

Assessment of Seedborne *Stagonospora nodorum* in New York Soft White Winter Wheat

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ABSTRACT

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Stagonospora nodorum, incitant of Septoria nodorum blotch, is the predominant foliar pathogen of winter wheat in New York. Previous field surveys indicated that seedborne inoculum may be a significant factor in the initiation of epidemics. To ascertain the extent of seedborne *S. nodorum* in New York winter wheat, 50 seed lots per year were selected randomly from those produced and submitted for certification (i.e., germination testing) in 1990 and 1991. Lots sampled in 1990 were screened for percentage of seed infected by *S. nodorum* using both a wet-blotter seedling symptom test and a fluorescence test on a selective medium. Results of both assays were highly correlated ($r = 0.82$). Consequently, lots sampled in 1991 were assayed by the fluorescence test only. Germination was not affected significantly by infection by *S. nodorum*, but was decreased significantly by infection by *Fusarium* species in several 1990 lots. *S. nodorum* was detected in all lots in 1990 and in all but nine lots in 1991. The average incidence of infected seed in the lots was 23% (range of 1-71%) in 1990, a moist season with moderate to severe Septoria nodorum blotch, and 2% (range of 0-19%) in 1991, an atypically dry season with low levels of Septoria nodorum blotch. All sampled lots met New York certification standards and were marketed to wheat producers. The results suggest that most winter wheat sown in New York has a potential source of seedborne inoculum of *S. nodorum*, with the extent of seed infection varying widely by the year in which seed was produced and the specific lot.

Additional keywords: *Leptosphaeria nodorum*, *Phaeosphaeria nodorum*, seed health test, *Triticum aestivum*

Septoria nodorum blotch, a foliage and glume disease of wheat (*Triticum aestivum* L.), is caused by the fungus *Stagonospora nodorum* (Berk.) Castellani & E.G. Germano = *Septoria nodorum* (Berk.) Berk. in Berk. & Broome (teleomorph: *Phaeosphaeria nodorum* (E. Müller) Hedjaroude = *Leptosphaeria nodorum* E. Müller). It occurs in most countries where wheat is grown (6,8,9,21,22,26), and it is responsible for significant reductions in yield (1,2,8,14,15). Seed infection by the pathogen

has been documented (5,6,11,12,13,17). However, the role of seedborne inoculum in the initiation of foliar epidemics is poorly understood, despite research on this aspect (3,16).

The soft white winter wheat production area of western New York is a relevant arena in which to assess the importance of seedborne *S. nodorum*, which is also the most prevalent foliar pathogen in the region (25). The physical and temporal isolation of wheat fields (provided by the lack of continuous wheat cropping and the presence of small, scattered wheat fields in long-term rotation with nonsusceptible crops) diminishes the epidemiological role of debris-borne conidia, an important inoculum source in areas where wheat is cropped continuously. Circumstantial evidence from wheat disease surveys in New York suggested that the wide varia-

tion in Septoria nodorum blotch severity among fields was due to variation in the level of seedborne *S. nodorum* (25).

The present study was undertaken to ascertain the extent of and the variation in occurrence of *S. nodorum* in winter wheat seed available for sowing by New York producers. A preliminary and additional objective was to identify the most efficient and cost-effective laboratory method for routine detection of *S. nodorum* in wheat seed lots.

