Aggressiveness of *Septoria Tritici* Isolates on Detached and Intact Leaves of Wheat Cultivars[a]

Sewalem A. Mebrate[1] and B.M. Cooke[2]
[1]Debre Zeit Agricultural Research Center
P.O.Box: 32, Debre Zeit, Ethiopia
[2]Department of Environmental Resources Management
Faculty of Agriculture, University College Dublin (UCD), Belfield, Dublin 4, Ireland.
[a]Part of MSc thesis study in plant pathology at the University College Dublin (UCD), Ireland.

Abstract

Septoria tritici blotch (STB) of wheat caused by *Septoria tritici* Roberge in Desmaz. (teleomorph: *Mycosphaerella graminicola* [Fuckel] J.Schrot in Cohn) is an important disease of wheat. Recently, STB has become one of the most common foliar diseases of the crop worldwide. Aggressiveness of isolates and isolate mixtures of *S. tritici* was compared on detached seedling leaves mounted on water agar (0.5%) and on intact seedlings of four wheat cultivars under laboratory and glasshouse conditions, respectively. The results obtained showed that there were significant differences between isolates/isolate mixtures of *S. tritici/Stagonospora nodorum* in all the parameters tested. Similar significant differences were observed between the isolates/isolate mixtures of the pathogen in their aggressiveness on the four cultivars tested in the two experiments. The Pearson's correlation coefficients (*r* values) between the parameters tested on detached and intact seedling leaves were positive and significant ($p < 0.05$). The high positive correlations observed between the parameters studied in the laboratory and glasshouse experiments suggest that the use of detached leaves of seedlings has considerable promise for quick screening of cultivars against the disease

Introduction

Septoria tritici blotch (STB) of wheat caused by the *Septoria tritici* Rob. ex. Desm. (teleomorph: *Mycosphaerella graminicola* [Fuckel] Schroeter) is an important disease of wheat worldwide (Eyal *et al.* 1987). *Stagonospora nodorum* (Berk.) Castellani & Germano [Synonymous: *Septoria nodorum* (Muller) Hedjaroude] also occurs throughout the world and is considered to be among the major diseases of wheat (Eyal *et al.*, 1987). In many wheat-growing areas in the world, the two diseases (STB and Septoria nodorum blotch or SNB caused by *Stagonospora nodorum*) are found together and mostly difficult to distinguish one from another based on symptomatology (Eyal *et al.*, 1987). In the central highlands of Ethiopia, the blotch disease complex is ranked the third most important disease of wheat next to stem and leaf rusts (Yeshi Andenow *et al.* 1995). Some of the many advantages detached leaf technique (DLT) has over the intact plant method are that it requires little space, saves time and resources, and it avoids contamination as it is conducted in a more asceptic conditions than the intact plant method.

Despite its advantages, DLT is not well exploited in testing the aggressiveness of isolates/isolate mixtures of *S. tritici/S. nodorum* on wheat cultivars probably due to the fear researchers have had that this method would not properly represent the intact plant tests. The main objectives of this study were therefore (1) to test the aggressiveness of the isolates/mixtures of the pathogens; and (2) compare DLT with the intact seedling test and thereby to see if this method can be used as a rapid option in testing the aggressiveness of isolates/mixtures of *S. tritici/S. nodorum* on wheat cultivars.

Materials and Methods

Two experiments, detached leaf and intact seedling tests, were carried out in the Department of Environmental Resources Management (ERM), University College Dublin, Ireland from March to June, 1998. In this study, three isolates of *S. tritici* (representing least, moderately and most aggressive isolates) and their mixture were used. Similarly, a mixture of three isolates of *S. nodorum* (Sn mixture) was used to form the fifth treatment with St mixture (St+Sn [3:1]) for the sake of comparisons. Two cultivars of wheat (*Triticum aestivum* L.) representing susceptible and resistant spring wheat varieties; and two other cultivars representing susceptible and resistant winter wheat varieties were used for the two experiments. Both experiments were laid out in a randomised complete block design (RCBD) with 4 replicates.

