Factors Affecting Infection of Wheat Seedlings by *Septoria nodorum*

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ABSTRACT

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Coleoptile infection of wheat by *Septoria nodorum* occurred over broad ranges of soil moisture, temperature, and planting depth. Increased frequency of watering significantly reduced the severity of the disease in seedlings. However, low soil water potentials, obtained by equilibration with polyethylene glycol solutions through dialysis membranes, did not enhance disease development. Seedlings became infected at temperatures of 10, 15, and 20 C in growth chambers. Sowing depths of 2.5, 5, and 7.5 cm in a gravel Peat-lite medium did not significantly affect seedling infection. There was a close positive correlation between percent seed infection and percent diseased seedlings. Increasing the concentration of the spore suspension used to inoculate the seed, from 10^3 to 10^6 spores per milliliter, increased percent seedling infection from 90 to 100%. Furthermore, high inoculum density on seeds resulted in more diseased and more severely diseased coleoptiles. Disease severity of seedlings increased until 4 wk after sowing.

Additional key words: epidemiology, glume blotch, *Leptosphaeria nodorum*, *Triticum aestivum*.

Septoria nodorum (Berk.) Berk. (perfect state *Leptosphaeria nodorum* Müller), cause of glume blotch in wheat, may be seedborne in the southeastern United States and causes damage to the seedlings (1,7,21). Brown lesions on coleoptiles of wheat seedlings, grown from seed infected with *S. nodorum*, were first described in 1945 by Machacek in Canada (15). In 1957, Hopp (12) recorded pycnidial production and in 1960 Ponchet (18) reported formation of swellings (knobs) on infected coleoptiles.

Effects of temperature on seedling infection have been studied by several investigators. Baker (2) reported severely diseased coleoptiles on seedlings grown in vermiculite at temperatures between 10 and 20 C and almost no disease developed at 5 and 25 C. Similarly, Holmes and Colhoun (11) found that 12 C was a favorable temperature for coleoptile infection in a sandy loam soil and that 8 and 17 C were less favorable. Hewett (10) reported that the number of diseased seedlings was reduced by 30% in a glasshouse at temperatures ranging from 15 to 25 C compared to colder conditions.

High soil moisture has been reported to decrease coleoptile infection. Holmes and Colhoun (11) found that infection of wheat coleoptiles was more severe when the seed was sown in a sandy loam soil at 30% maximum water holding capacity (MWHC) than in soil at 70% MWHC. Similarly, Baker (2) observed fewer diseased seedlings in water-saturated vermiculite than in unsaturated vermiculite.

Although the mechanism of enhancement of coleoptile infection by dry conditions is unknown, possible reasons could be either that dry conditions stress the plant, making it more susceptible to fungal invasion as reported for some soilborne fungi (6), or that microbial antagonism (3) is favored by high soil moisture.

This paper presents the results of investigations on the importance of seedborne inoculum of *S. nodorum* on the development of the glume blotch disease on wheat seedlings; and on the effects of soil moisture, temperature, depth of sowing, and inoculum density in seed on coleoptile infection.

MATERIALS AND METHODS

Effects of moisture. *Watering frequency.* Two seed lots of cultivar Coker 747 from a field inoculation experiment, one with 10% seed infection and the other with 82% seed infection, were used. Degree of seed infection with *S. nodorum* was determined by using the oxgall agar-NUV method (16). Some of the seed with 10% infection was also inoculated by immersion in a suspension of 10^6 spores of *S. nodorum* per milliliter under a vacuum (−68 kPa) for 10 min and then drying at room temperature for 24 hr. *S. nodorum* was detected on 100% of the inoculated seed.

Six seeds were sown 2.5 cm deep in Styrofoam cups (237-ml capacity) containing a gravel, Peat-lite (W. R. Grace Co., Traveler's Rest, SC 29690) mixture (2:1, v/v). Cups were placed in controlled environment chambers (9), maintained at the constant temperatures of 10, 15, and 20 C (±0.37 C) with 12 hr of light (30 klux, fluorescent and incandescent) alternating with 12 hr of darkness.

There were three moisture regimes. Plants were watered either every 12, 24, or 48 hr. The design was a completely randomized 3 (level of seed infection) × 3 (temperature) × 3 (watering frequency) factorial. Five seedlings from each cup were examined 1, 2, and 4 wk after sowing, and disease severity of the plants was rated according to the Horsfall-Barratt rating scale (13). Plants were also examined for pycnidial production using a microscope.

