Influence of Media on Pathogenicity and Morphology of Secondary Sporidia of *Tilletia indica*

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ABSTRACT

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Sporidia of *Tilletia indica* were produced in potato-dextrose broth with or without supplemental sucrose after 1 wk on a shaker. Secondary sporidia produced in liquid cultures were characteristically filiform and distinguishable from allantoid secondary sporidia produced on potato-dextrose agar plates. Boot and spray inoculations of bread wheat with filiform secondary sporidia resulted in lower levels of infection than similar inoculations with allantoid secondary sporidia. When screening germ plasm for Karnal bunt resistance, allantoid secondary sporidia produced in solid media should be used as inoculum.

Karnal bunt disease of wheat is caused by *Tilletia indica* (Mitra) (synonym *Neovossia indica* (Mitra) Mundkur). The teliospores of *T. indica* are deposited onto or into the soil at harvest or they may contaminate the surface of the seed (2,3,6). Upon germination at the soil surface, each teliospore produces a promycelium bearing filiform primary sporidia at its tip (11). These primary sporidia, or the secondary sporidia that develop subsequently, are carried to the wheat spike by air currents or splashing water (12).

The advantage of liquid cultures (1,4,8) over solid media is that they produce sporidia more rapidly for inoculation (1,4,8,14). In our laboratory studies, the liquid media that consistently produced abundant sporidia were potato-dextrose broth (PDB) and PDB supplemented with sucrose or sucrose plus dextrose. Sporidia produced in liquid cultures are considered secondary sporidia since they result from primary sporidia produced on the promycelium of the germinating teliospore. These secondary sporidia are similar to the primary sporidia morphologically (i.e., they are filiform); they are referred to as filiform secondary sporidia to distinguish them from allantoid secondary sporidia produced on solid media (5,9).

Our study compared the pathogenicity of filiform and allantoid secondary sporidia produced in liquid and on solid media, respectively.

MATERIALS AND METHODS

Inoculum. *Filiform secondary sporidia.* Sporidial and mycelial suspensions were prepared by placing a disk of water agar containing germinating teliospores into a test tube opposite a potato-dextrose agar (PDA) slant. Test tubes were kept for 1 wk at 20 C with alternating 12-hr periods of dark and light (0.9 $\mu E \cdot m^{-2} \cdot s^{-1}$ PAR [photosynthetically active radiation]). At the end of the week, sterile distilled water was added and the mycelium was scraped from the agar. One milliliter of the resultant sporidial and mycelial suspension was added to a flask containing 250 ml of PDB (35 g fresh potato, 250 ml water, 10 g dextrose). The flasks were placed on a shaker for 1 wk at 20 C, after which the number of filiform sporidia per milliliter was calculated using a hemacytometer. The sporidia concentration in the suspension was adjusted with distilled water to that required for each experiment.

Allantoid secondary sporidia. Production of allantoid secondary sporidia was started by taking a 6-mm disk of water agar covered with germinating teliospores and placing it facedown on the undersurface of the lid of a PDA