Experiment I: Detached seedling leaf test

Growth of seedlings

Seeds of Riband and Reaper,, representing susceptible and resistant winter wheat cultivars, respectively; and of Baldus and Alexandria, representing susceptible and resistant spring wheat cultivars, respectively (B.M. Cooke, personal communication) were sown in an isolation plant propagator (IPP) (Burkard Manufacturing Co. Ltd., UK). The varieties were sown in 13cm x 13cm pots(six pots for each cultivar) each filled with John Innes Number 2 compost. Ten seedlings were grown in each pot under conditions of ambient temperature with a 16 hr light and 8hr dark system for 23 days. At 3-leaf stage, the second leaves were harvested and excised at the ligule, removing their distal 2cm portions, and the next part into two segments of approximately 5cm each. The segments were then mounted adaxial side up on the surface of water agar (Benedikz *et al.* 1981) containing 0.5 % agar No. 3 and 2.5 g l^{-1} kinetin.

Preparation of water agar

Agar No. 3 (5 g l^{-1}) was prepared by sterilising it at 121 ^{0}C for 15 min in an autoclave. After cooling for 15-20 min in a water bath (60 ^{0}C), kinetin (2.5 g l^{-1}) was added to the water agar as leaf senescence retarder before pouring the agar into each Petri dish (one Petri dish per treatment and replicated four times). A dispenser adjusted to 15 ml in a manual delivery mode was used to pour the agar into Petri dishes.

Inoculum production

Leaf samples of cultivar Riband showing leaf blotch caused by *S. tritici* and glume blotch caused by *S. nodorum* symptoms were randomly collected from the Lyons Research Farm of the University College Dublin, Ireland. Then the diseased leaf samples were placed on moistened filter paper in Petri dishes. The Petri dishes containing the samples were kept in an incubator (20-23 ^{0}C) for 12 hrs to induce oozing of cirrhi from the pycnidia.

Then 20 monopycnidial isolates, each developed from a single pycnidiospore, from each of the two pathogens were isolated and cultured on Czapek Dox V-8 (CDV-8) agar medium to which chloramphenicol (250 mg/l) was added after autoclaving the CDV-8 to prevent bacterial growth. After subculturing, the new cultures were incubated on cool plates (Cooke 1980) for 21 days at 17 ^{0}C under a diurnal cycle of near-ultraviolet (NUV) radiation to promote sporulation. The isolates/mixtures of the pathogens were incubated onto detached leaves of cultivar Riband (data not shown). Based on the preliminary screening, three isolates; namely, St1, St10 and St8, representing most aggressive, moderately aggressive and least aggressive isolates of the *S. tritici* were selected for the two experiments. St mixture (1:1:1) was prepared by mixing equal amounts of spore suspensions of the three individual isolates of the pathogen grown separately. Similarly, three isolates of *S. nodorum* representing the least aggressive, moderately aggressive and most aggressive isolates were selected. Equal amount of spore suspension of the three isolates was mixed to form Sn mixture. Then St+Sn (3:1) mixture was formed, for comparison purposes, by mixing 75% of St mixture and 25% of Sn mixture.

Inoculation of detached leaves

Spore suspensions of the five isolates/isolate mixtures namely, St10, St8, St1, St mixture (1:1:1) and St+Sn (3:1) of the two pathogens were prepared as described by Baker & Smith (1978) and Benedikz *et al.* (1981). Spore concentration (1 x 10^6 spores ml^{-1}) was determined using a haemacytometer slide. Two drops of Tween 20 was added to each 50 ml spore suspension of the respective isolates or to each 50 ml sterile distilled water (SDW) for the control treatment. Inoculation was done in four replicates of

randomized complete block design (RCBD). Using a microlitre syringe (Benedikz *et al.* 1981), 10 il spore suspension or SDW (for the control treatment) was applied in the center of the two leaf segments in each Petri dish; the inoculated plates were incubated at 20 ^{0}C under 24 hr white light system in an incubator.

