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Alternative Measure for Assessing Incidence of Leaf Stripe on Barley

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***Pyrenophora graminea*, the causal agent of leaf stripe disease, is an economically important pathogen of barley found worldwide. It is critical to clearly define and standardize the leaf stripe assessment methods to avoid subjectivity and variability between assessors. Therefore, in this study, a comparison between the proportion of diseased plants (DP) and the proportion of diseased leaves (DL) per plant was investigated. Disease assessments were made visually at multiple sample sites in artificially and naturally inoculated research and production fields during four growing seasons. There were significant differences ($P = 0.001$) among cultivars in mean DP and DL averages, which are consistently higher in susceptible barley. However, DP values increased linearly as DL increased. The slopes and intercepts of the DP-DL relationship were consistent over the four growing seasons. This result might make a significant contribution for leaf stripe assessment in barley breeding programme.**

***Keywords* :** barley, incidence, leaf stripe, *Pyrenophora graminea*

Pyrenophora graminea Ito & Kuribayashi [anamorph *Drechslera graminea* (Rabenh. ex. Schlech. Shoem.)] the causal agent of leaf stripe, is exclusively seed borne and grows systemically within the developing barley plant (Mathre, 1997). The fungus survives in kernels as mycelium on the pericarp, and when a barley seed germinates, enters the plantlets through the coleorhiza (Platenkamp, 1976). Fungal hyphae grow intercellularly from the coleorhiza up all sides to the roots and scutellar node, where they initiate shoot infection (Haegi et al., 1998). In many barley growing areas of the world leaf stripe often leads to reduction in yield (Arabi et al., 2004; Porta-Puglia et al., 1986). The disease is particularly acute in Mediterranean's below 12 ºC during seed germination promote the infection of rootlet (Arru et al., 2003).

Efforts to minimize the impact of leaf stripe have been centered around the use of management strategies such as host resistance, crop rotation, tillage, and fungicide application (Pecchioni et al., 1999; Skou et al., 1994). From a management perspective, the comparison of leaf stripe epidemics across years and locations is necessary to determine the effects of the environment on the efficacy of a given management approach, under similar environmental conditions, and to develop or recommend management strategies or decision thresholds (Boulif and Wilcoxson, 1988; Pecchioni et al., 1996).

The first step to quantify the effect of leaf stripe is to develop a key that describes the growth and development of barley plants during the growing season. It should describe development from sowing to harvest, therefore, details drawings or photographs, showing characteristics of the various stages of development, including number of infected leaves are needed. Generally, barley reaction to leaf stripe is commonly measured either by incidence (proportion of diseased plants) or severity (proportion of infected leaf area per plant). However, in spite of the positive relationship found between these both criteria (Arabi and Jawhar, 2010), incidence is quicker and easier to measure than severity, and measures of incidence are often more accurate, precise, and reproducible than measures of severity (Campbell and Madden, 1990; Nutter and Schultz, 1995). In addition, assessment of leaf stripe severity under field conditions is tedious and time-consuming and may be prone to bias and experimental error (Tekauz and Chiko, 1980).

Leaf stripe assessment methods need to easily provide objective measurements for a specific growth stage of a crop so that data from different sources is comparable, and provide an adequate sample of the crop for assessment (Cockerell et al., 1995). However, to produce a disease assessment key, the development of disease over the whole disease cycle and at different stages of plant growth must be studied to make prototype standard diagrams and/or descriptions.

The purpose of this study was to develop a robust sampling and disease evaluation technique that tracked leaf stripe development in further field trials. Since a study by Arabi and Jawhar (2010) found a high correlation ($P < 0.001$) between number of diseased plants and infected leaf area per plant, assessment of disease incidence as the diseased leaves per plant was considered to be valid for this study. Therefore DP-DL relationship of leaf stripe was investi-

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gated to explore the possibility of simplifying disease assessment.

Disease assessment sites. In order to acquire data from leaf stripe epidemics of different intensity and to represent a range of environmental, cropping, and management conditions likely to influence the development of leaf stripe, several research plots and production fields were selected for leaf stripe assessment in Syria during 2007, 2008, 2009 and 2010, winter barley growing seasons. Commercial barley fields and research plots were naturally infected with *P. graminea*, while disease-screening nurseries were artificially infested.

Inoculum preparation. After an extensive screening for over ten years in the greenhouse and in the laboratory the *P. graminea* isolate (SY3) was proved to be the most virulent isolate to all barley genotypes available so far (Arabi et al., 2002; 2004). Therefore, it was selected for the DP-DL study. The fungal mycelia were transferred from a stock culture into Petri dishes containing potato dextrose agar (PDA, DIFCO, Detroit, MI, USA) with 13 mg/l kanamycin sulphate and incubated for 10 days at 21 ± 1 °C in the dark.