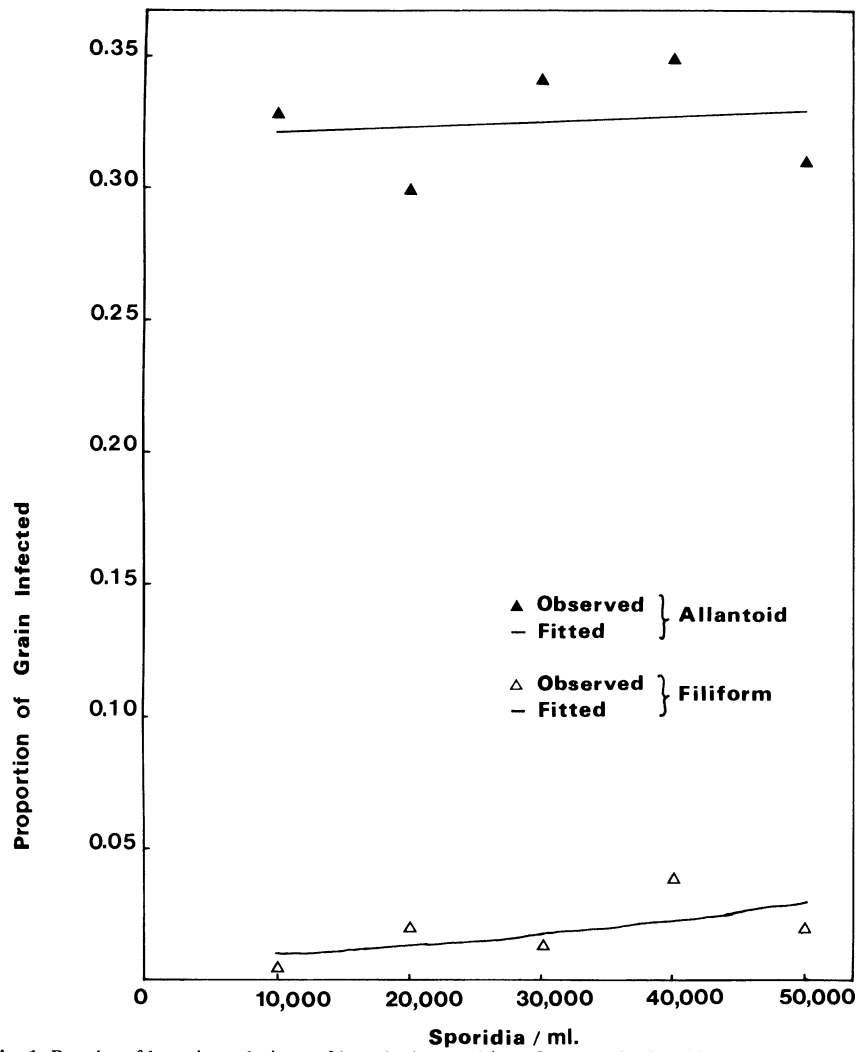

Fig. 1. Results of boot inoculations of bread wheat cultivar Genaro 82 with filiform or allantoid secondary sporidia of *Tilletia indica*.

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plate. Plates were kept for 7 days at 20 C with alternating 12-hr periods of dark and light (0.9 $\mu E \cdot m^{-2} \cdot s^{-1}$ PAR). Sterile distilled water (1 ml) was added to each plate, the mycelium was gently scraped, and the resultant suspension was distributed over the same plate. The plates were then incubated at 20 C, as previously. After 21 days, 10–20 ml of distilled water was added to each plate and the mycelium was scraped from the agar. The resultant suspension was filtered through four layers of gauze, after which the number of secondary sporidia per milliliter of solution was determined using a hemacytometer. The sporidia concentration in the suspension was then adjusted with distilled water to that required. Several plates were used to obtain an adequate volume of inoculum.

Boot inoculations. Filiform sporidia were produced in PDB after 7 days on a shaker and adjusted to concentrations of 10,000, 20,000, 30,000, 40,000, and 50,000 sporidia per milliliter. The equivalent concentrations of allantoid secondary sporidia were obtained from 4-wk-old PDA plates.

Each treatment consisted of 200 spikes of the bread wheat cultivar Genaro 82 (susceptible to Karnal bunt) in four replicates of 50 spikes each. Each spike was injected with 1 ml of sporidial suspension into the boot at midboot (GS 44) (16) using a hypodermic syringe and needle. The inoculations were carried out in the field in Obregon, Sonora, Mexico, with each replicate of 50 confined to one double 5-m row. At maturity, individual spikes were harvested and threshed by hand. The number of infected grains observed by visual inspection and the total number of grains were determined for each replicate.

Spray inoculations. The susceptible bread wheat cultivar Seri 82 was initially grown outside the greenhouse in pots (five plants per pot) containing a sterilized (methyl bromide, 1 lb/m^3) mixture of soil, sand, and peat (2:1:1, v/v). At 2 and 4 wk after sowing, 2 g of urea (46:0:0) was applied to each pot.

