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Relative importance of different types of inoculum to the establishment of *Mycosphaerella graminicola* in wheat crops in north-west Europe

F. Suffert* and I. Sache

INRA, UR1290 BIOGER-CPP, F-78850 Thiverval Grignon, France

The contribution of wheat debris to the early stages of septoria leaf blotch epidemics was assessed in a 3-year field experiment. First lesions were detected very early (December) in the case of an early sowing (mid-October), showing that the first contamination could occur as soon as the seedlings emerge. The tested debris management options (chopped debris, removal of debris followed by tillage, or tillage in absence of debris) had a strong effect, although transient, on the epidemic dynamic: the more debris present on the soil surface, the more severe initial disease was. The magnitude of differences between treatments differed substantially between years. The relative production of pycnidiospores and ascospores was measured on the chopped debris. Peaks in pycnidiospore and ascospore production coincided in October–November. Both types of spores can be involved as primary inoculum in north-west European conditions. The local amount of pycnidiospores available on debris in the field, estimated per square metre, was 1000-fold the local ascospore production. Moreover, inoculum production was quantified on debris exposed to different environmental conditions. Autumnal conditions, characterized by moderate temperature with alternating wet and dry periods, were favourable for the production of both pycnidiospores and ascospores, as shown by the high inoculum production on debris exposed to field or outdoor conditions. By late autumn, the canopy became the most important source of pycnidiospores, and this period, characterized by the decreasing role of debris as a local source of inoculum compared to distant potential sources, can be considered as the end of the early epidemic stages.

Keywords: ascospore, debris, *Mycosphaerella graminicola,* primary inoculum, pycnidiospore, *Triticum aestivum*

Introduction

Septoria leaf blotch, caused by *Mycosphaerella graminicola*, is one of the most damaging diseases of winter wheat worldwide. In less intensive wheat cropping systems, more and more popular in Western Europe, perspectives of disease management mostly rely on fungicide timing, cultivar choice and sowing date (Jordan & Hutcheon, 1999). Wheat debris management was identified as a factor controlling *M. graminicola* primary inoculum (Eyal *et al.*, 1987); accordingly, direct drilling and minimum tillage must influence disease development. Jordan & Allen (1984) showed that the development of barley net blotch (caused by *Pyrenophora teres*) was delayed and limited in ploughed areas compared to direct-drilled areas. Eyespot in wheat (caused by *Pseudocercosporella herpotrichoides*) was decreased by incorporating straw (Jenkyn *et al.*, 2001). Such an effect was not so clearly, or only marginally, reported in septoria leaf blotch (Suffert *et al.*, 2011). In some studies (e.g. Schuh, 1990), disease was assessed late in the season when, most probably, several infection cycles had already occurred; it can be assumed that the potential effect of crop debris, acting as a local source of primary inoculum, was blurred by the massive input of pycnidiospores acting as a local source of secondary inoculum. The build-up and overseasoning of inoculum involves various fungal (ascospores, pycnidiospores, mycelium) and plant (wheat seeds, stubble and debris; wheat volunteers; other grasses) material, as reviewed by Suffert *et al.* (2011). Among the potential mechanisms explaining year-to-year disease transmission and the onset of epidemics, primary infection by wind-blown ascospores released from pseudothecia borne by wheat debris is the most significant, assuming that local inoculum can be neglected (Sanderson & Hampton, 1978; Scott *et al.*, 1988; Hunter *et al.*, 1999; Eriksen & Munk, 2003). Shaw & Royle (1989) investigated the quantitative contribution of local and distant sources of inoculum using wheat seedlings as a biological trap. When protected from airborne inoculum (enclosed in tents), seedlings placed in a field previously sown with wheat (thus exposed to local inoculum) exhibited a much lower disease severity than seedlings left unprotected; the same result was obtained with seedlings kept away from

*E-mail: fsuffert@grignon.inra.fr

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local inoculum. Endogenous sources of inoculum for a given plot will also act as exogenous sources for neighbouring plots; no study appears to have considered explicitly that distant inoculum (ascospores) can be more abundant than local inoculum at the plot scale after a certain date. Some studies suggested that debris can also release a significant amount of pycnidiospores acting as local primary inoculum, especially in dry areas or where the sexual stage was not detected (Brokenshire, 1975; Djerbi, 1977; Obaedo *et al.*, 1999; Abrinbana *et al.*, 2010). The data collected by Eriksen & Munk (2003) suggested that during spring and to the end of June, only a few ascospores were produced in the crop and the majority of inoculum present during this period was pycnidiospores. No other studies have estimated the relative contribution of ascospores and pycnidiospores from local debris to first lesions.

The goal of the present study was to assess the contribution of crop debris and the relative importance of different types of inoculum to the early stages of septoria leaf blotch epidemics. In a 3-year field experiment, disease development was quantified as soon as wheat seedlings emerged and during the following winter period. The experimental treatments involved three debris management options: chopped debris, removed debris, or absence of debris. Production of ascospores and pycnidiospores was further quantified on debris exposed to different environmental conditions.

Materials and methods

Experimental design

A field experiment was carried out from 2007 to 2010 at the Grignon experimental station (Yvelines, France; 48°51′N, 1°58′E, 130 m a.s.l., 600 mm annual 30-year average rainfall). The soil type is an Orthic Luvisol with 250 g clay, 550 g silt and 200 g sand kg^{-1} in the topsoil (total $CaCO_3$: 21%; organic matter: 30 g kg^{-1}, total nitrogen: 1·6 g kg^{-1}, pH H_2O: 8·2). A 40- × 100-m plot, cropped with winter wheat during the 2006–07 season, was divided after harvest into two contiguous, 20- × 100-m subplots (Table 1). In the first subplot (CD), straw was chopped at harvest (mid-July) and left on the soil surface, and debris were chopped and partially buried to a depth of 10 cm with a disc harrow 6 weeks later (late September). In the second subplot (RD), straw was removed at harvest (mid-July), debris were chopped as in the first subplot then buried a second time and ploughed to a depth of 15–20 cm using a chisel cultivator just before subsequent wheat sowing (mid-October). During the second (2008–09) and third (2009–10) seasons, an additional plot previously cultivated with either maize (M) in 2008–09 or oilseed rape (OR) in 2009–10 was sown with wheat and used as a control plot, that is without wheat debris. Wheat cv. Soissons, moderately susceptible to septoria leaf blotch (resistance rating 5) was sown in mid-October (early in the season, wheat being generally sown in the area from the end of October to mid-November) at a sowing density of 225 seeds m^{-2} with an Amazone seed drill. The wheat crop emerged 10–15 days after sowing (Table 1). Subsequent crop management was performed according to locally recommended practices, except that no fungicide was applied.

