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First report of southern blight of common barley in Puerto Rico

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Abstract

In 1990–91, 96% of the test plants in a barley (*Hordeum vulgare* L.) winter nursery in Puerto Rico was lost to *Sclerotium rolfsii*. Small necrotic lesions (1–3 mm long) developed at the base of the stem, followed by wilting and general chlorosis as the lesions progressed and eventually girdled the base of the stem. Sclerotia then developed on the plants at the stem base. Two morphologically different strains of *S. rolfsii* that were mycelial incompatible were isolated. This is the first report of a destructive blight due to *S. rolfsii* on field barley in the Caribbean. The apparent rate of disease increase (r) was estimated to be 12.2% per day using the logistic model, with an initial disease incidence of 0.042% ($R^2 = 0.89$, $p \leq 0.001$).

Introduction

Barley (*Hordeum vulgare* L.) is one of the world's most widely-grown small-grain cereal crops (91 million hectares) which is used in malting and for animal feed [Mathre, 1982]. To expedite breeding programs for disease resistance, such as to stem rust [Steffenson, 1992], a winter nursery was initiated in October 1990 at Isabela, Puerto Rico by the USDA/ARS Tropical Agriculture Research Station and North Dakota State University. The plants began to show wilting after four weeks of growth in the field, due to infection by *S. rolfsii*.

The purpose of this study was to investigate the etiology and progression of the epidemic that destroyed the barley plants in the winter nursery of 1990.

Materials and methods

Field plot and pathogen isolation. The barley nursery was established at Isabela in north-west Puerto Rico, at latitude 18°30′ and at an elevation of 128 m with ambient temperatures ranging from 18.5 to 29.4 °C. The soil was a tropeptic haplorthox, clayey kaolinitic, isohyperthermic Coto clay with an organic matter content of 2.3% and soil pH of 5.4. The nursery consisted of 73 rows of barley plants (grown from carboxin-pretreated seeds) arranged north to south (perpendicularly to the direction of the wind) with 75 pairs of test plants between each pair of rows (a total of approx. 10,800 test plants in 144 rows). The test plants consisted of a varietal mixture in which each row represented a different genotype.

Barley plants which showed wilting, general chlorosis or small necrotic spots at the base of the stem were taken to the laboratory. A 3-cm long stem section was cut, washed in running tap water for 8 min, surface-disinfested with 0.5% sodium hypochlorite (NaOCl) for 6–8 min, rinsed in sterile distilled water (SDW) and plated on Difco-Bacto water agar (3%) and full-strength potato-dextrose agar (PDA). A total of 20 isolations were made over a 4-week period (beginning 30 days after establishing the barley nursery). Cultures were incubated at 26 °C and examined daily. A CMI description [Mordue, 1974] was used to confirm identity of the fungus.

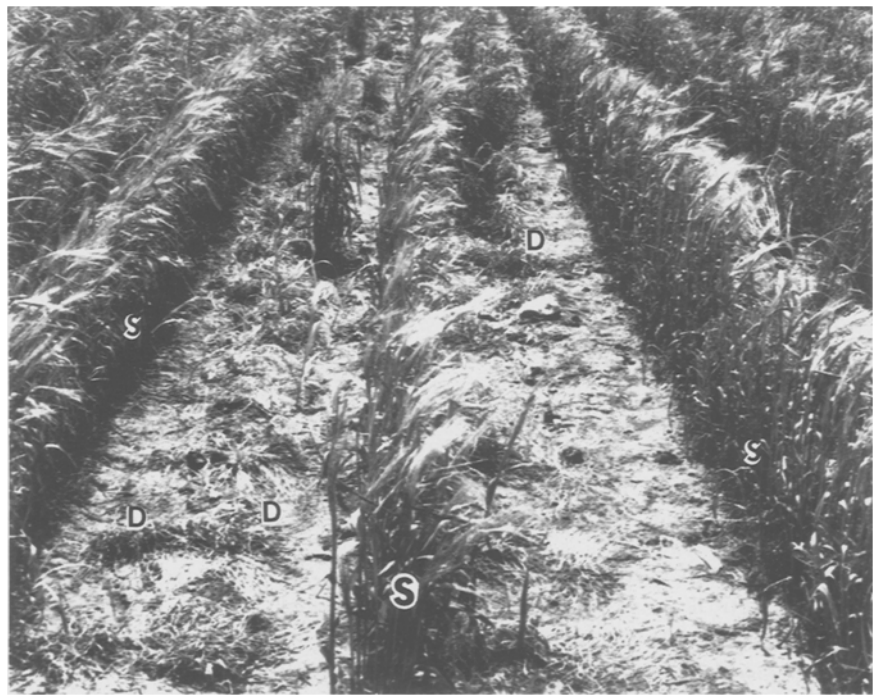

Fig. 1. Barley field after blight epidemic caused by *Sclerotium rolfsii*. S = rows grown from carboxin-treated seeds, D = diseased test plants (that were to be screened against stem rust).

