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Effectiveness of field isolation distance, tillage practice, cultivar type and crop rotations in controlling phoma stem canker on oilseed rape

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Abstract

Modern agriculture has led to simpler agricultural landscapes that favour the spread of pathogens and increase pressure from pests and diseases. Landscape-dependent interactions between crops and pathogens, including disease related dispersal patterns, and the benefits of reducing pathogen significance call for the design of disease-suppressive landscapes. Modelbased assessment is the most efficient method of choosing among management strategies. Based on a case study in France, we ranked the effectiveness of different crop mosaics for control of phoma stem canker on winter oilseed rape (WOSR). Assessed crop mosaics were developed from strategies defined by local stakeholders: (1) isolating target from source fields (all WOSR or only WOSR harbouring RlmX specific resistance), and (2) specifying tillage on WOSR stubble according to cultivar type (with or without RlmX). Model simulations highlighted the effectiveness of WOSR-isolation as compared to RlmX-isolation. Our analyses suggest that tillage (mouldboard ploughing) was the most important factor in

explaining the size and genetic structure of the pathogen population (determinant in explaining the breakdown of resistance), and yield loss. While the pathogen population and yield loss decreased with intensive management of non-RlmX-cultivars (85% of WOSR), the same management with RlmX-cultivars modified the genetic structure of the pathogen population. Increasing isolation distances led to reductions in pathogen population and yield loss only in the strategy of WOSR-isolation. Isolating source and target RlmX-cultivar had no effect on the evolution of the population's genetic structure. Although effective in phoma stem canker control, changing tillage can require significant changes for farms. Isolation distance would require extensive information on the landscape, and imply an aggregation of crops that might or might not be possible depending on a farm's spatial organization. This study could lead to the design of a Decision Support System targeting high risk (diseased) WOSR fields to be ploughed or isolated from the following year's cultivation.

Keywords: yield loss, cultivar resistance, pathogen population, strategy ranking, spatiallyexplicit model, mosaic design

Highlights

- Stakeholders designed tillage and isolation rules to design pest-suppressive crop mosaics.
- Mosaics were assessed on three complementary criteria with a spatially-explicit model.
- Intensive management of WOSR stubble was the most efficient factor.
- Isolation distances were efficient in decreasing yield loss and pathogen population.
- Implementing efficient strategy would require both individual and collective actions.

1. Introduction

 In recent decades, modern agriculture has led to the simplification of agricultural landscapes, both in terms of structure and crop composition (Stoate *et al*., 2001; Baessler and Klotz, 2006). This intensification process, linked with a simplification of cropping systems (Stoate *et al*., 2001), has strongly reduced crop genetic diversity in the field, thus favouring pathogen spread (Stuckenbrock and McDonald, 2008), and driving agrosystems towards increased vulnerability to pests and diseases (Meehan *et al*., 2011). With significant yield losses from pests and diseases (Oerke and Dehne, 2004; Oerke, 2006), crop arrangements in time and space (i.e., crop mosaics) represent a critical parameter to mitigate susceptibility to these losses. For instance, landscape composition and complexity have been identified as driving parameters of the rate of pollen beetle parasitism (Rusch *et al*., 2011), aphids and wheat diseases (Gosme *et al*., 2012), and the pathogen population structure responsible for wheat leaf rust (Papaix *et al*., 2011). These types of landscape-dependent crop-pathogen interactions and the desire to reduce pathogen significance call for the design of disease-buffering or disease-suppressive landscapes (Skelsey *et al*., 2010). For pathogens exhibiting a dispersal process (either active, e.g., insects, or passive, e.g., wind- or water-dispersed), pest-suppressive landscapes have to be designed both in terms of composition (e.g., proportion of the different crops/cultivars; Papaïx *et al.*, 2011), and configuration, including the exact and relative locations of crops and associated cropping systems (Leenhardt *et al*., 2010). In addition, landscape temporal evolution has to be characterized as crop-pathogen interaction exhibiting a year-to-year relationship (e.g., for pollen beetle in Rusch *et al*., 2011; for phoma stem canker in Bousset *et al*., 2015). The 23 consideration of spatial and temporal scales depends on processes and knowledge about the specific topic to address (e.g., crop-pathogen interactions), leading to rules defined in time (crop rotations and crop return time; Castellazzi *et al*. 2010), and/or in space (isolation

 distance or buffering zones) (e.g., Skelsey *et al*., 2010 on potato late blight; Colbach *et al*., 2009 on maize gene flow).

 Although disease-suppressive landscapes can theoretically be identified, their design and assessment remain challenging. Their design should begin by the identification of potentially efficient control methods (cultural, physical, biological or chemical), and their effect on pathogen populations, which have to be defined both in time and in space (Aubertot *et al*., 2006). Once identified, strategies that organize and coordinate these control methods on a landscape scale have to be built. Involving stakeholders in this step can help to develop and explore more suitable proposals (Brandenbourg *et al*., 1995), especially for agricultural landscapes where the choice and location of cropping systems are decided by local farmers (Primdahl, 1999), and influenced by local stakeholders (e.g., input providers, crop collector). Such involvement helps the integration of local specificities, providing more complete information on characteristics such as soil, climate, and markets (Reed, 2008; Voinov and Bousquet, 2008). Experimentation to assess the designed landscapes can be problematic, especially when exploring the effectiveness of several possible pre-identified alternatives, i.e. various arrangements of crops and control methods (Skelsey *et al*., 2010). Explorative modelling of the landscape system appears to be a suitable, and even necessary option. This method uses dynamic and spatially explicit models representing the necessary processes at field and landscape scales (e.g., Veldkamp *et al*., 2001; Lô-Pelzer *et al*., 2010b). Phoma stem canker of oilseed rape (causal agent *Leptosphaeria maculans* fungus) is responsible for major yield and economic loss worldwide (Fitt *et al*., 2006) and is characterized by crop-pathogen interactions, and potential control methods, which are defined in time and space (Aubertot *et al*., 2006; Lô-Pelzer *et al*., 2010b). Its epidemic cycle exhibits a year-to-year recurrence, and the primary inoculum (spores) is produced on winter oilseed rape (WOSR) stubble. These spores are wind-dispersed up to 5-8 kilometres (Bokor *et al*.,

