Host Genotype Effects on Inoculum Production by *Cephalosporium gramineum* from Infested Residue

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ABSTRACT

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Five winter wheat (*Triticum aestivum*) cultivars differing in resistance to Cephalosporium stripe were inoculated with *Cephalosporium gramineum*, and the mature infested straw was placed under conditions conducive to sporulation of the fungus. Cultivars Dodge and Newton had a significantly greater percentage of straw segments showing sporulation of *C. gramineum* than cultivar Sturdy, and dilution plating indicated that residue from those cultivars produced more inoculum per gram of straw. Straw from Plainsman V supported the production of inoculum at a level similar to that observed for Sturdy, whereas straw from Arkan supported a level of inoculum midway between those of Sturdy and Newton. Inoculum production was not related to the resistance response of the cultivar: Sturdy is highly susceptible, and Newton and Plainsman V are moderately resistant. These results suggest that Cephalosporium stripe decline induced by monoculture of moderately resistant cultivars is not the result of reduced ability of the infested residue of these cultivars to produce inoculum.

Additional keywords: *Hymenula cerealis*

Cephalosporium stripe of winter wheat (*Triticum aestivum* L.) is caused by *Cephalosporium gramineum* Nisikado & Ikata (=*Hymenula cerealis* Ell. & Ev.) (4). Infection occurs when the fungus passively or actively enters root wounds caused by alternate freezing and thawing of soil water (1,15). The pathogen then invades the plant systemically (18,23) and occludes vessels, thus disrupting plant-water relations and causing reduced grain yield (13). Parasitically colonized residue, which harbors the pathogen, is returned to the soil after grain harvest and serves as the source of primary inoculum (3,5,6,8). Conidia produced on the residue infect the next crop to complete the disease cycle (10,24).

The importance of the quantity of primary inoculum in disease development has been demonstrated for several monocyclic soilborne pathogens (2,9). In general, as the quantity of inoculum increases, disease increases until relatively high incidences are reached. Thus, decreasing initial inoculum density or inoculum efficiency is an important control strategy for monocyclic diseases. Similar relationships between inoculum density and disease incidence have been demonstrated for Cephalosporium stripe (7,16).

We recently showed that Cephalosporium stripe declined with continuous culture of moderately resistant cultivars, whereas a moderate level of disease persisted when susceptible cultivars were monocultured (21). For certain polycyclic leaf and stem diseases, the infection frequency is reduced in cultivars possessing rate-reducing resistance. Less inoculum results from these cultivars, leading to reduced disease development during a single season (11,19,20,22). We hypothesized that Cephalosporium stripe decline could result from reduced inoculum production on diseased residue originating from moderately resistant cultivars (21). The objective of this research was to determine whether host genotype affected the production of inoculum by *C. gramineum* on infested residue.

MATERIALS AND METHODS

Sources of systemically colonized straw. Two sources of infested residue were produced in the greenhouse. Five winter wheat cultivars were selected on the basis of their known reactions to Cephalosporium stripe. The cultivars (Sturdy, Arkan, Newton, Dodge, and Plainsman V) ranged from highly susceptible to moderately resistant. Plants were vernalized at 4 C for 35–40 days either as germinated seeds on moist filter paper in petri plates or as 10-day-old seedlings in vermiculite in plastic tubes 3 cm in diameter and 13 cm long. Vernalized, germinated seeds were then planted in vermiculite in tubes and placed in the greenhouse (20 ± 5 C), as were vernalized seedlings. At the four- to five-leaf stage, plants were removed from the tubes, inoculated, and replanted in pots 15 cm in diameter and 20 cm deep. Three to six plants were planted per pot in pasteurized growth medium containing one part sand, one part Chase silty clay loam soil, and one part vermiculite (v/v/v) (pH was 6.5). Two experiments using 10 or 15 replicate pots per cultivar were established.

Plants were inoculated with conidial suspensions of *C. gramineum* obtained from cultures grown for 30 days at 15 C in the dark on one-fourth strength cornmeal agar (CMA). Colonies were flooded with distilled water and rubbed with a glass rod, and the suspension was decanted into a suitable container. Roots of plants at the four- to five-leaf stage were severed 3 cm below the seed while under distilled water. Plants with cut roots were then placed in 500 ml of a suspension containing 10^6 or 10^8 conidia per milliliter and were incubated under greenhouse conditions for 12–24 hr before replanting.

At anthesis, tillers possessing leaves with characteristic chlorotic striping and necrotic veins were tagged at the highest leaf showing symptoms. After plants were mature, tagged tillers were severed at ground level and at tagged leaf level, stripped of sheaths, cut into segments 6 cm long, and stored air-dry under laboratory conditions.

