COMPARISON OF FOUR TAN SPOT RATING METHODS UNDER TWO FIELD INOCULATION TECHNIQUES IN MOROCCO

NASSERLEHAQ N. * and MERGOUM M.**

ملخص

إن تلقيح وتقييم المقاومة النباتية للتبقع الهلمنتسبوري (Pyrenophora tritici-repentis) في الحقل يمكن من تصفية عدد كبير من أنواع القمح المقاوم لهذا المرض. كان الهدف من هذه الدراسة هو مقارنة أربع طرق لتقييم المقاومة النباتية تحت طريقتين للتلقيح. أجريت عمليات التلقيح بالتبن المتعفن وبخلاصة البويغات الفطرية في حقول سيدي العايدي والعنوصر وكذلك في البيت الزجاجي لمركز سطات قيس حجم التبقع وكثرته في الأوراق العليا والسفلية في نبات القمح المتعفن لتسعة وتسعين نوعا في تجارب ذات ستة مكررات.

أظهرت النتائج أن قياس حجم التبقع في الأوراق العليا هو الأحسن في حين تبقى كثرته في الأوراق السفلى ثم العليا جائزة. أظهرت تقنتي التلقيح الحقلي فعالية متفارقة نسبيا عند الأوراق العليا واسلفلى. كما أن نتائج الحقل والبيت الزجاجي كانت متوافقة.

* Durum wheat breeder, CRRA/INRA, B.P. 589, Settat, Morocco.
** Breadwheat and triticale breeder, CRR/INRA, B.P. 589, Settat, Morocco.

RESUME

L'inoculation et l'évaluation de la tache helminthosporienne des blés (***Pyrenophora tritici-repentis***), sous conditions de champs est peu controlée mais peut inclure un nombre important de lignées. L'objectif de cette étude est de comparer quatre méthodes d'évaluation de la tache helminthosporienne sous deux techniques d'inoculation. Quatre vingt dix neuf génotypes de blé ont été testés par pulvérisation de spores et par épandage de paille infestée, à Sidi El Aydi et Annoceur au Maroc. Les tailles individuelles et la sévérité des taches ont été évaluées sur les feuilles des niveaux supérieurs et inférieurs. Les mêmes génotypes de blé ont été inoculés et évalués sous serre. Les tailles des lésions avaient une variance plus grande et mieux centrée sur les feuilles supérieures. La distribution des sévérités était légèrement plus large avec des moyennes mieux centralisées sur les feuilles inférieures. Les techniques d'inoculation ont montré de faibles différences entre les méthodes d'évaluation notamment des feuilles supérieures et inférieures. Les corrélations entre les résultats du champ et ceux de serre étaient positives et significatives. Cette expérience suggère que la taille des lésions des feuilles supérieures suivie de la sévérité sur toutes les feuilles de la plante sont les plus adéquates pour les expériences au champ.

MOTS CLES : Tan spot, Wheat, inoculation, rating technique

ABSTRACT

Field inoculation and evaluation of tan spot (***Pyrenophora tritici-repentis***) on wheats, is suggested for large numbers of entries. The objective of this study was to compare four tan spot disease reading methods under two field inoculation methods. Ninety nine spring wheat genotypes were tested under spray and straw mulch inoculation techniques in Sidi El Aydi and Annoceur Morocco. Lesion size and disease severity were evaluated on the upper and lower leaves of each plot. Greenhouse inoculation of the same wheat genotypes was compared with field inoculations. Lesion size was more variable and showed better centered means on the upper leaves. Disease severities were variable, but less than lesion size, and means were more centered on the lower leaves. Inoculation techniques showed small differences of the disease reading methoods, mainly between the upper and lower leaves. Correlations between field and greenhouse disease reactions were all positive and significant. It is suggested that lesion size on the upper leaves, followed by disease severities on all leaves are the best field disease reading methods.

INTRODUCTION

Pyrenophora tritici-repentis (Died). drechs. (PTR) attacks breadwheat (***Trtiticum aestivum l.***) and durum wheat (***T. turgidum var durum Desf.)*** and other Poaceae. In wheat, the disease is known as tn spot. leaf blight, or yellow sport. These lesions appear as tan diamond-shaped lesion on the leaves with a dark centrer surrounded by a yellow border (Hosford, 1967).

