Evolution of soil surface oilseed rape stubbles and their ability to produce spores of *Leptosphaeria maculans*: preliminary results

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ABSTRACT

Epidemics of phoma stem canker, a major disease for oilseed rape world wide, are initiated by ascospores produced on infected oilseed rape residues. The aim of this study was to characterise the effects of stubble age on primary inoculum production of *Leptosphaeria maculans.* Samples of oilseed rape stubbles, in fields from the area of St Florent (Cher, Center of France), were collected in early October, from the soil surface. In the sampled fields, oilseed rape had been cropped 1, 2 or 3 years before. The sampled fields were selected to represent a wide range of situations: shallow and deep soils with ploughing, superficial tillage or no tillage. The mean dry matter of the residues were 30 or 100 times higher in the n-1 year samples compared to the n-2 or the n-3 years samples respectively. After incubation in wet chambers, pseudothecia were mainly found in the n-1 samples, with a higher percentage of mature pseudothecia. The frequency of residues with pseudothecia was very low on n-3 samples, with no mature pseudothecia. The pycnidia frequency was similar on the n-1, n-2, and n-3 samples. Between 12 and 28% of the straw pieces had pycnidia. Germination tests were carried out for ascospores as well as for pycniospores. Rates of germination were always high for the pycnidiospores:(85 to 95%). The ascospore germination rate was 75% for the n-1 samples, and 55% for the n-2 samples. It was not possible to carry out the germination test for the n-3 samples because of a lack of mature pseudothecia. Ascospores, as well as pycnidiospores are able to induce new crop contamination. Nevertheless the n-1 samples have a much higher contamination potential, quantitatively with both types of spores, and qualitatively with ascospores. Because ascospores can be dispersed several hundreds meters or several kilometers, the spatial distribution of oilseed rape crops in a given region is may be as important to take into account as the field rotation itself, in the perspective of an integrated protection of the crop. Sound residue management and proper spatial distribution of oilseed rape crops are essential to contain the pathogen and contribute to the durable management of genetic resistances.

Key words: *Leptospheria maculans*, residues, pseudothecia maturation, crop succession, spores dissemination

INTRODUCTION

stubbles management has been, for a long time, one of the main recommendation to farmers to lower the contamination potential of *Leptosphaeria maculans*. Nevertheless, the disease is still a major problem for oilseed crops. The development of reduced tillage techniques and the breakdown of genotype specific resistances to phoma stem canker increase the interest to quantify the contamination potential of infected oilseed rape stubbles and to identify its major sources of variation. Understanding the levels and the frequency of ascospores emissions during autumn is also a major topic of interest to carry out efficient fungicides applications. The present study is a preliminary work aiming at quantifying surface oilseed rape residues from fields of different characteristics (soil depth, of tillage,), and to measure their ability to produce mature pseudothecia and pycnidia, ascospores and pycnidiospores, as well as their viability.

MATERIALS AND METHODS

Oilseed rape stubbles were collected from 18 fields chosen on three criteria: (i) duration since the previous oilseed rape crop (n-1, n-2, n-3), (ii) soil tillage (ploughing, reduced surface tillage, no tillage), (iii) soil type (deep or shallow soils). Residues were sampled from 8 x 0,25 m^2 randomised areas per location. Collected samples were stored in a cold chamber at 4°C. A sub-sample of 30 residues, representative of the different sizes observed, was collected for each location. Residues were placed in wet plastic boxes. Each sub-sample was then split in two different boxes. One was incubated at

15°C during 10 days under natural light from November to promote pseudothecia production and maturation. The second box was incubated between 20 and 24 °C under UV light during 12 hours per day during 10 days to promote pycnidia production. Production of pseudothecia and pycnidia was graded after 10 days incubation under a binocular according to 3 classes of abundance. For each box, 10 pseudothecia were sampled to check the maturation stage of pseudothecia, asci and ascospores. Spores viability was measured through germination tests carried out on glucose-agar medium in sterile Petri dishes. Germination was observed under microscope after 24 or 48 hours incubation for ascospores and pycnidiospores respectively.