Soil water potential. To study the effects of water potential on seedling infection, dialysis membranes (6 cm in diameter and 16 cm long) were tied with rubber bands at the bottom, then filled to a depth of 10.5 cm with a sterilized sandy loam soil which had been passed through a 2-mm sieve. The moisture retention curve for the sieved soil was determined by the pressure membrane technique (19), and this curve was used to determine water potential of the soil used in the treatments.

Polyethylene glycol 10,000 (PEG 10,000) (Fluka AG, Switzerland, supplied by Tendem Corp., New York, NY 11788) was used as the osmoticum. The molecular weight of PEG 10,000 ranged from 8,500 to 11,500, and the molecules were too large to pass through the membrane. Solutions of 25, 90, and 191 g of PEG 10,000 per liter of distilled water were used to vary the osmotic potentials (20). Soil columns in membranes were placed in 400-ml glass jars (No. 14400-66000; Ball Corp., Muncie, IN 47302) each containing 270 ml of one of the PEG 10,000 solutions mentioned above. The PEG solution initially was level with the soil surface and was readjusted to this level every 48 hr. The jars were maintained in a growth chamber with 12 hr of light (fluorescent and incandescent, 30 klux) alternating with 12 hr of darkness and day/night temperatures of 15 and 10 C.

To equilibrate the soil water potential with water potential of the PEG solutions, soil columns in membranes were maintained in the PEG solutions for 5 days before sowing. Wheat seed of cultivar Coker 747, with 75% infection by *S. nodorum*, and control seed free from *S. nodorum* were used in this experiment. Infected seeds were from plants that had been inoculated with *S. nodorum* and control seed was from uninoculated plants. All of the plants had been grown under the same conditions. Seed was assayed for *S. nodorum* by using the oxgall agar-NUV technique (16). Seed was either presoaked in distilled water for 12 hr just before sowing or was sown without soaking. Six seeds were sown 2.5 cm deep in each membrane tube. The experimental design was a split plot with PEG solution as the main plot, seed source as the subplot, and seed soaking as the sub-subplot with four replicates for each treatment.

Five seedlings from each membrane tube were examined for disease incidence and severity 22 days after sowing and seedlings were also rated for growth of roots and shoots on a scale of 1–5. Diseased plants were also examined for formation of knobs on the coleoptiles.

Samples of soil from the top, middle, and bottom of the columns were dried at 105 C for 24 hr to determine water content, and water potentials were determined by using the moisture retention curve. Also, an attempt was made to measure the water potentials of the coleoptiles with a thermocouple psychrometer, but this method failed to give consistent results.

Effects of inoculum density and depth of sowing. Two lots of seed of cultivar Coker 747, with 10 and 82% natural infection by *S. nodorum*, were selected for this experiment. In addition, four samples of the seed lot with 10% infection were inoculated with spore suspensions of *S. nodorum* containing 10^3, 10^4, 10^5, and 10^6 spores per milliliter of sterile water. *S. nodorum* was detected in all of the inoculated seed. Seed was sown at three depths (2.5, 5, and 7.5 cm) in 237-ml Styrofoam cups containing the gravel plus Peat-lite mixture mentioned previously. Six seeds were sown in each cup. The cups were maintained in three growth chambers with constant temperatures of 10, 15, and 20 C as described in the watering frequency experiment and were watered with deionized water once a day.

Five seedlings from each cup were examined for disease incidence and severity 1, 2, 4, and 6 wk after sowing. A random sample of the diseased plants was also examined for pycnidial production.

Effects of temperature. The data taken from the experiments on watering frequency and inoculum density and depth of sowing were analyzed to study the effects of temperature on symptom development on coleoptiles. This study included data taken from the plants: grown from the naturally infected seeds with 10 and 82% infection and from the seeds inoculated with a spore suspension containing 10^6 spores per milliliter of water; grown from seeds sown 2.5 cm deep; plants watered once each day; and plants examined for disease development 4 wk after sowing.

RESULTS

Effects of moisture. *Watering frequency.* Seedlings watered every 48 hr developed more severe symptoms on coleoptiles than those watered every 12 hr (Fig. 1). The slopes of the regression lines are 2.75, 6.10, and 7.70 for watering frequencies of every 12, 24, and 48 hr, respectively. The slopes of the regression lines for the 12- and 48-hr watering frequency treatments are significantly different, $P = 0.01$. Disease severity continued to increase for 4 wk after planting and pycnidia with pycnidiospores were observed on the coleoptiles and on the seed coats still attached to most of the diseased seedlings 4 wk after sowing.

