Plant Pathology (2013) Doi: 10.1111/ppa.12050

Assessment of quantitative traits of aggressiveness in *Mycosphaerella graminicola* on adult wheat plants

F. Suffert*, I. Sache and C. Lannou

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

Septoria tritici blotch, caused by *Mycosphaerella graminicola*, is a major foliar disease of wheat. The quantitative traits of pathogenicity are not comprehensively described in this pathosystem. The objective of this study was to identify and quantify the most relevant variables to describe traits of aggressiveness. Four wheat cultivars were inoculated in a greenhouse with four isolates. Inoculation was performed on a limited surface of the two uppermost leaves of adult plants. The dynamics of chlorotic, necrotic and sporulating areas were assessed twice a week. Pycnidia were counted at the same time. A Gompertz model was fitted to the resulting curves. Parameter combinations with easily interpreted biological relevance were examined further as descriptors of aggressiveness. Within each category of descriptor, those which were the most pairwise correlated and which explained the largest part of the variance were retained: incubation and latent period, development rate of sporulating area, maximal sporulating area, pycnidial density, and sporulation capacity. Correlations between these variables were discussed, assuming they reflect biological relationships between the corresponding aggressiveness quantitative traits. It is suggested that the selected variables, providing a good measure of *M. graminicola* fitness, can be used to estimate quantitative resistance of wheat to septoria tritici blotch, to characterize differences among isolates within a pathogen population, and to study quantitative adaptation of the pathogen to its host and to its environment.

Keywords: latent period, lesion development, pycnidiation, quantitative resistance, *Septoria tritici*, sporulation capacity

Introduction

Septoria tritici blotch, caused by the ascomycete fungus *Mycosphaerella graminicola* (proposed new name *Zymoseptoria tritici*; Quaedvlieg *et al.*, 2011), is a major foliar disease in many wheat-growing areas worldwide. Most recent attention has focused on qualitative interactions between *M. graminicola* and its host, i.e. complete resistance that follows a gene-for-gene relationship (Arraiano *et al.*, 2001; Czembor *et al.*, 2010; Ghaffary *et al.*, 2011). The description of quantitative interactions is still fragmentary and the source of much discussion (Chartrain *et al.*, 2004; Risser *et al.*, 2011), whether it is for applied purposes (e.g. developing quantitative resistance in breeding programmes) or more fundamental research (e.g. understanding the genetic basis of host defence mechanisms). Moreover, whilst quantitative traits of aggressiveness have been well characterized for rust and mildew pathogens, this is not the case for *M. graminicola*. This study undertakes an in-depth investigation of the quantitative traits of *M. graminicola*–wheat interaction with the aim of identifying those that are both measurable and of biological significance.

Aggressiveness, that is to say the quantitative component of pathogenicity, is often separated into elementary traits that reflect a biological function, such as infection efficiency, latent period, lesion size, lesion growth rate, sporulation capacity and infectious period (Van der Plank, 1963; Pariaud *et al.*, 2009). The experimental assessment of such traits requires methodological approaches that correspond to the pathosystem under study (Lannou, 2012). Moreover, some aggressiveness traits are directly reflected in a single observation (e.g. disease severity), whilst others need to be estimated by aggregation of temporal data (e.g. latent period).

In *M. graminicola* the assessment of traits such as latent period (Bouami *et al.*, 1996; Leyva-Mir *et al.*, 2009) or spore production (Eyal & Brown, 1976; Simon & Cordo, 1997) is complex. For each of these traits, different measurements are possible and some may be more informative than others. Moreover, the measurements of different traits may give redundant information when they are highly correlated. The most informative traits must then be carefully chosen, whether it is for measuring quantitative resistance, or pathogen adaptation to host or environment.

Kema *et al.* (1996b) have described the different phases of the infection cycle of *M. graminicola* on wheat seedlings under optimal conditions. Leaf penetration occurs within 3 days of inoculation. The fungus then develops without symptoms for 10–14 days. Necrotrophic feeding begins as disease symptoms appear, and from this point on, the growth of the fungus accelerates.

*E-mail: frederic.suffert@versailles.inra.fr

Chlorosis is the first visible symptom followed by necrosis. The necrotrophic phase involves the development of the asexual sporulation organs, called pycnidia.

In research investigating interactions between *M. graminicola* isolates and wheat cultivars under greenhouse or field conditions, two variables have been mainly used to assess disease severity on a leaf: the area of necrotic tissue (e.g. Bouami *et al.*, 1996; Cowger *et al.*, 2000; Czembor *et al.*, 2010) and the area covered by pycnidia (e.g. Chartrain *et al.*, 2004; Arraiano & Brown, 2006). Disease can be assessed at a sole time point or as the area under the disease progress curve (AUDPC). Variables characterizing the dynamics of the different processes (such as spore production or lesion development) were seldom assessed, in contrast to studies on biotrophic pathogens such as wheat rusts (Shaner, 1983; Robert *et al.*, 2004). The assessment of the pycnidial density (number of pycnidia cm^{-2}; Eyal & Brown, 1976; Simon & Cordo, 1997; Leyva-Mir *et al.*, 2009) and of the spore production (number of pycnidiospores produced by a single pycnidium; Eyal, 1971; Gough, 1978; Jeger *et al.*, 1984) was in limited use.

The objective of this investigation was to establish a phenotyping approach for assessing the development of *M. graminicola* lesions throughout a complete asexual cycle and to identify the most informative traits of aggressiveness for comparing *M. graminicola* isolates. To this end, an experiment was carried out, based on a combination of *M. graminicola* isolates × wheat cultivars. Different variables were measured and the most relevant for describing *M. graminicola* aggressiveness were selected. Finally, the correlation between those variables was evaluated.

Materials and methods

Plant material

A greenhouse experiment, set up in 2008–2009 and replicated in 2009–2010, involved the inoculation of four popular French wheat cultivars with four *M. graminicola* isolates. The experiment involved adult wheat plants having similar maturities. Seeds of cv. Apache, Soissons, Caphorn and Koreli were sown on 5 December 2008 (first replicate) and on 4 December 2009 (second replicate) in Jiffy peat pots kept for 2 weeks under greenhouse conditions to promote seedling emergence. Cultivars Apache and Soissons are considered to be moderately susceptible to septoria tritici blotch (resistance rating 5 on a 1–10 scale of decreasing susceptibility; Arvalis-Institut du Végétal/CTPS), while cvs Caphorn and Koreli are considered to be moderately resistant (resistance rating 6 and 7, respectively). Apache has resistance genes *Stb4* and *Stb11* (Ghaffary *et al.*, 2011), Caphorn and Koreli share *Stb6* (T. Marcel, BIOGER-CPP, INRA, Thiverval-Grignon, France, personal communication), while an *Stb* gene is still undetermined in Soissons.

Seedlings were vernalized in a growth chamber for 7 (Apache, Soissons) or 8 (Koreli, Caphorn) weeks at 8°C with a 10 h light period (30–40 μmol m^{-2} s^{-1}) and a 14 h dark period. Afterwards, seedlings were transferred back to the greenhouse and left to acclimatize for 1 week. Then, seedlings were transplanted into individual pots filled with 1 L commercial compost (Klasmann Peat Substrat 4; Klasmann France SARL). Four grams of slow-release fertilizer (Osmocote Exact 16-11-11N-P-K 3MgO Te) were added.

Plants were sprayed with Spiroxamine (Aquarelle SF at 2 mL L^{-1}; Bayer CropScience) as a preventive measure to control powdery mildew (*Blumeria graminis* f. sp. *tritici*), 5 weeks before inoculation at the latest. Moreover, plants were fertilized with Hydrokani C2 (Hydro Agri Spécialités), at a 1:100 dilution rate (total of 10 mL per plant) poured in the pot saucers 4, 5 and 7 weeks after transplantation. During plant growth, natural daylight was supplemented with 400 W sodium vapour lamps between 06:00 h and 21:00 h. A cooling system kept the air temperature below 20°C during the 15 h light period (300–600 μmol m^{-2} s^{-1}) and above 12°C during the 9 h dark period. During growth, plants were thinned to three stems per pot.

The air temperature in the greenhouse was automatically recorded every 15 min using a ventilated sensor (Greenhouse Humidity and Temperature Sensor type 224.401; RAM). 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.