There were no noticeable differences in crop development, plant vigour and size between treatments. Wheat density, estimated on 10 December 2009 as 200 plants m^{-2} in CD, 198 plants m^{-2} in RD, and 213 plants m^{-2} in C, was considered stable throughout the cropping season. During the three seasons, powdery mildew (*Blumeria graminis*) and leaf rust (*Puccinia triticina*) were hardly observed in the plots; tan spot (*Pyrenophora tritici-repentis*) severely attacked the crop in late 2009–10.

An automatic weather station (Enerco 516i; Cimel Electronique) located *c.* 500 m from the plots recorded hourly air temperature at a height of 2 m, and rainfall. The thermal time *t*, expressed in degree-days, was calculated, starting from the sowing date, by summing the daily mean air temperature using a 0°C base temperature.

Disease assessment

Disease was assessed at six sampling dates in 2007–08, 10 sampling dates in 2008–09, and 13 sampling dates in 2009–10, twice or thrice monthly at the beginning of the epidemics (autumn and winter) and thereafter monthly

Table 1 Synopsis of the field experiment: previous crop, wheat debris management and tillage practices during the three seasons

	2007–08		2008–09			2009–10		
Debris management[a]	RD	CD	RD	CD	M	RD	CD	OR
Preceding crop (year n – 1)	Wheat	Wheat	Wheat	Wheat	Maize	Wheat	Wheat	Oilseed rape
Ante-preceding crop (year n – 2)	Maize	Maize	Wheat	Wheat	Barley	Wheat	Wheat	Barley
Harvest	19 July	19 July	24 July	24 July	6 Oct.	28 July	28 July	17 July
Straw chopped and left on the soil	–	19 July	–	24 July	–	–	28 July	–
Stubble buried to a depth of 10 cm	29 Aug.	29 Aug.	11 Sep.	11 Sep.	–	14 Sep.	14 Sep.	–
Preceding crop debris ploughed 15–20 cm deep	16 Oct.	–	14 Oct.	–	16 Oct.	13 Oct.	–	19 Oct.
Sowing	16 Oct.	16 Oct.	17 Oct.	17 Oct.	17 Oct.	19 Oct.	19 Oct.	19 Oct.
Seedling emergence	31 Oct.	31 Oct.	30 Oct.	30 Oct.	30 Oct.	31 Oct.	31 Oct.	31 Oct.

[a]RD: removed wheat debris; CD: chopped wheat debris; M: maize debris (traces); OR: oilseed rape debris (traces).

during the epidemic phase (spring) (Table 2). At each sampling date, five plants were randomly collected in five 1-m^2 quadrats per plot and washed from soil particles. Disease intensity (incidence and severity) was assessed on the leaves of the main (M), first (T1) and second tillers (T2) of each plant. The leaf layers of the different tillers were identified according to their emergence order (L1 is the first leaf, L2 the second, etc.).

For a given layer, disease incidence was estimated as the percentage of leaves with visible symptoms. Severity was estimated as the average percentage of necrotic leaf area covered by pycnidia using a diagrammatic scale (1%, 2%, 3% and 5%, then 10%, 15%, 20% until 100%). Leaf area covered by pycnidia was considered a more accurate estimate of disease severity than necrotic area because natural senescence is particularly rapid on basal leaves and necrotic lesions caused by other leaf-spotting fungi (e.g. *Py. tritici-repentis* and *Phaeosphaeria nodorum*) interfere with septoria leaf blotch assessment (Blixt *et al.*, 2010).

In the early stages of the epidemics, the first lesions were detected on seedlings using a monocular magnifying glass swept along each leaf (Fig. 1a). During the second- (26 November and 12 December 2008) and third-season experiments (19 November and 24 November 2009), early, putative symptoms were systematically confirmed by plating *M. graminicola* on agar medium. Wheat leaves with putative lesions were placed on wet filter paper in a plastic box kept at room temperature (18–22°C) for 24 h to promote the exudation of cirrhi (Gough & Lee, 1985). A cirrhus from a single pycnidium per lesion was transferred to a Petri dish containing PDA (potato dextrose agar 39 g L^{-1}) and then streaked across the agar surface with a sterile glass rod to separate individual spores. Plates were incubated for 2 days at 18°C in the dark to promote yeast-like growth of the fungus.

Table 2 Wheat leaf layers attacked by septoria leaf blotch but still partially green on main (MT), first (T1) and second tillers (T2) at each sampling date during three seasons of experiments. For example, '3–5' means that layers 1 and 2 are completely senescent and that layers 3, 4 and 5 have at least one leaf neither completely senescent nor completely healthy, among the 3 × 25 plants sampled in RD (removed debris), CD (chopped debris) and M (maize debris traces)/OR (oilseed rape debris traces) plots

Date	°C-days[a]	MT	T1	T2
Season 2007–08				
16/10/2007	0			
14/01/2008	533	1–3		
05/02/2008	668	1–3	1	
03/03/2008	850	3–5	1–2	1
04/04/2008	1066	4–6	2–4	2–3
22/04/2008	1205	5–7	3–5	2–4
21/05/2008	1624	9–11	7–9	6–8
Season 2008–09				
17/10/2008	0			
26/11/2008	331			
12/12/2008	376	1–2		
18/12/2008	390	1–2		
14/01/2009	449	1–2		
30/01/2009	511	1–2		
11/02/2009	541	1–3		
24/02/2009	598	1–4	1	1
10/03/2009	675	3–4	1–2	1
30/03/2009	825	3–4	1–2	1–2
28/04/2009	1161	4–6	2–4	2–4
Season 2009–10				
19/10/2009	0			
12/11/2009	233			
19/11/2009	315			
24/11/2009	378	1–2		
30/11/2009	427	1–2		
08/12/2009	487	1–2		
22/12/2009	516	1–2		
14/01/2010	559	1–4		
03/02/2010	620	1–5	1–2	1–2
01/03/2010	728	2–5	1–3	1–2
31/03/2010	936	4–5	1–4	1–4
28/04/2010	1222	6–8	5–7	4–6
03/06/2010	1673	10	8	7
24/06/2010	2020	11–12	10–11	8–10

[a]°C-days: degree-days post sowing, base 0°C.

