European Journal of Plant Pathology **106:** 837–842, 2000.

Temperature dependent seed transmission of *Stagonospora nodorum* in wheat

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Accepted 7 September 2000

Key words: Triticum aestivum, *Septoria nodorum*, *Phaeosphaeria nodorum*, epidemiology

Abstract

The transmission of *Stagonospora nodorum* from four naturally infected winter wheat seedlots was quantified in controlled environment germination chambers at 9, 13, 17, 21, and 25 °C. Seedlings were harvested when the second leaf began to emerge. Coleoptiles and first seedling leaves were examined for the presence of lesions caused by *S. nodorum*. First leaves were incubated on Bannon's medium for 2 weeks, after which they were examined for pycnidia of *S. nodorum*. Transmission to the coleoptile occurred at all temperatures, but decreased from 100% to 72% as temperature increased from 9 to 25 °C. Transmission to the first leaf was less, dropping from 37% to 2% as temperature increased from 9 to 25 °C. At least 44% of infected first leaves were symptomless at all temperatures, with 96% of infected leaves showing no symptoms at 25 °C. Transmission to seedling leaves occurred over a broad temperature range. Under the high densities at which wheat is sown, a significant number of infected seedlings per unit area may originate from relatively low initial seed infection levels and transmission efficiencies.

Introduction

Stagonospora nodorum blotch, caused by *Stagonospora nodorum* (Berk.) E. Castell. & Germano syn. *Septoria nodorum* (Berk.) Berk. in Berk. & Broome (teleomorph *Phaeosphaeria nodorum* (E. Müll.) Hedjar. syn. *Leptosphaeria nodorum* E. Müll.) is one of the most economically important fungal diseases of wheat (*Triticum aestivum* L.). No cultivar is immune. Seed infection is common (Cunfer, 1983; Shah and Bergstrom, 1993), and infected seeds can be an important source of primary inoculum for foliar epidemics (Arseniuk et al., 1998; Babadoost and Hebert, 1984b; Góral et al., 1994; Luke et al., 1986; Milus and Chalkley, 1997; Rambow, 1990; Shah et al., 1995). The relationship between seed infection and early foliar disease is poorly understood. Polycyclic epidemics with high apparent infection rates are characteristic of seed-transmitted, necrotrophic pathogens, like *S. nodorum*. Therefore, foliar disease at late crop development stages is not necessarily correlated with the initial level of seed infection (Bretag et al., 1995; Shah et al., 1995). Transmission to coleoptiles has been documented in both laboratory and field experiments (Babadoost and Hebert, 1984a; Baker, 1970; Holmes and Colhoun, 1971), and lesions on the seedling leaves have been observed (Agrawal et al., 1986; Hewett, 1975; Holmes and Colhoun, 1971; Machacek, 1945). Transmission to true leaves has not been quantified previously. Our objective was to quantify the transmission of *S. nodorum* from naturally infected wheat seeds to seedlings at different temperatures. A preliminary report has been published (Shah and Bergstrom, 1999).

Materials and methods

Seed from three cultivars of soft white winter wheat, harvested in 1996, were used (Table 1). Each of the three cultivars was susceptible to *S. nodorum*. Seed infection incidence by *S. nodorum* was determined by assaying 200 surface-disinfested seeds per

Table 1. Characteristics of seedlots used in quantifying the transmission of *S. nodorum* from wheat seeds to seedlings

Lot	Cultivar	Germination (%)[2]	Seed infection incidence (%)[1]	
			S. nodorum	*Fusarium* spp.
1	Cayuga	87	54	5
2	Harus	86	68	12
3	Geneva	87	85	10
4	Geneva	91	96	0

[1] Based on an assay of 200 seeds per lot for *S. nodorum* and separate samples of 100 seeds per lot for *Fusarium* spp. and other seedborne fungi.

[2] Germination for lots 1–3 based on an assay of 200 seeds. Germination for lot 4 based on an assay of 400 seeds.

lot on SNAW medium (Manandhar and Cunfer, 1991). Separate samples of 100 seeds were assayed for infection by other seedborne pathogenic fungi by the freezing blotter method (Limonard, 1966).