Host genotypes. The 10 barley cultivars used in this study were chosen for their reaction to *P. graminea* from highly susceptible to highly resistant and diverse origins (Table 1). The universal susceptible cultivar WI2291 from Australia was included in each set as checks.

Experimental design. For inoculation, seed was surface-sterilized. Barley seeds of each cultivar were then placed on 8-day-old mycelial culture growing on PDA medium in Petri dishes and incubated at 6 °C for 14 days in the dark (Hammouda, 1986). In the control treatment, seeds were incubated on PDA medium alone. Commercial barley fields and research plots were naturally infected with *P. graminea*, while disease-screening nurseries were infested artificially with the isolate (SY3). In order to acquire data from leaf stripe epidemics of different intensity, both two research plots and production fields were selected for leaf stripe assessment in Syria during four growing seasons. The experimental design was a randomized complete block design with three replicates. Plot area was 1 × 1 m with a 1 m buffer zone. Each plot consisted of 5 rows with 25 cm apart having 50 seeds sown per row. Experimental design, cultural practices, inoculation methods, and mist irrigation were as previously described (Arabi et al., 2004). Weeds were controlled by pre- and post-emergence herbicides as appropriate. Soil fertilizers were drilled before sowing at a rate of 50 kg/ha urea (46% N) and 27 kg/ha superphosphate (33% P).

Disease assessment. At heading (ZGS 50, Zadoks et al., 1974), all healthy and diseased plants in the plots were counted. In each field/plot, disease assessments were estimated visually at several systematically selected sampling sites. Incidence (DP) was recorded as the proportion of diseased plants (number of diseased plants divided by the total number of plants sampled). Incidence (DL) was recorded as number of diseased leaves per plant expressed as a proportion of the total leaves.

Statistical analysis. The data for DP and DL were analyzed by analysis of variance (Newman-Keuls test), using the STAT-ITCF program (Anonymous, 1988). For all the experiments data, each pair of DP and DL values from each sampling site was considered as an observation for data analysis. The data were edited to remove observations with no diseased plants (i.e., DP = 0 and DL = 0), since the DP-

Table 1. Mean leaf stripe disease incidence DP; proportion of diseased plants and DL; proportion of diseased leaves per plant in 10 barley genotypes inoculated with isolate SY3 of *Pyrenophora graminea* under field conditions during 4 years

Cultivar	Source	DP	DL	
		% Diseased leaves	% Diseased leaves	Disease development
WI2291	Australia	100 a	96.67 a	Up to flag leaf
Arabi Abiad	Syria	100 a	91.33 a	Up to flag leaf
Arrivate	USA	95.0 b	89.21 a	Up to flag leaf
Furat1	Syria	82.3 c	58.67 b	Lower four leaves
Golf	England	61.7 d	55.33 b	Lower three leaves
Thibaut	France	21.7 e	26.67 c	First and second leaves
Igri	England	20.0 e	21.11 c	First and second leaves
PK30-126	Pakistan	16.0 f	22.67 c	First and second leaves
CI-5791	Ethiopia	14.0 f	4.33 d	First leaf
Banteng	Germany	1.1g[x]	1.33 d	First leaf

[x]Values within a column followed by different letters are significantly different at $P = 0.001$ according to Newman-Keuls test.

DL relationship is only defined when disease is present. The assumption of coincidence of the four regression lines for each year was tested using the ANOVA procedure implemented in the software package Statistica 6.1. Years were set as the categorical variable and coincidence was tested by simultaneously checking year's effect combined with its interaction with the incidence (Neter and Wasserman, 1974).

Leaf stripe was confined primarily to the lower leaves early in the seasons and progressed to the upper leaves, including the flag leaf, late in the seasons. The reactions of the 10 barley cultivars to *P. graminea* are presented in Table 1. Significant differences ($P = 0.001$) in mean DP and DL values were detected with values being consistently higher in the susceptible cultivars during the four growing seasons. The data showed that the highest mean incidence DP and DL were recorded in cultivars WI2291 and Arabi Abiad (DP = DL =100) and the disease development was up to flag leaf, whereas, the lowest was found in the resistant cultivars Banteng (DP $\approx$ DL $\approx$ 1) up to first leaf (Table 1).