Characterization of wheat debris on the soil surface

The distribution in length and weight of wheat debris after the different stubble treatments and tillage practices was compared between the RD and CD plots during the second- (17 November 2008) and third-season experiments (4 November 2009). All visible debris on the soil surface were collected from four randomly selected quadrats (30 × 30 cm), pooled and immersed into water for 15 min to promote the sedimentation of soil particles. The floating pieces of debris were sieved and drained on filter paper, and manually split into nine length classes (≤ 2 cm, > 2–3 cm, > 3–4 cm, > 4–5 cm, > 5–7·5 cm, > 7·5–10 cm, > 10–15 cm, > 15–20 cm, > 20 cm). Pieces in each length class were counted and weighed before (fresh weight) and after oven desiccation at 55°C for 48 h (dry weight). To assess the change of debris surface density in RD and CD, debris were collected again twice during the third-season experiment (10 December 2009 and 3 February 2010) and treated as described above.

Quantification of inoculum on wheat debris and leaves

Pycnidiospore and ascospore production on wheat debris

Wheat straw was collected at the soil surface from four randomly selected quadrats in the CD plot just after harvest at the end of the second experimental season (28 July 2009) and stored under an open farm shed (hereafter referred to as 'sheltered conditions'). Wheat debris still present on the soil surface in the CD plot was collected from four randomly selected quadrats on 29 October 2009 (2 weeks after debris had been ploughed to a depth of 15–20 cm; Table 1), split in six plastic grill boxes and weathered as follows. Two boxes were stored outdoors, partially exposed to rainfall and

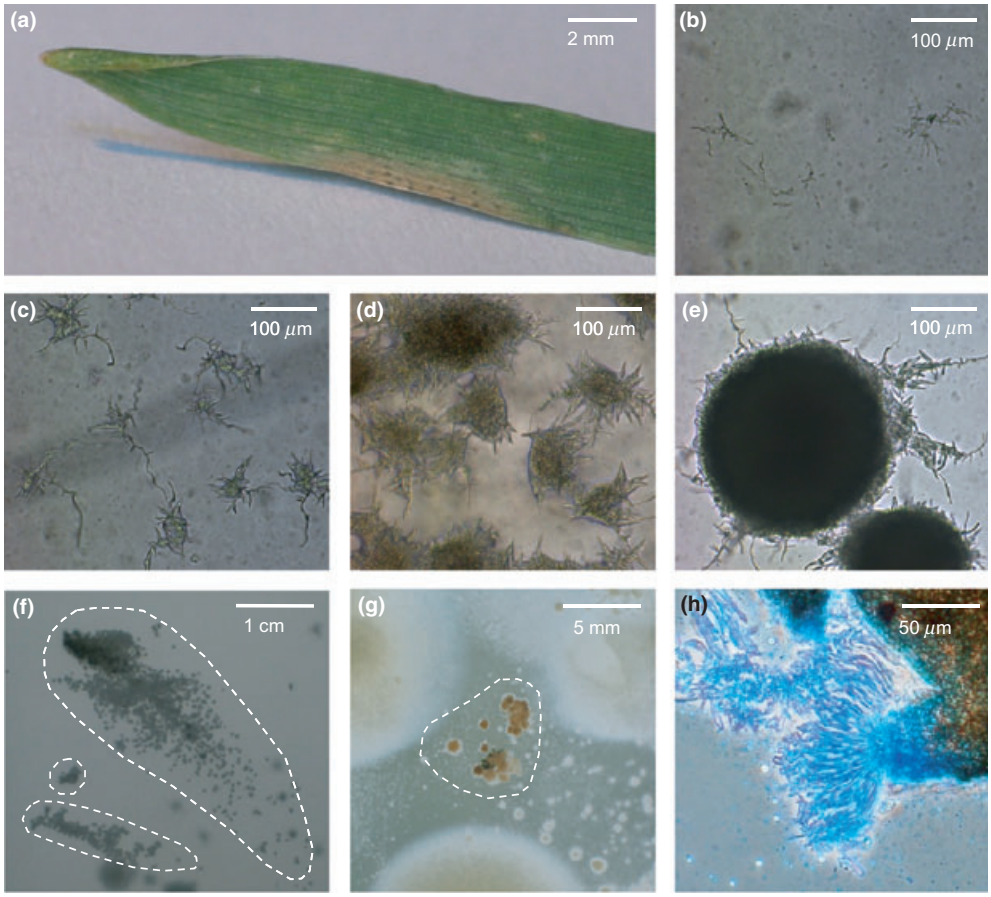

Figure 1 (a) Early septoria leaf blotch lesion on first basal wheat leaf. (b–g) *Mycosphaerella graminicola* colonies resulting from ascospore germination and conidiation on PDA after (b) 24 h, (c) 48 h, (d) 4 days, (e) 6 days, (f) 8 days and (g) 12 days. Dashed line (f–g) delimits a cluster of colonies. (h) *M. graminicola* pseudothecium (brown) and asci containing ascospores (blue) collected on wheat sheath debris.

wind ('outdoor conditions'). The four other boxes were stored in a laboratory room, two of them being wetted twice a month by spraying with 1 L water ('indoor wetted conditions') and the other two being kept untreated ('indoor non-wetted conditions'). The room air temperature was monitored with a thermohygrometer. An additional batch of debris, considered as the control, was kept at −15°C in a freezer ('frost conditions'); such conditions were expected to retain debris and inoculum in excellent condition.

Moisture content (MD) of debris placed in the different environmental conditions, except frozen conditions, was calculated every 3 weeks as:

$$\mathrm{MD} = \frac{WW - DW}{WW} \times 100$$

where WW is the wet weight (g) and DW the dry weight (g) after oven desiccation at 55°C for 48 h.

Production of pycnidiospores and ascospores was measured every 3 weeks from October 2009 to March 2010 on debris either directly collected from the CD plot (field conditions) or placed in conditions as described above.

Pycnidiospore production. Debris (20 g fresh weight) from outdoor conditions, sheltered conditions, indoor wetted conditions, indoor non-wetted conditions, and frost conditions, and 40 g debris from field conditions, was placed on a wet filter paper in moist chambers (plastic boxes 24 × 36 cm), water-sprayed with an Ecospray and incubated for 18 h at room temperature (18–22°C) to promote the exudation of cirrhi. The debris was then immersed in 1 L sterile water in an Erlenmeyer flask and mechanically shaken for 5 min. The average concentration of pycnidiospores in 10 replicate samples of each suspension was estimated with a haemocytometer (Malassez cell). The number of pycnidiospores released by 1 g dry debris (pycnidiospore release index, PRI) was calculated. The field pycnidiospore density was estimated as the number of pycnidiospores produced per gram of debris multiplied by the dry weight of debris per square metre.