Fungal material

The inoculum was obtained from stock conidial suspensions of four *M. graminicola* isolates stored at −80°C in a sterile 1:1 glycerol–water solution. Three of them were isolated from typical septoria tritici blotch lesions on leaves of cv. Soissons collected in 2008 in Grignon (Yvelines, France; isolate INRA08-FS0001 and INRA08-FS0002) and in Le Rheu (Ille-et-Vilaine, France; isolate INRA08-FS0003). For the sake of simplicity, the isolates will be hereafter referred to as FS1, FS2 and FS3, respectively. The fourth isolate was the reference isolate IPO323 (Kema & van Silfhout, 1997; Goodwin *et al.*, 2011). All four isolates share the specific avirulence genes *Avr-Stb4* and *Avr-Stb11* that match resistance genes in cv. Apache (T. Marcel, personal communication). In addition, IPO323 (Brading *et al.*, 2002) and FS1 (T. Marcel, personal communication) carry *Avr-Stb6* that matches the resistance gene in cvs Caphorn and Koreli.

Subcultures were grown in Petri dishes containing PDA (potato dextrose agar, 39 g L^{-1}) at 18°C and kept in the dark for 5 days. Conidial suspensions were prepared by first flooding the surface of the 5-day-old cultures with sterile distilled water and then by scraping the agar surface with a sterilized glass rod to release conidia. Suspensions were calibrated to 10^5 conidia mL^{-1} using a Malassez counting chamber. Two drops of surfactant (Tween 20; Sigma) were added to the suspensions to ensure adequate coverage of the inoculated leaf surface.

Experimental design and inoculation procedure

The experiment was set up as a randomized block design (four blocks, 128 pots), with the isolate (FS1, FS2, FS3 and IPO323), the wheat cultivar (Apache, Soissons, Caphorn and Koreli) and the spore collection (spores collected or not collected) as factors. Inoculations were carried out on 14 April 2009 and 27 April 2010 between wheat growth stages Z50 (first head spikelet visible) and Z59 (head fully emerged; Zadoks *et al.*, 1974). The conidial suspensions were applied to the median part of the leaf, along a 25 mm long section of the adaxial face of the flag leaf (F1) of the main tiller. A watercolour round paintbrush (French size #18, bristle length 25 mm; Auchan) was used to apply the suspension, using a square plastic frame (25 × 25 mm) to delimit the surface of inoculation. Because leaf width varies by

cultivar, inoculated surfaces ranged from 350 to 450 mm^2. The inoculated side of the leaf was immediately turned over to avoid any running of the inoculum suspension and enclosed in a transparent polyethylene bag containing a small amount of distilled water, thereafter sealed to maintain high humidity to promote infection. Bags were removed after 72 h and pots were arranged in a randomized block design, eight per saucer. A preliminary test comparing three durations of incubation (48, 72, 96 h) in polyethylene bags showed that 72 h was enough.

The sporulation capacity of each lesion was assessed by collecting spores on a weekly basis over 7 weeks, from the appearance of first pycnidia until total leaf senescence. The first collection was made 20 days post-inoculation (dpi) in 2009 and 28 dpi in 2010. Pycnidiospores were collected by placing the inoculated leaf surface in a 25 mm diameter tube containing 10 mL water and softly sweeping the area covered by pycnidia with a paintbrush. To avoid cross-contamination, a paintbrush was assigned to each isolate and rinsed in water after each collection. The inoculated surfaces were then dried using a paper towel to prevent smudging and avoid subsequent lesion expansion by secondary infection. The total number of spores produced weekly by a given lesion was estimated as the average spore concentration of three samplings of each washing suspension assessed with a Malassez counting chamber.

Preliminary validation of experimental conditions

A preliminary experiment, divided into three trials, was performed on adult wheat plants of cv. Apache in 2008 to test and optimize the inoculation procedure, the method of analysis of the lesion development, and the method of assessment of the sporulation capacity (Table S1; Fig. S1). The inoculation procedure, using either an atomizer or a paintbrush, had no significant effect on the lesion development. Accordingly, the paintbrush, which prevents smudging and allows neater lesions to be obtained, was used in all subsequent experiments. The variables characterizing lesion development were not significantly different between the two leaf layers (F1, F2), but variables characterizing disease severity differed when measured on F1 and F2. No significant interaction was observed between leaf layer and other factors. Only F1 was used in all subsequent experiments. The inoculum dose (10^3, 10^4, 10^5 and 10^6 conidia mL^{-1}) had a significant effect on all measured variables with, for most of them, a threshold effect when inoculum concentration exceeded 10^5 conidia mL^{-1}. Previously reported field studies and trials under controlled conditions used much higher concentrations (10^6–10^7 conidia mL^{-1}, except Shaw, 1990). Due to the threshold effect, the range in quantitative response for many aggressiveness traits might be reduced for such high concentrations. Moreover, high concentrations are not representative of field conditions (estimated to be 3×10^5 conidia mL^{-1} per droplet for *Phaeosphaeria nodorum*, the cause of wheat glume blotch disease; Brennan *et al.*, 1985). Accordingly, 10^5 conidia mL^{-1} was considered as the optimal concentration for maximizing differences in disease expression and used in all subsequent experiments.

Definition and assessment of aggressiveness variables

Sequence of symptoms and lesion development

Disease severity was visually assessed twice a week as the percentage of the inoculated leaf surface (1, 2, 3 and 5%, then increments of 5% up to 100%) which presented the following symptoms: chlorosis (the percentage of chlorotic area, CHL), necrosis (the percentage of necrotic area, NEC, including both sporulating and non-sporulating area), and visible pycnidia (the percentage of sporulating area, SPO) at the time of observation t. Here, the diseased area will be named 'lesion' for the sake of simplicity, although it is actually the result of the coalescence of several single lesions caused by multiple concomitant infection events. Chlorosis and necrosis of leaf margins not associated with pycnidia or outside the inoculated surface were excluded from the assessment, as such symptoms may be caused by natural senescence. As long as the apical senescence did not merge with the disease symptoms, the percentage of the inoculated surface which remained green (GRE) was estimated by subtracting chlorotic and necrotic areas from the total inoculated surface. Using a hand lens (magnification $\times 10$), the number of pycnidia (PYC) was visually estimated as the sum of pycnidia present on the adaxial and abaxial face of the inoculated surface. Disease severity was assessed every 3–4 days by the same experimenter from the time of inoculation until leaf senescence (15 and 17 assessments, from late April to mid-June, in 2009 and 2010, respectively).

A preliminary inspection of the data confirmed that disease progress curves of septoria tritici blotch are asymmetrical (Shaw, 1990). Correspondingly, the Gompertz equation was selected to fit the symptom curves (Berger, 1981).

NEC(t), the percentage of necrotic area at the time of observation t, was modelled as follows:

$$\mathrm{NEC}(t) = \mathrm{NEC}_{\max}\exp(-B_{\mathrm{NEC}}\, e^{-\mathrm{rNEC}t})$$

where $\mathrm{NEC}_{\max}$ is the maximum percentage of necrotic area, rNEC is the necrotic area development rate and B_{NEC} is a position parameter.

SPO(t), the percentage of sporulating area at the time of observation t, was modelled using the same equation and characterized by the variables $\mathrm{SPO}_{\max}$ and rSPO.

GRE(t), the percentage of the inoculated leaf surface which remained healthy at the time of observation t, was modelled as:

$$\mathrm{GRE}(t) = 100 - \mathrm{GRE}_{\min}\exp(-B_{\mathrm{GRE}}\, e^{-\mathrm{rGRE}t})$$

where $\mathrm{GRE}_{\min}$ is the minimum percentage of green area.

Equations of NEC(t), SPO(t) and GRE(t) were fitted to the observed values, excluding the time points when the disease was not yet recorded, as well as the points when the disease had already reached its maximum level.

CHL(t), the percentage of chlorotic area at the time of observation t, was calculated as:

$$\mathrm{GRE}(t) = 100 - \mathrm{CHL}(t) - \mathrm{NEC}(t)$$

Sequence of symptoms and lesion development over the assessment period were summarized through the computation of areas under disease progress curves (AUDPC) using the fit of the different symptom curves.