RESULTS

Samples received from fields grown with oilseed rape the previous year (n-1) were the biggest with a mean dry matter of 500 g about, mainly coming from stem residues. n-2 and n-3 samples were respectively 30 and 100 times less abundant, with a mean dry matter of 14 and 3 g and mainly composed of root residues. Stubbles from ploughed fields appeared to be smaller than those coming from fields with reduced or no tillage. However, this observation was only available for a limited number of locations and remain to be confirmed.

Observation of individual residues demonstrated a higher frequency of pseudothecia on n-1 samples than on older ones (Table 1). 65% of n-1 straw residues had an important quantity of pseudothecia. On the oldest residues (n-2 and n-3) there were only a very low number of pseudothecia for respectively 90 and 94% of the residues. Stubbles of different ages had similar numbers of pycnidia. From 72 to 88% of the stubbles had only a low number of pycnidia.

Table 1: Occurrence of pseudothecia and pycnidia on oilseed rape stubbles of different ages after 10 days incubation in wet chambers. Residues were graded according to 3 classes of pseudothecia or pycnidia abundance.

	Pseudothecia			Pycnidia		
Abundance	n-1 samples	n-2 samples	n-3 samples	n-1 samples	n-2 samples	n-3 samples
Only few	7%	90%	94%	88%	72%	80%
Intermediate	28%	10%	6%	8%	23%	17%
Abundant	65%	0%	0%	4%	5%	3%

The youngest residues had a quite high percentage of mature pseudothecia (66% for n-1 samples, 53% on n-2 samples, Table 2) . No mature pseudothecia, and no differenciated ascospores were found on n-3 samples.

Table 2: Number of observations within a given pseudothecia maturation stages from oilseed rape stubbles coming from n-1, n-2, or n-3 cropping seasons. A: unmature pseudothecia and undifferentiated ascospores; B: unmature pseudothecia with differentiated ascospores; C: mature pseudothecia with differentiated and mature ascospores.

	Pseudothecia maturation stage		
Stubble age	A	B	C
n-1 samples	10	4	27
n-2 samples	7	1	9
n-3 samples	6	0	0

Germination tests performed on ascospores respectively shown a higher viability of ascospores coming from the youngest residues compared to those coming from n-2 samples. It was not possible to perform the test on ascospores coming from the oldest stubbles due to a lack of differentiation and maturation of the ascospores. The pycnidiospores remained at a high viability level (pycniodispores germination rates ranged from 85 to 95% of germination, Table 3), even on the oldest residues where they were however less abundant.

Table 3: Rate of germination on Petri dishes of ascospores and pycnidiospores collected from residues of different ages.

	Residue age		
Type of spore	n-1 samples	n-2 samples	n-3 samples
Ascospores	75%	55%	not available
Pycnidiospores	85%	88%	95%

DISCUSSION

In this preliminary study, the results indicate that the main source of ascospores are infected residues of the previous cropping season. After being in the soil for more than one season, there was a quick decrease of residue available, a decrease of the potential number of mature pseudothecia produced on each residue, and a decrease of germination ability for ascospores coming from the oldest residues. These results are in agreement with Petrie (1995) or Turkington & al (2000) The combinaison of these parameters indicates that the contaminating potential through ascospores mainly comes from residues of the n-1 oilseed rape crop. In terms of risk limitation of phoma stem canker for farmers, this means that it is probably more efficient to take into account the spatial distribution of the neighbouring fields where oilseed rape was grown the previous year (n-1), than to increase the duration of the rotation in a given field.

On the other hand, pycniodispore contamination potential remain near stable for 3 years with no major differences in frequency of pycnidia, and germination ability. Nevertheless, those spores are dispersed by splashing and their dissemination is therefore limited. In addition, pycniodispores are smaller and more sensitive to environmental factors than ascospores.The pycnidiospore contamination potential is therefore lower than these of ascospores (Barbetti, 1976)..

No major differences could be observed among soils types and soil tillage. However each combination of residue age x soil type x soil tillage was only present in 1 field. In such instance, it is impossible to conclude and further studies on the effects of soil tillage on residue characteristics and their ability to produce spores are needed to analyse their influence on phoma stem canker contamination potential.

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