Tillers were selected and tagged when the spikes were just emerging from the boot (GS 55); in earlier studies, this growth stage was found to be most susceptible to spray inoculations (15). Tagged spikes (12 pots per treatment) were then sprayed with allantoid and filiform secondary sporidial suspensions at concentrations of 10,000, 20,000, 30,000, 40,000, and 50,000 sporidia per milliliter using a hand sprayer. The filiform and allantoid secondary sporidial suspensions were produced in the same way as for the boot inoculations. After inoculation, the pots were placed in a humidity chamber (95–100% relative humidity, 22 C) with alternating 15-min periods of mist and 45-min periods of light (92 $\mu E \cdot m^{-2} \cdot s^{-1}$ PAR) for 24 hr. Pots were then placed randomly on a table in the greenhouse (approximate conditions: 14–16 hr natural light; 22 C day, 12 C night; 45–60% relative humidity) until plant maturity, when individual spikes were harvested and threshed by hand. The number of infected grains and the total number of grains were then determined for each treatment.

Results of preliminary experiments using each inoculation technique showed unacceptable infection levels in the controls caused by soil problems (in the greenhouse) and problems with the inoculation technique (in the field). The data presented are from the second set of experiments.

Statistical methods. In both boot inoculation and spray inoculation experiments, the observed proportions of infected grains were analyzed by fitting linear logistic models as a special case of generalized linear models with binomial errors (10,13). Both experiments have one qualitative factor, type of sporidia, and a covariate, sporidia concentration. The analysis in both experiments was done using the number of infected grains and total number of grains per sporidia type by concentration. For boot inoculation, a separate analysis was done for each replicate.

By fitting successive models and taking the difference between the residual deviances, we tested: 1) whether the rate of increase in the proportion of infected grain caused by increased sporidia concentration was the same for filiform and allantoid sporidia, i.e., whether there was an interaction between sporidia type and sporidia concentration; 2) whether there was a difference in the proportion of infected grain by the two sporidia types, after a common adjustment for sporidia concentration; and 3) whether in the absence of interaction between sporidia type and sporidia concentration there was a significant rate of increase in the proportion of infected grain caused by an increase in sporidia concentration common to both types of sporidia.

The analyses were carried out using the Genstat package (7).