 1975), and can subsequently fall and infect young oilseed rape (Hall, 1992). A distance of 500 m between fields has been highlighted as theoretically efficient to avoid epidemics (Marcroft *et al.*, 2004). At field level, the main control method is the use of resistant cultivars. Two types of resistance can be used: quantitative (partial) resistance, controlling the extent of the disease (Delourme *et al*., 2006), or qualitative (specific) resistance (RlmX-gene), which prevents the disease if a common resistance gene is harboured by both the landing pathogen and the cropped WOSR cultivar (Plissonneau *et al.*, 2016). However, large-scale cultivation with a qualitatively-resistant cultivar can quickly lead to the breakdown of its specific resistance (Rouxel *et al*., 2003), and require other associated control methods. Field control methods include WOSR sowing date, fertilization (Aubertot *et al*., 2004), tillage for WOSR stubble management (Schneider *et al*., 2006), and fungicide applications that are only effective during a limited time span (Gladders *et al*., 2006). These methods can help control the disease by two means: reducing the size of the pathogen population, and limiting the selection pressure on pathogen populations (Aubertot *et al*., 2006). To be efficient, these control methods have to be combined and organized in space and time through 'integrated' strategies that combine agronomic practices and/or the deployment of cultivar genotypes (e.g., minimum between-field distance) (Gladders *et al*., 2006; Sprague *et al*., 2006). Integrating results (i.e., processes, scales) of empirical studies in a modelling framework can help to understand and tackle the many interactions between crop and pathogen and their spatio-temporal dynamics (e.g., on potato late blight in Skelsey *et al.*, 2009, 2010). Indeed, such strategies cannot be tested in the real world because of their necessarily large spatio- temporal scales (Legg, 2004). Spatially explicit modelling is thus seen as very useful to assess performances of strategies designed at large spatial and temporal scales (Hijmans and van Ittersum, 1996; Vinatier *et al.*, 2016). Such models can then be used as virtual laboratories (Charnell, 2008) to conduct *ex ante* simulation experiments (i.e. strategy testing) at large

 scales. Using this type of models in combination with expert knowledge can improve the realism of such simulation experiments (e.g., Sadok *et al.*, 2009).

 For phoma stem canker of oilseed rape, SIPPOM-WOSR is, up to our knowledge, the only spatially explicit model taking into account the effects on disease development, in time and space, of the whole set of cropping practices impacting disease control, i.e., proportion and location of oilseed rape, cultivar type, sowing date and rate, fertilization and tillage practices, and fungicide application (Lô-Pelzer *et al*., 2010a, 2010b). This model was applied on "extreme situations", by testing the effect on pathogen population size of two contrasted crop management plans (limited vs. good disease control) and two virtual landscapes (random location of spores' sources/targets vs. maximizing the distance between sources and targets) (Lô-Pelzer *et al.*, 2010b). These simulations confirmed the general effect of crop management (tillage practices, sowing date and density) and source/field distances on *Leptosphaeria maculans* pathogen population (Lô-Pelzer *et al.*, 2010b). As the implementation of integrated pest control strategies requires the participation of stakeholders (Rusch *et al.*, 2010), SIPPOM-WOSR was then used in a participatory scenario approach, where local stakeholders numerically designed future cropping systems that could happen in case of contextual changes (Hossard *et al*., 2013). These cropping systems were simulated with SIPPOM-WOSR to assess their effect on phoma stem canker control, with regards to indicators describing the pathogen population (size, genetic structure) and subsequent yield loss. Simulations were analysed in order to (1) identify efficient scenarios (Hossard *et al.*, 2015b), (2) highlight, rank and quantify the effect of the most impacting cropping practices (Hossard *et al.*, 2013, 2015a, 2015b), and (3) identify the spatial scale at which cropping practices influence the pathogen genetic structure (Hossard *et al.*, 2015a). However, the simulations performed in these studies mostly corresponded to model 'testing' by the stakeholders, and led to a kind of "sensitivity analysis" on cropping practices, more than to the design coherent strategies. Indeed, the

 designed scenarios included extreme values for key variables (e.g., crop rotation, cultivar characteristics, random crop allocation) leading to a low chance that such scenarios would happen in reality (Hossard *et al.*, 2013, Hossard *et al.*, 2015b), and thus provided a limited support for local stakeholders. Nevertheless, such models are of interest for local stakeholders as they can assess the effects of coordinated actions aiming at solving a local issue (Souchère *et al.*, 2010). Following the previous studies on the most sensitive model variables, parameters and inputs (Lô-Pelzer *et al.,* 2010a; Hossard *et al.*, 2015a, 2015b), SIPPOM- WOSR could then help local stakeholders to foresee the consequences on phoma control of different coherent strategies of cropping systems and their spatial distribution, contributing to support their strategic thinking by an *ex ante* assessment of multi-plot and multi-years strategies. Based on a real-world case study located in France, this paper is aimed at characterizing, comparing, and ranking the effectiveness of different types of crop mosaics for phoma stem canker control. The designed crop mosaics were built from different cropping strategies, defined by local stakeholders: (1) isolating target fields from source fields in time and/or space, and (2) specifying tillage practices according to their cultivar type. These mosaics, describing both annual cropping plans and cropping systems, were assessed with SIPPOM- WOSR (Lô-Pelzer *et al.*, 2010a, 2010b).