MATERIALS AND METHODS

Media assessment. The following media were compared for their efficacy in the detection of *S. nodorum* in wheat seed: 1) oxgall medium (19), modified by the addition of streptomycin sulfate (100 mg/L) after autoclaving (18); 2) oxgall medium modified by the addition of streptomycin sulfate as above, and by the substitution of the agar component with Gelrite gellan gum (8 g/L, Schweizerhall Inc., South Plainfield, NJ); 3) *S. nodorum* agar for wheat (SNAW) (18); and 4) SNAW modified by substitution of the agar component with Gelrite (8 g/L). Five seed lots, representing four cultivars, two production years, and five production locations in New York, were assayed. The experimental design was a one-way randomized complete block, with the seed lot as the blocking factor. Two hundred seeds of each lot were assayed on each medium. Seeds were placed (12 per plate) on the medium in 9-cm-diameter glass petri plates. Plates were incubated at room temperature (20-24 C), arranged singly 13.5 cm below cool-white fluorescent lights (GTE F40/CW) with a 12-hr photoperiod for 7 days. Seed were examined after 4 and 7 days of incubation for the presence of colonies of *S. nodorum* growing out from the seeds. The colonies were detected by their bright green fluorescence under near ultraviolet light, colony morphology, or

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the production of pycnidial initials (18). Data were analyzed by analysis of variance (ANOVA) using the general linear procedure of SAS Statistical Analysis System (23), and treatment means were compared by Fisher's least significant difference test.

Seed-lot survey. Winter wheat seed samples from lots produced in 1990 and 1991 in New York, and submitted for certification (i.e., germination testing), were obtained from the New York State Seed Testing Laboratory, New York Agricultural Experiment Station, Geneva, New York. Fifty samples were chosen randomly each year. In 1990, four cultivars were represented (25 samples of Geneva, 15 of Harus, five of Frankenmuth, and five of Houser); and in 1991, six cultivars were represented (22 samples of Geneva, 21 of Harus, three of Frederick, two of Houser, and one each of Augusta and Frankenmuth).

In 1990, subsamples of 200 seeds from each lot were screened for the presence of *S. nodorum*, both by a fluorescence test on Gelrite SNAW medium and by a seedling symptom test; in 1991 the seeds were screened by the fluorescence test only. For the seedling symptom test, 100 seeds were placed on a wet blotting paper on a cafeteria tray, two blotters per tray. The trays were enclosed in clear plastic bags and incubated at room temperature (20–24 C) for 7 days. Blotters were re-moistened periodically to prevent the germinating seedlings from drying out. After 7 days, the seedlings were evaluated for infection by *S. nodorum*, manifested as elongate necrotic lesions on the coleoptiles. The proportion of seed infected by *S. nodorum* was calculated for each lot assayed. From the seedling symptom test, data were also collected on the percentage of seed germinated in each lot and the percentage of seed infected by *Fusarium* species. Seeds infected by *Fusarium* species were surrounded by characteristic fast-growing, fluffy, white fungal colonies that produced a red pigment. Identifications were confirmed by conidial morphology. Results of the seedling symptom test were regressed against those of the fluorescence assay using the REG procedure of SAS (23).

A questionnaire was sent to the producers of the 50 seed lots assayed in 1990 to obtain information on cropping practices used in the production of winter wheat seed lots for certification. For 35 of the 50 sampled lots, information was obtained on the geographic locations of production fields, previous crops grown, use of seed-applied and foliar-applied fungicides, and tillage practices. The percentage of seed infected by *S. nodorum* was plotted against these data to determine any correlations.

RESULTS AND DISCUSSION

Media assessment. The four media did not differ significantly in the detection

of *S. nodorum* in wheat seed (Table 1). However, significantly more pycnidial initials were produced on SNAW media than on oxgall media ($P > 0.05$), and the production of pycnidial initials was highest on Gelrite SNAW (Table 1).

Mathur and Lee (19) developed oxgall medium to detect *S. nodorum* in wheat seed based on the characteristic, bright green fluorescence of colonies under near ultraviolet light. Oxgall medium provided a quicker and less tedious assay

Table 1. Relative efficacy of four media for detection of *Stagonospora nodorum* in wheat seed

Seed lot	Percentage of seed producing colonies of <i>S. nodorum</i> ^w							
	Total				With pycnidial initials			
	Oxgall ^x		SNAW ^y		Oxgall		SNAW	
	Agar	Gelrite	Agar	Gelrite	Agar	Gelrite	Agar	Gelrite
A	75	74	78	79	1	4	30	47
B	51	38	46	45	0	2	4	25
C	23	20	15	24	1	1	6	12
D	15	9	11	9	1	0	2	3
E	35	36	45	37	1	1	5	9
Mean	40a ^z	35a	39a	39a	1b	2b	9c	19d

^wBased on 200 seeds from each seed lot assayed on each medium for 7 days.