Seeds were sown 2.5 cm deep in a commercial greenhouse soil mix (Metromix; Scotts-Sierra Horticultural Products Company, Marysville, OH, USA) in plastic germination trays (98 wells per tray, one seed per well). The experimental unit was a half tray of 49 contiguous wells sown to one lot. The trays were incubated in germination chambers (SG 30 Seed Germinator; Hoffman Manufacturing, Inc., Albany, OR, USA) at 90% relative humidity and a 16 h photoperiod. Light was supplied by three vertically oriented cool white fluorescent bulbs (General Electric F40CW) on either side of the shelves. Two germination trays were positioned on each of the four shelves per chamber. Position of experimental units on each shelf was assigned at random. All four seedlots were thus represented on each shelf within a chamber, giving a total of 196 seeds sown per lot within the chamber. Five temperature treatments (9, 13, 17, 21, 25 °C) were used. Two chambers were available for the study, so temperature treatments were investigated once per chamber, yielding two sets of data for each temperature. Seedlings were watered periodically to keep the soil moist.

The number of seedlings emerged and the number of days from sowing were recorded when the second leaves of seedlings became visible. Seedlings were then harvested and washed to remove soil particles. Coleoptiles and first leaves were examined visually for the presence of lesions indicative of infection by *S. nodorum*. First leaves were excised and placed on Bannon's medium (Bannon, 1978) without prior surface disinfestation. Leaves of seedlings grown at 9 °C were short enough to be placed whole on the medium. At the other temperatures, leaves were too long to fit whole, so 5 cm sections from both the leaf apex and base regions were plated onto Bannon's medium. Leaves were inspected for pycnidia and cirrhi of *S. nodorum* after plates had been incubated for 14 days at room temperature (21–24 °C) under near-ultraviolet light (12 h photoperiod). A leaf was scored positive for infection if it bore pycnidia of *S. nodorum*.

Transmission of *S. nodorum* to the coleoptile or first leaf was calculated as the percentage of coleoptiles or first leaves, respectively, infected by *S. nodorum* divided by the percentage of sown seeds infected by *S. nodorum*. Percentage of seedlings emerged was calculated from the proportion of seedlings which emerged above the soil line relative to the number of seeds sown.

Seedling emergence and *S. nodorum* transmission percentages were arcsine square root transformed before analysis by the MIXED procedure of SAS (SAS Institute Inc., Cary, NC, USA). The MIXED procedure computes restricted maximum likelihood and maximum likelihood estimates of variances, which are preferable to the ANOVA estimates particularly when random and fixed effects are both present in an experimental design (Searle et al., 1992). Temperature was the only fixed effect. Random variation in transmission is potentially attributable to seedlot, germination chamber, or the position of the trays within the chambers. Seedlings on upper shelves were larger than those on lower shelves. Proximity to the fluorescent bulbs had no visible effect on seedling growth. Seedlot, germination chamber and position of the shelf within a chamber were therefore treated as random effects. A second set of models included the number of days between sowing and seedling harvest (DAYS) as an additional random effect since elapsed time of the host-pathogen interaction may potentially affect transmission. The effect of DAYS was assessed by examining changes in the log-likelihood; if l_1 and l_2 represent the log-likelihoods for models with and without DAYS, respectively, then $2 \cdot \{l_1 - l_2\}$ is approximated by a χ^2 distribution with 1 degree of freedom for the null hypothesis that DAYS has no effect on transmission.

Results and discussion

Transmission of *S. nodorum* from seeds to coleoptiles and first seedling leaves occurred at all temperatures studied. Temperature significantly affected emergence

($P = 0.0021$), coleoptile transmission ($P = 0.0001$) and first leaf transmission ($P = 0.0001$). Seedlot accounted for 16%, 18%, and 28% of the random variation in percent emergence, coleoptile transmission and first leaf transmission, respectively. There was virtually no random variation in the data due to germination chamber or shelf position within a chamber.

Transmission to the coleoptiles was significantly higher at 9 °C compared to all other temperatures ($P < 0.05$), and was lower at 25 °C ($P < 0.05$) than at all other temperatures. Mean coleoptile transmission was 100% at 9 °C, decreasing to 72% as temperature increased to 25 °C (Figure 1B). Transmission frequencies higher than 100% were attributed to the inherent error associated with estimating the true incidence of seed infection at sowing. Coleoptile transmission was recalculated using the upper 95% confidence limit for seed infection incidence (based on the binomial distribution) for the respective lots. In this instance mean transmissions to the coleoptiles at 9 °C were 1.0 for lots 1–3, and 0.87 for lot 4. Estimates of transmission to the coleoptiles were based solely on visual symptoms. Other seedborne, seed-transmitted fungi such as *Fusarium* spp. and *Pyrenophora tritici-repentis* can produce lesions on coleoptiles (Schilder and Bergstrom, 1995). The levels of *Fusarium* spp. in the lots used in this study were too low to have a significant impact (Table 1) however, and *P. tritici-repentis* was not detected in any of the freezing blotter seed assays. The relatively high transmission frequencies of *S. nodorum* to wheat coleoptiles agreed with previous observations (Babadoost and Hebert, 1984a; Bateman, 1977; Hewett, 1975; Holmes and Colhoun, 1971).