Inoculum production assay. Infested residue from the greenhouse was used to investigate effects of cultivars on inoculum production. Experiments were repeated using residue from each of two greenhouse studies, for a total of four experiments.

Ten 6-cm segments were weighed, placed on 30 ml of air-dry vermiculite in a plastic petri dish 9 cm in diameter, and moistened with 25 ml of distilled water. Six plates, constituting six replications, were prepared for each cultivar for each experiment. All plates were randomized in a covered plastic box containing a beaker filled with water to maintain high relative humidity and incubated at 15 C in the dark for 4–5 wk.

After incubation, the surface of each straw segment was inspected for sporu-

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lation and/or sporodochia of *C. gramineum* with a dissecting microscope (×8.0), and the percentage of sporulating segments was recorded. The contents of each plate was then blended in distilled water in a total volume of 1,000 ml for 5 min and serially diluted. A sample (0.1 ml) of each 10^{-3}, 10^{-4}, and 10^{-5} dilution was plated on each of two plates containing a semiselective medium. One liter of the medium contained 4.25 g of Difco CMA, 15.75 g of agar, 0.1 g of $CuSO_4 \cdot 5H_2O$, 50 mg of streptomycin sulfate, 20 mg of chloramphenicol, and 1,000 ml of distilled water. The CMA, agar, and water were autoclaved, and then the filter-sterilized $CuSO_4 \cdot 5H_2O$ and antibiotics were added. The medium was acidified to pH 4.0 with 1.0 M HCl when the medium attained 55 C. After about 7 days of incubation at 21 ± 2 C, colonies characteristic of *C. gramineum* were counted under a dissecting microscope (×8.0).

Data analysis. The number of colonies per plate from the 10^{-5} dilution was used in conjunction with the air-dry weight of straw to calculate colony-forming units (cfu) per gram of straw and per gram of straw exhibiting sporulation. Thus, the capacity of infested straw to produce inoculum and the inoculum production from a sporulating piece of infested straw were assessed. Raw data for percentage of straw segments showing sporulation and log-transformed data for colony-forming units per gram were analyzed by analysis of variance using Fisher's protected least-significant-difference test for mean separation.

RESULTS

No significant interactions between experiment and cultivar were detected for any of the three parameters measured. When sporulation from the first source of residue was quantified, more than 50% of the straw segments of all cultivars except Sturdy exhibited sporulation (Table 1). For all cultivars, an average of 68% of the segments exhibited sporulation.

The cultivars Newton and Dodge had a significantly greater ($P = 0.05$) percentage of segments exhibiting sporulation than Sturdy (Table 1). Plainsman V was not significantly different from Sturdy in experiments using the second source of residue. Over all four experiments, Newton and Dodge had the highest percentage of segments exhibiting sporulation (77 and 76%, respectively), Sturdy had the fewest (53%), and Arkan and Plainsman V supported intermediate levels (70 and 63%, respectively).

Combining all experiments and both sources of residue, we found that infested straw produced an average of 3.4×10^9 cfu/g of straw (Table 2). Straw from all cultivars supported overall average sporulation levels greater than 1.5×10^9 cfu/g. In each experiment, cultivars differed significantly in the capacity of *C. gramineum* to sporulate from infested residue (Table 2). Straw from Newton and Dodge produced significantly more inoculum than that from Sturdy and Plainsman V. Over all four experiments with both sources of residue, infested residue of Newton and Dodge produced the highest levels of inoculum (5.6 and 4.1×10^9 cfu/g of straw, respectively), Sturdy and Plainsman V the lowest (1.7 and 2.3×10^9 cfu/g, respectively).

For both sources of residue, sporulating straw for all cultivars produced a minimum of 1×10^9 cfu/g, and the overall average was 4.7×10^9 cfu/g (Table 3). Sporulating segments of Newton produced significantly more inoculum than those of Sturdy and Plainsman V. Although other cultivars differed significantly among themselves in inoculum production, the differences were not as consistent between experiments and sources of residue. Over all four experiments, inoculum production per gram of sporulating straw was greatest for Newton (7.0×10^9 cfu) and lowest for Sturdy (2.9×10^9 cfu) and Plainsman V (3.5×10^9 cfu).