Incubation and latent periods

Incubation period can be defined as the interval between inoculation and the appearance of first symptoms (Van der Plank, 1963). Chlorosis, the first expression of the infection visible to the naked eye, is a transitory symptom. In this investigation, the time elapsed from inoculation to the appearance of first chlorosis (TCHL_5), based on the threshold CHL = 5%, and the time elapsed from inoculation to the maximum percentage of chlorotic area ($\mathrm{TCHL}_{\max}$), were retained as two practical definitions of incubation period. Alternative definitions of incubation

period, the time elapsed from inoculation to 5% of maximum necrotic area NEC_{max} ($LatNEC_5$) and the time elapsed from inoculation to 50% of NEC_{max} ($LatNEC_{50}$), were also retained.

Latent period was defined in septoria tritici blotch as the interval between inoculation and the first appearance of pycnidia (Shearer & Zadoks, 1972). In this case, because the accuracy of the disease dynamic measurement was not based on a high number of infected leaves (four replicates per treatment), but on the high number of assessment time points (15 and 17), latent period ($LatSPO_5$) was estimated for a given leaf as the time elapsed from inoculation to 5% of the maximum percentage of area covered by pycnidia SPO_{max}. $LatSPO_{50}$, defined as the time elapsed from inoculation to 50% of SPO_{max}, was also retained.

Incubation and latent periods, expressed in thermal time, were calculated for each leaf from the fitted Gompertz models of SPO(t) and CHL(t).

The parameter DelaySPO, defined as the time elapsed from the appearance of first chlorosis ($TCHL_5$) to $LatSPO_{50}$, corresponds to the delay between the first visible symptoms and the peak of pycnidiation.

Spore production

The number of pycnidia produced on a lesion (PYC_{max}) was estimated as the maximal count of pycnidia, usually reached just before the leaf became entirely senescent. The pycnidial density (PYC_{dens}) was calculated as the surface density of pycnidia of the sporulating area (PYC_{max}/SPO_{max}). The total number of pycnidiospores produced by a lesion was calculated by summing the number of spores produced weekly by one lesion from the first to the last collection. To calculate the average spore production per pycnidium (nbSPO), the total number of spores produced by a lesion was divided by PYC_{max}.

Data analysis

Eighteen variables were defined and categorized according to biological functions (Table 1): incubation (CHL_{max}, $TCHL_5$, $TCHL_{max}$), latency ($LatNEC_5$, $LatNEC_{50}$, $LatSPO_5$, $LatSPO_{50}$), lesion development (rNEC, rSPO, DelaySPO), disease severity (GRE_{min}, NEC_{max}, SPO_{max}, audpcNEC, audpcSPO), pycnidiation (PYC_{max}, PYC_{dens}) and sporulation capacity (nbSPO). Because variables within the same category are intrinsically cor-

Table 1 Definition and categorization of 18 aggressiveness variables

Category	Variable		Definition	References
Incubation	Maximum chlorotic area	CHL_{max}	Maximum percentage of chlorotic area	Bouami *et al.*, 1996
	Incubation period	$TCHL_5$	Time elapsed from inoculation to CHL(t) = 0·05 (first signs of chlorosis)	
		$TCHL_{max}$	Time elapsed from inoculation to CHL(t) = CHL_{max}	
Latency		$LatNEC_5$	Time elapsed from inoculation to NEC(t) = 0·05 × NEC_{max} (first signs of necrosis)	
		$LatNEC_{50}$	Time elapsed from inoculation to NEC(t) = 0·5 × NEC_{max}	
	Latent period	$LatSPO_5$	Time elapsed from inoculation to SPO(t) = 0·05 × SPO_{max} (appearance of first pycnidia)	Bouami *et al.*, 1996; Lovell *et al.*, 2004; Viljanen-Rollinson *et al.*, 2005; Leyva-Mir *et al.*, 2009
		$LatSPO_{50}$	Time elapsed from inoculation to SPO(t) = 0·5 × SPO_{max}	
Lesion development	Necrosis development rate	rNEC	Necrotic area development rate	
	Sporulation development rate	rSPO	Sporulating area development rate	
		DelaySPO	Time elapsed from $TCHL_5$ to $LatSPO_{50}$	
Disease severity	Minimum green area	GRE_{min}	Minimum percentage of green area	Parker *et al.*, 2004
	Maximum necrotic area	NEC_{max}	Maximum percentage of necrotic area	Bouami *et al.*, 1996; Kema *et al.*, 1996a; Cowger *et al.*, 2000; Czembor *et al.*, 2010
	Maximum sporulating area	SPO_{max}	Maximum percentage of area covered by pycnidia	Bouami *et al.*, 1996; Kema *et al.*, 1996a; Chartrain *et al.*, 2004; Risser *et al.*, 2011;
	Area under the necrotic area progress curve	audpcNEC		Ahmed *et al.*, 1996; Mojerlou *et al.*, 2009
	Area under the sporulating area progress curve	audpcSPO		Arraiano *et al.*, 2001
Pycnidiation	Maximum number of pycnidia	PYC_{max}		
	Pycnidial density	PYC_{dens}	$PYC_{dens} = PYC_{max}/SPO_{max}$	Eyal & Brown, 1976; Simon & Cordo, 1997; Leyva-Mir *et al.*, 2009
Sporulation capacity	Spore production	nbSPO	Average number of pycnidiospores produced by a pycnidium	Gough, 1978; Simon & Cordo, 1997

related, correlations were estimated and used as first criterion to select the variables considered as the most informative for each category (those which were the most pairwise correlated). In parallel, the percentage of variance explained by the cultivar and isolate factors, and their interaction, were estimated for each of the 18 variables and used as secondary criterion; the variables which explained the larger part of the variance were selected because they potentially account well for the genetic variability of aggressiveness. In a second step, correlations between the selected variables (for each category) were assessed. These correlations were assumed to reflect biological relationships (positive or negative) between traits of the host–pathogen interaction. Correlation tests and ANOVA were performed with the S-PLUS 6.0 software (Lucent Technologies, Inc.).

Results

Lesion development

In many compatible interactions, chlorosis, characterized by small light-green or yellow spots covering 1–3% of the inoculated surface, was observed very early, usually at 15–23 dpi (300–450 degree-days). With time, these chlorotic spots increased in size, became more homogeneous, progressively coalesced, and then turned yellow. The visual assessment of the chlorotic area became difficult when it reached a maximum of 20–30% of the inoculated surface (Fig. 1a).

The first signs of necrosis (brown, dry and senescent tissue) were usually visible at 18–28 dpi (350–500 degree-days), when the chlorotic area was around 10–15%, i.e. on average 3–6 days after the first chlorotic spots. Necrosis ($LatNEC_5$) was well established a few days (about 50 degree-days) before the peak in chlorotic area ($TCHL_{max}$). The necrotic area increased and reached its maximum (NEC_{max}), usually 50–100%, at 40–55 dpi (800–1000 degree-days), while the chlorotic area reached a peak ($TCHL_{max}$) at 25–35 dpi (450–650 degree-days) and then decreased (Figs 1a & 2).

Because disease did not cover the whole inoculated surface due to the development of the apical senescence, SPO_{max} was usually lower than 100%. In compatible interactions, first pycnidia appeared concomitantly to the first necroses. In some cases (e.g. FS3 × Caphorn), the first pycnidia appeared within still green or chlorotic tissues, and the area surrounding these pycnidia turned into small necrotic spots 24–48 h later. In such interactions, pycnidia appeared quickly in comparison to the development rate of other symptoms. After 700–800 degree-days the necrotic area was usually not entirely covered by pycnidia, especially on the outer edge of the lesion (Fig. 2); late differences between NEC and SPO were most likely due to natural senescence.