Transmission to the first leaves was lower than to the coleoptiles (Figure 1C). First leaf transmission decreased sharply from that at 9 °C as temperature increased, but transmission was not significantly different ($P > 0.05$) at 13, 17 and 21 °C. Transmission to the first leaf base region was higher than transmission to the leaf apex. The ratio of leaf tip to base transmission decreased as temperature was increased (Figure 1D). This agreed with the observations of Agrawal et al. (1986) who found that symptoms on the first leaves always progressed from the leaf base upwards.

There was a significant temperature effect ($P = 0.0001$) on the proportion of infected first leaves with symptoms by the time the seedlings were harvested (Figure 2). The number of days between sowing and seedling harvest had no effect on symptom expression. There was no difference in the proportion of asymptomatic but infected first leaves at 9, 13, 17, and

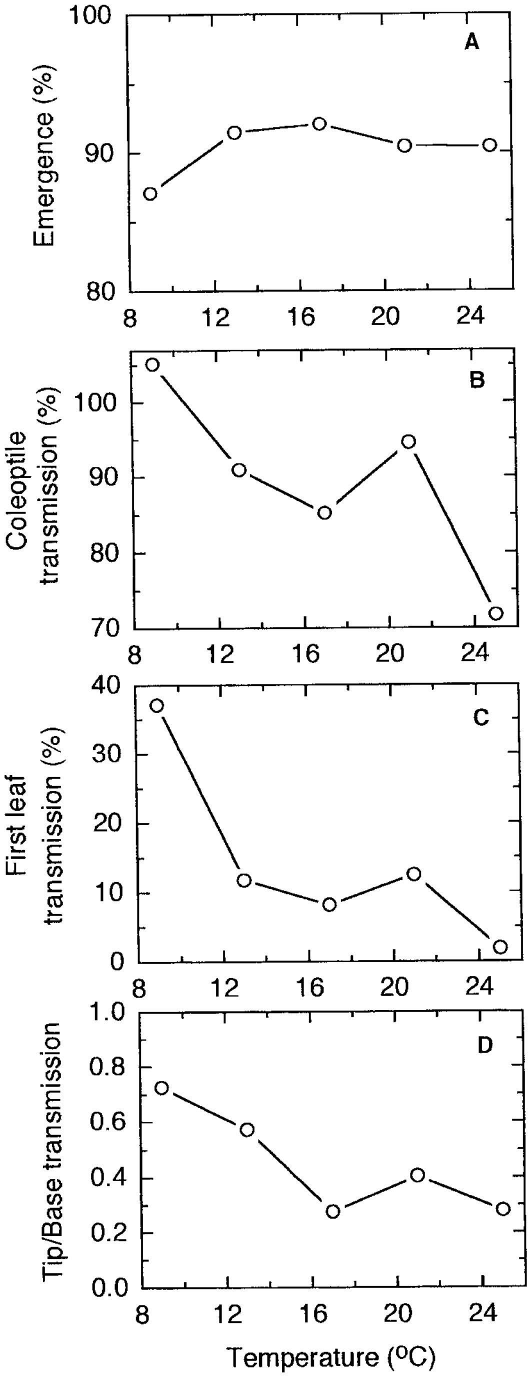

Figure 1. Relationship of A: emergence, B: coleoptile transmission, C: first leaf transmission, and D: the ratio of first leaf tip to base transmission to temperature for winter wheat seedlots naturally infected by *S. nodorum*. Data points are the least-squares means over four seedlots. Standard errors of the means were too small to be seen on the graphs.