Pathogenicity test. A mixture of sand, loam soil and sugarcane bagasse (1:2:1, v/v) was autoclaved at 121 °C for 1 h at 1 atm. Barley seeds from the winter nursery were immersed in 0.5% NaOCl for 90 s, rinsed for about 3 min in 100 ml SDW, and then soaked in 250 ml of SDW for 30 min to moisten the seed coats. Two seeds were placed in the soil mixture at a depth of 2 cm in each of 20 pots (12-cm diameter), which were placed in a greenhouse (temperature range of 26–30 °C). Soil was moistened daily.

After 4 weeks, plants in 10 pots were inoculated with sclerotia of *S. rolfsii* isolated from diseased barley plants grown on 1% Difco-Bacto agar for 8 days. Sclerotia (6 per plant) were placed on the soil surface in contact with the stem base and covered with a thin layer of sand. Uninoculated barley plants served as controls. Reisolations were made from stem lesions after 12 days as described above on PDA.

The isolate from barley plants (designated strain 'B') was compared to an isolate ('M') obtained from crop residue of the legume *Mucuna deeringiana* (Bort.) Merr. in the same nursery. Mycelial plugs (0.6-cm diam.) taken from the edge of a 5-day-old culture of each isolate were placed 5 cm apart on PDA in a 9-cm Petri dish and incubated at 28 °C to detect the presence of a barrage (aversion zone).

Analysis of disease progression. Seven measurements of disease incidence in the barley field were taken over a 3-month period to obtain a disease progress curve. Disease incidence was expressed as a percentage after scoring the number of symptomatic plants among 1500 random test plants in 10 randomly chosen rows. The logistic regression model was used to analyze disease progress and to calculate estimates of initial disease proportion (Y_0) and the apparent infection rate (r) [Burdon, 1987; Leonard and Fry, 1986; Vanderplank, 1963]. The final disease level (Y_{max}) was obtained from field data.

Results and discussion

Symptomatology. Approximately 96% of the barley plants were destroyed by *S. rolfsii* ('B' strain) that reached destructive epidemic proportions in the 1990–91 winter nursery (Fig. 1). All isolations from 20 diseased plants on water agar or PDA consistently yielded *S. rolfsii* ('B' strain). The barley isolate was pathogenic

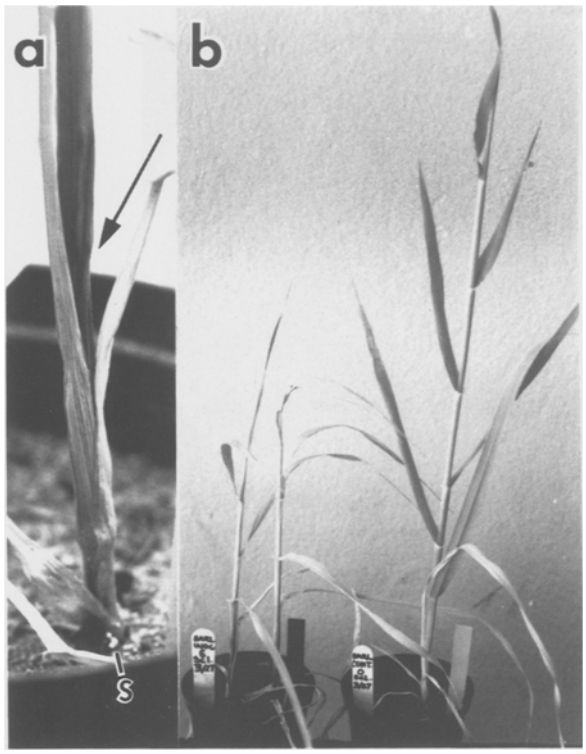

Fig. 2. Inoculated plant (a) showing necrotic lesion (arrow) completely girdling stem base with immature sclerotia (S). (b) Inoculated plants (left) showing retarded growth and chlorosis; uninoculated barley plant (right).

to barley plants in greenhouse studies. All field symptoms were reproduced on barley plants inoculated with *S. rolfsii* in the greenhouse, except for wilting which was probably circumvented because of an adequate water supply.