2. Material and methods

2.1. Method overview

 The design, assessment, and comparison of strategies combining cropping practices and their allocation for efficient phoma stem canker control were performed in four steps that combine a participatory approach and numerical simulations with a spatially-explicit model (Figure 1):

 A. Stakeholders designed strategies to control phoma stem canker in their region. These strategies consisted of rules for combining and allocating crops and cropping practices to fields. Two main strategies were designed: isolation of crops or specific cultivars, and setting of specific tillage practices (to manage WOSR stubble) according to cultivar types. B. In the second step, these rules were implemented to build mosaics of crops and cropping practices at the landscape scale. Based on a map of the studied region (Figure 2), between- fields distances were calculated to assign WOSR, specific cultivars and tillage practices annually to each field for each strategy. C. In the third step, these mosaics were simulated with the spatially-explicit model SIPPOM- WOSR (Lô-Pelzer *et al*., 2010a, 2010b) to assess their impacts on phoma stem canker. D. In the fourth step, statistical analyses were performed to assess and rank the strategies according to their effectiveness for phoma stem canker control, i.e., the ability of strategies to control phoma epidemics and preserve resistance. Strategies were compared with three complementary criteria (size and genetic structure of the pathogen population, and yield loss due to phoma). 141 # Figure 1 approximately here $#$ 2.2. Study area Our case study was located in the Picardie region (NUTS-2), in northern France. In this region, an agricultural context (Oise NUTS-3) was defined as corresponding to specific soil

characteristics and cropping systems. Local stakeholders defined cropping systems (Hossard

- *et al*., 2015b) characterized by a 7-year crop rotation (with one WOSR every 7 years, i.e.
- 14.3% of WOSR each year). WOSR is sown at the end of august, at a mean density of 45
- 149 seeds m⁻², with 15% of sown WOSR being RlmX-cultivars. Stakeholders associated RlmX-

 of year y) (Hall, 1992). The isolation of target fields therefore means not sowing either WOSR or RlmX-cultivars in the neighbourhood of source fields (Figure 2). Two different types of fields were considered for isolation: all winter oilseed rape crops (whatever their cultivar type) or only one specific cultivar type (cultivars with the RlmX-gene). Stakeholders defined three isolation distances: adjacent fields (0 m), and buffer zones of 500 m or 1,000 m around source fields.

 For each isolation distance and isolation target (WOSR or RlmX-cultivars), stakeholders wanted to explore two frequencies of WOSR or RlmX-cultivars in the landscape: (1) the reference of 14.3% (corresponding to 7-year rotation), and (2) a higher frequency of 20% (corresponding to a 5-year rotation). The rule was thus to grow WOSR on each field every 5 or 7 years. For the WOSR isolation strategy, the annual frequency of RlmX-cultivars corresponded to a reference of 15% of WOSR that was provided by local stakeholders. For RlmX isolation strategy, the stakeholders defined two options characterizing the temporal synchrony of RlmX-cultivars. The first option was to keep a constant frequency across time (15%, as for WOSR isolation strategy). The second option was to set a temporal asynchrony for RlmX-cultivar resulting in a frequency of RlmX-cultivar three times higher in year y than 191 in year y+1. For this option, the multi-year frequency was kept to the reference (15%) , so annual RlmX frequency was 25% for year y and about 8.33% (25/3) for year y+1.

2.3.2. Cultivar-specific stubble management

 In combination with the isolation rules, stakeholders designed strategies of WOSR stubble management. Three options of tillage were designed: mouldboard ploughing (MB), two passages of stubble breaking (SB), or the reference (1/3 of the fields with one SB passage and 2/3 with two SB passages). Tillage options were applied either on all WOSR stubble or separately for cultivar types (with and without RlmX-gene). This led to nine stubble

 management combinations (3 options for RlmX-cultivars, and 3 options for the cultivars without the RlmX gene).

2.3.3. Implementation rules

 Distances between fields were calculated with the R package 'rgeos' (Bivand and Rundel, 2016). Ten thousand multi-year crop mosaics were realized for each of the twelve combinations of the three criteria: isolation distance (0 m, 500 m, or 1,000 m), isolation target 207 (WOSR or RlmX-cultivars), and WOSR frequency (14.3% or 20%). For each mosaic, the $1st$ year crop allocation to fields was random, and isolation rules were applied for the following years. For each year, the WOSR acreage objective was constant. For the mosaics with 0 m isolation distance (adjacent fields), the fields selected for WOSR cropping in year y-1 had to be non-adjacent, and at a distance lower than 500 m of WOSR of year y. Similarly, field selection for the 500 m isolation distance incorporated WOSR fields located between 500 m and 1,000 m from the source fields. For each factor combination, three crop mosaics were chosen for simulation with the spatially-explicit model SIPPOM-WOSR. These three replicates were chosen in order to minimize the acreages of fields where no rapeseed could be grown during the 5 or 7 years. Tillage (residue management) was then assigned to WOSR fields according to the 9 options, with a random allocation of one or two passages of stubble breakers for the reference tillage option in WOSR fields. Isolation rules for WOSR fields, combined with tillage options, led to 162 mosaics (3 replicates x 9 tillage options x 3 isolation distances x 2 WOSR frequencies; Table 1). Isolation 221 rules for RlmX-cultivar fields, combined with tillage options, led to 324 simulated mosaics (3 replicates x 9 tillage options x 3 isolation distances x 2 WOSR frequencies x 2 temporal options –synchronous/asynchronous; Table 1).

225 $\#$ Table 1 approximately here $\#$

2.4. Model-based simulation of the strategies

 SIPPOM-WOSR simulates the effects of cropping practices and their spatial locations on the evolution of phoma stem canker disease (Lô-Pelzer *et al*., 2010b). The model uses daily weather data and spatially distributed information on field soils, pathogen population, and cropping system characteristics. Cropping system information is thus provided at the field scale, with details on winter oilseed rape frequency and management (cultivar, sowing date and rate, autumnal fertilization, fungicide application and tillage on stubble after WOSR harvest). SIPPOM-WOSR combines population, epidemiological and crop modelling approaches through five sub-models: (i) the production of primary inoculum in source fields, (ii) the dispersal of ascospores produced in source fields, (iii) the genetic compatibility between ascospores dispersed from source to target fields, (iv) the growth of winter oilseed rape, and (v) the infection of WOSR by the pathogen and associated yield loss. Using process-based equations, SIPPOM-WOSR simulates three indicators of phoma stem canker management: two epidemiological indicators (size and genetic structure of the pathogen population) and one agro-economical indicator (yield loss due to phoma disease). Model functioning, equations and parameters are detailed in Lô-Pelzer *et al.* (2010a, 2010b). Each mosaic was run with the model SIPPOM-WOSR for a five-year simulation (one year for model initialization and four years of simulations; as performed in Hossard *et al*., 2013; 2015a; 2015b), with initial model parameters (see details in Lô-Pelzer *et al*., 2010a; 2010b). 2.5. Analyses of strategy performance Mosaics were evaluated with three criteria: the size of the pathogen population (number of

249 spores), the fraction of virulent pathotypes on $R \text{Im} X$ -gene (hereafter called avrlmX, in %),

 and the yield loss due to phoma epidemics (% of potential yield). For each simulated mosaic, these criteria were averaged over the simulation years, at the landscape scale, in terms of annual area-weighted values (annual area-weighted sum for population size and annual area-weighted average for yield loss and avrlmX).