^xOxgall medium (19), modified by addition of streptomycin sulfate (100 mg/L) after autoclaving (18), or modified by addition of streptomycin sulfate after autoclaving and substitution of the agar component with Gelrite gellan gum (8 g/L).

^ySNAW medium, original (18), and modified by substitution of the agar with Gelrite (8 g/L).

^zMeans followed by the same letter are not significantly ($P = 0.05$) different using Fisher's least significant difference test.

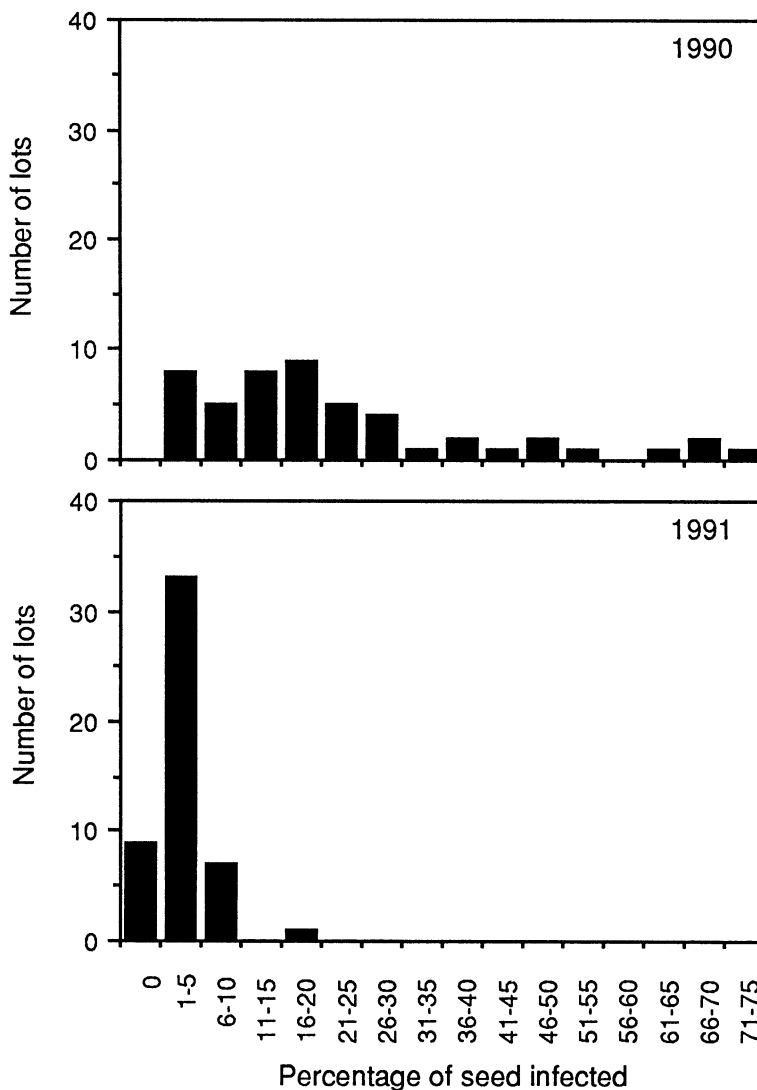


Fig. 1. Occurrence of *Stagonospora nodorum* in seed of 50 random lots of certified winter wheat produced in New York in 1990 and 1991. For each lot, 200 seeds were assayed on *S. nodorum* agar for wheat (SNAW) medium with Gelrite gellan gum substituted for the agar.