DISCUSSION

Winter wheat cultivars differ significantly in the amount of inoculum of *C. gramineum* produced from straw known to be infested with the fungus. In our experiments, Sturdy and Newton differed the most. Infested straw of Sturdy produced 70% fewer conidia than

Table 1. Percentage of straw segments of five winter wheat cultivars infested with *Cephalosporium gramineum* that produced sporodochia or spore masses in the laboratory

	Source of residue					
	Greenhouse 1			Greenhouse 2		
	Experiment			Experiment		
Cultivar	1	2	Average[z]	1	2	Average[z]
Newton	65	75	70 a	82	85	84 a
Dodge	75	67	71 a	88	73	81 a
Arkan	72	68	70 a	75	65	70 b
Plainsman V	68	53	61 a	72	57	65 b
Sturdy	35	38	37 b	73	65	69 b
Average[z]	63 A	60 A		78 B	69 B	

[z] Means within a column or column averages within each residue source followed by a common letter do not differ significantly ($P = 0.05$) according to Fisher's protected least-significant-difference test.

Table 2. Number of conidia of *Cephalosporium gramineum*[y] produced in the laboratory from infested straw of five winter wheat cultivars

	Source of residue					
	Greenhouse 1			Greenhouse 2		
	Experiment			Experiment		
Cultivar	1	2	Average[z]	1	2	Average[z]
Newton	5.7	5.1	5.4 a	7.2	4.3	5.8 a
Dodge	3.1	4.1	3.6 ab	5.7	3.5	4.6 a
Arkan	2.7	1.7	2.2 bc	3.7	4.4	4.1 a
Plainsman V	2.4	1.6	2.0 c	3.0	2.0	2.5 b
Sturdy	0.9	0.4	0.7 d	3.6	1.6	2.6 b

[y] Colony-forming units per gram of straw (× 10^9).
[z] Means within a column followed by a common letter do not differ significantly ($P = 0.05$) according to Fisher's protected least-significant-difference test.

Table 3. Number of conidia of *Cephalosporium gramineum*[y] produced in the laboratory from sporulating, infested straw of five winter wheat cultivars

	Source of residue					
	Greenhouse 1			Greenhouse 2		
	Experiment			Experiment		
Cultivar	1	2	Average[z]	1	2	Average[z]
Newton	7.8	6.6	7.2 a	8.7	4.9	6.8 a
Dodge	4.4	6.1	5.3 a	6.5	4.5	5.5 ab
Arkan	3.8	3.1	3.5 b	4.8	7.0	5.9 a
Plainsman V	3.4	2.8	3.1 b	4.4	3.4	3.9 c
Sturdy	2.4	1.0	1.7 c	5.6	2.6	4.1 bc

[y] Colony-forming units per gram of sporulating straw (× 10^9).
[z] Means within a column followed by a common letter do not differ significantly ($P = 0.05$) according to Fisher's protected least-significant-difference test.

that of Newton; that is, infested straw of Newton produced 229% more conidia than that of Sturdy.

Martin et al (14) described a positive correlation between inoculum density and disease development regardless of the cultivar. Thus, differences in inoculum production of the magnitude reported here would be expected to affect the severity of Cephalosporium stripe in the field. However, because our experiments used infested straw from the greenhouse and involved an artificial environment for sporulation, additional research is needed to determine whether similar differences among cultivars occur under natural conditions.

Two components contributed to differences among cultivars in inoculum production: the percentage of infested straw segments with visible signs of sporulation and the number of conidia produced from segments that did support sporulation. Thirty-one percent fewer segments of Sturdy than of Newton showed sporulation, and 59% fewer conidia were produced from these sporulating segments; that is, Newton had 45% more sporulating segments than Sturdy, and 141% more conidia were produced.

Several sources of resistance to Cephalosporium stripe have been identified (7,12,14,17). These resistant lines and cultivars of wheat show either lower disease incidence or less severe disease compared with susceptible cultivars exposed to the same inoculum densities. The results presented here suggest a third mechanism whereby host genotype may affect disease: the capacity for sporulation from infested tissue. However, whether the cultivar response described here can be manipulated in a breeding program is not known.

One goal of this study was to determine whether cultivars ranging in resistance to stripe also differed in the capacity of their infested residue to support inoculum production. An inability or reduced ability of infested residue of Newton, Dodge, and Plainsman V to produce inoculum would help explain the decline in Cephalosporium stripe observed following monoculture of these moderately resistant cultivars (21). However, results of this study suggest that disease decline is not the result of reduced ability of infested residue to produce inoculum, because infested residue of all cultivars supported fairly profuse sporulation. In fact, two cultivars that displayed disease decline (Newton and Dodge) produced significantly more inoculum than the susceptible cultivar Sturdy. The fact that the data presented here represent the amount of inoculum present at a single point in time may account for this discrepancy. Further research is needed to determine whether the total amount of inoculum produced by infested straw over time varies among cultivars.