 Two complementary analyses were performed to assess the effectiveness of isolation strategies for phoma management. We first developed linear models to test the effect of the different factors on the three evaluation criteria for each of the two isolation strategies (WOSR or RlmX). The included explanatory factors were WOSR frequency, isolation distances, tillage option for RlmX-cultivars and for the other cultivars, and temporal synchrony (for the RlmX-isolation strategy only). Analysis of variance was performed for each strategy, considering all factors as categorical. The contribution of each factor was assessed by the Mean Squared Error (MSE), which corresponds to the sum of squares divided by the associated number of degrees of freedom (df). The significance of each factor was 263 tested using F-tests, and model goodness-of-fit was evaluated by the adjusted R^2 and the error size of the residuals (RMSE, Root Mean Square Error). Model residuals were checked for symmetry and normality, and independence from fitted values.

 In the second set of analyses, we built regression trees to identify the best performing combinations of factors, using the same explanatory factors as for variance analysis. One regression tree was built for each evaluation criteria, considering the two isolation strategies separately, using the CART method. This method recursively partitions the data in two groups to minimize within-group variability while maximizing between-group variability, choosing one factor at a time (Breiman *et al*., 1984). Trees were selected using a modal tree based on 100 cross-validations (Breiman *et al*., 1984), and final trees were pruned according to the 1- standard error rule to avoid data over-fitting. For each explanatory factor in the final tree, factor splitting was applied (Breiman *et al*., 1984; Therneau *et al*., 2015), calculating an index

 of factor importance that corresponds to the sum of the deviance decrease at each node. Six final trees were built, corresponding to the three evaluation criteria for the two isolation strategies. New analyses of variance were performed for these six final trees, using the groups as explanatory factors. The difference between groups was tested with Bonferroni least significance difference (LSD) when the group-based analysis of variance was significant $(p<0.05)$.

Analyses were performed with the R software version 3.2.3 (R Development Core Team,

2015), using the package "rpart" (Therneau *et al*., 2015) for regression tree and the package

"agricolae" (de Mendiburu, 2016) for LSD tests.

3. Results

3.1. Crop mosaics

 The two crop rotations designed by local stakeholders led to an objective of 334 ha of winter oilseed rape (WOSR) grown annually for the 5-year rotation, and 239 ha of WOSR for the 7- year rotation. Slightly higher WOSR acreages over the simulation years could be achieved (e.g., for 0 m isolation distance, see Table 2). The field map used for simulation is a real- world map (Figure 2), with field size ranging between 0.91 ha and 37.61 ha, for an average of 10.58 ha \pm 6.85 ha.

For WOSR isolation strategy, the WOSR acreage objective was only achieved for the three

replicates of the 7-year rotation of the 0 m isolation distance (i.e., contiguous fields), and it

was associated with low between-year variability (3-9 ha, Table 2). For the 5-year rotation,

the contiguous fields forbidding rule (0 m isolation distance) led to WOSR acreages that were

about 10% lower than the objective (annual average of 301 ha compared to a 334 ha

objective, Table 2), and were associated with a high between-year variability (33-60 ha, Table

2). WOSR acreage objectives for WOSR isolation strategy were not met for the 500 m and

 1,000 m isolation distances, for the two rotations. For the 5-year rotation, WOSR acreages were lower than the objective by 26-30% and 30-31% respectively, for the 500 m and 1,000 m isolation distances. These mosaics also highlighted high between-year variability, of about 70 ha for and 140 ha for 500 m and 1,000 m isolation distances, respectively (Table 2). Similarly, the WOSR acreage objective associated with the 7-year rotation was not met for the 500 m and 1,000 m isolation distances. However, the obtained acreages were closer to the objective of 14.3% WOSR annually, with on average 12-13% WOSR for the 500 m isolation distance, and 11-12% for the 1,000 m distance. For all isolation distances, the WOSR between-year variability was lower in the 7-year rotation mosaics than in the 5-year rotation mosaics (Table 2). For WOSR isolation strategy, the 15% of RlmX-cultivars were based on WOSR acreages, and were lower than the objective for all isolation strategies, except for the 0 m isolation distance in the 7-year rotation. Between-replicates variability increased with the isolation distance (Table 2). For RlmX isolation strategy, WOSR acreage objectives were achieved for the two rotations and the three isolation distances (Table 2), because the isolation distances were only applied to 15% of WOSR fields (those grown with RlmX-cultivars). Thus both WOSR and RlmX- cultivar acreages were stable throughout the rotations and the isolation distances for the RlmX isolation strategy. Between-year and between-replicate variabilities were lower in this strategy as compared to the WOSR isolation strategy.

320 $\#$ Table 2 approximately here $\#$

3.2. Performance of WOSR isolation strategy

The simulations of WOSR-isolation strategy that were performed with the model SIPPOM-

WOSR resulted in a wide range of values for the three evaluation criteria, averaged over the

325 simulation years. Pathogen population size ranged between 4.5 x 10^{12} spores and 1.6 x 10^{15} spores; the fraction of virulent pathotypes on RlmX-gene ranged between 9.5% and 81.3%, and yield loss ranged between 1.5% and 7.6%. Yield loss increased with an increase of the pathogen population size, but not with an increase of the fraction of virulent pathotypes on RlmX-gene (Figure 3).