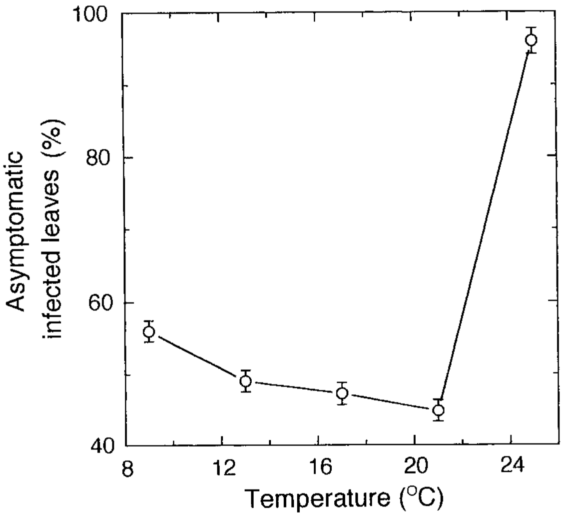

Figure 2. Percentage of asymptomatic *S. nodorum*-infected first leaves versus temperature. Data points are the least-squares means over four seedlots. Standard errors of the means are shown.

21 °C ($P > 0.4$). At 25 °C, 96% of infected first leaves were asymptomatic. Therefore, monitoring the transmission of *S. nodorum* from infected seeds solely on visual symptoms is at best unreliable.

Emergence exceeded 80% at the five temperatures tested (Figure 1A), but was significantly lower at 9 °C than that at all other temperatures ($P < 0.05$). There were no significant differences in emergence among the other temperatures. Though emergence was partly a function of seedlot, seedborne *S. nodorum* does not generally reduce wheat seedling emergence (Gilbert et al., 1995).

The number of days between sowing and seedling harvest improved the temperature effects model only for transmission to the coleoptiles ($P = 0.00072$). However, only 3.5% of the random variation in coleoptile transmission was explained by including the number of days between sowing and seedling harvest. This result possibly reflects the duration of the interaction between the wheat coleoptile and *S. nodorum* in determining transmission. Time between sowing and seedling harvest did not affect transmission to the first leaf, which indicated that at the time seedlings were harvested (leaf 1 already fully emerged) potential pathogen transmission to the first leaf was complete.

Our experiments were done in a light soil mix with little mechanical impedance and that was kept near maximum water holding capacity. Transmission to the first leaves is expected to be higher under conditions which delay emergence, and thus increase the time of interaction between pathogen and host. Rates of emergence in wheat decrease as temperature is lowered from 17 to 5 °C and as soil strength increases (Addae et al., 1991). Deeper sowing increases the time to emergence (Kirby, 1993). Time to emergence increases as soil water potential decreases (Gan et al., 1996; Lafond and Fowler, 1989), which may help to explain why wet soils are associated with lower transmission of *S. nodorum* to wheat coleoptiles (Holmes and Colhoun, 1971). We did not record time to emergence, but wheat cultivars do differ in speed of emergence (Lafond and Baker, 1986), and transmission frequencies of *S. nodorum* may be related to this genetically based host character.

Transmission varied nonlinearly with increasing temperature. *S. nodorum* has a maximum *in vitro* growth rate at 20 °C (Djurle and Yuen, 1991). The optimal temperature for winter wheat growth varies between 20 and 24 °C (Jame et al., 1999). Leach (1947) showed that pre-emergence damping-off at different temperatures could be predicted by the ratio of a soilborne pathogen's growth rate and the coefficient of velocity of seedling emergence, a measure of the host growth rate. This concept has been used with varying degrees of success with fungal pathogens involved in pre-emergence damping-off (Gleason and Ferriss, 1987), but potentially can be extended to the transmission of seedborne pathogens. Transmission to the first leaves can be thought of as being directly proportional to the ratio of the growth rates of the pathogen and of the host plant. Faster growth rates for the pathogen may lead to higher transmission, whereas a faster host growth rate may result in leaves escaping infection before they emerge.

Previous studies quantified transmission to the coleoptiles (Babadoost and Hebert, 1984a; Babadoost and Hebert, 1984b; Hewett, 1975; Holmes and Colhoun, 1971). Reports of lesions on the first leaves were solely observational (Agrawal et al., 1986; Hewett, 1975; Holmes and Colhoun, 1971; Machacek, 1945). Our study is the first to quantify the transmission of *S. nodorum* to the first seedling leaf in wheat. The coleoptile stops growing once it emerges and so remains largely underground (Simmons, 1987). The first leaf then pushes through the coleoptile tip and is therefore the first wholly above-ground plant organ. Coleoptiles generally do not emerge from sowing depths greater than 70 mm (Kirby, 1993). Recommended sowing depths for winter wheat in

New York are 25–38 mm, which means that 5–15 mm of coleoptile emerges above-ground. In many other wheat production regions where soil moisture is more limited, seeds are sown at depths of 50 to as much as 200 mm (Schillinger et al., 1998). Transmission of *S. nodorum* to coleoptiles may provide primary inoculum for foliar epidemics in areas such as New York where seeds are sown at depths less than 50 mm. Transmission to leaves may be of epidemiological significance in most wheat production areas.