The first disease symptom appearance was assessed using a binocular microscope with 12x magnification. Incubation period (IP) was then recorded in hours from the time of inoculation to symptom appearance. Size of necrotic lesions (mm) was measured starting from the 11th days after inoculation using a millimetre rule.

Experiment II: Intact seedling test

Growth of seedlings

Growth of the selected cultivars Riband, Reaper, Baldus, and Alexandria were sown in 13 x 11 cm pots filled with John Innes Number 2 compost. Four seedlings of the wheat cultivar were accommodated in each pot. The pots were arranged in four replicates of randomized complete block design in a green house.

Inoculation of intact seedling leaves

The isolates/isolate mixtures used and method of preparation of the spore suspension of each isolate or isolate mixture were the same as described for the detached leaf test. Two drops of Tween 20 was added to each 50 ml spore suspensions of each isolates/mixtures or to each 50 ml of SDW for the control treatment. At the 3-leaf stage (GS 13 of the Zadok's modified Cereal Growth Stages, and Tottman and Makepeace 1979) the four plants per pot were placed on a turntable revolving at 45 rpm. Then 5ml spore suspension of each of the treatments were sprayed onto four seedlings, in each pot, using a pressure-assisted, 3 bars, SAGOLA (MOD-475) atomiser. Immediately after inoculation, each pot was covered by a clear polythene bag for 96 hr to provide humid environment for infection.

Incubation period was recorded in hrs beginning from the time of inoculation to symptom appearance. Disease severity (% leaf area covered with necrotic lesions) was recorded 21 days after inoculation using a Septoria Key (Anonymous 1976).

The General Linear Model (GLM) of the SAS Program for Windows Version 6.12 (SAS Institute Inc., 1989-1996) designed for two-factor factorial treatment structure in an RCBD design with interactions was used for analysis of variance (ANOVA). Log (x+1) transformed data were used for analysis to stabilize the variances.

Results

Effects of isolates/mixtures

As indicated in Figure 1A, isolates/mixtures of significantly differed in their incubation periods (IP) in both detached and intact leaf tests. In the intact leaf test, St8 is significantly different from the rest having the longest IP recorded in the experiment. Similarly, St8 had the longest IP followed by St10 in the detached leaf test. The two mixtures and St1 did not significantly differ in their IPs in the detached leaf test. The difference between the isolates/mixtures was clearly demonstrated in the percentage disease severity (DS) and in the size of necrotic lesion (NLS) developed on detached and intact seedling leaves, respectively (Figure 2A).

Isolates St1 and St10 did not significantly differ in the DS they caused on the four wheat cultivars tested. The two isolate mixtures, St mixture and St+Sn (3:1), did not also significantly differ in the NLS they caused in the laboratory experiment.

Effects of cultivars

The four wheat cultivars significantly differed in their effect on IP, NLS, and DS caused by the isolates/mixtures tested in two experiments (Table 1).

Isolate x cultivar interaction effects

No significant isolate x cultivar interactions were observed for IPs both in the detached and intact seedling tests and for the NLS caused by the isolates/mixtures tested on detached seedling leaves (Table 2). However, significant isolate x cultivar interaction was observed in disease severity (DS). Relatively higher DS were recorded on cultivar Baldus inoculated with St1 or St mixture or St+Sn (3:1), followed by DS caused by St mixture or St+Sn (3:1) on cultivar Riband. Generally, slightly higher DS were recorded on all four cultivars inoculated with St+Sn (3:1) than the most aggressive *S. tritici* isolate (St1) or St mixture. This might be due to the 25% *S. nodorum* inoculum in the St+Sn (3:1) mixture.

