SUMMARY

Conidia of *Pyrenophora graminea*, the causal agent of barley leaf stripe, were simultaneously exposed outdoors to direct solar radiation or placed in an adjacent ventilated enclosure in the dark for four months. After exposure, conidia were placed on water agar in closed Petri dishes and allowed to germinate for 24 h. Shaded conditions were always more favourable to conidia germination and mycelial growth than sunlight conditions. Significant decreases (P<0.05) in conidia germinability (60.6%) and mycelial growth (41.46%) were detected in light-exposed conidia in comparison with the non-exposed control. Exposure also decreased the pathogenicity of fungus on different cultivars. The possibility that sunlight exposure may reduce conidia germinability over the hottest four months and aerial transport distances should be considered.

Key words: *Pyrenophora graminea*, barley, leaf stripe, spore survival, solar radiation.

INTRODUCTION

*Pyrenophora graminea* Ito & Kuribayashi [anamorph *Drechslera graminea* (Rabenh. ex. Schlech. Shoem.)] is the seed-borne agent of barley leaf stripe (BLS), a disease responsible for a 73% reduction of annual yield in highly susceptible barley varieties in Syria (Arabi et al., 2004). Aerial dispersal of this fungus is considered to be an important factor in the spreading of BLS epidemics (Davis and Jackson, 2005). When conditions are wet or humid, spores are produced on the leaf surface at about the time when spikes of healthy plants in the field begin to flower. Spores are dispersed by wind to developing spikes, germinate, and cause infections. The recent resurgence of BLS in Syria appeared to be linked with the arrival of more aggressive genotypes of the causal agent (Arabi and Jawhar, 2007), thus calling for renewed and improved disease management. In addition to the differential levels of pathogenicity of diverse *P. graminea* strains, variation in the occurrence of the pathogen linked with changes in weather conditions has contributed significantly to the sporadic nature of BLS. In the 1990’s, *P. graminea* was not detectable in many Syrian locations. Clearly, forecasts could be improved if they comprised not only information on the presence/absence of the pathogen in an area, but also the risks associated with varying levels of inoculum.

Since our long-term goal is to identify the factors that favour BLS development, the aerial dispersal of fungal spores was investigated, being a likely determinant of disease outbreaks. To this aim, the effects of sunlight on conidial survival under field conditions was studied, for these effects are likely to be important in determining *P. graminea* survival of during dispersal.

MATERIALS AND METHODS

Experimental material. The isolate of *P. graminea* Sy3 used in this study was chosen among 15 isolates, as the most virulent isolate on barley differential genotypes (Arabi et al., 2005). At the end of the growing season, conidia of Sy3 were produced on the leaf surface of barley (cvs WI 2291, Golf and Igri) by wetting plants twice a day, using a high-pressure sprayer.

Exposure to sunlight. The exposure of conidia to sunlight was performed between June to October 2006 under field conditions approximately 22 km west Damascus. Two sets of samples were used, kept under direct sunlight and in the dark. After harvesting, plants infected with Sy3 were exposed to full sunlight under field conditions and four leaves were taken from each plant at the end of exposure for further experiments. Sy3 conidia in the second set were transferred to glass microscope-slide coverslips (18 mm × 18 mm × 0.14 mm) by touching lightly a BLS lesion with a coverslip. Conidia-laden coverslips were then placed on screens in a darkened enclosure with ventilation. The experiment consisted of 10
coverslips (six replicates) for each treatment.

Meteorological data. Global solar irradiance (watts per square meter), air temperature, relative humidity (RH) and wind speed were measured during the experiment using a data logger (model 1020, COMBILOG, Theodor Friendrichs & Co. Hamburg, Germany). Air temperature and RH were measured with a platinum resistance thermometer sensor (pt100, DIN 60751 B) and wind speed with a cup anemometer (model 014A, COMBILOG, Germany). These instruments were located in the test site, at a height of 1.1 m above the ground. The data were recorded at 1 h intervals.

Germinability assessment. At the end of the experiment sunlight-exposed and shaded conidia were collected. Microscope coverslips were placed conidia-side down on 1.5% water agar medium in 9-cm plastic Petri dishes (6 Petri dishes per treatment), incubated at 20-22°C for 24 h to allow germination. Percentages of germinated conidia were counted in random fields at 100X with a light microscope. A total of 200 to 400 conidia were examined in six replicates (Petri dish/replicate), one replicate matching a replicate in the field, with the higher number of conidia counted when germinability was low. A conidium was considered germinated if the length of the germ was greater than or equal to its length.

Pathogenicity tests. Pathogenicity tests of all exposed and non-exposed conidial samples were done using three barley cultivars (WI 2291, Golf and Igri), selected for their different resistance levels to BLS and inoculated with Sy3 as described by Hammouda (1986). One-hundred and fifty seeds of each cultivar were surface sterilized in 5% NaOCl for 5 min, washed several times in sterile deionized water, and left to dry between sterilized filter papers. Seeds of each cultivar were transferred to Petri dishes (50 seed each) containing an actively growing mycelium (8-day-old) cultured on potato dextrose agar (PDA, DIFCO, USA) and incubated at 6°C for 14 days in the dark. After inoculation the seeds were removed carefully and planted in the field.