331 # Figure 3 approximately here $#$

 All models used for variance analysis explained more than 82% of the evaluation criteria variability, and were associated with low root mean squared errors (Table 3). The analyses of variance performed on simulations for WOSR isolation strategy highlighted a highly significant effect (p<0.001) of isolation distance (0 m, 500 m, or 1,000 m), tillage on WOSR stubble of both RlmX- and non-RlmX-cultivars, and rotation (5 and 7-year). Tillage on non- RlmX-cultivars was the most explanatory factor (Table 3). The size of the pathogen population was significantly lower for this factor when the stubble of non-RlmX-cultivars (i.e. 85% of WOSR) was ploughed (Modality "MB" of the factor "Till.other" of Figure 4A; Appendix A). For the fraction of virulent pathotypes on RlmX-gene (avrlmX), tillage on both 342 types of cultivars had a highly significant impact $(p<0.001)$, while the isolation distance and 343 the rotation had significant ($p<0.05$) and non-significant effects ($p>0.05$), respectively (Table 3). Ploughing the stubble of RlmX-cultivars significantly decreased the fraction of virulent pathotypes on RlmX-gene, as compared to the two other tillage options, while ploughing the stubble of non-RlmX-cultivars significantly increased this fraction (Appendix A, Figure 4C). Isolation distance and tillage on non-RlmX-cultivars were the most important factors (highest MSE) for yield loss; as with tillage on RlmX-cultivars, they had a highly significant impact (Table 3). For instance, changing from 0 m to 1,000 m distance decreased average yield loss

353 # Figure 4 approximately here $#$

355 $\#$ Table 3 approximately here $\#$

 The results of the variance analyses revealed that the most efficient combinations for minimizing simulated values differed between the three evaluation criteria. However, the tillage option on non-RlmX-cultivars always constituted the main factor, and therefore the first node for the three regression trees (Figure 5). In this node, the 'mouldboard ploughing' modality was opposite to the 'reference' and '2 passages of stubble breaker' modalities. The tillage option on RlmX-cultivars was the second factor explaining the fraction of virulent pathotypes on RlmX-gene. The second explaining factor for the evaluation criteria of Population size and Yield loss was the isolation distance (Figure 5, Table 4). Isolation distance was not selected as a main explanatory factor of the RlmX-virulent fraction. Rotation length was selected as an important factor only for the Size of the Pathogen Population (Figure 5, Table 4). Overall, the smallest pathogen population was simulated when ploughing stubble of non-RlmX-cultivars. Combining isolation distance of 0 m and the most intensive rotation (5-year) led to the highest population (Figure 5). Ploughing stubble of RlmX- cultivars but not those of non-RlmX-cultivars led to the lowest RlmX-virulent fraction, and the inverse ploughing rule led to the highest. Note that no significant differences of avrlmX were detected when the stubble management of both types of cultivar did not differ (Figure 5). Finally, yield loss was significantly lower (3.2%) for the largest isolation distance (1,000

m) and ploughing of non-RlmX-cultivars, as compared to low isolation distance (0 m) and no

ploughing of non RlmX-cultivars (yield loss of 6.4%) (Figure 5).

377 $\#$ Figure 5 approximately here $\#$

379 $\#$ Table 4 approximately here $\#$

3.3. Performance of RlmX-cultivar isolation strategy

This strategy also led to a wide range of values for the three evaluation criteria. Although the

range was not as wide as in the WOSR isolation strategy, the simulated values were generally

384 higher, with the size of the pathogen population ranging between 9.9 x 10^{12} spores and 2 x

385 10^{15} spores, the fraction of virulent pathotypes on RlmX-gene ranging between 14% and 81%,

and yield loss ranging between 3.7% and 7.9% (Figure 6). Similar to the WOSR isolation

strategy, higher yield losses were associated with larger pathogen population sizes, but not

always with larger fractions of virulent pathotypes on RlmX gene (Figure 6).

390 $\#$ Figure 6 approximately here $\#$

 The ranking of the importance of explanatory factors in the RlmX-cultivar isolation strategy differed from that in the WOSR-isolation strategy, however tillage on non-RlmX-cultivars remained the main factor (highest MSE, Table 5). Tillage on RlmX-cultivars also had a significant effect on the three evaluation criteria. Isolation distance had a significant impact on yield loss only (p<0.05, Table 5), which varied, as distance increased (Figure 7, Appendix B). Increasing rotation length significantly decreased both yield loss and the size of the 398 pathogen population ($p<0.001$) (Table 5). Synchronism of RlmX-cultivars significantly

4. Discussion

4.1. Tillage and cultivar effects

 This study assessed three complementary evaluation criteria in order to quantify the effect of various stakeholder-designed strategies for the control of phoma stem canker of winter oilseed rape. We found that the effectiveness of the strategies varied according to the evaluation criteria in a manner consistent with Hossard *et al*. (2015b), who assessed mosaics with random crop allocations, unlike the rule-based mosaics assessed in our study. In our study, deep tillage (mouldboard ploughing of stubble) on non-RlmX-cultivars (i.e. not harbouring the specific resistance gene RlmX) was the main lever for decreasing the size of the pathogen population and yield loss due to phoma stem canker, but conversely, led to increased breakdown in RlmX resistance (i.e. higher frequency of virulent pathotypes on RlmX-gene). This opposing result is primarily linked to the high level of quantitative resistance harboured by non-RlmX-cultivars, leading to less disease, lower yield loss, and in turn, to fewer pathogens in the following year (Lô-Pelzer *et al*., 2009) as compared to RlmX-cultivars (here associated with a lower level of quantitative resistance). In parallel, within a unique mosaic, 436 deep tillage applied only on fields grown with RlmX-cultivars was better at preserving RlmX resistance (i.e. smaller fraction of resistant pathogen population), while deep tillage on non- RlmX-cultivars led to less preservation of RlmX resistance gene by mechanically favouring spores resistant to RlmX (since only resistant spores can develop), as suggested by Hossard *et al*. (2015a). Similarly, in a four-year experiment, Daverdin *et al.* (2012) found a rapid evolution of pathogen population towards resistance to Rlm7-gene when residues of Rlm7- cultivars were chiselled (minimum tillage) and sensitive cultivars (i.e. without Rlm7-gene) were ploughed. This opposite result highlights the independence between the two evaluation criteria regarding the genetic structure and the size of the pathogen population, in accordance with findings of previous modelling studies on phoma stem canker (Hossard *et al*., 2015b), and on other crop pathogens. For instance, Papaix *et al.* (2015) highlighted that landscape

 structures promoting large pathogen populations led to lower adaptation potential in their host.