Ascospore production. Debris from field conditions, outdoor conditions, sheltered conditions, indoor wetted conditions, indoor non-wetted conditions, and frost conditions was cut into 1-cm fragments and kept at 18°C for 24 h. Seven grams of debris fragments were evenly spread

on wet filter paper in two moist boxes (24 × 36 cm). In each box, eight Petri dishes (90 mm in diameter) containing PDA medium were placed upside down 1 cm above the debris. The boxes were placed for 6 h at 18°C in the dark; thereafter Petri dishes were closed and incubated in the same conditions.

After 24 h, mature ascospores ejected onto the Petri dishes germinated with one or two hyphae extending terminally, followed by lateral hyphae, as described by Halama (1996) (Fig. 1b). Ascospores produced yeast-like colonies consisting of numerous conidia with short hyphae (Fig. 1c,d). Six days later, longer hyphae emerged from the colony margin (Fig. 1e). Colonies appearing as a cream-coloured, slimy, convoluted heap, as described by Scott *et al.* (1988) were indistinguishable from those growing on PDA from pycnidiospores collected in the field. Ten days later, colonies developed black stroma-like structures as described by Halama (1996).

Distribution of colonies on Petri dishes was clustered. Each colony was visually assigned to a cluster (Fig. 1f,g) and the number of colonies was counted under a microscope (× 200 magnification) 3, 5 and 7 days after ascospore collection. Using a binocular microscope, several pseudothecia similar to those described by Halama (1996) were detected on a leaf sheath above which a high density of colonies had appeared. Pseudothecia were collected with a sterile needle, crushed and examined microscopically by mounting them in water and lactophenol-cotton blue. Asci and ascospores were found (Fig. 1h). Accordingly, it was assumed that a colony resulted from the germination of a single ascospore, and that a cluster appeared just above active pseudothecia from which several ascospores had been ejected.

A pseudothecia index (PI), assumed to be proportional to the amount of active pseudothecia borne by 1 g dry debris, was calculated as:

$$\mathrm{PI} = \frac{C}{dw}$$

where C is the average number of clusters per Petri dish and dw is the dry weight of debris exposed to a Petri dish:

$$dw = DW \cdot \frac{\pi \cdot r^2}{x \cdot y}$$

where DW = 7 g (dry weight of debris spread in a moist box), r = 4·5 cm (radius of a Petri dish), x = 24 cm and y = 36 cm (width and length of the box, respectively).

An ascospore release index (ARI), equal to the number of ascospores released per gram of dry debris, was calculated as:

$$\mathrm{ARI} = \frac{1}{dw} \sum_{i=1}^{C} n_i$$

where n_i is the number of colonies in cluster i.

Pycnidiospore production on wheat plants

Pycnidiospore production was assessed on the 25 plants sampled in the RD, CD, M and OR plots used for disease assessment during the second (2008–09) and third experiment (2009–10). Plant total fresh weight (roots excluded) was measured. All leaves (including basal senescent leaves if any) and stems were placed on a wet filter paper in a moist box (24 × 36 cm) and incubated for 18 h at room temperature (18–22°C). Leaves and stems were then immersed in sterile water (0·1–3 L, depending on the quantity of leaves) in an Erlenmeyer flask and mechanically shaken for 5 min.

The number of pycnidiospores was assessed as the average concentration of five replicate samples of each suspension estimated with a haemocytometer (Malassez cell). The field pycnidiospore density was estimated as the number of pycnidiospores produced per plant multiplied by the plant density measured on 2 December 2008 and 10 December 2009, respectively.

Results

Characterization of wheat debris on the soil surface

Debris collected in November was mainly composed of tiller and leaf sheath fragments because such parts decomposed less rapidly than leaf blades (Scott *et al.*, 1988). The debris surface density recorded in 2008 and 2009–10 was higher in the CD than in the RD plot (Table 3). In the 2009–10 season, debris surface density decreased over time in both these plots, the decrease being sharper in the CD plot. Accordingly, the ratio of debris surface density between CD and RD decreased from 2·4 in November 2009 to 1·5 in February 2010.

Debris coverage in the CD plot from November 2009 to April 2010 was estimated using a linear regression taking into account results of the three debris collections (4 November 2009, 10 December 2009, 3 February 2010): $y = a.x + b$, where x is thermal time (expressed in °C-days post-sowing) and y is the debris surface density (g m^{-2}), with a = −0·17 and b = 237·5 (R^2 = 1·000) in RD, and a = −0·68 and b = 616·5 (R^2 = 0·999) in CD.

The CD plot was covered by smaller pieces of debris, whereas the RD plot contained longer pieces: 90% of the pieces of debris in CD had a length ≥ 4 cm (2008) or

Table 3 Debris surface density in the removed debris (RD) and chopped debris plots (CD) assessed during the 2008–09 and 2009–10 seasons

	Date			
Plot	17/11/2008 (277°C-days)	4/11/2009 (174°C-days)	10/12/2009 (507°C-days)	3/02/2010 (620°C-days)
RD (g m^{-2})	59·2	207·8	151·1	131·7
CD (g m^{-2})	143·9	497·6	275·6	193·3
Ratio CD/RD	2·4	2·4	1·8	1·5

≥ 5 cm (2009), while 90% in RD had a length ≥ 5 cm (2008) or ≥ 7·5 cm (2009) (Fig. 2). The impact of the debris management in CD (straw chopped and spread out on the soil in mid-July and debris ploughed to a depth of 15–20 cm) on its distribution in length and weight was high, and more pronounced in 2009–10 than in 2008–09.

Mean daily air temperature in the field ranged from 5 to 19°C from September to December 2009 and from −6 to 9°C from December 2009 to February 2010. Mean daily room air temperature (indoor wetted and non-wetted conditions) ranged from 17 to 21°C. Average debris moisture content was high (57·9%) in field conditions, moderate (20·3%) in indoor wetted conditions, and low (15·8%, 15·2% and 15·0%, respectively) in sheltered, indoor non-wetted, and outdoor conditions.