Lesions were first observed at the base of the stem near the soil level two days after inoculation. Small, irregularly shaped or elongated necrotic spots developed on the stem, and general chlorosis followed as the spots enlarged, coalesced and eventually girdled the plant (Fig. 2a). The necrosis extended 2.5–3.75 cm above and below the soil line. Infected plants were stunted and chlorotic (Fig. 2b). *S. rolfsii* produces oxalic acid and pectinolytic and cellulolytic enzymes [Bateman and Beer, 1965; Punja and Rahe, 1992] which cause cellular maceration. *S. rolfsii* has been previously reported on barley in California, but was not considered to be an important pathogen on this crop [Sprague, 1950] in the temperate regions. This is the first report of *S. rolfsii* on barley in Puerto Rico.

From the Isabela farm, 20 isolations were recovered from barley plants ('B' strain) and 10 from rotting pods of the legume *M. deeringiana* ('M' strain). On PDA, the 'M' strain produced thick aerial mycelium that formed a dense inner ring (Fig. 3a). A distinct zone of inhibition developed when the two isolates were paired, and sclerotia formed (Fig. 3b), but not when the same strain was grown together (photograph not shown). *S. rolfsii* is comprised of many strains which can be distinguished by aversion or barrage zone formation when grown in proximity [Epps *et al.*, 1951; Nakata, 1925a, b; Punja and Grogan, 1983]. Although the pathogenicity of the 'M' strain was not tested, strain 'M' was never isolated from barley.

Sclerotia averaged 1.5–2.0 mm in the 'M' strain, were light brown and fewer (20–50/plate). The 'B' strain produced dark brown spherical sclerotia, 0.8–1.0 mm in diameter and in higher numbers (90–120/plate). A single pathogenic isolate may not be adequate to index the presence of resistance genes in barley plants due to variation in *S. rolfsii* strains in a particular area. *S. rolfsii* infects more than 500 species of plants belonging to about 100 families [Aycock, 1966; Mordue, 1974]. The fungus causes economic damage mostly to crops grown under warm humid conditions in tropical and subtropical regions and areas with mild winter [Punja and Rahe, 1992]. In Puerto Rico *S. rolfsii* exists in all agroecological zones but disease incidence is < 10% (personal communication, P. R. Hepperly, Former Research Plant Pathologist, USDA/ARS, TARS, Puerto Rico).

Disease progression. When observed disease incidence was plotted, a sigmoid disease progress was obtained (Fig. 4). The disease developed to epidemic proportions, reaching $Y_{max} = 96\%$ within 97 days (Fig. 4). The logistic model was used to characterize the epidemic. The regression of logits, $\ln[Y/(1-Y)]$, on time t (in days from planting) where Y represents percentage of plants diseased, was used to calculate initial disease incidence ($Y_0 = 0.042\%$) and apparent rate of increase of disease ($r = 12.2\%$ per day). The predicted progress curve for function Y is shown in Fig. 4.

The r and R^2 values calculated (where $R^2 = 0.89$, $P \leq 0.001$) for Southern blight on barley in Puerto Rico were reminiscent of those for destructive foliar diseases [Leonard and Fry, 1986] (e.g. coffee rust where $r = 12\%$, $R^2 = 0.86$–0.97 and southern corn leaf blight, where $r = 10$–11%, $R^2 = 0.91$–0.98). Campbell *et al.* [1984] suggested that epidemics of soil-borne diseases may not differ totally from foliar or shoot diseases but that different explanations (such as interactions of plant maturation, differences in root or multistem growth,

