Effect of Wheat Host Cultivars on Pycnidiospore Production by Septoria tritici

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ABSTRACT

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Pycnidia of *Septoria tritici* in leaves of moderately-resistant wheat cultivar TAM W-101 and susceptible cultivars Improved Triumph and Triumph 64 liberated 2.0-2.5 times more pycnidiospores per pycnidium than did pycnidia in leaves of resistant cultivar Oasis. This relationship of pycnidiospore liberation to disease reaction class of the cultivar occurred whether plants were incubated in a greenhouse or a growth chamber. However, the average pycnidium produced in a growth chamber released about twice as many pycnidiospores as the average pycnidium produced in a greenhouse regardless of cultivar.

Additional key words: Septoria leaf blotch, speckled leaf blotch. *Triticum aestivum*, resistance, susceptibility.

Reactions of wheat (*Triticum aestivum* L.) cultivars to infection by *Septoria tritici* Rob. ex. Desm. commonly are rated on the numbers of pycnidia (pycnidium density) and the amount of leaf necrosis that develops (2, 3, 4, 5, 6, 7, 8, 9). Differences in percent leaf necrosis among cultivars usually correlate positively with numbers of pycnidia formed. Shaner et al. (9) showed that significantly fewer pycnidia formed in tissue of resistant 'Oasis' than in susceptible cultivars Abe, Arthur, and Arthur 71 under field conditions. Eyal et al. (2) proposed that the existence of significant differences in pycnidium production among *S. tritici* culture/wheat cultivar combinations is evidence for physiologic specialization within the fungus species. Thus, pycnidium density is an important criterion of cultivar resistance.

The effect of host genotype on numbers of pycnidiospores produced by individual pycnidia has not been studied. Eyal and Brown (3) observed a decrease in pycnidium size as density increased in heavily infected flag leaves from field-grown plants. However, Eyal (1) reported that in two highly susceptible cultivars (designated as FA 8193 and M 652) no correlation existed at $P = 0.05$ between total spore numbers within a pycnidium and the volume of pycnidia produced.

The objective of this study was to determine if a relation exists between the numbers of pycnidiospores produced by individual pycnidia and field ratings of cultivars designated as susceptible, moderately resistant, and resistant.

MATERIALS AND METHODS

The experiment was performed twice. Pycnidia used in the first trial developed in plants kept in a greenhouse with natural light (approximately 12-13 hr) and with temperature varying from 15 C at night to as high as 30 C during the day. Pycnidia used in the second trial developed in plants kept in a growth chamber programmed for 20 ± 2 C with 12 hr of 17,500 lux and 12 hr of darkness. In the first trial Improved Triumph, Tam W-101, and Oasis were selected as representatives of susceptible, moderately resistant, and resistant cultivars, respectively. In the second trial, cultivar Triumph 64 was substituted for Improved Triumph.

Nonvernalized 30-day-old plants (third- to fourth-leaf stage) of each cultivar were inoculated with a mixture of two cultures of *S. tritici*. One culture (MT-5), obtained from A. L. Scharen (SEA, USDA, Plant Pathology, Montana State University, Bozeman, MT 59715) grew as a proliferating pink spore mass. The second culture (Oasis-3), obtained from G. Shaner (Botany and Plant Pathology, Purdue University, W. Lafayette, IN 47907) produced dark mycelium and pink pycnidia in culture.

The cultures were grown singly in a liquid medium [2 g of malt extract (Difco Laboratories, Detroit, MI 48232) and 0.5 g of yeast extract (U.S. Biochemical Corp., Cleveland, OH 44128) in 1 liter of distilled water] at about 22 C for 8-12 days. Flasks containing the cultures were manually shaken vigorously once each day. Equal volumes of medium containing the respective cultures were combined to produce the inoculum. Nonflavored gelatin (0.5 g dissolved in 25 ml of warmed distilled water) was added to 100 ml of inoculum as a sticker. The inoculum was finger-spread on the surfaces of all leaves.

