of tested input variables for this sub-model was 120 (Table 4).

Parameter associated with recombination was tested. We used three values of the parameter representing physical genetic linkage between avirulence genes, from almost complete linkage to complete independence (Table 5).

**Table 5. Description of parameters of SIPPOM-WOSR subjected to sensitivity analysis and their values. The base for thermal times is 0°C.**

Sub-Model	Parameter	Description	Nominal value	Boundary values	Reference
Dispersal	$\mu_{\text{cauchy}}$	Parameter associated with wind intensity	10 s	5; 50	Diggle <i>et al.</i> , 2002
	Pixel size	Size of each pixel of the grid	50 m	10; 100	
Genetic	$C_{\text{linkage}}$	Genetic linkage between avirulence gene (Recombination)	0.25	0.05; 0.5	Lô-Pelzer <i>et al.</i> , 2009

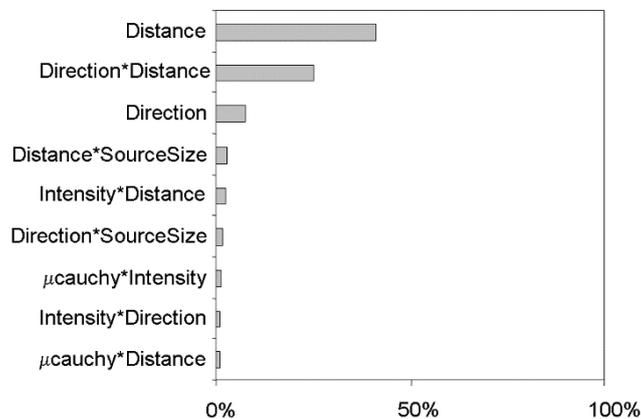
The output variables analysed was the frequency of the double virulent pathotype. The effect of variations in parameter values, as well as the effect of variations in input variables (and interactions) were entered in the variance analysis.

## Results

### *Dispersal sub-model*

The effects of parameters and input variables as well as their interactions were introduced into the variance analysis model ( $R^2 = 77\%$ ). The variation in distance between source and target fields had the greatest impact on the “number of spores landing on target field”, as well as the interaction between distance and wind direction, and the wind direction itself (Figure 3). The effects of variation in both parameters  $\mu_{\text{Cauchy}}$  and pixel size were negligible.

**Fig 3. Sensitivity analysis of the dispersal sub-model to several variables or parameters: Distance: distance between source and target fields, SourceSize: source field size, Direction/Intensity: wind direction/intensity,  $\mu_{\text{Cauchy}}$ : parameter of the dispersal function (Table 4). Sensitivity of the output variable “number of ascospores per target field surface unit” to parameter and input variable variations ( $R^2 = 77\%$ ).**



### *Genetic sub-model*

The effects of parameters and input variables were introduced into the variance analysis ( $R^2 = 69\%$ ). The effect of the initial frequency of the double virulent pathotype had the greatest impact on the “final frequency of the double virulent pathotype” ( $P < 0.0001$ ) and the variation in this input variable explained 70% of the coefficient of determination. Effects of initial population size and frequency of the simple pathotype were also significant ( $P < 0.0001$  and  $P = 0.0015$  respectively). On the other hand, the effect of the genetic linkage between avirulence genes was not significant. This shows that the initial frequencies of pathotypes have a big effect on their final frequencies during a year.

## Discussion

Distances between fields as well as wind direction had a big effect on spore flow between two fields, as expected. Even for the longest distance (10 km), the number of ascospores reaching the target field was not zero. This indicates the need to consider a large region for simulations. The variation in the parameter  $\mu_{\text{Cauchy}}$ , which is difficult to estimate experimentally because of the difficulty of trapping spores at long distances due to their dilution in the air, had little effect on the number of spores landing on the target field. The estimation of this parameter is therefore not a priority. The effect of pixel size was also negligible and pixel size was fixed at 50 m \* 50 m in SIPPOM.

The initial frequencies of pathotypes had a big effect on the final frequencies. This suggests the need to enter correct initial frequencies at the beginning of simulations in order to

follow the breakdown of a specific resistance. Nevertheless, these data are not obtainable by experimentation when frequencies are very low, *e.g.*, for frequencies of virulent pathotypes when a new resistance gene is introduced into a region. The use of resistant cultivars is considered in the management strategies whereas the frequencies of pathotypes at very low level are not measurable *e.g.*, virulent pathotypes when a new resistance gene is introduced in the region. This is why further studies have to be done on how the new virulent pathotypes appear, *i.e.*, what are the mechanisms involved in the loss of avirulence genes that will maybe allow an easier detection of this pathotypes at very low frequencies. The model therefore helps to point out the necessity to improve the techniques of detection of low pathotype frequencies, as the simulation of impact of management strategies on disease control and resistance durability seems to be highly dependent on these initial frequencies.

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