than did the blotter methods (7), but the amount of infection could be underestimated because of competition by other seedborne fungi such as species of *Alternaria*, *Fusarium*, and *Epicoccum*. In addition, the high concentration of oxgall (15 g/L) made the medium expensive. Manandhar and Cunfer (18) recently developed SNAW medium, which facilitated the detection of *S. nodorum* by suppressing the growth of interfering fungi and bacteria with fungicides and antibiotics. It also enhances detection by promoting the production of pycnidial initials (18). SNAW is less expensive than oxgall medium because it utilizes only 1.5 g/L of oxgall. Our data corroborate the conclusions of Manandhar and Cunfer (18) that SNAW medium is efficacious in the detection of *S. nodorum* in wheat seed, except that we did not observe the significant increase over oxgall medium that they reported. We found

that the modification of SNAW by substituting Gelrite for agar further reduced the cost of the medium without reducing its efficacy. In fact, the substitution further enhanced the production of pycnidial initials. Gelrite SNAW was therefore utilized for our seed-lot survey.

Seed-lot survey. In 1990, each seed lot was infected by *S. nodorum*. The mean infection level of 1990 lots was 23% (range of 1–71%), based on results of the fluorescence assay on Gelrite SNAW medium (Fig. 1). Results of the seedling symptom test were highly correlated with those of the fluorescence assay on Gelrite SNAW medium (Fig. 2), with the seedling symptom assay indicating more infection. Isolations from coleoptile lesions indicated that more than 90% of the lesions were associated with seedborne *S. nodorum*, whereas less than 10% were associated with seedborne *Fusarium* species or bacteria. In 1991, all but nine lots

were infected by *S. nodorum*, but they exhibited a mean infection level of only 2% (range of 0–19%). The extent of and the variation in occurrence of *S. nodorum* in New York seed lots is consistent with reports from other wheat-growing regions. In England, Hewett (13) encountered seed-infection levels by *S. nodorum* of up to 40%. Cunfer (6) found a wide range (0–59%) in the incidence of seed infected by *S. nodorum* in Georgia.

Climatic conditions, especially rainfall during grain formation, greatly influence the progression of *Septoria nodorum* blotch in wheat (10,27). The gross difference in infection level between seed produced in 1990 and 1991 can be understood by contrasting conditions during the two production seasons. *Septoria nodorum* blotch was moderate to severe in 1990, a season with greater than normal precipitation in May and June (period of flag leaf emergence to grain formation); whereas foliar symptoms were infrequently observed in 1991, a season with atypically dry conditions in May and June. However, it is significant that 82% of seed lots were infected, albeit at low levels, even in a dry year. This suggests that foliar fungicide sprays aimed at reducing disease on the flag leaves and glumes may not be sufficient to produce seed with no or low infection by *S. nodorum* in a moist season. Applications of propiconazole (Tilt 3.6E) to a winter wheat field at initiation of anthesis and at kernel early dough stage (20 days before harvest) in 1990 significantly reduced *Septoria nodorum* blotch on flag leaves and glumes, but failed to reduce seed infection by *S. nodorum* to below 15% (Shah and Bergstrom, unpublished). Soft white winter wheat seed sown by grain producers in western New York is derived largely from certified lots produced in the same region. Based on the responses of seed producers to our questionnaire in 1990, certified seed is produced by the same cultural practices used in the production of wheat grain for milling (20,24). Seed infection level was not correlated with previous crop, tillage practice, use of carboxin plus thiram (Vitavax 200) seed treatment, or foliar application of propiconazole prior to flag leaf emergence.

All sampled lots met New York certification requirements (including laboratory germination in excess of 85% and tolerances for cultivar purity, weed seeds, foreign material, and infection by smut and bunt fungi) and were marketed to grain producers. Infection of seed by *S. nodorum* was not correlated with laboratory germination. However, reduction in germination was significantly correlated with infection of seed by *Fusarium* species (Fig. 3), a serious problem in New York in years with excessive moisture during wheat flowering (4).