Tan sport occurrence is erratic and is well studied in most countries. Yield losses consequent to this disease are reported to be as high as 45% under favorable conditions and a maximum of 72% loss have been observed in Kenya (Hosford, 1982). The use fungicides can reduce tan spot but is costly an requires timely monitoring of the disease; Therefore genetic resistance is the best strategy for reducing tan spot losses (Gilchrist, 1982).

Greenhouse inoculation and disease evalutation techniques of tan spot are well developed and give consistent data that are suitable for scoring resistance (Hosford, 1982). Field inoculation techniques have been improved for screening and selecting breeding lines ; however, homogeneous infection is difficult to obtain because of greater environ mental variability. The most important aspect in field inoculation techniques is covering the experimental plot with plastic and providing free moisture, using a fine mist sprayer for a predetermined period of time.

Various rating scales have been devised to evaluato tan spot in wheat (Hosford, 1982). The Saari an d Prescott (1975) rating scale was applied for large field experiments (Couture, 1980; Elias et al. 1989). Criteria used in these early rating systems were disease severity (Misra and Singh, 1972), number of lesions per leaf (Gilchrist et al., 1984), and percent infection and leaf position combined with lesion size (Raymond et al. 1985). lesion size alone (Coxe and Hosford, 1987) and lesion size combined with the number of lesions per unit area also wer used (Gilchrist et al. 1984). Lesion size might contribute to a simple reliable method for evaluating resistance. Larez (1985) reported that lesions on susceptible wheats were larger than those on resistant ones. The length of the wet period also affected lesion size. Luz and Bergstroum (1986) found that the temperature in the post-inoculation period also affected lesion size. Cox and Hosford (1987) found that lesion size on the flag leaf was correlated most to field reaction and permitted separation among different wheat genotypes.

Takauz (1986) successfully used lesion type to rate net blotch of barley (***Pyrenophora teres Drechs.***). Lamari an Bernier (1989) used lesion type of tan spot and found consistent differences in all the wheat genotypes studied. Rating systems based on disease severity are suited for rapid screening in field experiments while those based on lesion size and reaction type are more suited for greenhouse experiments. The development of a system based on lesion size and/or lesion type is desirable for a more precise and simple assessment of tan spot on wheats.

The objectives of this study were to evaluate four tan spot rating techniques under spray and straw mulch field inoculations and to compare them with more

precise greenhouse inoculation and disease evaluation technique. The disease reading methodes are lesion size and disease severity separately on the upper and lower leaves.

MATERIALS AND METHODS

The Hosts

Ninety nine durum and bread wheat genotypes wer selected and classified uppon their reactions to tan spot inito three different groups. The first group was composed of 56 advanced lines selected from ICARDA's (International Center for Agricultural Research in the Dry Areas ; Aleppo, Syria) an CIMMYT's (Centro International de Mejoramiento de Mais Y Trigo, Distrito Federal, Mexico) 1986-87 Durum Observation Nurseries (low rainfall : DON-LR and moderate rainfall : DON-MR. , respectively). Among the 56 entries, 26 entries (10 resistant and 16 susceptible to tan spot) were selected from the DON-LR, and 30 entries (13 resistant and 17 F2-derived F5 durum wheat lines from the cross "Edmore x PI184526" and from the cross "Calvin x PI184526". These lines were previously tested in field and greenhouse studies with subsequent selection for this study of 11 resistant and 6 susceptible lines from each cross for this study. The third group consisted of four durum wheat cultivars : "WELLS" , "Monroe", "Lloyd", "Vic", tow durum wheat accessions : "PI184526", "PI166308", and three beadwheat genoypes "BH1146", "Chris" and "ND495".

Two experiments were planted in Sidi El Aidi and Annoceur, Morocco, in 1988-89 and 1989-90. One experiment for spray inoculation and another for straw mulch inoculation were planted in hill plots, five seeds/hill, in a randomized complete blok disign with six blocks. The distance between hill plots was 40 cm, and the entire experiment consisted of a 24 by 25 hill-plot grid.

The Pathogen

A single conidium culture of ***Pyrenophora tritici-repentis*** isolated in 1988 from Sidi El Aidi was used for spray inoculation. The straw used for inoculating was westhere and artificially infested with conidial and mycelial suspensions.