 Previous studies on phoma stem canker of winter oilseed rape, or on other wind-dispersed fungus diseases (e.g., *Puccinia triticina* responsible for wheat leaf rust), highlighted the major effects of landscape composition in terms of acreages of the considered crop and/or of the different host cultivars (level and type of resistance) on disease spread and impacts (e.g., Hossard *et al*., 2015a; Papaïx *et al*., 2011). In our study, only the frequency of the host crop was tested (linked to the rotation period). Increasing WOSR acreages led to a significant increase of yield loss and the size of the pathogen population in the two tested strategies (WOSR- or RlmX-cultivar isolation), but never on the frequency of virulent pathotypes on RlmX-gene (as RlmX frequency remains stable in our simulations). The frequency of RlmX- cultivars in the landscape was highlighted as the main explanatory factor of the pathogen population genetic structure of *Leptosphaeria maculans* (Hossard *et al*., 2015a using a modelling approach; Rouxel *et al.*, 2003 using field survey). Adding this variable to the simulation framework, co-designed here with stakeholders, could highlight the effectiveness of other combinations of cropping practices defined in time and space. In particular, Hossard *et al*. (2015a) showed that the fraction of virulent pathotypes on RlmX-gene depended primarily on the tillage option on RlmX-cultivars at low incidences of these cultivars in agricultural landscapes, and reciprocally by tillage options on non-RlmX-cultivars at higher incidences of RlmX-cultivars, as found here. The behaviour of the genetic structure in the pathogen population could be explored by combining the rules tested here with higher proportions of RlmX-cultivars at the landscape scale.

4.2. Spatial effects

 Simulations of crop mosaics involving isolation distances are strongly dependent on the modelling framework used, in our case, the SIPPOM-WOSR (Lô-Pelzer *et al*., 2010a, 2010b). This model had previously revealed its potential for simulating the impact of spatial distribution of WOSR and associated cropping practices on disease severity in winter oilseed rape with preliminary simulations of WOSR-isolation on simplified landscapes (Lô-Pelzer *et al*., 2010b). The dispersal curve produced in SIPPOM-WOSR has been found to be consistent with dispersal kernels of *Leptosphaeria maculans* determined in field experiments, especially for distances exceeding 100 m (Bousset *et al*., 2015), and depending on weather conditions (wind speed). Spatial effects also depend on landscape configuration, e.g., hedges potentially acting as barriers. These elements were not taken into account in our study, while they could decrease dispersal, and consequently the size of the pathogen population and yield loss.

4.2.1. WOSR isolation strategy

 Based on the rapid decrease in spore dispersal and disease severity that was observed over the first hundred meters (Salam *et al*., 2001 and Marcroft *et al*., 2004, respectively), Marcroft *et al*. (2004) recommended distances between WOSR stubble (source) and sown WOSR (target) of at least 100 m, and preferably 500 m. Our work identified isolation distances as the second most important factor (after tillage of stubble) affecting the size of the pathogen population and yield loss in the simulations of a WOSR-isolation strategy. Adjacent fields (0 m isolation distance) led to higher yield loss and larger pathogen population, when compared to 500 m and 1,000 m isolation distance. These findings are consistent with the systematically higher infection levels of adjacent fields observed by Marcroft *et al.* (2004). For the WOSR-isolation strategy, regression trees highlighted lower yield loss for 1,000 m isolation distance when compared to 500 m, and the analysis of variance revealed a significant decrease of virulent pathotypes on the RlmX-gene between 500 m and 1,000 m (Appendix A). These results differ from the observations of Marcroft *et al*. (2004), who found a decline in disease severity from

 100 m to 500 m, but no significant decrease between 500 m and 1,000 m. They however did not detail cultivars and resistance characteristics, while, in our simulations, lower resistance breakdown at 1,000 m could explain the lower yield loss. Other explanatory factors could be linked to weather conditions (wind intensity and direction), which were not detailed in Marcroft *et al*. (2004), but are important parameters for dispersal (Bousset *et al*., 2015) and subsequent phoma evaluation criteria (Hossard *et al*., 2015b).

4.2.2. RlmX-cultivars isolation strategy

 For the RlmX-isolation strategy, isolation distances were significant only in terms of yield loss, and were not highlighted as a main factor in the corresponding regression tree. In this case, simulated yield loss was significantly higher for the 500 m isolation distance than for the 0 m isolation distance, while no significant differences were found between 0 m and 1,000 m, and between 500 m and 1,000 m. This potentially inconsistent result may be an artefact of mosaics design (non-RlmX field location) since the isolation distance for this strategy was only set for RlmX-cultivars (15% of the winter oilseed rape cultivation). In this strategy, the main effect expected by local stakeholders concerned the genetic structure of the pathogen population (i.e., the RlmX-virulent pathotypes) considered to be an indicator of resistance durability. We found that isolating RlmX-cultivars had no effect on this evaluation criterion. This may be consistent with previous findings of Travadon *et al*. (2011) who found high gene flow among *Leptosphaeria maculans* populations in France, which could be due to high dispersal rates or large population sizes.