However, DP values increased linearly as DL increased. There was no difference in the slopes and intercepts of the DP-DL relationship among the four years, as it was shown by the test coincidence ($F_{3,32} = 0.236$, $P = 0.870$). Subsequently, data from all years were pooled to calculate a single regression line (Fig. 1). These findings are in agreement with the results of Nyvall et al. (1995) for the seed borne *Bipolaris oryzae* and *B. sorokiniana* pathogens on wild rice. In some cases DP = DL for one or more observations such as in the susceptible cultivars WI2291 and Arabi Abiad (Table 1). The differences in the means of increase in incidence parameters have been attributed to the occurrence of two distinct types of infection, allo- and auto-infection for polycyclic diseases (Seem, 1984). An increase in

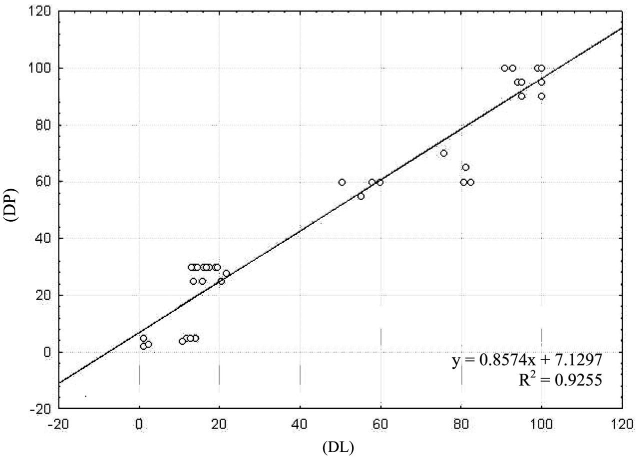

Fig. 1. Relationship between diseased plants (DP) and diseased leaves (DL) of barley leaf stripe. Data points shown as (○) represent many hidden observations.

incidence DP results from allo-infection (spread among plants), whereas an increase in mean DL within a sampling unit results from both allo- and auto-infection (spread within infected plant units - leaves). Using simulation models, Willocquet and Savary (2004) demonstrated that the time taken for maximum disease incidence ($I = 100$) to occur decreased with increasing allo-infection. In the case of leaf stripe, which normally functions as a monocyclic disease, the infection of new disease-free plants from primary inoculum (analogous to allo-infection) was probably higher than the spread within infected plants (analogous to auto-infection). This might be attributed to that leaf stripe, is a monocyclic disease, inoculum density certainly will influence incidence level.

The cultivars planted during the four growing seasons of this study varied greatly in resistance to leaf stripe. However, cultivars that are resistant to leaf stripe may in fact have different resistante response to the spread of the fungus hence, for any given DP incidence value, a wide range of DL values observed across the cultivars. McRoberts et al. (2003) reported that incidence analysis is directly useful in evaluating resistance response. In particular, the DP-DL relationship could be used to draw conclusions about the relative rate of disease increase among cultivars with different levels of resistance.

We were interested in how DP-DL relationship might change as the season progressed. The results of test coincidence revealed that differences in weather conditions during the four growing seasons and geographical locations did not result in different patterns in the DP-DL relationship. From a meteorological standpoint, the average climate may not change from year to year in the temperate regions. This may contribute to the consistent in the DP-DL relationship between growing seasons found in this study. In addition, the number of plants sampled and the small distance among locations did not affect DP-DL relationship either. It is noteworthy that in the DP-DL relationships among locations have been reported for powdery mildew of apple (Seem and Gilpatrick, 1980).

Using the relationship between DP-DL, management decision thresholds may be established. For instance, Dillard and Seem (1990) proposed DP of 0.8, which corresponds to a mean DL of 0.01, as an action threshold for rust of maize. A 0.1 mean DL value was used by De Wolf et al. (2003) as being indicative of the occurrence of a major epidemic of Fusarium head blight of wheat. According to the result of our study DP = DL = 0.99 for susceptible cultivars and DP DL 0.1 for resistant ones (Table 1).

We undertook this study to determine a general DP-DL relationship for leaf stripe and then to establish whether that relationship would remain the same for different years, locations and cultivars. The results of this study show that there was a positive correlation between leaf stripe parameters DP and DL in barley which was consistent among seasons and locations. Therefore, it is recommended that leaf stripe be evaluated from measures of DP or DL which may be beneficial for many types of studies on this disease. The estimation of mean DL from DP would substantially reduce the work load in leaf stripe quantification in field surveys and treatment comparisons. It is less time-consuming and generally requires less training of assessors (Campbell and Madden, 1990).

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