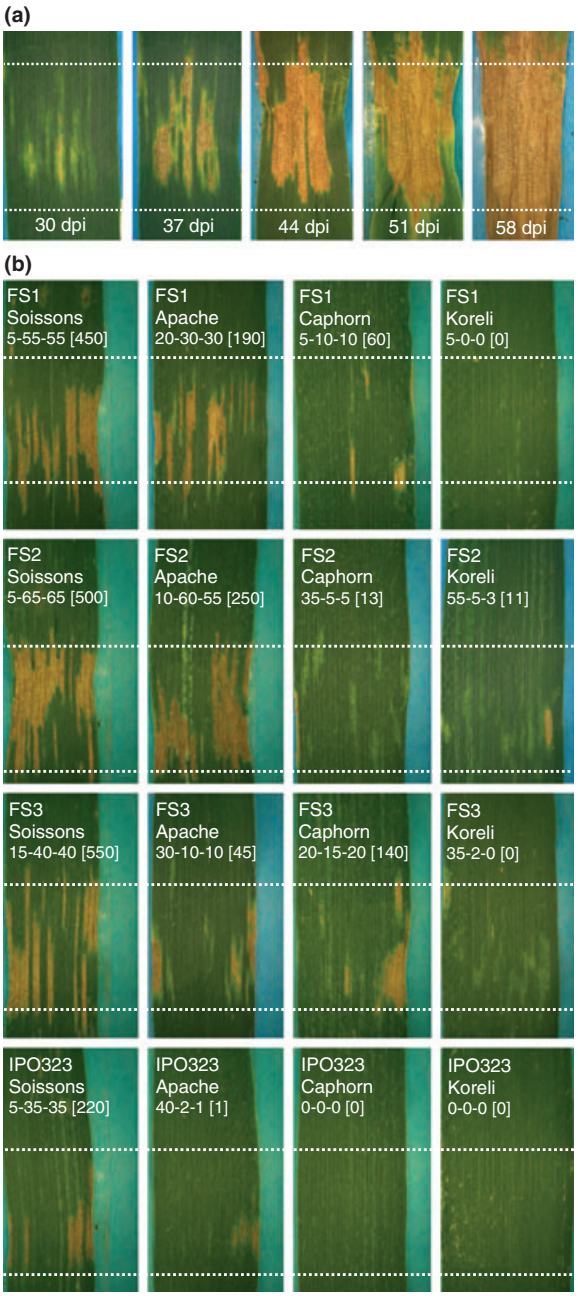

Figure 1 Symptoms of septoria tritici blotch on a wheat flag leaf. (a) Cultivar Apache inoculated with *Mycosphaerella graminicola* isolate FS3. (b) Four cultivars (Soissons, Apache, Caphorn, Koreli) inoculated with four isolates (FS1, FS2, FS3, IPO323); disease was visually assessed at t = 35 days post-inoculation (dpi) and is presented as follows: CHL(t) – NEC(t) – SPO(t) [PYC(t)] (see Table 1 for definitions of variables). Dotted lines delimit the inoculated leaf surface (350–450 mm^2). Inoculation was carried out with a paintbrush and the inoculum dose = 10^5 conidia mL^{-1}.

Effect of cultivars and isolates

Lesion development was significantly affected by the host, the pathogen and their interaction (Fig. 3). The first chlorotic spots appeared earlier on Apache, Caphorn and Soissons in 2009, and earlier in Caphorn than on Apache in 2010 (Table 2). Differences in the incubation period ($TCHL_5$) between isolates were highly significant in 2010. The necrosis development rate (rSPO) ranged between 0·14 and 0·16% per degree-day for Soissons

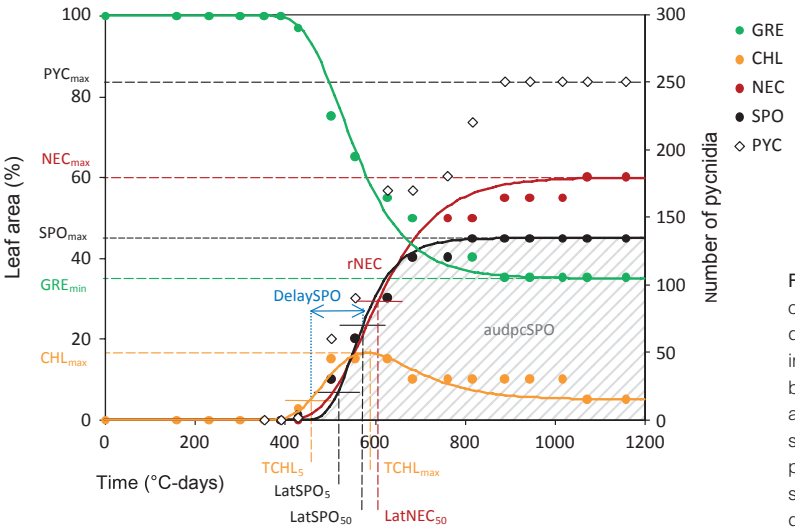

Figure 2 Development of a septoria tritici blotch lesion on a wheat flag leaf. Points indicate the experimental data (2010 experiment, isolate FS1 × cv. Apache, inoculation with paintbrush, without spore collection, block c). Lines correspond to the fitted curves (green area, GRE; chlorotic area, CHL; necrotic area, NEC; sporulating area, SPO, expressed in %; and number of pycnidia, PYC). Time is expressed in degree-days, starting from the sowing date. See Table 1 for definitions of variables.

and Caphorn in 2009 and 2010 and for Apache in 2010, while it was significantly higher for Koreli. Differences in rSPO between isolates were not significant, except for isolate FS3 in 2010.

The latent period ($LatSPO_5$) ranged between 450 and 570 degree-days for Soissons, Apache and Caphorn, whilst it was significantly longer for Koreli: first pycnidia appeared on average 320 degree-days later. DelaySPO ranged between 190 and 280 degree-days for Soissons, Apache and Caphorn, whilst it was longer for Koreli. Differences in DelaySPO were significant between isolates.

The differences in SPO_{max} and audpcSPO between cultivars and isolates were significant (Table 2). The pycnidial density (PYC_{dens}) was also significantly different between cultivars and isolates: in 2010 PYC_{dens} on Soissons was 1·5 times higher than on Apache and Caphorn, and three times higher than on Koreli. PYC_{dens} was the lowest in the cases of Koreli and IPO323 during the second experiments (2010). This was due to the late start in lesion development, which was limited when the apical senescence merged with the inoculated surface. The sporulation capacity, estimated by the average number of spores produced by a single pycnidium (nbSPO), ranged from 4000 to 8500 spores per pycnidium (Table 2). On Koreli the pycnidia appeared too late to allow an accurate estimation of the spore production. Differences were significant between cultivars and isolates in the 2009 experiment but not in 2010.

Because of cultivar × isolate interactions, means of the different aggressiveness variables were compared for each cultivar separately. Interactions were significant in 11 of the 18 variance analyses (Table 2); interactions were significant for latent period ($LatSPO_5$ and $LatNEC_{50}$) and DelaySPO both in the 2009 and 2010 experiments. Other significant interactions were only established in the 2010 experiment. Cultivar Koreli and isolate IPO323 were responsible for the largest contribution to these interactions. When isolate IPO323 was excluded from the analysis of the 2010 experiment, the cultivar × isolate interaction for SPO_{max} and audpcSPO were no longer significant. The other contributions to the cultivar × isolate interaction for latent period were moderate and can be assigned to cvs Apache and Soissons, and isolates FS2 and FS3: in the 2009 and 2010 experiments, $LatSPO_5$ and $LatSPO_{50}$ on Apache were significantly longer for isolate FS3 than FS2, while there was no difference on Soissons. Similar results were obtained for DelaySPO. In contrast, nbSPO on Soissons was significantly higher for isolate FS3 than FS2, while there was no difference on Apache.

Selection of variables most relevant for describing aggressiveness

Because variables within the same category of biological function (Table 1) are intrinsically correlated, a selection was made using two criteria. The first selection was based on the coefficient of correlation among those variables. This led to the selection of $TCHL_5$, $LatSPO_5$, rSPO, SPO_{max} and PYC_{dens}, which were the best correlated with all other variables in the same category. The coefficient of correlation ranged from 0·64 to 0·98 in 2009 and from 0·84 to 0·99 in 2010 with all P values <0·01 (data not shown). The second criterion was the part of the variance explained by the cultivar, the isolate and their interaction (Table 3). This led to the selection of the same variables, except SPO_{max}. These five variables, along with nbSPO, were considered as the most informative for assessing aggressiveness traits in *M. graminicola*.