The plants then were covered with a supported opaque polyethylene film and kept moist by a time clock-controlled mist blower for 96 hr.

Twenty days after inoculation (pycnidia first appeared 16 days after inoculation), 16 leaf segments (1-3 cm long) bearing dense pycnidia were excised from each cultivar and clasped separately in spring-loaded pin-curl clips (alligator type). Four leaf segments from each cultivar were placed in each of four petri dishes containing filter paper moistened with deionized distilled water. A glass rod placed on the filter paper, and beneath the points of the clips, prevented contact of the leaf segments with the filter paper. Before placement in the petri dishes, leaf segments were submerged in deionized distilled water for about 15 sec to wet them and the pycnidia. Treatments (four leaf segments of each cultivar within a petri dish) were assigned to each of four replications in a randomized complete block design on a laboratory bench. The laboratory temperature varied from 18-25 C. The filter papers were wetted periodically to maintain high humidity. Pycnidiospores (hereafter called spores) produced in greenhouse- and growth-chamber-cultured plants, respectively, were harvested and counted after 24-26 hr and 48-50 hr in the petri dish moist chambers. After the first harvest, the dishes were left open for 30 hr; the leaf segments and filter papers then were rewetted and high humidity was maintained by periodically moistening the filter paper for another 24-26 hr, when a second spore harvest and count was made.

Spores were harvested by dipping each leaf segment 10 times into 300 μliters of deionized distilled water deposited in spot glass depressions with an Eppendorf syringe. Disposable micropipettes were used to stir the water and spores and to transfer drops of the suspensions to a hemacytometer slide. An estimate of the total number of spores produced in each petri dish chamber was derived arithmetically from the hemacytometer counts.

The numbers of pycnidia in leaf segments from greenhouse plants (trial 1) were counted immediately after the second harvest. Pycnidia in leaf segments from growth chamber plants (trial 2) were counted prior to the first wetting and again after the second harvest. There was a small and statistically nonsignificant increase in numbers of pycnidia between harvests. In both trials, the numbers of pycnidia counted after the second harvest were used to calculate the number of spores liberated per pycnidium. To facilitate counting of pycnidia, the leaf segments were placed between microscope slides held together at the ends with an adhesive cellophane tape. Counts were made using a binocular dissecting scope and transmitted light. Because the leaf segments were chosen for a high density of pycnidia and the leaf area sampled was not constant, the number of pycnidia counted did not relate to a cultivar-culture interaction. In an earlier experiment, significantly ($P = 0.01$) more pycnidia developed per square centimeter of inoculated second leaves of Improved Triumph (193 pycnidia) than were observed with TAM W-101 (84 pycnidia) and Oasis (93 pycnidia).

Analysis of variance and Tukey's test (10), $P = 0.05$, were used to compare treatment means.

RESULTS AND DISCUSSION

Pycnidia of *S. tritici* in leaves of resistant Oasis released significantly fewer spores per pycnidium than those in leaves of moderately resistant TAM W-101 and susceptible Improved Triumph and Triumph 64 (Table 1). The average pycnidium produced in TAM W-101, Improved Triumph, and Triumph 64 released about 2.0-2.5 times more spores than the average pycnidium produced in Oasis. This greater release of spores from pycnidia in leaves of susceptible cultivars occurred in both trials. The cultivar effects on the number of spores released per pycnidium were significant for spore count totals after two wettings in both trials, and after the first wetting in the second trial. Although Tukey's test indicated that the number of spores released per pycnidium in the three cultivars during the first wetting of the first trial was not significant, the average pycnidium in

TABLE 1. Effect of wheat cultivar on pycnidiospore production by *Septoria tritici*