Effect of wheat debris as local source of inoculum on epidemic development

In the three seasons, the early disease symptoms consisted of small typical lesions (Fig. 1a), sometimes bearing only one pycnidium; disease severity ranged from 1% to 3%. At each sampling date, disease intensity at the tiller scale was estimated on one to five leaf layers, excluding the totally senescent and totally healthy layers (Table 2). During the 2007–08 season, the first observation of the crop was made on 14 January 2007 (533°C-days post-sowing, i.e. 416°C-days after seedling emergence; Table 2). Disease incidence on the main tiller (MT) was already very high (100% on L1, 40% on L2 and < 5% on L3 in CD; 80% on L1, 10% on L2 and < 5% on L3 in RD; data not shown). Disease severity on MT-L1 was moderate (35% in CD and 10% in RD; Fig. 3). During the following seasons, the first sampling was performed earlier, when wheat plants were still entirely healthy. During the 2008–09 season, no disease was found on 26 November and the first symptoms were detected on 12 December (376°C-days post-sowing, i.e. 263°C-days after seedling emergence). Disease incidence was already high (90% on L1 and 10% on L2 in CD; 5% on L1 in RD; 15% in M; data not shown). Disease severity on MT-L1 was low (< 5% in CD, RD and M; Fig. 3). During the 2009–10 season, no disease was found on 12 November and the first symptoms were detected on 24 November 2009 (378°C-days post-sowing, i.e. 245°C-days after seedling emergence). Disease incidence was moderate (50% on L1 in CD; 55% on L1 and < 5% on L2 in RD; < 5% in OR; data not shown). Disease severity on MT-L1 was low (< 5% in CD, RD and OR; Fig. 3).

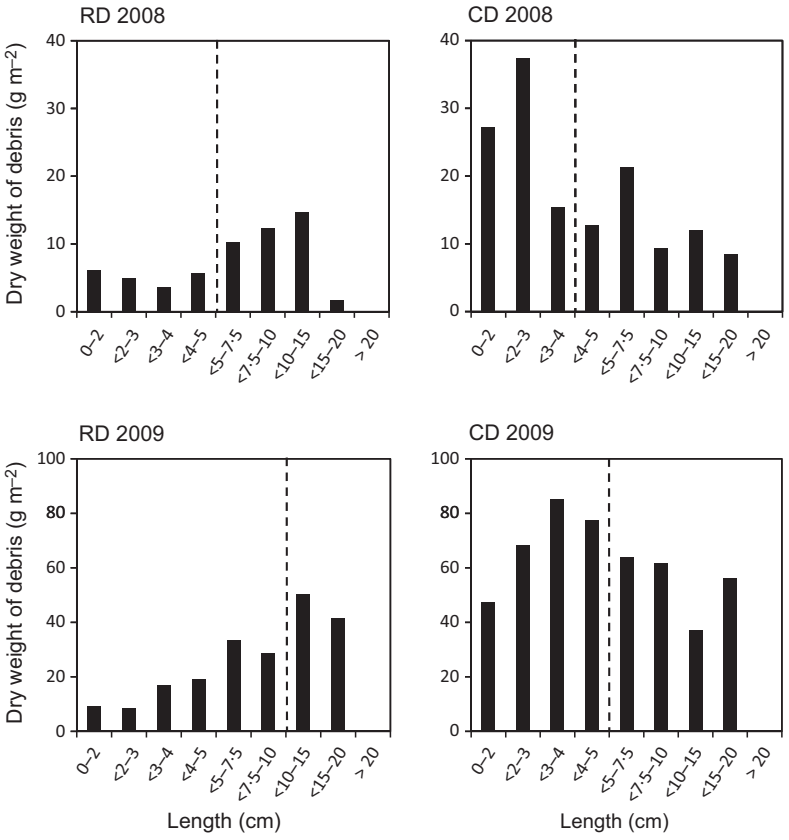

Figure 2 Distribution in length and weight of wheat debris collected in removed debris (RD) and chopped debris plots (CD) in autumn 2008 (17 November) and 2009 (4 November). Dashed line is positioned at the ninth decile, i.e. the class above which 10% of the longest debris (in number) are assigned.

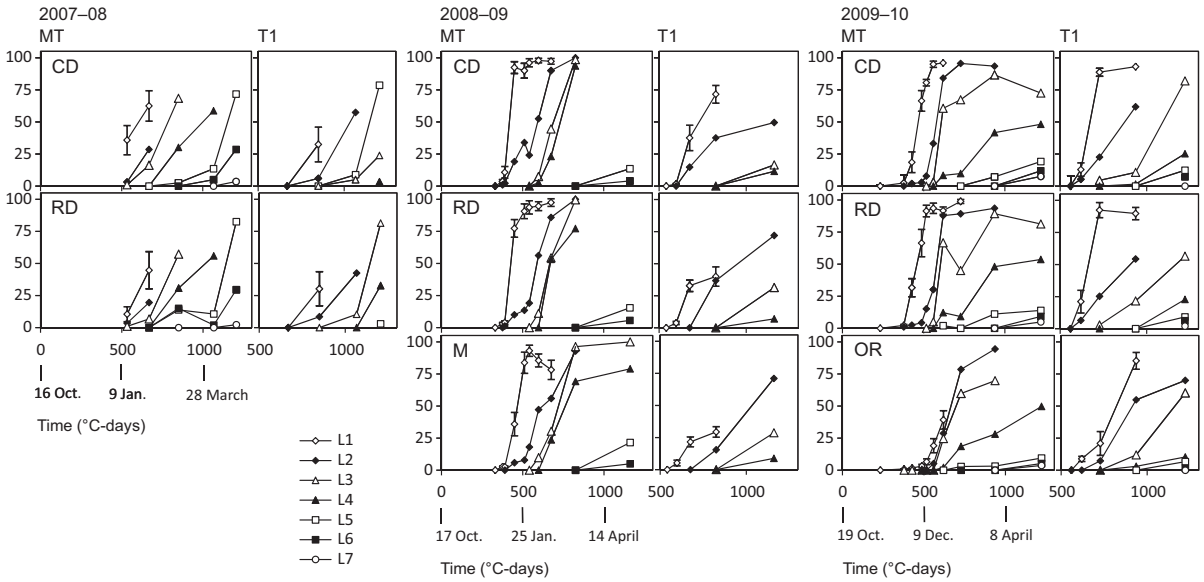

Figure 3 Wheat leaf area covered by pycnidia of *Mycosphaerella graminicola* (disease severity) on leaf layers L1 to L7 of main tiller (MT) and first tiller (T1) in chopped debris (CD), removed debris (RD), maize (M) and oilseed rape plots (OR) during three seasons of field experiments (2007–08, 2008–09, 2009–10). Standard deviation (vertical bars) is shown only on first leaves of MT and L1 for the sake of clarity; standard deviation on the other leaves is in the same range (5–10% of mean).