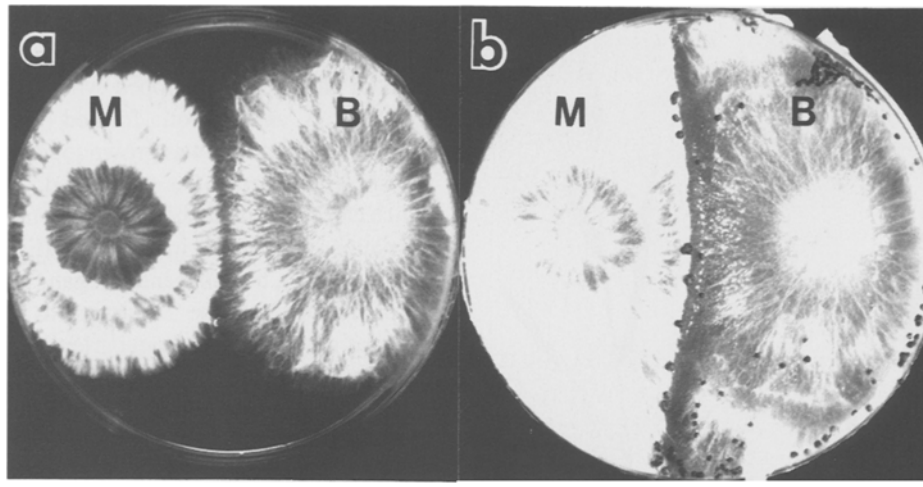

Fig. 3. Sclerotium rolfsii – (a) Early growth of 'M' and 'B' strains on PDA medium (2½ days). (b) Sclerotia and aversion zone formation between the 'M' and 'B' strain of *S. rolfsii* (8 days).

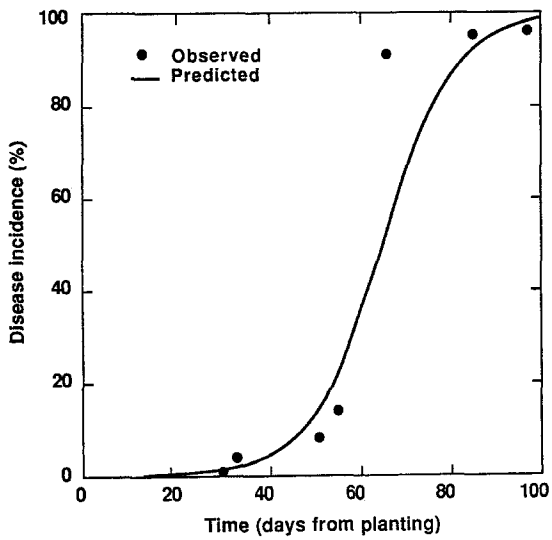

Fig. 4. Southern blight incidence (•) in the 1990–1991 barley winter nursery on Puerto Rico and predicted values (–) obtained by fitting the logistic model to the data. Estimates of parameters: Y_0 = 0.042%, r = 12.2% (S.E. = 0.019) with observed Y_{max} = 96%. Coefficient of determination (R^2) = 0.89 at $p \leq 0.001$.

host/pathogen response to environment and others) may be more appropriate to account for similarities in shoot and soil-borne pathosystems. We describe these factors as site-specific conducive interactions. Initial inoculum density was fairly high (Y_0 = 0.042%) at the time of sowing the barley seeds in the tilled field that was previously planted to taniers *Xanthosoma sagittifolium* (L.) Schott. The sclerotial fungus was observed in the tanier debris.

Four percent of the test plants survived symptomless. These plants either escaped infection or are more tolerant with age. The plants grown from carboxin treated seeds (Fig. 1, S) were not affected by *S. rolfsii*. The potential of carboxin as an effective control for the blight needs testing.

Acidic soils at Isabela could be conducive to the growth of *S. rolfsii* [Zillinsky, 1984] in addition to the warm, wet soil, overcrowding and shading from the multistems, bridges formed by senescing lower leaves of nearby plants, and sufficiently high initial inoculum density.

Deep burial of crop residues during field preparation [Punja and Jenkins, 1984], use of fertilizers with bicarbonate/carbonate anions and NH_3 [Punja, 1989], utilization of *Trichoderma harzianum* as an antagonist to *S. rolfsii* [Chet *et al.*, 1979], and avoidance of field plots previously planted to susceptible crops were recommended for disease management.

The impact of *S. rolfsii* diseases on barley in future winter nurseries in the tropics is the loss or reduction of valuable germplasm having resistance to other diseases, such as stem rust.

Conclusions

Sclerotium rolfsii caused an epidemic in the 1990–91 barley winter nursery in Puerto Rico. Symptoms of

the disease are characterized by wilting and general chlorosis of barley plants. Small lesions form at the stem base, coalesce and girdle the base of the stem followed by the formation of sclerotia. This is the first report of a destructive blight due to *S. rolfsii* on field barley in the tropics. Apparent rate of disease increase (r) was estimated to be 12.2% per day while a final disease incidence of 96% was observed.

Acknowledgements

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