Seed-to-seedling transmission of *S. nodorum* occurred over a broad range of temperatures representative of many wheat production areas. Our results suggest that seed transmission of 10% or greater to first leaves can be expected when infected seeds are sown into soil at 17 °C, a typical soil temperature at sowing in New York. Under these conditions, a seedlot with 5% infection incidence sown to achieve a stand of 200 plants m^{-2} would average one infected seedling m^{-2}. One infection focus m^{-2} places every plant in the stand within the documented range of splash dispersal of *S. nodorum* pycnidiospores (Fitt et al., 1989). There is therefore the potential for a rapid increase in the incidence of diseased plants relatively early in the crop's growth. Even after ear emergence, further disease spread up to 2 m is still possible (Griffiths and Ao, 1976).

Further experiments are needed to establish whether genetic resistance to seed transmission exists in wheat cultivars. Stevens et al. (1996) found that *S. nodorum* activated certain genes during incompatible reactions with barley coleoptiles, suggesting that genetic resistance to transmission occurs in barley. Differential resistance of wheat cultivars to seed infection by *S. nodorum* was recently demonstrated (Shah et al., 2000). Such resistances could be exploited together with seed fungicides and agronomic practices that optimize seedling emergence to reduce the contribution of seed-transmitted inoculum to foliar epidemics of stagonospora nodorum blotch.

Acknowledgements

This research was supported in part by Cornell University Hatch Project NYC-153472 and a Graduate Research Assistantship from the Department of Plant Pathology, Cornell University, to the senior author. We thank C.E. McCulloch for advice on statistical analyses and P.A. Arneson for comments on the manuscript.

References

Addae PC, Collis-George N and Pearson CJ (1991) Over-riding effects of temperature and soil strength on wheat seedlings under minimum and conventional tillage. Field Crops Research 28: 103–116

Agrawal K, Singh T, Singh D and Mathur SB (1986) Studies on glume blotch disease of wheat II. Transference of seed-borne inoculum of *Septoria nodorum* from seed to seedling. Phytomorphology 36: 291–297

Arseniuk E, Góral T, Sowa W, Czembor HJ, Krysiak H and Scharen AL (1998) Transmission of *Stagonospora nodorum* and *Fusarium* spp. on triticale and wheat seed and the effect of seedborne *Stagonospora nodorum* on disease severity under field conditions. Journal of Phytopathology 146: 339–345

Babadoost M and Hebert TT (1984a) Factors affecting infection of wheat seedlings by *Septoria nodorum*. Phytopathology 74: 592–595

Babadoost M and Hebert TT (1984b) Incidence of *Septoria nodorum* in wheat seed and its effects on plant growth and grain yield. Plant Disease 68: 125–129

Baker CJ (1970) Influence of environmental factors on development of symptoms on wheat seedlings grown from seed infected with *Leptosphaeria nodorum*. Transactions of the British Mycological Society 55: 443–447

Bannon E (1978) A method of detecting *Septoria nodorum* on symptomless leaves of wheat. Irish Journal of Agricultural Research 17: 323–325

Bateman GL (1977) Effects of seed treatments on *Septoria nodorum* infection of winter wheat. Plant Pathology 26: 127–134

Bretag TW, Price TV and Keane PJ (1995) Importance of seed-borne inoculum in the etiology of the ascochyta blight complex of field peas (*Pisum sativum* L.) grown in Victoria. Australian Journal of Experimental Agriculture 35: 525–530

Cunfer BM (1983) Epidemiology and control of seed-borne *Septoria nodorum* on wheat. Seed Science and Technology 11: 707–718

Djurle A and Yuen JE (1991) A simulation model for *Septoria nodorum* in winter wheat. Agricultural Systems 37: 193–218

Fitt BDL, McCartney HA and Walklate PJ (1989) The role of rain in dispersal of pathogen inoculum. Annual Review of Phytopathology 27: 241–270

Gan Y, Stobbe EH and Njue C (1996) Evaluation of selected nonlinear regression models in quantifying seedling emergence rate of spring wheat. Crop Science 36: 165–168