Cultivars and statistical parameters	Number of pycnidia sampled	Spores released/pycnidium[a] First wetting ($\times 10^3$)	Second wetting ($\times 10^3$)	Total ($\times 10^3$)
Trial 1 (Greenhouse test):				
Improved Triumph	5,256	4.2 x[b]	5.3 x	9.5 x
TAM W-101	4,187	3.0 xy	6.4 x	9.4 x
Oasis	4,910	1.6 xy	2.4 x	4.0 y
hsd[c], $P = 0.05$		2.3		5.0
CV		35.9 %	43.9 %	30.0 %
Trial 2 (Growth-chamber test):				
Triumph 64	2,988	14.3 x	7.7 x	22.0 x
TAM W-101	4,717	10.6 xy	5.0 x	15.6 y
Oasis	1,444	4.9 y	3.5 x	8.4 z
hsd[c], $P = 0.05$		7.0		6.2
CV		32.3 %	54.1 %	18.4 %

[a]Pycnidia produced in the greenhouse and growth chamber were kept moist in the first wet period for 24 and 48 hr, respectively. Pycnidia were kept moist for 24 hr in the second wet period in both greenhouse and growth chamber tests.

[b]Numbers followed by the same letter are not significantly different according to Tukey's w - procedure. Abbreviations hsd = honestly significant difference and CV = coefficient of variability.

Improved Triumph released 2.6 times as many as one in Oasis. The number of spores released during the second wetting did not differ significantly in either trial regardless of host cultivar. These data indicate that using spore counts to compare cultivar reactions may lead to erroneous conclusions if the pycnidia have been wet prior to their collection and subsequently induced spore emission.

The length and width of 100 dry pycnidia in four or more randomly selected lesions in each cultivar used in trial 1 were measured. The pycnidia averaged 151 × 107 μm in Improved Triumph, 133 × 92 μm in TAM W-101, and 127 × 88 μm in Oasis. The height of 50 randomly selected pycnidia in Improved Triumph and 30 in Oasis averaged 40 and 35.3 μm, respectively. To measure heights of pycnidia, strips of dry-lesion tissue about 0.5 mm wide were placed on edge in small mounds of petroleum jelly on microscope slides. Pycnidia exposed along the upper edges of the strips were illuminated with incident light and measured with an ocular micrometer in a compound light microscope. By using these measurements, and assuming infinitely thin pycnidium walls, the average volume of a pycnidium in Improved Triumph was estimated at 335,230 μm^3, and of one in Oasis at 204,811 μm^3; the ratio of these volumes is 1.6:1. The equation $V = 4/3\ \pi$ abc was used to calculate the volume. In the equation, V = volume in μm^3, and a, b, and c = one-half of the length, width, and height, respectively, of the pycnidia in μm. Because height measurements of pycnidia were few, tests for significant differences between volumes of pycnidia produced in the cultivars were not made. However, the differences suggest a correlation between pycnidial volume and the number of spores released per pycnidium.

Approximately twice as many spores were released from a pycnidium produced in the growth chamber as were released from one produced in the greenhouse, regardless of cultivar. This difference presumably derived in part from differences in one or more environmental factors such as light quality, humidity, leaf water potential and temperature. Also, the longer duration (48-50 hr vs. 24-26 hr) of the first wetting of pycnidia produced in the growth chamber may have contributed to the difference. Eyal (1) reported that the pycnidia from flag leaves of susceptible plants in the field and from inoculated susceptible seedlings in the greenhouse produced about 4,000 released and unreleased spores per pycnidium. In my tests, the total spores released per pycnidium substantially exceeded 4,000, except for those released by pycnidia in leaves of Oasis incubated in the greenhouse.

The fact that the quantity of pycnidia (2, 3, 9) and the spores produced per pycnidium are greater in the susceptible host than in a resistant one suggests that counting spores liberated by pycnidia in a given unit of diseased tissue may be an expedient method of differentiating resistant and susceptible wheats. The results also indicate that the number of spores released by a pycnidium in a given host cultivar or line may vary widely among environments. Thus, a screening method based on spore counts per unit of diseased tissue would require strict control of environmental conditions and adherence to standardized techniques.

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