PTR cultures were grown on potato dextrose agar medium for eight days at 20°C under continuous white and near ultraviolet light (Sylvania F40 and Norelco F40 WW). Plugs (5 mm in diameter) were removed from the actively growing regions on the plate (white-gray colored), transplanted into modified V8-agar medium (PDA, 15% V8) and returned to the growth chamber, conidia production was initiated by incubation under dark for eight hours (Odvody and Boosalis, 1978; Gilchrist, 1982).

Spore cellection was made by flooding the plates with sterile distilled water and dislodging the spores with a small plastic racket. The resulting suspension was blended for two minutes at low speed, and the spore concentration was adjusted, using a hemacymeter to 20,000 to 30,000 propagules/ml by adding sterille distilled water. Three drops of Tween 20 were added per liter of prepared suspension.

Inoculation

Spray inoculation was made before the heading stage. The field experiment was watered (20 mm) and covered with plastic. Plant inoculation was made using a knap sprayer and completed before sunset.To maintain high air relative humidity and to keep moisture on the leaves, the experiment was sprayed with water at intervals during the night in the morning to avoid heat buil-dup. In the straw inoculation, the plots were mulched with weathered and artificially infected straw at the two to three leaf growth stages.

All wheat genotypes were also planted in 20 cm pots in the greenhouse and inoculated at the flowering stage with the ***P. tritici repentis*** isolates SEA1 and SEA2 (from the Sidi El Aydi experiment station). Plants were placed in a mist chamber for 30 hours. Disease reaction (lesion size) was evaluated on a O (immune) - 5 (lesionsize larger than 4 mm and/or coallescent) scale after an eight days stay in th greenhouse.

Data collection and analysis

Data were collected when leaf spotting differences between th entries were apparent. Plants were evaluated at the upper level (flag leaf and flag leaf-1, for plants with four leaves or more, flag leaf only for plants with less than four leaves) and the lower level (the remaining leaves). Average lesion size on a scale of 0 to 5 (Cox and Hosford, 1987. 0: immune and 5: lesion larger than 4 mm and/or coales cent) and average disease severity on a scale of 1 to 9 (Saari and Prescott, 1975. 0: immune 9: maximal severity.) were evaluated for each level and hill plot. These four different disease readings will be called: disease severity on the upper leaves, and disease severity on the lower leaves. Heading date (number of days from seeding to when approximately 50% of the spikes on the plants were completely emerged) and plant height (awns excluded) also were taken.

Data were analyzed, using the SAS General linear Model procedure (SAS Institute Inc., 1985) with the following statistical models :

$Yijk = f + Li + R(L)i(j) + Gk + GLik + nijk$

where :

Yijk = Observed value for the ijk-th entry.

f = Overall mean.

Li = Effect of the i-th location/year, i = 1, 2.

R(L)i(j) = Effect of the k-th replication within the i-th location, j = 1 to 6.

Gk = Effect of the k-th genotype, k = 1 to 99.

GLik = The interaction effect of the k-th genotype and the j-th location.

nijk = Effect of the random error with the assumption that the nijk are NID (0, s2).

Combined analyses of variance across locations were computed after using the Bartlett test for the homogeneity of variances (Steel and Torrie, 1980). The range and standard deviation of cultivar/line mean disease reaction, were

computed for each technique of field inoculation. Correlation of field with greenhouse disease reactions is used to evaluate the reliability of each field disease reading method within the different inoculation techniques.

RESULTS AND DISCUSSION

At Annoceur, the 1988-89 experiment was abandonned because of excessive water stress that enhanced senescence before optimal disease development. In 1989-90, the spray inoculation was applied during cool and humid weather conditions that were followed by intermittent periods of drought and high temperature. In the post inoculation period daily temperatures ranged from 33° to 37° and relative air humidity was high at night (60 to 80%). Tan spot development was slow at the beginning but seemed to increase at the end in both spray and straw mulch experiments.

At Sidi El Aidi, days wer hot and dry outside the rainy periods, and nights were cool and humid. After inoculation, daily temperatures ranged from 32 to 38° C. In 1988-89, late rains enhanced a natural infection of tan spot throughout the country. In 1989-90, stem and leaf rust (***Puccinia graminis Pers. f. sp. tritici Eriks.*** & E. Henn. , and ***Puccinia recondita Rob.*** ex Desm. f.sp. ***tritici***) epidemics developed. This experiment is not considered.