In the three seasons, the percentage differences in disease severity between the CD plots and the M/OR plots were 30–100% on the five leaves from 300 to 800°C-days and then decreased to zero (Fig. 4; black dots). In 2007–08 and 2008–09, percentage differences in disease severity between the CD plots and the RD plots were 0–80% on the five leaves from 300 to 800°C-days and then decreased to zero (Fig. 4; white diamonds). In 2009–10,

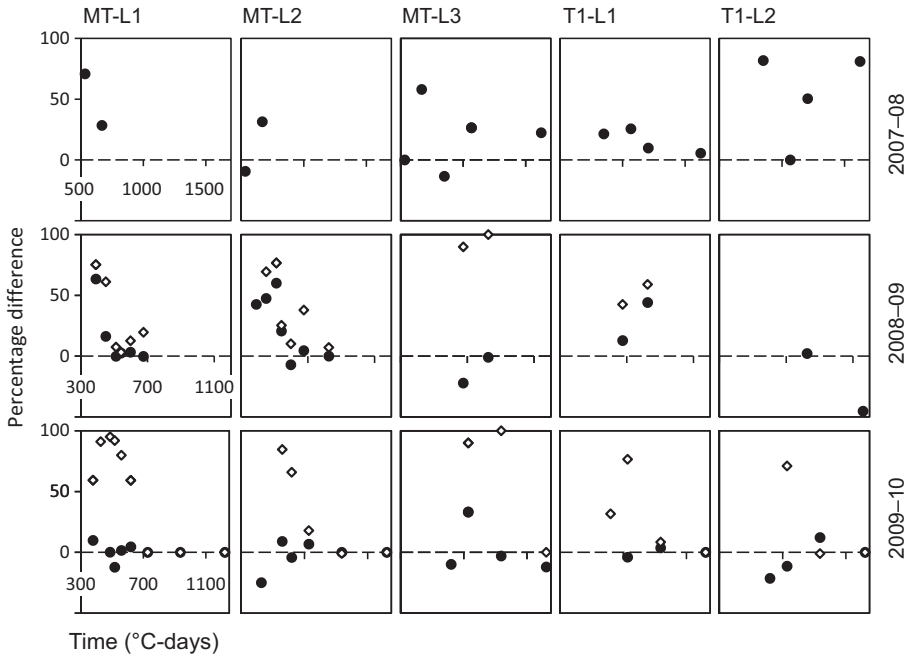

Figure 4 Percentage difference in disease severity between chopped debris (CD) and removed debris (RD) plots in 2007–08, 2008–09 and 2009–10 (solid points) and between CD plot and M (maize) plot in 2008–09 or OR (oilseed rape) plot in 2009–10 (open diamonds). Disease severity was assessed on leaves MT-L1, MT-L2, MT-L3 (main tiller), and T1-L1, T1-L2 (first tiller).

percentage differences were low and even negative. Similar results were obtained when considering disease incidence (data not shown).

Pycnidiospore and ascospore production on wheat debris exposed to different environmental conditions

The amount of pycnidiospores available on debris collected from the field peaked in early November 2009 (10^6 pycnidiospores g^{-1}; Fig. 5a); moisture content of the debris was high (60%; Fig. 5e) as a result of mild and rainy weather conditions (Fig. 5f). The amount of pycnidiospores on debris exposed to outdoor conditions was lower than in the field and peaked in early December 2009 (5×10^5 pycnidiospores g^{-1}; Fig. 5a). The amount of pycnidiospores on debris exposed to sheltered or indoor non-wetted conditions was even lower, but still higher than production on debris exposed to indoor wetted or frozen conditions.

The amount of active pseudothecia on debris collected from the field, which was estimated by the number of clusters of *M. graminicola* colonies on Petri dishes, decreased from September to December, peaked again in early January and then decreased to hardly measurable levels in March (Fig. 5b). Production of ascospores on debris collected from the field peaked in early November 2009 (1300 ascospores g^{-1}; Fig. 5c) and sharply decreased in November (*c.* 150 ascospores g^{-1}); few ascospores were then produced until March. Production of ascospores on debris exposed to outdoor conditions was similar to that on debris left in the field in November and December, and then increased until early January, when it was similar to the peak recorded in early November. Production of ascospores on debris exposed to sheltered,

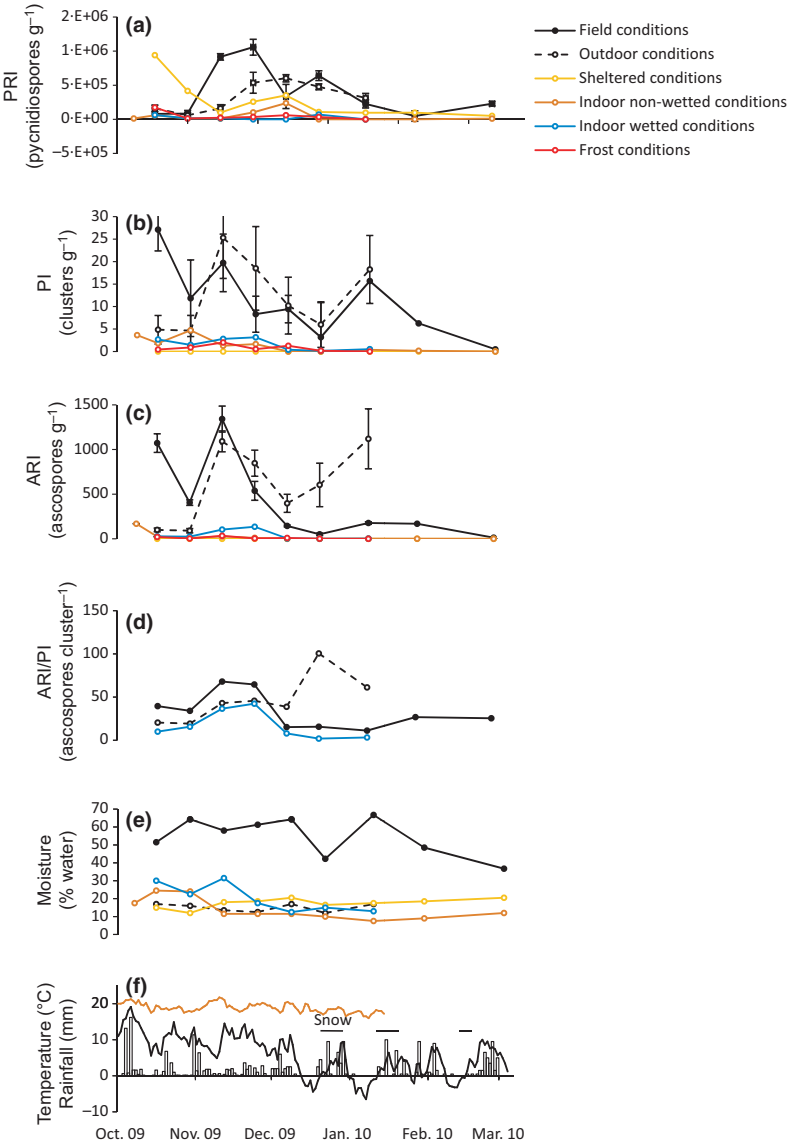

Figure 5 Quantification of *Mycosphaerella graminicola* pycnidiospore and ascospore production on wheat debris exposed to different environmental conditions during the early stages of a septoria leaf blotch epidemic (September 2009 to March 2010). (a) Pycnidiospore release index (PRI). (b) Pseudothecia index (PI). (c) Ascospore release index (ARI). (d) Ratio ARI/PI. (e) Moisture content of debris (% water). (f) Mean daily air temperature (°C; curves) and daily rainfall (mm; histogram). Vertical bars represent standard deviation.