Soil water potential. Higher concentrations of PEG significantly reduced growth of roots and shoots. However, soil water potentials in the range of −0.7 to −9.6 bars (measured in middle of the tubes) had no effect on either disease incidence or symptom severity of coleoptiles. In this experiment, 39% of the diseased coleoptiles had one to three knobs.

Effects of inoculum density and depth of sowing. Increased inoculum density increased disease incidence on the coleoptiles (Fig. 2). Seedlings from the lot containing 82% infected seed had much higher disease incidence than seedlings from the lot containing 10% infected seed. Likewise, more infected plants were obtained from the seeds inoculated with 10^4 spores per milliliter than from the seeds inoculated with 10^3 spores per milliliter. However, inoculation of seed with $>10^4$ spores per milliliter did not result in a greater percentage of infected plants.

Increased inoculum density also increased disease severity (Fig. 3). The seed lot with 82% infection gave rise to seedlings much more severely diseased than seedlings from the seed lot with 10% infection. Disease severity was also significantly greater on seedlings from the inoculated seed than on those from naturally infected seed. Similarly, disease severity on seedlings increased as

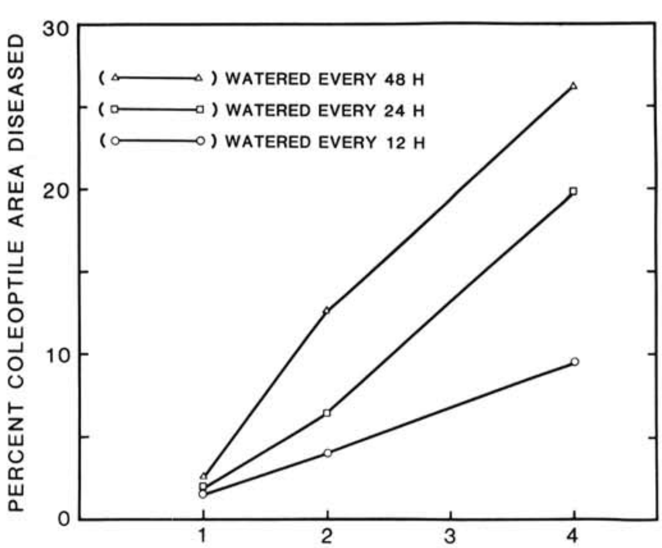

Fig. 1. The effect of watering frequency on severity of disease caused by *Septoria nodorum* on coleoptiles of wheat seedlings.

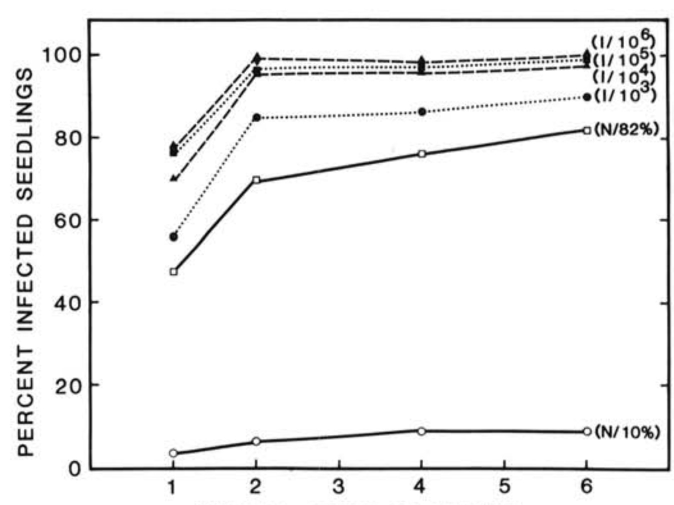

Fig. 2. The effect of inoculum density of *Septoria nodorum* in wheat seed on coleoptile infection. (N/10%) represents naturally infected seed with 10% infection; (N/82%) represents naturally infected seed with 82% infection; and (I/10^3), (I/10^4), (I/10^5), and (I/10^6) represent seed inoculated with *S. nodorum* spore suspensions of 10^3, 10^4, 10^5, and 10^6 spores per milliliter of water, respectively.

the inoculum concentration increased. Depth of sowing had no significant effect on disease development.

There was no significant change in disease incidence beyond the second week after sowing (Fig. 2). Disease severity of coleoptiles, however, increased significantly until 4 wk after sowing. At this time, pycnidia were observed on the coleoptile and on the seed of most of the diseased plants.