The correlations among the six selected variables are listed in Table 3. Correlations between incubation ($TCHL_5$) and latent period ($LatSPO_5$) were, as expected, positive and high. Correlations between latent period and lesion development rate (rSPO) were also positive and high; the later the first symptom, the higher its development rate. Correlations between disease severity (SPO_{max}) and incubation or latent period were negative;

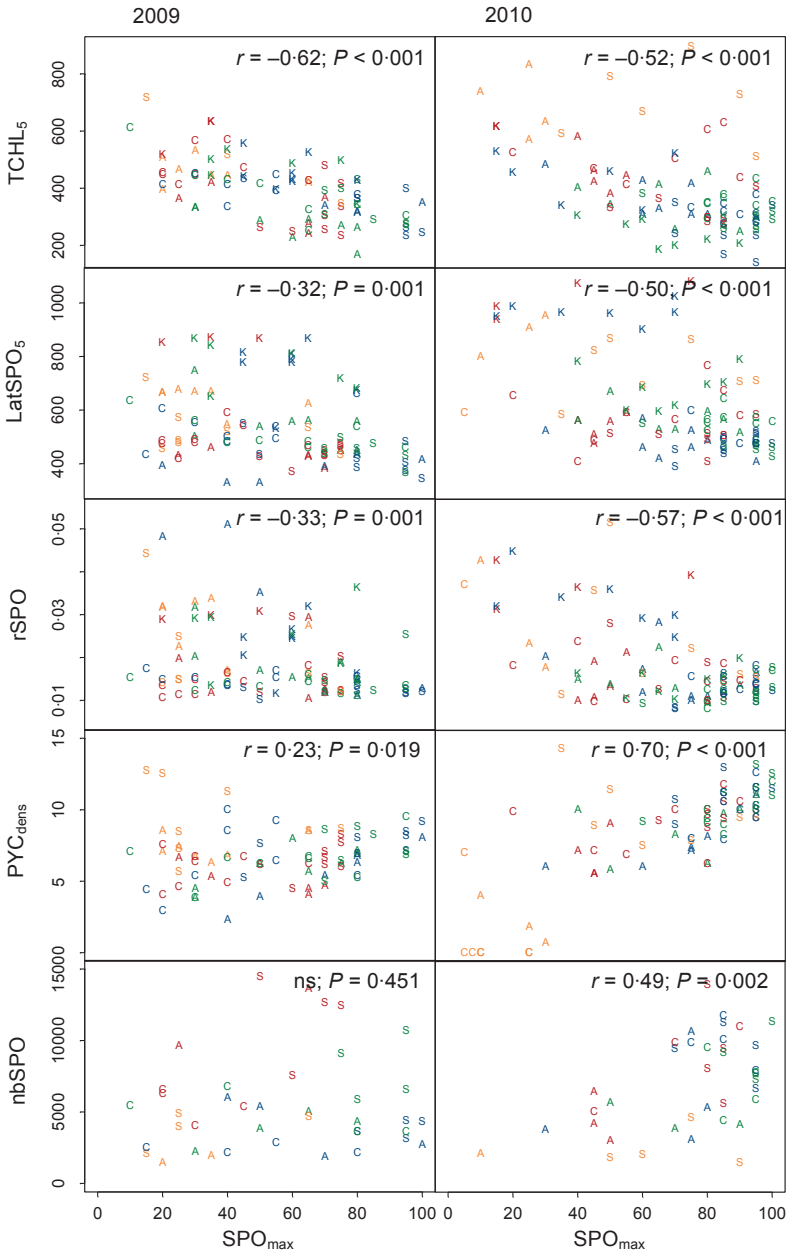

Figure 3 Plots of maximum sporulating area (SPO_{max}, in %) against five other relevant aggressiveness quantitative traits of four *Mycosphaerella graminicola* isolates on four cultivars (second set of experiments 2009 and 2010): incubation period ($TCHL_5$, in degree-days); latent period ($LatSPO_5$, in degree-days); sporulating area development rate (rSPO); maximum pycnidial density (PYC_{dens}); sporulation capacity (nbSPO, in number of spores per pycnidium). Each data point is one replicate of an isolate × cultivar interaction. Letters (A, S, C, K) indicate the cultivars (Apache, Soissons, Caphorn and Koreli, respectively) and colours correspond to the isolates (red for FS1, blue for FS2, green for FS3 and yellow for IPO323).

the earlier the first signs of a lesion (chlorosis or necrosis), the greater the sporulating area (SPO_{max}). Correlations between pycnidiation (PYC_{dens}) or sporulation (nbSPO) and incubation ($TCHL_5$) or latent period ($LatSPO_5$) were negative. Therefore, the earlier the first symptoms (chlorosis or necrosis), the higher the final density of pycnidia, and the higher the single-pycnidium spore production.

Figure 3 displays correlations between SPO_{max}, one of the most used aggressiveness variables in the literature (Table 1), and the five other variables taken to be the most relevant. Table 4 is a simplified version of Table 2 and confirms that the six selected variables provide the complementary information needed to characterize the different isolates and cultivars (isolate IPO323 and cv. Koreli were excluded from this table because of their low aggressiveness and susceptibility, respectively). For example, isolate FS1 presented a shorter latent period and a higher sporulation capacity but a lower pycnidiation than FS3 on Apache, while SPO_{max} was low for both isolates. Inversely, SPO_{max} was different between FS1 and FS3 on Caphorn while the other aggressiveness variables, except $TCHL_5$, were similar. Whatever the given variable, the overall aggressiveness of FS2 and FS3 was similar on the three cultivars. Taken trait by trait, the aggressiveness of both isolates was similar on Soissons but differences between several traits existed on Apache and Caphorn.

Table 2 Mean values of selected aggressiveness variables for each wheat cultivar and *Mycosphaerella graminicola* isolate (2009 and 2010 replicates)

Category	Variable[a]	Year	Cultivar					Isolate					Cultivar × isolate interaction
			Soissons	Apache	Caphorn	Koreli	P^b	FS3	FS2	FS1	IPO323	P^b	P^b
Incubation	$TCHL_5$	2009	351·9a[c]	345·9a	421·5a	482·1b	<0·001	358·3ab	386·3ab	405·2a	471·2b	<0·001	0·308
		2010	367·0ab	427·8a	388·4b	362·6ab	0·005	307·7a	331·9b	435·8c	692·9bc	<0·001	<0·001
Latency	$LatSPO_5$	2009	456·4a	505·8a	510·6a	773·9b	<0·001	553·8a	528·0a	504·3a	578·9b	<0·001	0·001
		2010	539·4a	570·7a	551·4a	856·5b	<0·001	571·2a	588·3ab	619·5c	770·6bc	<0·001	<0·001
	$LatNEC_{50}$	2009	578·8a	602·4a	647·2a	815·3b	<0·001	655·7a	634·6a	636·2a	655·2b	<0·001	0·001
		2010	669·8a	691·9a	678·2a	900·1b	<0·001	697·8a	697·4ab	722·1b	828·8ab	<0·001	<0·001
Lesion development	rSPO	2009	0·0154a	0·0225b	0·0142a	0·0247b	<0·001	0·0175a	0·0187a	0·0170a	0·0235a	0·057	0·429
		2010	0·0150a	0·0165a	0·0151a	0·0259b	<0·001	0·0131a	0·0180b	0·0186b	0·0261b	<0·001	<0·001
	DelaySPO	2009	188·6ab	243·2ac	190·5b	535·7c	<0·001	284·9a	237·3ab	196·6bc	172·9c	<0·001	0·001
		2010	277·0a	251·3a	259·7a	528·4b	<0·001	382·4a	347·8a	250·5b	131·5c	<0·001	0·002
Disease severity	SPO_{max}	2009	66·0a	52·0b	43·5c	48·6c	<0·001	61·0a	60·8a	47·2b	35·0c	<0·001	0·452
		2010	80·3a	52·6b	63·7c	51·8b	<0·001	79·0a	72·9a	54·7b	34·7c	<0·001	0·002
	audpcSPO	2009	24451a	16623b	12705c	4737d	<0·001	17793a	18590a	14863a	6654b	<0·001	0·212
		2010	34360a	20640b	26082b	9308c	<0·001	32242a	31312a	18195b	6000c	<0·001	0·004
Pycnidiation	PYC_{dens}	2009	7·7a	6·3b	6·3a	2·5c	<0·001	5·7a	5·9a	4·9b	8·4a	<0·001	0·677
		2010	10·4a	6·8b	7·6b	4·0c	<0·001	9·4a	7·9b	6·8b	4·8c	<0·001	<0·001
Sporulation capacity	nbSPO	2009	6854a	4793b	4236c	ns	0·001	5535a	3393b	9216c	3120b	<0·001	0·012
		2010	7427a	4700a	8415a	ns	0·011	6923a	8249a	7614a	2363a	0·005	0·841

[a]See Table 1 for definitions of variables.