4.3. Mosaic realism and feasibility

 In our study, local stakeholders designed management strategies that involved two different scales: (1) the field, mainly through stubble management depending on cultivar choice, and (2) the landscape, through isolation distances between source and target fields. The rotation

 length was defined at field scale, but also affected the landscape scale through the frequency of winter oilseed rape crops within the region. WOSR acreage has continuously increased in the Picardie NUTS-2 region since 1993, and currently accounts for about 9.6% of arable land (Agreste, 2013, 2014). This indicates a trend of shorter crop rotations, leading to more frequent cultivation of winter oilseed rape, which is consistent with the hypotheses of stakeholders involved in this study. In terms of WOSR stubble management, local stakeholders built rules involving either small changes (two passages of stubble breaker) or major changes (use of mouldboard ploughing) for comparison to the designed reference situation, where WOSR stubble was not ploughed before the following crop, as WOSR results in a good soil structure (Chan and Heenan, 1996). Although very efficient for burying stubble, and thus limiting inoculum production (Schneider *et al*., 2006), the implementation of mouldboard ploughing after WOSR would represent a significant change in management, implying the need for new ploughing equipment, and the reorganization of work at farm scale. This points to one of the limits of this study in that farms are not represented in the simulation model used. Farms could be represented, as suggested for another spatially-explicit model by Vinatier *et al.* (2012), by adding a sub-model considering farmers as agents with their specific decision rules, concerning work organization (e.g., Attonaty *et al.*, 1993; Jeuffroy *et al.*, 2012), and crop choices and allocation (e.g., Matthews, 2006). Indeed, the crop isolation rules tested here led to crop clustering effects from one year to the next, an effect that increased with the isolation distance. Such crop aggregation could be difficult to set up in practice, since crop mosaics are primarily built through crop clustering effects due to the organization of crop rotation at the farm scale (Thenail *et al*., 2009; Schaller *et al*., 2012). In the Picardie NUTS-2 region, the average farm size increased from 88 ha to 102 ha between 2000 and 2010 (Agreste, 2013), and frequent merging of fields has occurred in the region since the 1950s (Philippe and Polombo, 2009), leading to farms with grouped fields. This means that the

 isolation distance strategy would require the farmer to dedicate a large part of his land to WOSR in one given year, and forego growing it the next year. This could lead to work organization issues, to increase the farmer's risks and dependence on market prices, and also to negative impacts on other pest and diseases (e.g., on attacks by root maggot and pollen beetle in Valantin-Morison *et al.*, 2007), and thus does not seem realistic. Implementing isolation practices would also require increased coordination in year-to-year planning, and coordination between neighbouring farmers' management and cropping plans, which is not currently the case. Moreover, designed crop mosaics for the WOSR-isolation strategy did not all lead to the annual acreage objective, thus highlighting the difficulty of organizing WOSR in time and space to respect the constraints of isolation distances. As suggested by Castellazzi *et al*. (2010), this points to the need to test the 'physical' feasibility of spatio-temporal constraints, which depend on field patterns within the landscape, in order to evaluate the 'optimality' of various crop arrangements. Taking explicitly into account farms could help to assess the 'real' world feasibility of efficient crop mosaics for phoma control, and in turn lead to the design of a landscape-based Decision Support System representing farms explicitly. Two concepts are usually included in farm or landscape decision support models, i.e. the cropping plan (spatial dimension) and the decisions for crop rotation (temporal dimension) (Dury *et al*., 2012). Farmers' decisions are defined at different temporal scales, from tactical (intra-) annual decisions (e.g. crop allocation, crop management) to structural multi-year decisions (e.g. crop rotation) (Risbey *et al.*, 1999), and drive the dynamic spatial distribution of crops and associated practices at landscape scale (Dury *et al.*, 2012). Our results could be included in a decision support system bases on this information and field observations, in order to advise individual farmers on the potential phoma risk due to previous year WOSR and cultivar locations. This type of tool could then be used to facilitate the identification of infected fields for mouldboard ploughing (i.e., to limit epidemics expansion), and the

 implementation of isolation strategies with neighbouring farms, depending on other local pests (e.g., pollen beetle), and farm constraints.

5. Conclusion

 We showed in this study that stubble management was a key practice for phoma control, although displaying opposite results with ploughing of non-RlmX residues leading to lower yield loss and size of the pathogen population, but higher resistance breakdown. Combining intensive stubble management while isolating WOSR source and target fields was even more efficient in controlling the disease, while RlmX-cultivar isolation was not. Large isolation distances between WOSR fields increased phoma control, but did not allow achieving annual WOSR acreages' objectives.

 These results indicate possible trade-offs on phoma control between short- and long-term objectives, i.e. yield loss (impacting mostly farmers and crop collectors) and resistance durability (impacting mostly crop collectors and crop breeders), respectively. They also highlight that the combination of field (tillage) and landscape (isolation) factors are promising levers for phoma control. These two management strategies contribute to different types of advice for farmers: cultivar-dependent WOSR management for tillage, and spatial rules for isolation. These types of strategies are proposed when compared to current one, mostly relying on cultivar choice (resistance) and delay return (of both WOSR and resistance), with no technical information with regards to spatial deployment. A promising perspective to this study would be the design of a decision support tool to facilitate phoma risk quantification, and further advice priority (infected) fields to be either ploughed or isolated for next year WOSR cultivation.

Acknowledgments

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- Figure 1. Steps for the design and comparison of control strategies
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- Figure 2. Location of (A) the study district (in grey) and landscape modelling support (red
- dot), and (B) example of landscape composition resulting from the rules for spatial allocation
- of WOSR fields in year y+1
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- Figure 3. Simulation results for WOSR isolation strategy. Yield losses are plotted against size
- of the pathogen population (A) and frequency of virulent pathotypes on RlmX-gene (avrlmX,
- B).
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- Figure 4. Boxplots of simulated values for Population size (A), Frequency of the virulent
- 826 pathotypes on RlmX-cultivars (B), and Yield loss (C) for the WOSR isolation strategy.
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- Figure 5. Regression tree models of the three evaluation criteria for the WOSR isolation
- 829 strategy: Population size (A), avrlmX (B) and Yield loss (C).
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- Figure 7. Boxplots of simulated values for Population size (A), Frequency of the virulent
- 836 pathotypes on RlmX-cultivars (B), and Yield loss (C) for the RlmX isolation strategy.
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- Figure 8. Regression tree models of the three criteria for the RlmX-cultivar isolation strategy:
- Population size (A), avrlmX (B) and Yield loss (C).
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 Figure 6. Simulation results for RlmX isolation strategy. Yield losses are plotted against size of the pathogen population (A) and frequency of virulent pathotypes on RlmX-gene (avrlmX, B).