Gilbert J, Tekauz A and Woods SM (1995) Effect of *Phaeosphaeria nodorum*-induced seed shrivelling on subsequent wheat emergence and plant growth. Euphytica 82: 9–16

Gleason ML and Ferriss RS (1987) Effects of soil moisture and temperature on Phomopsis seed decay of soybean in relation to host and pathogen growth rates. Phytopathology 77: 1152–1157

Góral T, Arseniuk E, Sowa W, Czembor HJ and Krysiak H (1994) Effect of seedborne inoculum on septoria (stagonospora) nodorum blotch incidence in triticale and wheat under field conditions. Hodowla Roslin Aklimatyzacja I Nasiennictwo 38: 281–285

Griffiths E and Ao HC (1976) Dispersal of *Septoria nodorum* spores and spread of glume blotch of wheat in the field. Transactions of the British Mycological Society 67: 413–418

Hewett PD (1975) *Septoria nodorum* on seedlings and stubble of winter wheat. Transactions of the British Mycological Society 65: 7–18

Holmes SJL and Colhoun J (1971) Infection of wheat seedlings by *Septoria nodorum* in relation to environmental factors. Transactions of the British Mycological Society 57: 493–500

Jame YW, Cutforth HW and Ritchie JT (1999) Temperature response function for leaf appearance rate in wheat and corn. Canadian Journal of Plant Science 79: 1–10

Kirby EJM (1993) Effect of sowing depth on seedling emergence, growth and development in barley and wheat. Field Crops Research 35: 101–111

Lafond GP and Baker RJ (1986) Effects of genotype and seed size on speed of emergence and seedling vigor in nine spring wheat cultivars. Crop Science 26: 341–346

Lafond GP and Fowler BD (1989) Soil temperature and water content, seeding depth, and simulated rainfall effects on winter wheat emergence. Agronomy Journal 81: 609–614

Leach LD (1947) Growth rates of host and pathogen as factors determining the severity of pre-emergence damping-off. Journal of Agricultural Research 75: 161–179

Limonard T (1966) A modified blotter test for seed health. Netherlands Journal of Plant Pathology 72: 319–321

Luke HH, Barnett RD and Pfahler PL (1986) Development of septoria nodorum blotch on wheat from infected and treated seed. Plant Disease 70: 252–254

Machacek JE (1945) The prevalence of *Septoria* on cereal seed in Canada. Phytopathology 35: 51–53

Manandhar, JB and Cunfer, BM (1991) An improved selective medium for the assay of *Septoria nodorum* from wheat seed. Phytopathology 81: 771–773

Milus EA and Chalkley DB (1997) Effect of previous crop, seedborne inoculum, and fungicides on development of stagonospora blotch. Plant Disease 81: 1279–1283

Rambow M (1990) Importance of infected seed as a source of infection of *Septoria nodorum* Berk. Nachrichtenblatt für den Pflanzenschutz in der DDR 44: 153–155

Schilder AMC and Bergstrom GC (1995) Seed transmission of *Pyrenophora tritici-repentis*, causal fungus of tan spot of wheat. European Journal of Plant Pathology 101: 81–91

Schillinger WF, Donaldson E, Allan RE and Jones SS (1998) Winter wheat seedling emergence from deep sowing depths. Agronomy Journal 90: 582–586

Searle SR, Casella G and McCulloch CE (1992) Variance Components. Wiley, New York

Shah D and Bergstrom GC (1993) Assessment of seedborne *Stagonospora nodorum* in New York soft white winter wheat. Plant Disease 77: 468–471

Shah D, Bergstrom GC and Ueng PP (1995) Initiation of septoria nodorum blotch epidemics in winter wheat by seedborne *Stagonospora nodorum*. Phytopathology 85: 452–457

Shah DA and Bergstrom GC (1999) Seed transmission of *Stagonospora nodorum* in wheat. Phytopathology 89: S71

Shah DA, Bergstrom GC and Sorrells ME (2000) Differential seed infection of wheat cultivars by *Stagonospora nodorum*. Plant Disease 84: 749–752

Simmons, SR (1987) Growth, development, and physiology. In: Heyne EG (ed) Wheat and Wheat Improvement (pp 77–113) American Society of Agronomy, Inc., Madison

Stevens C, Titarenko E, Hargreaves JA and Gurr SJ (1996) Defence-related gene activation during an incompatible interaction between *Stagonospora* (*Septoria*) *nodorum* and barley (*Hordeum vulgare* L.) coleoptile cells. Plant Molecular Biology 31: 741–749