[b]*P* value associated with the analysis of variance. Sources of variation are 'cultivar' (Apache, Soissons, Caphorn, Koreli), 'isolate' (FS1, FS2, FS3, IPO323), 'spore collection' (yes, no) and 'block' (a, b, c, d). Effect of spore collection and block was not significant ($P > 0·05$). None of the interactions tested in the model was significant ($P > 0·05$), except cultivar × isolate interactions for several aggressiveness variables.

[c]Data followed by different lower case letters indicate significant differences between means ($P < 0·01$).

ns, not significant.

Table 3 Correlation between categorized aggressiveness variables (2009 and 2010 replicates)

Category	Aggressiveness variable[a]	% of variance explained by cv + iso + cv × iso (2009/2010)	% of variance explained by all factors and interactions[b] (2009/2010)	Coefficient of correlation[c] (2009/2010)					
				$TCHL_5$	$LatSPO_5$	rSPO	SPO_{max}	PYC_{dens}	nbSPO
Incubation	CHL_{max}	81·8/88·4	98·3/97·8						
	$TCHL_5$	81·2/90·6	97·2/99·2	1/1					
	$TCHL_{max}$	80·2/92·6	98·1/99·1	0·84/0·76					
Latency	$LatNEC_5$	90·7/92·8	99·1/99·4	0·66/0·61					
	$LatNEC_{50}$	89·0/86·5	99·1/99·1	0·67/0·57					
	$LatSPO_5$	91·4/95·8	99·2/99·5	**0·61/0·59**	1/1				
	$LatSPO_{50}$	89·3/94·0	99·0/99·3	0·62/0·55	0·98/0·98				
Lesion development	rNEC	76·9/73·9	97·8/98·1	0·50/0·52	0·68/0·65				
	rSPO	63·7/83·5	95·4/98·2	**0·38/0·49**	**0·42/0·71**	1/1			
	DelaySPO	84·9/92·2	97·3/99·0	−0·31/−0·54	0·56/0·33	ns/ns			
Disease severity	GRE_{min}	80·3/92·4	98·0/99·1	0·72/0·62	0·47/0·62	ns/0·60			
	NEC_{max}	80·2/91·1	97·3/98·8	−0·66/−0·57	−0·41/−0·64	ns/−0·63			
	SPO_{max}	69·8/86·2	97·3/98·6	**−0·62/−0·52**	**−0·32/−0·50**	**−0·33/−0·57**	1/1		
	audpcNEC	81·4/88·8	98·7/99·7	−0·73/−0·61	−0·72/−0·77	ns/−0·61	0·74/0·83		
	audpcSPO	83·5/91·1	98·5/99·1	−0·72/−0·60	−0·70/−0·76	−0·37/−0·62	0·83/0·84		
Pycnidiation	PYC_{dens}	86·5/92·8	98·5/99·5	**ns/−0·39**	**−0·48/−0·78**	**−0·32/−0·58**	**0·23/0·70**	1/1	
	PYC_{max}	78·1/94·1	97·6/99·2	−0·60/−0·49	−0·56/−0·71	−0·41/−0·57	0·86/0·88	0·64/0·90	
Sporulation capacity	nbSPO	88·3/75·3	98·5/98·9	**ns/−0·52**	**−0·39/−0·56**	**ns/ns**	**ns/0·49**	**ns/0·47**	1/1

[a]See Table 1 for definitions of variables. The selected variables (in bold) are those which were the most pairwise correlated within the same category: $TCHL_5$, $LatSPO_5$, rSPO, SPO_{max} and PYC_{dens}. Only the correlations between these variables (by column) and the 18 others (by line) are presented.

[b]cv + iso + collect + block + (cv × iso) + (cv × collect) + (cv × block) + (iso × collect) + (iso × block) + (collect × block).

[c]Underlined correlations are presented in scatter plots (Fig. 3), with a distinction between cultivar and isolates; ns, not significant ($P > 0{\cdot}01$).

Table 4 Overview of the aggressiveness variables of three *Mycosphaerella graminicola* isolates (FS1, FS2, FS3) assessed on wheat cv. Apache (A), Soissons (S) and Caphorn (C)

Category	Agressiveness variable[a]	Isolate[b]								
		FS1			FS2			FS3		
		A	S	C	A	S	C	A	S	C
Incubation	$TCHL_5$	+	+	−−	+	++	−	++	+	+
Latency	$LatSPO_5$	+	+	−	+	+	−	−	+	−
Lesion development	rSPO	+	+	+	++	−−	+	+	−	+
Disease severity	SPO_{max}	−−	+	−−	+	++	+	−	++	+
Pycnidiation	PYC_{dens}	−−	+	−	−	++	+	+	++	−
Sporulation capacity	nbSPO	++	++	+	+	+	+	−	+	+

[a]See Table 1 for definitions.

[b]The variable is noted + or − when it contributes to increasing or decreasing the aggressiveness of the pathogen, respectively. Large effects are denoted by ++ or −−.

Discussion

The approach proposed in this study made it possible to quantitatively characterize the development of septoria tritici blotch lesions on adult wheat plants throughout a complete infection cycle. The approach includes procedures for inoculation, symptom assessment and data analysis to extract relevant aggressiveness variables. The method is based on the analysis of the temporal dynamics of lesion growth, as opposed to a sole time point assessment. All the aggressiveness variables provided information about differences between isolates or cultivars. The direct quantification of only four symptoms (CHL, NEC, SPO, PYC), completed by spore counting (nbSPO), led to the estimation of eighteen variables. Curve fitting allowed variable estimation

without the burden of daily observations, as shown for latent period by Shaner (1980). The high number of time point assessments was compensated by the low number of required replicates. Four replicates were enough to identify differences among isolates or cultivars. These differences were consistent from one experiment to another, indicating a good repeatability of the measurements. The interest of the approach is to assess concomitantly a complete set of aggressiveness traits, rather than selecting one a priori as in many studies (Table 1).

NEC is the easiest symptom to assess visually: the outline of necrosis is neater than chlorosis, while the detection of pycnidia, which can require a hand lens, is not necessary. NEC_{max} would be a highly relevant aggressiveness trait provided that the whole necrotic area is actually caused by *M. graminicola*. However, disease lesions may coalesce with natural apical senescence, especially when lesions appear near the leaf end (Ben Slimane *et al.*, 2012). As the presence of pycnidia is the only unequivoqual symptom of septoria tritici blotch, SPO_{max} should be preferred, especially under field conditions, although the part of explained variance was smaller than for NEC_{max}. Although NEC_{max} and SPO_{max} are the variables most commonly used to characterize wheat–*M. graminicola* interaction, other variables are informative and should also be considered.

Latent period is usually taken to be the time necessary for a population of lesions, grown under the same conditions, to sporulate (Lovell *et al.*, 2004). Because the appearance of septoria tritici blotch lesions is not completely synchronous (Shaw, 1990), and to limit the number of replicates, latent period was assessed here as the time by which SPO reaches a given percentage of SPO_{max} (5 or 50%). Obtained from the SPO equation, $LatSPO_5$ and $LatSPO_{50}$ are strongly dependent. $LatSPO_5$ was retained, being closer to the original definition of latent period (Van der Plank, 1963). Under field conditions, the latent period cannot be assessed because the date of contamination is often unknown. An alternative could be to measure DelaySPO, the delay between the observation of the first symptoms and the peak of pycnidiation, which is fairly correlated with $LatSPO_5$ (Table 3).