The location of the experiment was favorable for the development of BLS. Inoculated seeds were sown under rainfed conditions (500 mm annual rainfall) in a completely randomized block design, with three replicates (50 plant/replicate/cultivar). Plots were 1 × 1 m in size with a 1 m buffer. Each replicate consisted of five rows, 25 cm apart with 10 seeds sown per row. At the heading stage (GS 50) diseased (showing leaf stripe) and healthy plants were counted. The degree of resistance to BLS was assessed as the percentage of infected plants according to Delogu et al. (1989).

Measure of mycelial growth. Mycelial growth was measured at the end of sunlight exposure by culturing the exposed conidia on Petri dishes containing PDA with 13 mg/l kanamycin sulphate. The plates were incubated at 21±1°C in the dark to allow fungal growth. Comparisons with controls were made by measuring mycelial growth of three or four colonies per plate 8 days after culturing (six replicates).

Statistical analysis. All experiments were performed three times. An F-test was used to determine if the three runs of each experiment were homogeneous and if the data could be pooled, which showed that this was possible. Thus, all further analyses were conducted on pooled data. Statistical analysis was performed using the STAT-ITCF program (Anonymous, 1988). Analysis of variance (Newman-Keuls test) was used to test for differences among exposed and non exposed conidia. Analysis of pathogenicity tests was done on the percentage of infected plants, to determine the effect of sunlight exposure.

RESULTS AND DISCUSSION

Table 1 shows the range of environmental conditions encountered during exposure of P. graminea conidia to sunlight. Germinability of conidia decreased (60.6%) significantly (P<0.05) after sunlight exposure in comparison with the non-exposed controls. In addition, exposure to sunlight significantly (P<0.05) decreased (41.46%) mycelial growth 8 days after exposure in comparison with the non-exposed control (Table 2). There were significant differences (P<0.05) in pathogenicity

<table>
<thead>
<tr>
<th>Variable</th>
<th>Range (Unit)</th>
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<tbody>
<tr>
<td>Outdoor air temperature</td>
<td>25-36 (°C)</td>
</tr>
<tr>
<td>Relative humidity</td>
<td>35-49 (%)</td>
</tr>
<tr>
<td>Average wind speed at sample height</td>
<td>0.8-3.3 (m/s)</td>
</tr>
<tr>
<td>Irradiance of incident solar radiation</td>
<td>690-900 (w/m²)</td>
</tr>
</tbody>
</table>

Conditions in the lab were stable

Table 2. Percent germination and mycelium growth of P. graminea isolate Sy3 exposed (G_S) and not exposed to sunlight (G_NS) (six replicates).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>% Germination (24h of culture)</th>
<th>Growth rate (cm) (8 days of culture)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G_NS</td>
<td>87.5a ± 3.421</td>
<td>4.1a ± 0.132</td>
</tr>
<tr>
<td>G_S</td>
<td>34.47b ± 7.96</td>
<td>2.4b ± 0.260</td>
</tr>
</tbody>
</table>

Means followed by different letters differ significantly at (P<0.05) by Newman-keuls's test.
between exposed and non-exposed fungus (Table 3).

Survival of conidia is of paramount importance in the build-up of *P. graminea* inoculum, as conidia transported from infested residue by the wind or rain can infect healthy plants. Although there may have been differences in the ability of conidia at different ages to germinate, we used the germination of non-exposed conidia as a base-line to compensate for these differences.

The present study has shown that exposure to sunlight decreases both germinability and pathogenicity of *P. graminea* conidia, in agreement with the results obtained by Arabi and Jawhar (2003) with Cochliobolus sativus, and by Hughes et al. (2003) with antarctic terrestrial fungi. Rotem et al. (1985) and Gates (1980) attributed the inhibiting effect to the strongly increases amount of biological activity per unit of energy following exposure to shorter wavelength light (254 nm). Mohammed and El-Hassi (1986) suggested that conidial survival under sunlight may increase the production of secondary metabolites that may play a role in protection from solar irradiation. Swan (1974) reported that melanin can offer an excellent protection against sunlight in many fungi. Conidia of *P. graminea* are dark brown, thus these pigments might be one of the reasons behind their tolerance of solar irradiation during the first periods of exposure.

To our knowledge, this is one of the few studies on the effects of sunlight on the viability of conidia and aerial dispersal of a seed-borne pathogen. The sunlight apparently poses no strong crucial impediment to the survival of conidia of *P. graminea* under field conditions during the four hottest months in Syria. Therefore, although conidia of this pathogen are able to travel several kilometers in the atmosphere, decrease in their germinability due to sunlight may be relevant when starting inoculum levels are low. However, aerial dispersal alone cannot explain the presence BLS in new areas due to the fact that *P. graminea* is seed-borne (Zriba and Harrabi, 1995). Biological information will help determining spore dispersal and infection probabilities when regional levels of inoculum are sufficiently low, so as to postpone or forego chemical control. The present work is part of an on-going project on the epidemiology of leaf stripe in Syria. A model framework will be developed to accommodate readily new information on the biology of the fungus and the susceptibility of barley cultivars to BLS as it becomes available.

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## REFERENCES


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