Table 1. Summary of the characteristics of performed simulations for the two isolation targets (fields grown with WOSR or RlmX-cultivars)

MB: Mouldboard ploughing; 2SB: 2 passages of stubble breaker; ref: reference tillage; ¹WOSR annual frequency correspond to the rotations designed by stakeholders: 5-year rotation (20%) and 7-year rotation (14.3%) with only one WOSR cultivation during the rotation.

Table 2. WOSR acreages of the three replicates of each isolation distance for RlmX- and WOSR-isolation strategies. Annual mean and standard deviation over the simulation years are indicated for each replicate.

Table 3. Analysis of variance of yield loss, frequency of virulent pathotypes on RlmXcultivars, and size of the pathogen population for the strategies of WOSR field isolation.

d.f.: number of degrees of freedom; MSE: Mean Squared Error; RMSE: Root Mean Squared Error; Tillage Other : tillage on non-RlmX cultivars.

Table 4. Tree main characteristics (importance of each factor and global relative error) for the three evaluation criteria of WOSR isolation strategies.

-: this factor was not retained as informative in the final tree.

d.f.: number of degrees of freedom; MSE: Mean Squared Error; RMSE: Root Mean Squared Error; Tillage Other : tillage on non-RlmX cultivars.

Table 6. Tree main characteristics (importance of each factor and global relative error) for the three evaluation criteria of RlmX isolation strategies.

-: this factor was not retained as informative in the final tree.

Figure 1. Steps for the design and comparison of control strategies.

¹Stakeholders designed two rotations (7- and 5-years) corresponding to an annual WOSR frequency of 14.3% and 20%, respectively.

²Stakeholders designed three isolation distances corresponding to buffer zones of 0 m (contiguous fields), 500 m and 1,000 m between source fields (WOSR of year y) and target fields (WOSR of year y+1). Isolation distances considered either all WOSR fields, either only RlmX-cultivars.

³Stakeholders designed specific tillage practices (to manage WOSR stubble) according to cultivar types (with or without RlmX qualitative resistance).

Figure 2. Location of (A) the study district (in grey) and landscape modelling support (red dot), and (B) example of landscape composition resulting from the rules for spatial allocation of WOSR fields in year y+1.

Town and wooded area are indicated in grey and green, respectively.

Maps were done with the R software (R Development Core team, 2015) and the R packages 'maptools' (Bivand and Lewin-Koh, 2016) and 'shapefiles' (Stabler, 2013).

Figure 3. Simulation results for WOSR isolation strategy. Yield losses are plotted against size of the pathogen population (A) and frequency of virulent pathotypes on RlmX-gene (avrlmX, B).

Figure 4. Boxplots of simulated values for Population size (A), Frequency of the virulent pathotypes on RlmX-cultivars (B), and Yield loss (C) for the WOSR isolation strategy.

Extreme values correspond to minimum and maximum simulated values. Red lines indicate the global median (bold line), $25th$ and $75th$ quantiles of simulated values for the corresponding variable and isolation strategy. Till. RlmX: tillage on RmX-cultivars; Till. Other: tillage on non-RlmX cultivars; Ref: reference tillage; 2SB: 2 stubble breakers; MB: Mouldboard ploughing; 7y: 7-year rotation; 5y: 5-year rotation. Colours of boxplot distinguish the modalities of a factor. Figures were realized with 'ggplot2' R package (Wickham, 2009).

Figure 5. Regression tree models of the three evaluation criteria for the WOSR isolation strategy: Population size (A), avrlmX (B) and Yield loss (C). For each group, the 1st number indicates the group mean, the $2nd$ number (n) indicates the

number of simulations, and the 3rd its corresponding percentage. The criterion distribution for each group is indicated below each tree. For each criterion, groups sited with a same letter on top of boxplot were not statistically different at $\alpha = 0.05$ (LSD test). Till.other: tillage on non-RlmX cultivar residues; Till.RlmX: tillage on RlmX-cultivars residues; MB: Mouldboard ploughing; ref: reference; 2SB: two passages of stubble breaker; 7y: 7-year rotation. Tree figures were realized with the R package 'rattle' (Williams, 2011). Darkest box colours correspond to higher group means.

Figure 6. Simulation results for RlmX isolation strategy. Yield losses are plotted against size of the pathogen population (A) and frequency of virulent pathotypes on RlmX-gene (avrlmX, B).

Figure 7. Boxplots of simulated values for Population size (A), Frequency of the virulent pathotypes on RlmX-cultivars (B), and Yield loss (C) for the RlmX isolation strategy.

Extreme values correspond to minimum and maximum simulated values. Red lines indicate the global median (bold line), $25th$ and $75th$ quantiles of simulated values for the corresponding variable and isolation strategy. Till. RlmX: tillage on RmX-cultivars; Till. Other: tillage on non-RlmX cultivars; Ref: reference tillage; 2SB: 2 stubble breakers; MB: Mouldboard ploughing; 7y: 7-year rotation; 5y: 5-year rotation; Synch: RlmXcultivar synchrony; Asynch: RlmX-cultivar asynchrony. Colours of boxplot distinguish the modalities of a factor. Figures were realized with 'ggplot2' R package (Wickham, 2009).

Figure 8. Regression tree models of the three criteria for the RlmX-cultivar isolation strategy: Population size (A), avrlmX (B) and Yield loss (C).

For each group, the 1st number indicates the group mean, the $2nd$ number (n) indicates the number of simulations, and the $3rd$ its corresponding percentage. The criterion distribution for each group is indicated below each tree. For each criterion, groups sited with the same letter on top of boxplot were not statistically different at $\alpha = 0.05$ (LSD test). Till.other: tillage on non-RlmX cultivar residues; Till.RlmX: tillage on RlmX-cultivars residues; MB: Mouldboard ploughing; ref: reference; 2SB: two passages of stubble breaker; 7y: 7-year rotation. Tree figures were realized with the R package 'rattle' (Williams, 2011). Darkest box colours correspond to higher group means.