The number of spores produced by a pycnidium (nbSPO) differed between isolates and cultivars (Table 2). From only two collection events, Gough (1978) established that pycnidia released 2–2·5 times more spores in moderately resistant and susceptible cultivars than in resistant cultivars. The method used in the present study, requiring 5–6 collection events as in the case of Eyal's (1971), probably allows a more realistic estimation of the average sporulation capacity of a pycnidium. Moreover, pycnidia are not homogeneous in age. Taking into account the dynamics of pycnidium cohorts would improve the estimation of sporulation capacity. The variation in the quantity of pycnidia and spores between isolates and cultivars suggests that spore production is a variable of interest when seeking to characterize aggressiveness.

Infection efficiency, defined as the ratio of the number of lesions to the number of deposited spores, is an important aggressiveness trait (Lannou, 2012). It was not assessed in the present study because it requires controlling the number of spores deposited on the leaf (Azzimonti *et al.*, 2013), which is technically very difficult with *M. graminicola*.

The identification of the variables considered as the most informative per category of biological function is one of the main results of this study. $TCHL_5$ (incubation period), $LatSPO_5$ (latent period), rSPO (sporulating area development rate), SPO_{max} (maximum sporulating area), PYC_{dens} (pycnidial density) and nbSPO (spore production) were selected among 18 variables as the most relevant for describing aggressiveness, based on their capacity to best represent the other variables of the same category and on their capacity to discriminate isolates and cultivars.

Because septoria tritici blotch epidemics are the consequence of several embedded infection cycles (Lovell *et al.*, 1997), they can be highly impacted by a small variation in one of the six selected aggressiveness traits investigated at the single monocycle scale. $LatSPO_5$, which could appear in an estimate of the pathochron (the number of phyllochrons per latent period; Beresford & Royle, 1988), and SPO_{max}, PYC_{dens} or nbSPO, which reflect the amount of secondary inoculum, can highly impact the potential for the disease to build up on upper leaves. From an epidemiological point of view, the most useful aggressiveness variables are $LatSPO_5$, SPO_{max} and nbSPO, because they provide complementary information (Table 4). While PYC_{dens} and SPO_{max} are moderately correlated, these two aggressiveness traits, as well as rSPO and $LatSPO_5$, show differences between isolates and cultivars (Table 4).

Significant positive correlations were found in this study between several aggressiveness traits of *M. graminicola*, extending the range of already reported correlations, for example between necrotic area and pycnidium number (Eyal *et al.*, 1985), latent period, necrotic area and density of pycnidia (Bouami *et al.*, 1996), and sporulation capacity and density of pycnidia (Simon & Cordo, 1997). Oddly enough, no negative correlation was identified, while trade-offs between aggressiveness traits are common in a wide range of pathosystems (Pariaud *et al.*, 2009; Lannou, 2012). This result does not necessarily mean that such trade-offs do not exist in *M. graminicola*; rather, the experimental design was not intended to reveal them because biological material consisted only of four isolates and because the presence of resistance factors in the host could mask the expression of trade-offs.

Cultivar × isolate interactions were found for most aggressiveness variables. Significant interactions were identified between two virulent isolates (FS2 and FS3) and two susceptible cultivars (Apache and Soissons). Both significant interactions (e.g. Cowger & Mundt, 2002) and the absence of interaction (e.g. Van Ginkel & Scharen, 1988) between isolates of *M. graminicola* and

wheat cultivars have previously been reported. The occurrence of cultivar × isolate interactions confirms that the assessment of several variables, rather than a single one, is necessary, if the aggressiveness of an isolate or the resistance level of a wheat cultivar is to be accurately characterized.

The approach proposed in this study is recommended to accurately measure quantitative traits of aggressiveness in *M. graminicola*. Such a measurement is required for instance to accurately characterize the level of quantitative resistance of wheat cultivars, to more fully characterize pathogen response to environment and host factors, and to better assess differences in aggressiveness of *M. graminicola* isolates within a pathogen population.

The approach detailed in this study can be useful for characterizing partial resistance and identifying QTL in controlled conditions. Plants need examining only twice a week, based on a continuous thermal scale. The accurate assessment of aggressiveness quantitative traits of *M. graminicola* by phenotyping on adult wheat plants can be seen as complementary to seedling (Czembor *et al.*, 2010; Ghaffary *et al.*, 2011) or detached (Arraiano & Brown, 2006) leaf tests, which make it possible to carry out high-throughput phenotyping, but are not adapted for detecting low-effect resistance QTLs at the adult plant stage.

From another point of view, research carried out to characterize *M. graminicola*–wheat interactions and defence mechanisms requires the accurate assessment of the transitions between the absence of symptoms, the chlorotic phase, the necrotic phase, and the appearance of the first pycnidia (e.g. Keon *et al.*, 2007). Such transition periods are short when compared to the overall time scale of disease dynamics and they are not easily quantifiable. To relate gene expression to specific symptoms or symptom transition, these periods must be accurately characterized.

The approach proposed in this study can also be useful for the study of *M. graminicola* quantitative adaptation to its host (Cowger *et al.*, 2000) or to its environment, including temperature (Zhan & McDonald, 2011), atmospheric gas emission (Shaw *et al.*, 2008), light intensity and relative humidity (Shaw, 1991). Such studies have to include different fitness traits that can be assessed by the selected aggressiveness variables. The fact that a host effect as well as a host–isolate interaction has been easily identified for several of these variables suggest that they could provide a good measure of the fitness of *M. graminicola* in different conditions.

Acknowledgements

This work was supported by a grant 2009–2011 of the Fonds de Soutien à l'Obtention Végétale en blé tendre (FSOV) *Évaluation de la résistance du blé à la septoriose provoquée par M. graminicola*. The authors are grateful to Nathalie Galet, Christian Lepoulennec and Majed Tliha for their technical assistance, Dr Thierry Marcel for helpful discussion about *M. graminicola*–wheat interactions, and Dr Suzette Tanis-Plant for discussions and editorial advice in English. The authors thank the anonymous reviewers for both general and specific comments on the manuscript.

References

Ahmed HU, Mundt CC, Hoffer ME, Coakley SM, 1996. Selective influence of wheat cultivars on pathogenicity of *Mycosphaerella graminicola* (anamorph *Septoria tritici*). *Phytopathology* **86**, 454–8.

Arraiano LS, Brown JKM, 2006. Identification of isolate-specific and partial resistance to septoria tritici blotch in 238 European wheat cultivars and breeding lines. *Plant Pathology* **55**, 726–38.

Arraiano LS, Brading PA, Brown JKM, 2001. A detached seedling leaf technique to study resistance to *Mycosphaerella graminicola* (anamorph *Septoria tritici*) in wheat. *Plant Pathology* **50**, 339–46.

Azzimonti G, Lannou C, Sache I, Goyeau H, 2013. Components of quantitative resistance to leaf rust in wheat cultivars: diversity, variability and specificity. *Plant Pathology*. doi: 10.1111/ppa.12029.

Ben Slimane R, Bancal P, Suffert F, Bancal MO, 2012. Localized septoria leaf blotch lesions in winter wheat flag leaf do not accelerate apical senescence during the necrotrophic stage. *Journal of Plant Pathology* **94**, 543–53.

Beresford RM, Royle DJ, 1988. Relationships between leaf emergence and latent period for leaf rust (*Puccinia hordei*) on spring barley, and their significance for disease monitoring. *Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz* **95**, 361–71.

Berger RD, 1981. Comparison of the Gompertz and logistic equations to describe plant disease progress. *Phytopathology* **71**, 716–9.

Bouami Fe, Jlibene M, Mazouz H, 1996. Partial resistance and interaction in the *Triticum aestivum–Septoria tritici* association. *Al Awamia* **95**, 29–38.

Brading PA, Verstappen ECP, Kema GHJ, Brown JKM, 2002. A gene-for-gene relationship between wheat and *Mycosphaerella graminicola*, the septoria tritici blotch pathogen. *Phytopathology* **92**, 439–45.

Brennan RM, Fitt BDL, Taylor GS, Colhoun J, 1985. Dispersal of *Septoria nodorum* pycnidiospores by simulated rain and wind. *Journal of Phytopathology* **112**, 291–7.