Significant differences among wheat genotype disease reactions were observed with all inoculation techniques at all locations (Table I).

Variances were homogeneous across locations. Coefficients of variation ranged from 4 to 37%.

Wheat genotype by location/year interaction was significant in both inoculation techniques but produced very low magnitude mean square errors compared to the main effects. In addition, mean cultivar/line disease reactions were ranked similarly at both locations years. Spearman rank correlation coefficients of wheat genotype mean disease reactions between locations were positive and highly significant, indicating that genotype by environment interraction was due to magnitude rather than to the ranking of cultivars (Table II). In addition, field disease readings from the two inoculation techniques were positively correlated to greenhouse testing of the wheat genotypes (Table III) and, highly significant (Table IV). These results indicate that the two inoculation techniques effectively created tan spot epidemics and that genotype response as described by the four disease reading methodes was consistent across locations and with greenhouse testing. In consequence, discussion of wheat genotype disease reactions was based on the means across locations.

From the breeding standpoint, a successful inoculation and evaluation technique should lead to maximal variation among host genotypes reactions rather than maximal development of the disease on all tested material. Although similar conclusions were drawn from all the disease reading methods (lesion sizes and disease severities on upper and lower leaves), differences in the variation and the distribution of data collected for each disease reading methode were observed in Annoceur and Sidi El Aidi (Table III). These observed differences may be relevant to the value of these disease reading methods and their use in future experiments.

Table I : **Combined analysis of variance for tan spot reaction of 99 wheat genotypes, expressed in four disease reading methods to two field inoculation techniques in Sidi El Aydi, 1988-1989 and Annoceur. 1989-1990.**

Disease reading	Inoculation method	Mean square errors (1)		
		G.MSE	GE. MSE	CV
				%
Lesion size on upper leaves	Spray	17.1 **	1.9 **	35
	Straw mulch	17.0 **	1.6 **	22
Lesion size on lower leaves	Spray	1.9 **	0.4 **	10
	Straw mulch	1.7 **	1.1 **	4
Severity on upper leaves	Spray	14.6 **	1.0 **	37
	Straw mulch	13.7 **	1.8 **	27
Severity on Lower leaves	Spray	18.4 **	2.5 **	23
	Straw mulch	13.7**	1.8 **	20

1 : G.MSE : Genotype mean squared errors : GE. MSE : Genotype environment mean squared erros: and, CV : Coefficient of variation.

** : Significant at the .01 levels of probability.

Table II : **Spearman coefficients of rank correlation (r) between wheat genotype mean tan Spot reaction ranks at and between reaction ranks at Sidi El Aidi, 1988-89 and Annoceur, 1989-90. (n = 99).**

Disease reading	Inoculation methods	
	Spray	Straw mulch
Lesion size, upper leaves	0.85 **	0.80 **
Lesion size, lower leaves	0.66 **	0.22 *
Severity, upper leaves	0.82 **	0.85 **
Severity, lower leaves	0.73 **	0.81**

*, ** : Significant at the .05 and . 01 levels of probability respectively

Table III : **Variation in disease reaction of 99 wheat genotypes under two field inoculation techniques of Pyrenophora tritici-repentis at Sidi El Aidi, 1988-89 and Annoceur, 1989-90.**

Disease reading	Inoculation method	Mini-mum	Maxi-mum	Mean	SD(1)
Lesion size on upper leaves	Spray	0.7	4.8	2.8	1.3
	Straw mulch	1.3	5.0	3.6	1.1
Lesion size on lower leaves	Spray	3.2	5.0	4.7	0.4
	Straw mulch	4.6	5.0	4.9	0.1
Severity on upper leaves	Spray	0.3	4.3	2.1	0.9
	Straw mulch	0.9	4.1	2.5	0.8
Severity on Lower leaves	Spray	1.8	7.3	4.3	1.3
	Straw mulch	2.2	6.7	4.5	1.1

(1) SD : Standard deviation of host genotype mean disease reaction
Lesion size on a scale of 0 : immune, to 5 : size > 4mm and/or coalescent.
Disease severity on a scale of 0 : immune to 9 maximum leaves coverage.