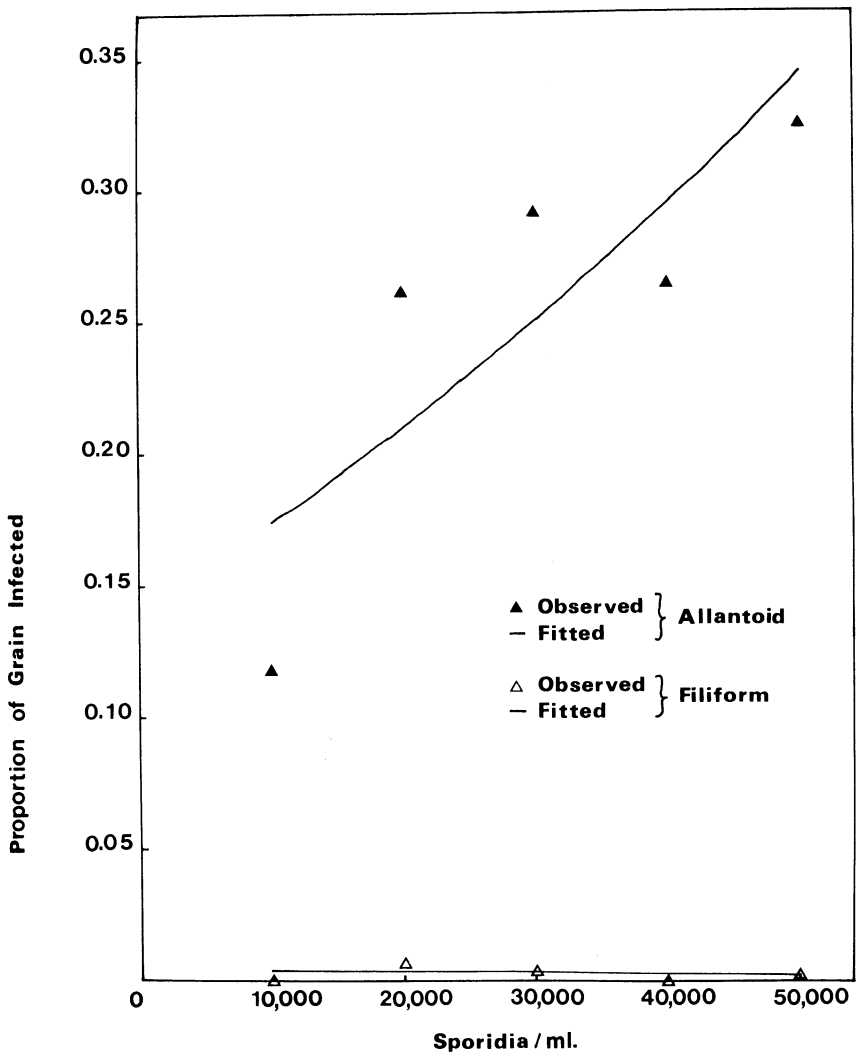

Fig. 2. Results of spray inoculations of bread wheat cultivar Seri 82 with filiform or allantoid secondary sporidia of *Tilletia indica*.

RESULTS AND DISCUSSION

The results of fitting the logistic models to the boot inoculation data show

a highly significant interaction between sporidia type and sporidia concentration (chi-square = 103, df = 1). For allantoid sporidia, the proportion of infected grain was always higher than that for filiform sporidia, and its rate of increase with increasing sporidia concentration was smaller (Fig. 1). If p is the probability of infection, differences on a logistic scale can be interpreted in terms of the odds for infection against those for noninfection, namely $p/(1 - p)$. The exponential of a logistic difference is thus a ratio of odds. In this case, the ratio of the odds for infection of allantoid over filiform for a given sporidia concentration was exp $(4.106 - 0.0270c)$, where c is the concentration of sporidia per milliliter (in thousands). The standard error of the difference in slope (−0.0270) was 0.00272. A negative coefficient of c means that the ratio of odds decreases as the sporidia concentration increases. In other words, the difference in the proportion of infected grain between the allantoid and filiform sporidia types decreased with an increase in sporidia concentration.

The results for the boot inoculation experiment also showed a highly significant difference in the proportion of infected grain caused by different sporidia types after a common adjustment for sporidia concentration (chi-square = 17,215, df = 1).

As with boot inoculation, the results of fitting the logistic models to the spray inoculation data also revealed a significant interaction between sporidia type and sporidia concentration (chi-square = 6.3, df = 1). For allantoid sporidia, the proportion of infected grain was always higher than that for filiform sporidia. Where the slope for filiform sporidia was almost zero, the slope for the allantoid sporidia was greater and positive (Fig. 2). The ratio of the odds for infection of allantoid over those for filiform for a given sporidia concentration was exp $(3.849 + 0.0359c)$ where c is the sporidia concentration per milliliter (in thousands) and the standard error of the difference in slope (+0.0359) was 0.0144. A positive coefficient of c means that the ratio of odds increased with the concentration; that is, the difference in the proportion of infected grain between the allantoid and flliform sporidia types increased as the sporidia concentration increased.

The results of the spray inoculation experiment also showed a highly significant difference in the proportion of infected grain caused by the different sporidia types after a common adjustment for sporidia concentration (chi-square = 3,658, df = 1).

In both boot inoculation and spray inoculation experiments, the filiform secondary sporidia caused considerably less infection than the allantoid secondary sporidia, indicating that filiform secondary sporidia are less reliable for use in Karnal bunt inoculations. Earlier published reports on inoculation techniques using inoculum produced in liquid cultures did not compare the two sporidia types (1,4,8,14).

Our conclusion is that when screening germ plasm for Karnal bunt resistance, allantoid secondary sporidia produced in solid media should be used as inoculum. If filiform secondary sporidia produced in liquid cultures are used instead, there is a high risk of false negatives caused by the lower pathogenicity of these sporidia.

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