Chartrain L, Brading PA, Widdowson JP, Brown JKM, 2004. Partial resistance to Septoria tritici blotch (*Mycosphaerella graminicola*) in wheat cultivars Arina and Riband. *Phytopathology* **94**, 497–504.

Cowger C, Mundt CC, 2002. Aggressiveness of *Mycosphaerella graminicola* isolates from susceptible and partially resistant wheat cultivars. *Phytopathology* **92**, 624–30.

Cowger C, Hoffer ME, Mundt CC, 2000. Specific adaptation by *Mycosphaerella graminicola* to a resistant wheat cultivar. *Plant Pathology* **49**, 445–51.

Czembor PC, Radecka-Janusik M, Mankowski D, 2010. Virulence spectrum of *Mycosphaerella graminicola* isolates on wheat genotypes carrying known resistance genes to septoria tritici blotch. *Journal of Phytopathology* **159**, 146–54.

Eyal Z, 1971. The kinetics of pycnospore liberation in *Septoria tritici*. *Canadian Journal of Botany* **49**, 1095–9.

Eyal Z, Brown MB, 1976. A quantitative method for estimating density of *Septoria tritici* pycnidia on wheat leaves. *Phytopathology* **66**, 11–4.

Eyal Z, Scharen AL, Huffman MD, Prescott JM, 1985. Global insights into frequencies of *Mycosphaerella graminicola*. *Phytopathology* **75**, 1456–62.

Ghaffary SMT, Faris JD, Friesen TL *et al.*, 2011. New broad-spectrum resistance to septoria tritici blotch derived from synthetic hexaploid wheat. *Theoretical and Applied Genetics* **124**, 125–42.

Goodwin SB, Ben M'Barek S, Dhillon B *et al.*, 2011. Finished genome of the fungal wheat pathogen *Mycosphaerella graminicola* reveals dispensome structure, chromosome plasticity, and stealth pathogenesis. *PLoS Genetics* **7**, e1002070.

Gough FJ, 1978. Effect of wheat host cultivars on pycnidiospore production by *Septoria tritici*. *Phytopathology* **68**, 1343–5.
Jeger MJ, Jones DG, Griffiths E, 1984. Sporulation of *Septoria nodorum* (and *S. tritici*) on spring wheat cvs Kolibri and Maris Butler in relation to growth stage, plant part and time of season. *Annals of Applied Biology* **104**, 31–9.
Kema GHJ, van Silfhout CH, 1997. Genetic variation for virulence and resistance in the wheat–*Mycosphaerella graminicola* pathosystem III. Comparative seedling and adult plant experiments. *Phytopathology* **87**, 266–72.
Kema GHJ, Annone JG, Sayoud R, van Silfhout CH, van Ginkel M, de Bree J, 1996a. Genetic variation for virulence and resistance in the wheat–*Mycosphaerella graminicola* pathosystem. I. Interactions between pathogen isolates and host cultivars. *Phytopathology* **86**, 200–12.
Kema GHJ, Yu D, Rijkenberg FHJ, Shaw MW, Baayen RP, 1996b. Histology of the pathogenesis of *Mycosphaerella graminicola* in wheat. *Phytopathology* **86**, 777–86.
Keon J, Antoniw J, Carzaniga R *et al.*, 2007. Transcriptional adaptation of *Mycosphaerella graminicola* to programmed cell death (PCD) of its susceptible wheat host. *Molecular Plant–Microbe Interactions* **20**, 178–93.
Lannou C, 2012. Variation and selection of quantitative traits in plant pathogens. *Annual Review of Phytopathology* **50**, 319–38.
Leyva-Mir SG, Gilchrist-Saavedra LI, Huerta-Espino J, Villaseñor-Mir HE, 2009. Effect of the interaction of isolates of *Septoria tritici* Rob. ex. Desm. inoculated into different genotypes of wheat (*Triticum aestivum* L.), in the latency period. *Revista Mexicana de Fitopatologia* **26**, 15–20.
Lovell DJ, Parker SR, Hunter T, Royle DJ, Coker RR, 1997. Influence of crop growth and structure on the risk of epidemics by *Mycosphaerella graminicola* (*Septoria tritici*) in winter wheat. *Plant Pathology* **46**, 126–38.
Lovell DJ, Hunter T, Powers SJ, Parker SR, van den Bosch F, 2004. Effect of temperature on latent period of septoria leaf blotch on winter wheat under outdoor conditions. *Plant Pathology* **53**, 170–81.
Mojerlou S, Safaie N, Alizadeh A, Khelghatibana F, 2009. Study of latent period and interactions between different *Septoria tritici* genotypes and different wheat cultivars and lines in greenhouse. *Trakia Journal of Sciences* **7**, 7–17.
Pariaud B, Ravigné V, Halkett F, Goyeau H, Carlier J, Lannou C, 2009. Aggressiveness and its role in the adaptation of plant pathogens. *Plant Pathology* **58**, 409–24.
Parker SR, Welham S, Paveley ND, Foulkes J, Scott RK, 2004. Tolerance of septoria leaf blotch in winter wheat. *Plant Pathology* **53**, 1–10.
Quaedvlieg W, Kema GHJ, Groenewald JZ *et al.*, 2011. *Zymoseptoria* gen. nov.: a new genus to accommodate *Septoria*-like species occurring on graminicolous hosts. *Persoonia* **26**, 57–69.
Risser P, Ebmeyer E, Korzun V, Hartl L, Miedaner T, 2011. Quantitative trait loci for adult-plant resistance to *Mycosphaerella graminicola* in two winter wheat populations. *Phytopathology* **101**, 1209–16.
Robert C, Bancal M-O, Lannou C, 2004. Wheat leaf rust uredospore production on adult plants: influence of leaf nitrogen content and septoria tritici blotch. *Phytopathology* **94**, 712–21.
Shaner G, 1980. Probits for analyzing latent period data in studies of slow rusting resistance. *Phytopathology* **70**, 1179–82.
Shaner G, 1983. Growth of uredinia of *Puccinia recondita* in leaves of slow- and fast-rusting wheat cultivars. *Phytopathology* **73**, 931–5.
Shaw MW, 1990. Effects of temperature, leaf wetness and cultivar on the latent period of *Mycosphaerella graminicola* on winter wheat. *Plant Pathology* **39**, 255–68.
Shaw MW, 1991. Interacting effects of interrupted humid periods and light on infection of wheat leaves by *Mycosphaerella graminicola* (*Septoria tritici*). *Plant Pathology* **40**, 595–607.
Shaw MW, Bearchell SJ, Fitt BDL, Fraaije BA, 2008. Long-term relationships between environment and abundance in wheat of *Phaeosphaeria nodorum* and *Mycosphaerella graminicola*. *New Phytologist* **177**, 229–38.
Shearer BL, Zadoks JC, 1972. The latent period of *Septoria nodorum* in wheat. 1. The effect of temperature and moisture treatments under controlled conditions. *Netherlands Journal of Plant Pathology* **78**, 237–47.
Simon MR, Cordo CA, 1997. Inheritance of partial resistance to *Septoria tritici* in wheat (*Triticum aestivum*): limitation of pycnidia and spore production. *Agronomie* **17**, 343–7.
Van der Plank JE, 1963. *Plant Diseases: Epidemics and Control*. New York, USA: Academic Press.
Van Ginkel M, Scharen AL, 1988. Host–pathogen relationships of wheat and *Septoria tritici*. *Phytopathology* **78**, 762–6.
Viljanen-Rollinson SLH, Marroni MV, Butler RC, Deng Y, Armour T, 2005. Latent period of Septoria tritici blotch on ten cultivars of wheat. *New Zealand Plant Protection* **58**, 256–60.
Zadoks JC, Chang TT, Konzak CF, 1974. A decimal code for growth stages of cereals. *Weed Research* **14**, 415–21.
Zhan J, McDonald BA, 2011. Thermal adaptation in the fungal pathogen *Mycosphaerella graminicola*. *Molecular Ecology* **20**, 1689–701.

Supporting Information

Additional Supporting Information may be found in the online version of this article.

Figure S1 Effect of inoculation procedure on selected aggressiveness variables.

Table S1 *P* values associated with the analysis of variance for selected aggressiveness variables performed in order to test and optimize experimental conditions.