Table IV : **Correlation coefficients (r) among four field disease reaction readings under two field inoculation techniques at Sidi El Aydi, 1988-89 and Annoceur, 1989-90. (n=99). (1).**

Disease readings	Lesion size on lower leaves	Severity on upper leaves	Severity on lower leaves
Lesion size on upper leaves	0.8 [0.5]	1.0 [0.9]	1.0 [0.9]
Lesion size on lower leaves		0.8 [0.5]	0.8 [0.5]
Severity on upper leaves			1.0 [1.0]

(1) No parenthesis and [ ] : observed at spray and straw mulch inoculation, respectively.

NB. All correlations observed were highly significant.

Lesion size

Lesion size on the lower leaves showed less variation and higher means than lesion size lesion size on the upper leaves. The range of variation (see maximum -minimum, Table III) and the standard deviation (Table III) are respectively wider and larger for lesion size on the upper leaves. The smaller variation on the lower leaves is due to higher minimum values. In addition, mean genotype disease reactions were higher for the lower leaves than they were for the upper leaves.

The difference between upper and lower leaves may be caused by the nature of the disease progression. Tan spot development, in both artificial and natural inoculation, is greater on the lower leaves than on the upper leaves. Lower leaves have older tissues and experience greater ambient air humidity, longer free moisture periods and milder temperatures (Hosford et al. , 1987; Luz and Bergstrom, 1986; Tekauz, 1986 and Misra and Singh, 1972). Lesion size on the lower leaves may reach its maximum before significant differences start to show on the upper leaves and in this study, all disease readings were made when differences between genotypes were most apparent. These are probable reasons why in this study, lesion size of lower leaves sowed smaller variances and higher averages. Correlations with greenhouse disease data were less marked than those of the upper leaves and could be due to the same reason ; when mean lesion size becomes closer to the maximum allowed by the scale used (0 - 5), variation is small and does not precisely reflect plant disease reaction. Correlation with more precise greenhouse disease data becomes restricted (Table V).

The effect of the inoculation technique also is apparent on the means and the variation of lesion sizes of both the upper and lower leaves (Table III). The range of variation and the mean genotype reaction standard deviation are smaller, while mean genotype disease reaction is larger for the straw mulch inoculation technique. This may be explained by the better success of the straw mulch inoculation technique. The straw mulched on the plot may act as a reservoir of inoculating spores that are released every times the environmental conditions are favorable. The lower leaves seem to be more exposed to this inoculum pressure than the upper leaves. Under these assumptions, the lesion size of the lower leaves will be near maximum and therefore not reflect maximum genetic variation.

Disease severities

Disease severities ou upper and lower leaves showed less marked differences than lesion sizes. Disease severity on the lower leaves showed wider variation, more centered means, and better correlations to greenhouse disease readings than severity on the upper leaves (Tables I and III). Disease severity in this study is a function of the number and size of the lesions. Lesion size of the lower leaves was always near the maximum. However, lesion number must have showed greater variation, which may compensated for lesion size small variability. The scale used to score disease severity is from 0 to 9, while that of lesion size was only from 0 to 5. This wider scale seems to help in a better eye evaluation of the disease. Disease severity in this study had a wider range of variation primarily due to higher maximum values and secondarily to smaller

munimum values. In parallel, mean genotype disease severity was higher for the lower leaves (Table III).

The effect of the inoculation technique is not apparent in the case of disease severity between the upper and the lower leaves : the differences are small (considering the scale 0 - 9), and do not follow the same trend (Table III).

These results indicate that lesion size on the upper leaves, and disease severity on both the upper and lower leaves all can be alternative choices for disease evaluation under different conditions. When condtions are favorable to disease development, lesion size probably should be preferred, but when unfavorable, disease severity on the lower leaves or on all leaves should be preferred.

Correlation studies

Correlation among all field disease reading methods and between field and greenhouse disease data were analysed to compare field data, (where conditions and inoculum are not controlled), with those of the greenhouse (conditions where all these factors are controlled).