Our results suggest that most winter

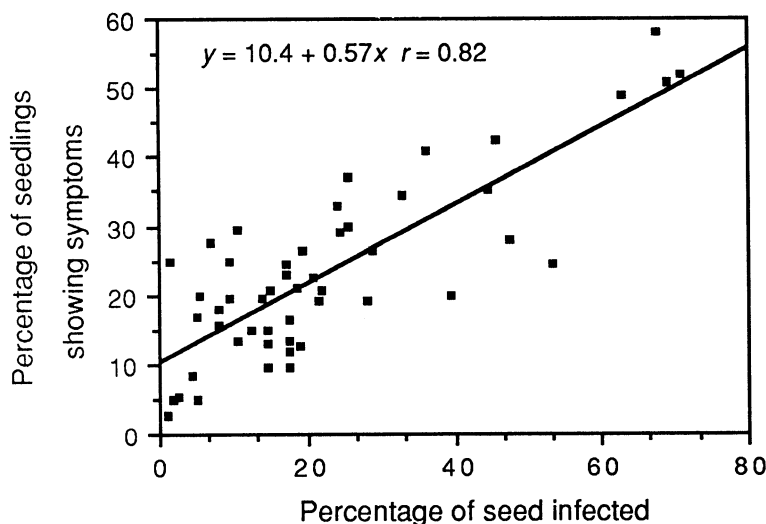


Fig. 2. Correlation of results of a fluorescence assay (*Stagonospora nodorum* agar for wheat [SNAW] medium with Gelrite gellan gum substituted for the agar) with those of a seedling symptom assay for detection of *S. nodorum* in wheat seed. Fifty random lots of certified winter wheat seed produced in New York in 1990 were assayed by each method, 200 seeds per lot.

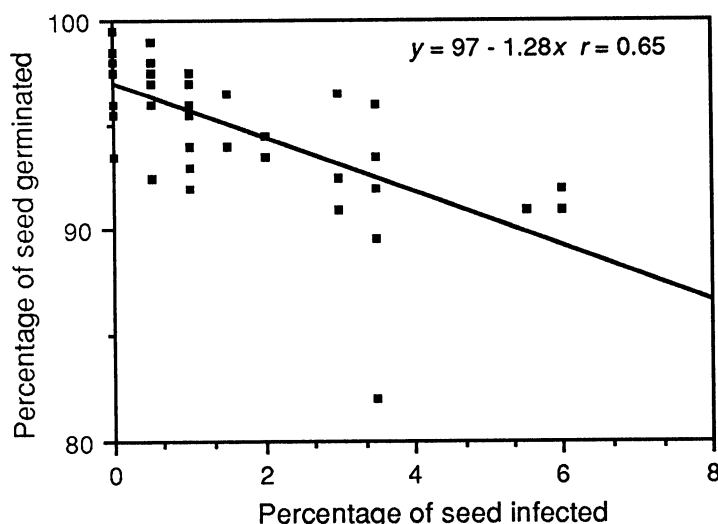


Fig. 3. Correlation of germination of winter wheat seed with seed infection by *Fusarium* species. Fifty random lots of certified winter wheat seed produced in New York in 1990 were assayed by a blotter test, 200 seeds per lot.

wheat sown for grain production in New York has a potential source of seedborne inoculum of *S. nodorum*, with the extent of seed infection varying widely according to the year in which the seed was produced and the specific seed lot. The role of seedborne inoculum in *Septoria nodorum* blotch epidemics is poorly understood. The percentage of seed infected by *S. nodorum* has been associated with seedling infection (3) and with *Septoria nodorum* blotch at later stages of wheat development (16). Current field research in New York is aimed at relating the level of seed infection by *S. nodorum* to the progress of foliar epidemics in isolated plots in the absence of wheat debris. If and where seedborne *S. nodorum* is shown to be important in epidemic development, strategies may need to be developed to reduce or eliminate seed infection by a combination of disease control in seed-production fields, seed testing for *S. nodorum* as a part of official certification, and the use of effective seed-applied fungicides.

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