Effects of temperature. There were no significant differences either in disease incidence or severity of disease among plants grown at 10, 15, or 20 C (*unpublished*), although those grown at 15 C were more severely diseased than those grown at the other two temperatures.

DISCUSSION

Wheat seedlings watered every 48 hr were more severely diseased than those watered every 12 hr. These results agree with those of Baker (2) and Holmes and Colhoun (11) indicating that dry soil conditions favor development of the disease. In these experiments, as well as in those reported by Baker (2) and by Holmes and Colhoun (11), moisture levels were controlled by adding water to the growing medium. However, when soil water potential was controlled by equilibration with solutions of polyethylene glycol, soil water potentials low enough to significantly reduce the growth of wheat seedlings had no significant effect on disease development. It seems that the effect of moisture level on disease development is more closely associated with the addition of water to the soil than with soil water potential. When water is added to soil, wet soil conditions may prevail for some time after watering. These periods of high soil moisture do not occur when soil water potential is controlled by equilibration with osmotic solutions. Since increasing water stress of wheat seedlings by low soil water potentials did not increase disease development, it seems unlikely that the enhancement of disease by dry soil conditions is due to an effect of water stress on the host plant.

It has been suggested that the toxin ochracine, which is produced by *S. nodorum* and which can reduce the growth of wheat seedlings, may play a role in disease development (5,8). If this toxin does play a role in disease development, frequent watering could dilute the toxin and reduce its effects. However, an attempt to decrease disease incidence in seedlings germinated on germination paper by frequent washing of the seedlings was not successful (data not presented). Bateman (3) reported that infection by *S. nodorum*

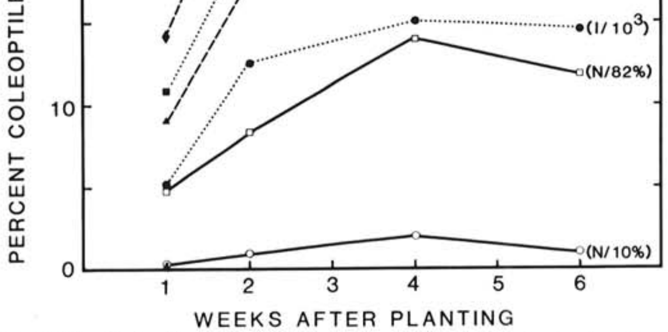

Fig. 3. The effect of inoculum density of *Septoria nodorum* in wheat seed on disease severity of coleoptiles. (N/10%) represents naturally infected seed with 10% infection; (N/82%) represents naturally infected seed with 82% infection; and (1/10^3), (1/10^4), (1/10^5), and (1/10^6) represent seed inoculated with *S. nodorum* spore suspensions of 10^3, 10^4, 10^5, and 10^6 spores per milliliter, respectively.

may be reduced by antagonistic microorganisms. The effect of soil moisture level on disease development may be due to an effect on antagonists. Periods of high moisture which occur when water is added to soil to adjust the moisture level may be important in promoting the growth of antagonistic microorganisms. The length of time the soil moisture remains high is affected by both the frequency of watering and the quantity of water added. The fact that different soil water potentials obtained by equilibration failed to influence disease development may be due to the absence of periods of high moisture. At this time, the most attractive hypothesis to explain the enhancement of disease development by dry soil conditions seems to be that antagonistic microorganisms are suppressed under these conditions, resulting in less antagonism and more disease.

Temperatures of 10–20 C were found to be favorable for coleoptile infection. This is in agreement with the reports of several investigators (2,10,11,14). Since soil temperatures at wheat-sowing time in North Carolina are usually in this temperature range, temperature is probably not a limiting factor for seedling infection.

In this study there was a close positive relationship between percent seed infection and percent diseased seedlings as reported by Hewett (10). Almost every seed infected by *S. nodorum* gave rise to an infected seedling and pycnidia were produced on most of the diseased coleoptiles. Consequently, even a very low percent seed infection must be considered important because pycnidia produced on the coleoptiles could serve as a source of inoculum for infection of foliage of the plants during the growing season in the field as reported by Obst (17).

Hewett (10) reported that deeper sowing of seed, 5 cm deep, in medium-to-heavy loam soil reduced emergence of diseased seedlings by 10% compared to the seed sown at a depth of 2.5 cm. Block (4) concluded that sowing at a depth that hastens emergence could be effective in reducing seedling damage caused by *S. nodorum*. In this study, in a gravel plus Peat-lite medium, depth of sowing did not affect emergence of the seedlings, disease incidence, or disease severity. Therefore, the effect of deeper sowing of seed on disease development may be related to soil texture and environmental conditions.