Correlation between detached and intact leaf tests

As shown in Table 3, all the parameters tested on detached leaves and intact seedlings were significantly correlated. The correlation between IP of isolates/mixtures tested in the two experiments was positive and significant ($r = 0.85$; at $P < 0.05$). Similarly, the correlation between NLS and DS was positive and highly significant ($r = 0.97$; at $P < 0.01$). On the other hand, IP on detached seedling leaves and DS on intact seedlings were negatively and significantly correlated ($r = -0.94$; at $P < 0.01$). Conversely, IP on intact seedlings and NLS on detached seedling leaves were also negatively and significantly correlated ($r = -0.86$; at $P < 0.05$).

Table 1. Cultivar effects on the components of partial disease resistance of the *S. tritici* isolates/mixtures.

Wheat cultivars	Parameters[a]			
	IP	IPG	NLS	DS
Riband	5.17**b**	6.04**c**	2.33**b**	2.86**b**
Reaper	5.17**b**	6.04**c**	2.33**b**	2.86**b**
Baldus	4.76**c**	5.97**d**	2.61**a**	3.04**a**
Alexandria	5.28**a**	5.28**b**	2.23**c**	2.49**c**

[a] ***IP: incubation period in the detached leaf test;.significant at P < 0.01.***
NLS: necrotic lesions size in the detached leaf test; significant at P < 0.01.
IPG: incubation period in the intact leaf test; significant at P < 0.01.
DS: disease severity in the intact leaf test; significant at P < 0.01.

Table 2. Isolate/mixture x cultivar interaction effects on the components of partial disease resistance tested on the wheat cultivars.

Isolate/mixture x cultivar interaction	Parameters tested[a]			
	IP	*NLS*	*IPG*	*DS*
Stl0 x Riband	5.17	2.26	6.00	2.81**b**
St10 x Reaper	5.40	1.97	6.24	2.20**d**
St10 x Baldus	4.79	2.56	5.96	2.88**b**
St10 x Alexandria	5.30	2.17	6.13	2.44**cd**
St8 x Riband	5.37	2.02	6.14	2.58**c**
St8 x Reaper	5.50	1.66	6.31	1.69**d**
St8 x Baldus	4.92	2.39	6.07	2.58**c**
St8 x Alexandria	5.42	1.93	6.20	1.96**d**
St1 x Riband	5.05	2.49	5.97	2.90**b**
St1 x Reaper	2.16	6.20	2.53**c**	4.67
St1 x Baldus	2.71	5.94	3.25**a**	5.25
St1 x Alexandria	2.35	6.07	2.44**cd**	5.13
St mixture x Riband	2.43	6.04	3.01**ab**	5.30
St mixture x Reaper	2.16	6.24	2.58**c**	4.72
St mixture x Baldus	2.67	5.94	3.25**a**	5.33
St mixture x Alexandria	2.36	6.12	2.72**bc**	5.16
St+Sn x Riband	2.46	6.05	3.01**ab**	5.30
St+Sn x Reaper	2.14	6.26	2.77**bc**	4.69
St+Sn x Baldus	2.70	5.96	3.22**a**	5.11
St+Sn x Alexandria	2.37	6.12	2.88**b**	
Probability of significance	NS	NS	NS	0.05

[a] ***IP: incubation period in the detached leaf test; not significant at P < 0.05.***
NLS: necrotic lesions size in the detached leaf test; not significant at P < 0.05.
IPG: incubation period in the intact leaf test; not significant at P < 0.05.
DS: disease severity in the intact leaf test; significant at P < 0.05.
NS: not significant at P < 0.05; figures in a column followed by the same letter(s) Are not significantly different at P < 0.05.

Table 3 Correlation coefficients (*r* values) of *S. tritici* isolates/mixtures for the components of partial disease resistance tested.

Parameters tested		*r* values	Significance test [$P(r \ldots 0)$][1]
Detached leaves	Intact seedlings		
Incubation period (*IP*)	Incubation period (*IPG*)	+0.85	0
Incubation period (*IP*)	Disease severity (*DS*)	-0.94	**
Necrotic lesion size (*NLS*)	Incubation period (*IPG*)	-0.86	0
Necrotic lesion size (*NLS*)	Disease severity (*DS*)	+0.97	**

[1] *: *Significant at* $P < 0.05$; and **: significant at $P < 0.01$.