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LITERATURE CITED

1. Bailey, J. E., Lockwood, J. L., and Wiese, M. V. 1982. Infection of wheat by *Cephalosporium gramineum* as influenced by freezing of roots. Phytopathology 72:1324-1328.
2. Baker, R. 1978. Inoculum potential. Pages 137-157 in: Plant Disease: An Advanced Treatise, Vol. II: How Disease Develops in Populations. J. G. Horsfall and E. B. Cowling, eds. Academic Press, New York.
3. Bockus, W. W., O'Connor, J. P., and Raymond, P. J. 1983. Effect of residue management method on incidence of Cephalosporium stripe under continuous winter wheat production. Plant Dis. 67:1323-1324.
4. Bruehl, G. W. 1957. Cephalosporium stripe disease of wheat. Phytopathology 47:641-649.
5. Bruehl, G. W., and Lai, P. 1966. Prior colonization as a factor in the saprophytic survival of several fungi in wheat straw. Phytopathology 56:766-768.
6. Bruehl, G. W., and Lai, P. 1968. The probable significance of saprophytic colonization of wheat straw in the field by *Cephalosporium gramineum*. Phytopathology 58:464-466.
7. Bruehl, G. W., Murray, T. D., and Allan, R. E. 1986. Resistance of winter wheats to Cephalosporium stripe in the field. Plant Dis. 70:314-316.
8. Christian, D. G., and Miller, D. P. 1984. Cephalosporium stripe in winter wheat grown after different methods of straw disposal. Plant Pathol. 33:605-606.
9. Fry, W. E. 1982. Principles of Plant Disease Management. Academic Press, New York. 378 pp.
10. Lai, P., and Bruehl, G. W. 1966. Survival of *Cephalosporium gramineum* in naturally infested wheat straws in soil in the field and in the laboratory. Phytopathology 56:213-218.
11. Lee, T. S., and Shaner, G. 1984. Infection processes of *Puccinia recondita* in slow- and fast-rusting wheat cultivars. Phytopathology 74:1419-1423.
12. Martin, J. M., Mathre, D. E., and Johnston, R. H. 1983. Genetic variation for reaction to *Cephalosporium gramineum* in four crosses of winter wheat. Can. J. Plant Sci. 63:623-630.
13. Martin, J. M., Mathre, D. E., and Johnston, R. H. 1986. Water use in winter wheat as influenced by *Cephalosporium gramineum*. Crop Sci. 26:14-20.
14. Martin, J. M., Mathre, D. E., and Johnston, R. H. 1986. Winter wheat genotype responses to *Cephalosporium gramineum* inoculum levels. Plant Dis. 70:421-423.
15. Mathre, D. E., and Johnston, R. H. 1975. Cephalosporium stripe of winter wheat: Infection processes and host response. Phytopathology 65:1244-1249.
16. Mathre, D. E., and Johnston, R. H. 1975. Cephalosporium stripe of winter wheat: Procedures for determining host response. Crop Sci. 15:591-594.
17. Morton, J. B., and Mathre, D. E. 1980. Identification of resistance to Cephalosporium stripe in winter wheat. Phytopathology 70:812-817.
18. Morton, J. B., Mathre, D. E., and Johnston, R. H. 1980. Relation between foliar symptoms and systemic advance of *Cephalosporium gramineum* during winter wheat development. Phytopathology 70:802-807.
19. Parlevliet, J. E. 1979. Components of resistance that reduce the rate of epidemic development. Annu. Rev. Phytopathol. 17:203-222.
20. Shaner, G. 1973. Reduced infectability and inoculum production as factors of slow mildewing in Knox wheat. Phytopathology 63:1307-1311.
21. Shefelbine, P. A., and Bockus, W. W. 1989. Decline of Cephalosporium stripe by monoculture of moderately resistant winter wheat cultivars. Phytopathology 79:1127-1131.
22. Statler, G. D., and McVey, M. A. 1987. Partial resistance to *Uromyces appendiculatus* in dry edible beans. Phytopathology 77:1101-1103.
23. Wiese, M. V. 1972. Colonization of wheat seedlings by *Cephalosporium gramineum* in relation to symptom development. Phytopathology 62:1013-1015.
24. Wiese, M. V., and Ravenscroft, A. V. 1975. *Cephalosporium gramineum* populations in soil under winter wheat cultivation. Phytopathology 65:1129-1133.