LITERATURE CITED

1. Babadoost, M., and Hebert, T. T. 1982. Seed-borne incidence of *Septoria nodorum* and the effects of environmental conditions on wheat seedling infection. (Abstr.) Phytopathology 72:975.
2. Baker, C. J. 1970. Influence of environmental factors on development of symptoms on wheat seedlings grown from seed infected with *Leptosphaeria nodorum*. Trans. Br. Mycol. Soc. 55:443-447.
3. Bateman, G. L. 1979. Relationship between *Fusarium nivale* and other micro-organisms on seed of wheat and barley. Trans. Br. Mycol. Soc. 72:245-249.
4. Block, G. 1957. Ergebnisse von Untersuchungen über die Braunfleckigkeit (Spelzenbräune) des Weizens (*Septoria nodorum* Berk.). Pflanzenschutzberichte 9:112-114.
5. Bousquet, J. F., Skajennikoff, M., Bethenod, O., and Chartier, P. 1976. Effet de l'ochracine (ou melléine) métabolite isolé des filtrats de culture de *Septoria nodorum*, sur la résistance stomatique de plantules de blé. C. R. Acad. Sci., Ser. D., Paris 283:1053-1055.
6. Cook, R. J. 1973. Influence of low plant and soil water potential on diseases caused by soilborne fungi. Phytopathology 63:451-458.
7. Cunfer, B. M. 1978. The incidence of *Septoria nodorum* in wheat seed. Phytopathology 68:832-835.
8. Devys, M., Bosquet, J. F., Skajennikoff, M., and Barbier, M. 1974. L'ochracine (melléin), phytotoxine isolée du milieu de culture de *Septoria nodorum* Berk. Phytopathol. Z. 81:92-94.
9. Downs, R. J., and Bonaminio, V. P. 1976. SEPEL Phytotron Procedural Manual for Controlled Environment Research at the Southeastern Plant Environment Laboratories. N. C. Agric. Exp. Stn. Tech. Bull. 244. 36 pp.
10. Hewett, P. D. 1975. *Septoria nodorum* on seedlings and stubble of winter wheat. Trans. Br. Mycol. Soc. 67:7-18.
11. Holmes, S. J. I., and Colhoun, J. 1971. Infection of wheat seedlings by *Septoria nodorum* in relation to environmental factors. Trans. Br. Mycol. Soc. 57:493-500.
12. Hopp, H. 1957. Untersuchungen über die Braunfleckigkeit des Weizens und ihren Erreger *Septoria nodorum* Berk. (Syn. *Macrophoma*

hennebergii Kuhn). Phytopathol. Z. 29:395-412.
13. Horsfall, J. G., and Barratt, R. W. 1945. An improved grading system for measuring plant diseases. (Abstr.) Phytopathology 35:655.
14. Kietreiber, M. 1961. Die Erkennung des *Septoria*-befalles von Weizenkörnern bei der Saatgutpüfung. Pflanzenschutzberichte 26:129-157.
15. Machacek, J. E. 1945. The prevalance of *Septoria* on cereal seed in Canada. Phytopathology 35:51-53.
16. Mathur, S. B., and Lee, S. L. N. 1978. A quick method for screening wheat seed samples for *Septoria nodorum*. Seed Sci. Technol. 6:925-926.
17. Obst, A. 1977. Unterschungen zur Epidemiologie, Schadwirkuny und prognose der Spelzenbräune (*Septoria nodorum*) des Weizens. Bayer. Landwirtsch. Jahrb. 54:72-117.
18. Ponchet, J. 1960. La détection des parasites transmis par les semences de blé. Proc. Int. Seed Testing Assoc. 25:539-553.
19. Richard, L. A. 1947. Pressure membrane apparatus, construction and use. Agric. Eng. 28:451-454.
20. Sikurajapathy, M. 1982. Quantitative evaluation of moisture stress on mungbean (*Vigna radiata* L.) and rice (*Oryza sativa* L.). Ph.D. dissertation. North Carolina State University, Raleigh. 136 pp.
21. Thomas, M. H., Jr. 1962. Factors affecting glume blotch development on wheat and variation in the causal organism, *Septoria nodorum*. Ph.D. dissertation. North Carolina State University, Raleigh. 58 pp.