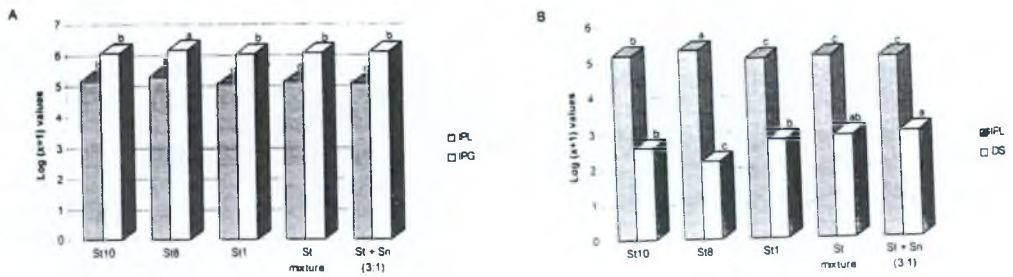

Figure 1. Comparison between the isolates/mixtures tested in terms of their *IP*, *IPG*, and *DS* tested on detached and intact seedling leaves.

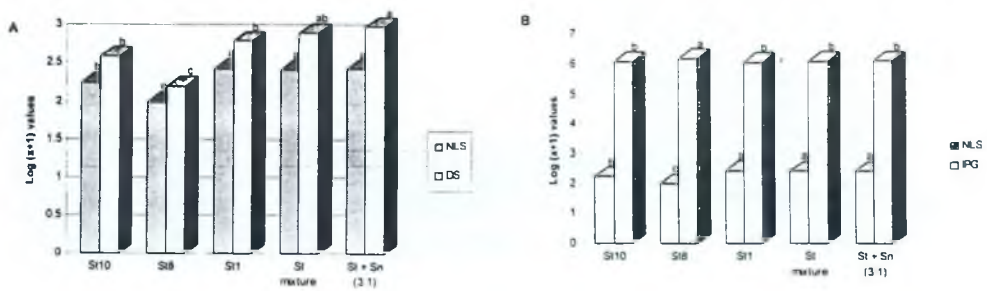

Figure 2. Comparison between the isolates/mixtures tested in terms of their NLS, DS and IPG.

Discussion

Effects of isolates/mixtures

The observed difference in IP was mainly due to the long IP of St8 (Figure 1A). The remaining isolates/mixtures did not significantly differ from each other in terms of their IPs. This might be an indication that IP had relatively poor efficiency in differentiating between the isolates/mixtures tested. It might also have limited importance to the overall aggressiveness of the isolates/mixtures tested.

As shown in Figure 2A, the highest DS (2.97) recorded on intact seedling leaves inoculated with St+Sn (3:1) might be due to the presence of the 25% *S. nodorum* inoculum within the St+Sn (3:1). This might, in turn, be due to the shorter IP of *S. nodorum* inoculum compared to *S. tritici* isolates as described by Benedikz *et al.* (1981) and Eyal *et al.* (1987) and/or due to faster multIPication rate of *S. nodorum* (Shaw 1997). The reasonably high aggressiveness exhibited by the two isolate mixtures (St mixture or St+Sn) in the two experiments might also be due to the additive action of genes for virulence in *S. tritici* isolates (Arama 1996). Hence, the isolate mixtures would have advantage over the individual isolates tested in that they have more genes for aggressiveness, contributed by each isolate in the mixtures (St mixture or St+Sn), to tackle more genes for resistance in the host cultivars.