indoor wetted, and indoor non-wetted conditions was consistently low throughout the experiment (< 100 ascospores g^{-1}). The number of ascospores liberated by a pseudothecium during a single experimental release event, which is proportional to the ratio ARI/PI, evolved similarly for field, outdoor, and indoor wetted conditions in October and November (Fig. 5d). The ratio ARI/PI peaked in early November (50–60 ascospores on average), then decreased on debris collected from the field (< 20 ascospores after late November), while it continued to increase on debris exposed to outdoor conditions (50–100 ascospores g^{-1} from early December to January).

During the early stages of the epidemics (October–February), the peaks in pycnidiospore and ascospore production coincided; the amount of pycnidiospore produced by 1 g debris was 500- to 1000-fold the ascospore production. This ratio increased to 10 000-fold during the epidemic stage (March).

Effect of wheat debris management on pycnidiospore production in the field

Pycnidiospore production on wheat plants started on 18 December 2008 in the three field plots, coinciding with the detection of first symptoms; production was high but remained below 2×10^8 spores m^{-2} until late February, and then peaked in late March (2×10^9 spores m^{-2}) (Fig. 6). At the end of April, pycnidiospore production decreased to the amount observed in December–February. From mid-December 2008 to late February 2009, differences in the number of pycnidiospores produced were observed among the CD, RD and M plots. Until 14 January, pycnidiospore production was higher in the CD and RD plots than in the M plot (6×10^7, 10^7 and 5×10^5 spores m^{-2}, respectively, on 18 December). No statistical test was performed because pycnidiospore production was assessed using the same spore suspension. On 14 January, pycnidiospore production was equivalent in the CD and RD plots, and still higher than in the M plot. From late January to mid-February 2009, pycnidiospore production was higher in M than in RD (respectively 2×10^8 vs. $1{\cdot}6 \times 10^8$ spores m^{-2}), and higher in RD than in CD (10^8 spores m^{-2}). Later on, pycnidiospore production was equivalent in the three plots.

Pycnidiospore production on wheat plants started on 30 November 2009 in the three field plots, coinciding with the detection of first symptoms; production peaked on 8 December in the CD and RD plots, whilst it remained very low in the OR plot. From late November 2009 to early January 2010, pycnidiospore production decreased in the CD and RD plots. Later on, pycnidiospore production resumed in a similar pattern in the three plots and peaked in March ($1{\cdot}5 \times 10^9$ spores m^{-2}). By the end of November 2009, more pycnidiospores were collected from wheat plants (> 2×10^8 pycnidiospores m^{-2}) than on debris (< 10^8 pycnidiospores m^{-2}) and the canopy became the most important source of pycnidiospores in CD and RD plots.

Discussion

In the 3 years of the experiment, epidemics began very early during the crop season (late November to mid-December) in the case of an early sowing (mid-October). This finding is consistent with the early field detection of disease in the UK (Shaw & Royle, 1989) during the 1985–86 and 1986–87 seasons. During the 2007–08 season, the first symptoms were observed when disease incidence on the main tiller was already very high; however, in all probability, the epidemic had begun late December or early January. During the 2008–09 and 2009–10 seasons, the first symptoms occurred *c.* 250°C-days after

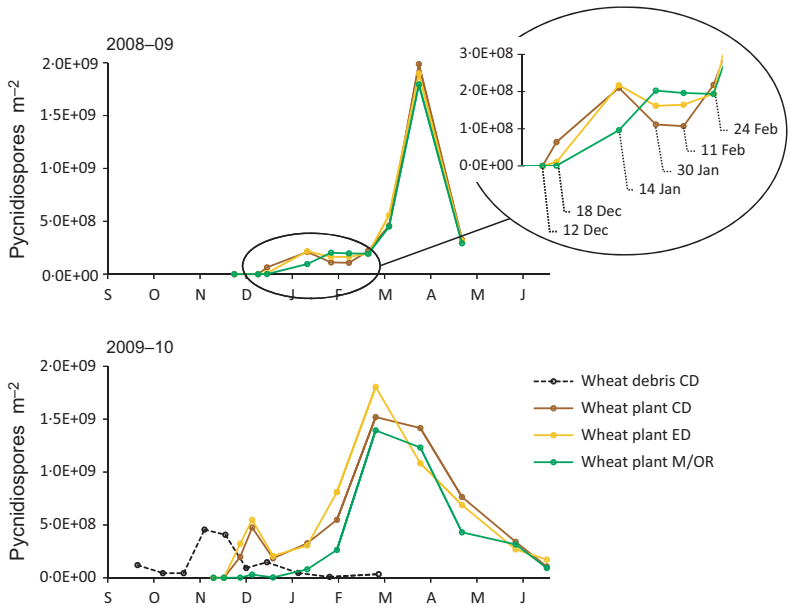

Figure 6 Quantification of *Mycosphaerella graminicola* pcynidiospore production in the field in chopped debris (CD), removed debris (RD), maize (M) and oilseed rape plots (OR) during the early stages of septoria leaf blotch epidemics on wheat. Numbers of pycnidiospores produced on wheat residues left on soil surface (primary inoculum) (2009–10) were distinguished from those produced on wheat plants (secondary inoculum) (2008–09, 2009–10).

seedling emergence; 330°C-days after seedling emergence, disease incidence lesions on the first leaves reached 100%. Such time spans are similar to the minimal latent period assessed in different conditions (Armour *et al.*, 2004; Lovell *et al.*, 2004; Viljanen-Rollinson *et al.*, 2005), while an increase in latent period was even observed at low temperatures (< 4·3°C), perhaps reflecting a physiological trade-off in which ability to grow fast at high temperatures is sacrificed for ability to grow fast at low temperatures (Shaw, 1990). This supports the occurrence of the first contamination by *M. graminicola* as soon as the first leaf emerges.