Table V : **Correlation coefficients (r) between genotype mean tan spot reaction and greenhouse mean disease reaction for two field inoculation techniques in Sidi El Aidi, 1988-89 and at Annoceur, 1989-90. (n = 99).**

Field disease reading	Field Inoculation technique	Greenhouse inoculated isolates (1)	
		SEA1	SEA2
Lesion size on upper leaves	Spray	0.55 **	0.71 **
	Straw mulch	0.53 **	0.72 **
Lesion size on lower leaves	Spray	0.53 **	0.62 **
	Straw mulch	0.36 **	0.31 **
Disease severity upper leaves	Spray	0.53 **	0.70 **
	Straw mulch	0.65 **	0.75 **
Disease severity lower leaves	Spray	0.56 **	0.71 **
	Straw mulch	0.62 **	0.71 **

(1) ** : Significant at the 0.01 level of probability.

Highly significant correlations were recorded among all field disease reading methods (Table IV). Lesion size on the lower leaves showe the least amount of variation explained by the remaining technique. Field disease readings were positively correlated to greenhouse testing of the wheat genotypes for the two inoculation techniques (Table V).

Lesion size on the lower leaves again showed the lowest variation explained by the greenhouse disease data. The technique of field inoculation did not affect the amount of covariation in other disease reading methods than lesion size on the lower leaves (Tables IV and V).

Disease data were negatively correlated with plant height and the number of days to heading in the three field inoculation techniques (Table VI). Correlations between tan spot reactions and plant growth characteristics were reported in other studies (Élias et al., 1989).

Table VI : **Correlation coefficients (r) between field disease reaction and plant growth traits under two field inoculation techniques with Pyrenophora tritici-repentis at Sidi El Aydi, 1988-89 and at Annoceur, 1989-90. (n = 99). (1)**

Disease reading	Inoculation method	Plant height	Days to heading
Lesion size on upper leaves	Spray	- 0.73 **	- 0.72 **
	Straw mulch	- 0.84 **	- 0.71 **
Lesion size on lower leaves	Spray	- 0.62 **	- 0.75 **
	Straw mulch	- 0.51 **	- 0.44 **
Disease severity upper leaves	Spray	- 0.72 **	- 0.62 **
	Straw mulch	- 0.85 **	- 0.74 **
Disease severity lower leaves	Spray	- 0.84 **	- 0.61 **
	Straw mulch	- 0.83 **	- 0.77 **

(1) ** : Significant at the 0.01 level of probability.

Plant height in cm. from ground to spike tips, excluding awns.

Days to heading : Number of days from seeding to approximately 50 % of the plants with their spikes completely emerged.

This study indicates that they are consistent regardless of the circumstances in which the disease is created.

The effectiveness of a field inoculation method depends on the primary inoculum and the weather conditions (mild temperatures, good humidity, and free moisture) that prevail during inoculation and development of the disease (Adee and Pfender, 1989). Field spray inoculations in this study provided the primary inoculum and only 8 to 10 hours of favorable environmental conditions for infection. Incubation, disease development, and secondary inoculations are largely affected by the weather conditions following inoculation (Larez, 1985). In addition to the unfavorable environmental conditions that may hinder disease development, natural tan spot epidemics can affect the quality of the data by creating different inoculum amounts with probable differences in virulence. Naturel infection was moderate at Annoceur and high at Sidi El Aidi. Natural tan spot epidemic may have interferred significantly with the artificial inoculation. All these factors contribute to the potential quality of an environment in regards to selection for tan spot resistance.

CONCLUSIONS

This study shows that:

(1) Under environments favorable to disease development, spray inoculation may show more differences in host reaction than straw mulch inoculation. Straw inoculation was equally effective in this study but generally does not use specific pathogen isolates. In addition, straw inoculation may indirectly bring inoculum of other diseases.

(2) Under dry conditions, straw inoculation may perform better than spray inoculation. Straw inoculation has the probable advantage of releasing inoculum whenever conditions are favorable. The increased inoculation success and the simplicity of the straw inoculation technique probably overcome the disadvantage of its nonspecificity.

(3) Disease reading methodologies should be chosen according to the technique of field inoculation and the conditions of the development of the disease.

(4) Lesion size on the lower leaves was a pour estimator of genetic vaiance. Under conditions favorable to disease development, lesion size of the upper leaves seems to be the best indicator of disease reaction.

(2) Disease severity of both upper and lower leaves can be used effectively in the case of poorer disease development.

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