The least DS (2.20) was caused by St8 (Fig. 1B) which was expected from an isolate with longest IPs of 5.30 and 6.18 on the four wheat cultivars tested in the detached and intact leaf tests, respectively (Figures 2A and 1B). St10 caused significantly larger necrotic lesions than St8 but significantly smaller necrotic lesions than those caused by St1. Based on the parameters tested in the two experiments, St1 is found to be the most aggressive individual isolate tested followed by St10; whereas St8 was the least aggressive isolate tested. The two mixtures were as equally aggressive as St1 as measured by IPs in both experiments and NLS in the detached leaf test; however, the mixtures were more aggressive than St1 in terms of DS. The additive action of genes for aggressiveness in the isolate mixtures seemed to be more evident in DS than in the rest of the components of partial disease resistance tested.

Effects of cultivars

The results obtained (Table 1) suggested that cultivar Baldus was the most susceptible cultivar to the isolates/mixtures tested. The shortest IPs (4.76 and 5.97) in the detached and intact tests, respectively; the largest NLS (2.61) in the laboratory experiment and the highest DS (3.04) in the glasshouse experiment were recorded on cultivar Baldus. Based on the same parameters tested (Table 1) in the two experiments, the second most susceptible wheat cultivar to infection by the isolates/mixtures of the pathogen tested was Riband followed by cultivar Alexandria. The most resistant wheat cultivar tested to the infection by the five isolates/mixtures of *S. tritici* was Reaper.

The least aggressive isolate, St8, caused the least DS recorded on the four cultivars tested. Similarly, disease symptoms caused by St10, the moderately aggressive isolate, were of intermediate in severity. The low aggressiveness of St8 demonstrated by the least DS (2.20) on intact seedling leaves and the smallest necrotic lesions (2.00) on detached seedling leaves seemed to be associated with its longest IPs of 5.30 and 6.18 in the laboratory and glasshouse experiments, respectively (Figure 1A).

Isolate x cultivar interaction effects

The significant ($P < 0.05$) isolate x cultivar interaction for DS (Table 2) might be an indication that there was specificity in the *S. tritici* isolate/mixture x wheat cultivar pathosystem. However, the magnitude of specificity was not sufficient to warrant classification of these isolates into physiologic races of the pathogen. Eyal *et al.* (1973), Yechilevich-Auster *et al.* (1983), McKendry & Henke (1994) also found significant *S. tritici* isolate x wheat cultivar interaction but these authors did not classify the isolates tested into physiologic races because the observed differences in specificity were too low. According to Eyal *et al.* (1973), such isolates with low magnitude of specificity do not demonstrate "true physiologic specialization". Therefore, the differences between the isolates/mixtures observed in this investigation were attributed to differential aggressiveness rather than to differences in specific virulences. This conclusion is in line with the one made previously (Van Ginkel and Scharen 1988) after recording pathogenic variability between *S. tritici* isolates.

Though no significant isolates/mixtures x wheat cultivars interactions were observed for the NLS on detached seedling leaves in the detached leaf test, similar trends of aggressiveness were exhibited by the isolates/mixtures of the pathogen on the four cultivars tested.

Correlation between detached and intact leaf tests

The trend of aggressiveness of isolates/mixtures tested was therefore similar in both experiments showing detached leaf test could effectively represent the intact plant method in testing the aggressiveness of isolates of the pathogen.

More meaningfully, correlations between parameters tested on detached and intact seedling leaves were made (Table 3) to see whether detached leaf tests has a considerable promise in testing the aggressiveness of isolates/mixtures of *S. tritici*.

While there were no significant interactions in IP and NLS, the significant interaction ($P < 0.05$) in DS suggest that it was a better component of partial disease resistance to differentiate between the isolates/mixtures tested. However, due to low magnitude of differences it was not possible to classify the isolates of *S. tritici* studied in the current investigation into distinct physiologic races of the pathogen. Therefore, the differences observed in this investigation were attributed to differential aggressiveness rather than to differences in specific virulences.

The results of the two experiments and the correlation analyses strongly suggest that detached leaf test can represent intact plant method in testing the aggressiveness of isolates. Detached leaf test can therefore be used as a rapid option in screening large sets of wheat cultivars against *S. tritici* isolates especially when researchers are limited by time and greenhouse space.

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