The quantity of wheat debris remaining on the soil affected the availability of *M. graminicola* inoculum and the earliness and severity of first infections (Fig. 4). Debris acting as the local source of primary inoculum had a strong, although transient, effect on the early stages of epidemics. The effect, detected on first leaves, vanished after 700°C-days (mid-March). At a given sampling date from November to March, this effect similarly decreased with increasing leaf layer, vanishing beyond the fifth leaf. Shaw & Royle (1989) found little difference in disease severity between first and second wheat by late autumn; there was no remaining effect by the spring. In barley net blotch caused by *Py. teres*, however, Jordan & Allen (1984) found an effect of straw disposal and cultivation method (plough versus direct drill) on early disease incidence and severity comparable to the findings here.

Crop debris can modify the soil local microclimate (Sharratt *et al.*, 1998; Hatfield & Prueger, 2004) and the phylloclimate (temperature perceived by the plant; Chelle, 2005), especially during winter. A 1°C difference on seedling leaves located close to the soil in the CD plot compared to the M or OR plots would have increased the thermal time scale by 40°C-days in 2008–09 and by 25 in 2009–10 at the occurrence of first symptoms. Such a temperature effect as an accelerator of early epidemic development could partially explain the percentage differences in disease severity in the different plots.

A simple method was designed to assess ascospore production on wheat debris in laboratory conditions. The decrease in the ratio ARI/PI on debris collected from the field after mid-December could be interpreted as the partial exhaustion of pseudothecia (Fig. 5d). Assuming that the number of 'clusters' was proportional to the number of fertile pseudothecia before this date, it was estimated that a pseudothecium could release 40–70 ascospores (maximum) per collection event, which is consistent with the estimates given by Eriksen & Munk (2003). Ascospores (maximum *c.* 10^3 spores g^{-1} per collection event) and pycnidiospores (maximum *c.* 10^6 spores g^{-1} per collection event) were released from wheat debris collected in the field from September to March. Peaks in ascospore and pycnidiospore potential production coincided in November, when wheat seedlings emerged. This result is consistent, firstly with those obtained by Scott *et al.* (1988), who showed that the number of pseudothecia on wheat debris peaked in December–January and declined to a low level thereafter, whilst the number of pycnidia declined to zero by December, and also with the seasonal pattern of ascospore trapping of Hunter *et al.* (1999) and Eriksen & Munk (2003).

The present results confirm that both types of *M. graminicola* spores can be involved as primary inoculum in Western European conditions. This is consistent with the fact that regional or climatic conditions can modify the relative importance of the different forms of inoculum involved in the early stage of septoria leaf blotch epidemics (Suffert *et al.*, 2011). As estimated in laboratory conditions, the pycnidiospore pool was 1000-fold the ascospore pool; the method used allowed estimation of the ascospore and pycnidiospore amounts available at a given time, but their actual liberation (instantaneous versus cumulated production) in field conditions was not addressed. Ascospores have a dispersal advantage over pycnidiospores because they are carried away over long distances during relatively dry periods, when dispersal of pycnidiospores is limited. Like pycnidiospores produced on green leaves during spring, pycnidiospores produced on wheat debris can be dispersed by rain-splash over short distances (Holmes & Colhoun, 1975) and could act as a significant local primary inoculum source in stubble-sown wheat fields.

Autumnal field conditions (temperature 5–15°C), characterized by alternating wet and dry periods, were favourable to the production of both pycnidiospores and ascospores; constant high moisture content of debris was not required, as shown by the production of ascospores in outdoor conditions. Warm (indoor wetted or non-wetted conditions) or constant dry (sheltered) conditions did not increase sporulation. Such conditions were also reported as favourable for pycnidial production in *Ph. nodorum* (Scharen, 1966; Harrower, 1974). Dry and freezing conditions should be considered only as controls and cannot be compared with field and outdoor conditions, because no new pycnidia were formed on debris incubated in a moist chamber at room temperature (18–22°C) for 18 h.

Late November can be considered as the end of the early epidemic stages. First, the canopy becomes at that time the most important source of pycnidiospores, rather than crop debris, and pycnidisopores act as secondary rather than primary inoculum (Fig. 6). Similar seasonal variations in sources of primary inoculum were reported for *Rhynchosporium secalis* on winter barley (Davis & Fitt, 1992). The decrease of the number of pycnidiospores on crop debris was caused both by the decay of debris (Table 3) and by the decomposition of pycnidiospores (Brokenshire, 1975) and of pycnidia by soil microorganisms (Baker, 1969). Secondly, debris in distant plots must have been then the most important source of ascospores, the production of which peaked in November (Fig. 5). Shaw & Royle (1989) demonstrated that ascospores act as remote, airborne inoculum.

During the winter period, as expected, the development of the epidemic slowed down. From late January to late February 2009, spore production on plants that had been infected early (in the CD plot, and then in the RD plot) became, unexpectedly, lower than on plants that

had been infected later (in the M plot) (Fig. 6). First, most of the leaves where the earliest lesions had appeared became totally or partially senescent, were detached on the soil, and were, therefore, not collected for the quantification of pycnidiospore production, although still producing pycnidiospores. Secondly, the earliest, oldest lesions, when incubated in moist chambers, were probably exhausted, while the latest, youngest lesions continued to sporulate.

Reducing the quantity of wheat debris on the soil surface over the autumn and winter months has been suggested as a means of protecting the next crop from early disease attacks (e.g. Yarham & Norton, 1981; Jordan & Allen, 1984; Jenkyn *et al.*, 1994). For some cereal diseases, especially septoria leaf blotch, this proposal is not fully supported by research results, which are quite inconsistent (Eyal *et al.*, 1987; Schuh, 1990; Sutton & Vyn, 1990; Bailey *et al.*, 2001; Gilbert & Woods, 2001). These reports assessed the effects of crop rotations and tillage at different periods, mostly late epidemic stages, when several infection cycles had already occurred. However, experiments performed by Shaw & Royle (1989) during the early epidemic stages *sensu stricto* did not rule out local sources of primary inoculum for seedlings (wheat debris); the apparently negligible effects of cropping history argue for a major role of distant source of primary inoculum. The present study showed that wheat debris can act as a source of primary inoculum (both ascospores and pycnidiospores), and that debris management can reduce the local amount of primary inoculum. The decrease in primary inoculum delayed the disease dynamics during the winter period; later on, disease progress resumed because of the input of inoculum of remote origin (ascospores). Accordingly, management of wheat debris at the plot scale cannot be expected to decrease final disease severity and yield loss. Such a decrease, however, is likely to be obtained by limiting primary inoculum on a larger scale, considering that local inoculum sources in a given plot will also act as inoculum sources for distant plots. Defining the scale relevant for management by cropping practices requires a better knowledge of the rate at which concentrations of viable ascospores decline with distance from a source.

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