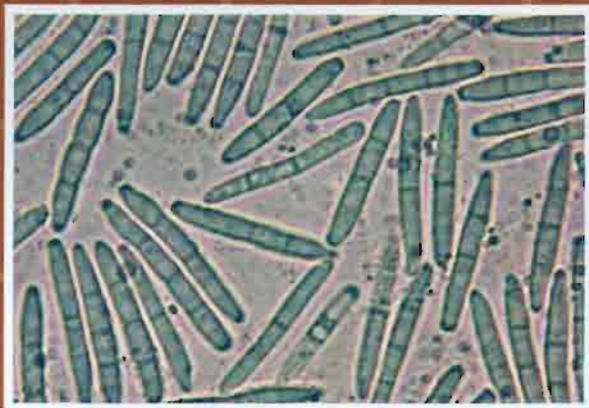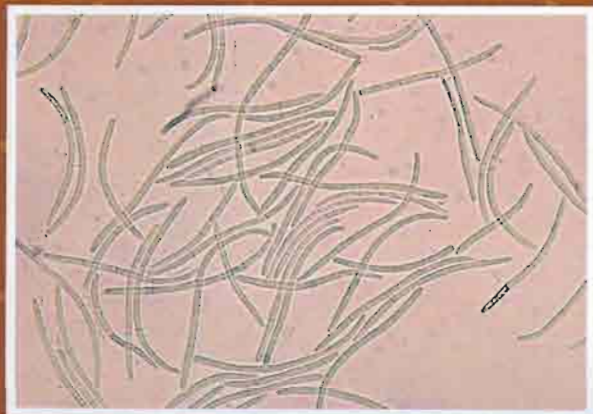# The Septoria Diseases of Wheat
Concepts and methods of disease management

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Concepts and methods of disease management

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Abstract
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figures, 20 color plates)

In the last 25 years, attention to the
septoria diseases of wheat has intensified.
The two pathogens of the septoria group
that have the greatest impact on global
wheat production are *Septoria tritici* and
Septoria nodorum. Annual yield losses
worldwide due to both diseases are
estimated at about 9 million metric tons.
Breeding for resistance has obtained a
preeminent place in a number of research
and crop improvement programs
worldwide.

In this introduction, emphasis is placed
on summarizing the more *pertinent*
scientific reports for managing the two
major septoria pathogens. Research data
are interpreted into concepts and
procedures. Topics include the biology of
the fungi, infection process, collection
and handling of infected material,
isolation and maintenance of the fungi,
inoculum production, artificial
inoculation, disease assessment,
epidemiology, pathogen specialization,
breeding for resistance, and means of
cultural and chemical control.

Each treatment of a topic or group of
alternative methods is followed by the
recommendation of one or more preferred
techniques or approaches. This
information is intended for wheat
scientists in developed and developing
countries who are unfamiliar with these
diseases.

Contents

Preface

The economic impact of the septoria diseases of cereals on wheat production in certain parts of the world has caught the attention of increasing numbers of growers, scientists, policy makers, and administrators. This intensified interest has lead to more funds allocated to plant pathological research and cultivar development programs. This in turn has lead to a better understanding of the diseases and the release of a number of high-yielding, disease-resistant cultivars for the septoria-prone areas. Though much scientific literature has accumulated, no publication, to date, has gathered together the basic information necessary for a *practical* approach to understanding the diseases, the methodology for screening resistance, and other control measures.

In this publication, we have reviewed the literature and presented it in a format concentrating on the biology of the pathogens, processes associated with infection, isolation and maintenance of the fungi, inoculum production, inoculation, disease assessment, epidemiology, breeding for resistance and means of cultural and chemical control. We have not intended to present an intensive, detailed overall review of the literature, but to bring attention to the more relevant scientific reports pertaining to the various topics covered. The information elaborates on concepts and methods employed in septoria research and their implementation.

The practical information is intended for wheat scientists who are unfamiliar with these diseases in both developed and developing countries.

Introduction

Septoria is the name commonly applied to more than 1,000 species of fungi, most of which are plant parasites. Approximately 100 species are parasitic on cereals and grasses. Many are economically important on crops other than cereals (123).

Distribution

There are two major septoria diseases that cause problems in wheat in many parts of the world. These are septoria tritici blotch (Plate 1, p.19) (syn. septoria leaf blotch, speckled leaf blotch of wheat) incited by the fungus *Septoria tritici* (sexual state: *Mycosphaerella graminicola*) and septoria nodorum blotch (syn. septoria glume blotch of wheat) caused by the fungus *Septoria nodorum* (sexual state: *Leptosphaeria nodorum*). The world distribution of these diseases is shown in Figure 1.

Economic Importance to Wheat Growers

Both diseases cause serious yield losses (35, 54, 97, 111, 137, 139). Yield losses attributed to heavy incidences of septoria tritici blotch and septoria nodorum blotch of wheat have been reported to range from 31% (4) to 53% (35). In 1982, worldwide loss was estimated to be 9 million metric tons with a value of over U.S. $1 billion (123). The average yearly losses in yield in the United States due to septoria tritici blotch and septoria nodorum blotch were estimated at 1% in 1965 (2). The other few available national loss estimates range between 1 and 7% annually (35). Both diseases are capable of reducing yields by as much as 30-40%, values usually obtained from numerous fungicide control comparisons (18). Under severe epidemics, the kernels of vulnerable wheat cultivars are shrivelled and are not fit for milling (Figure 2).
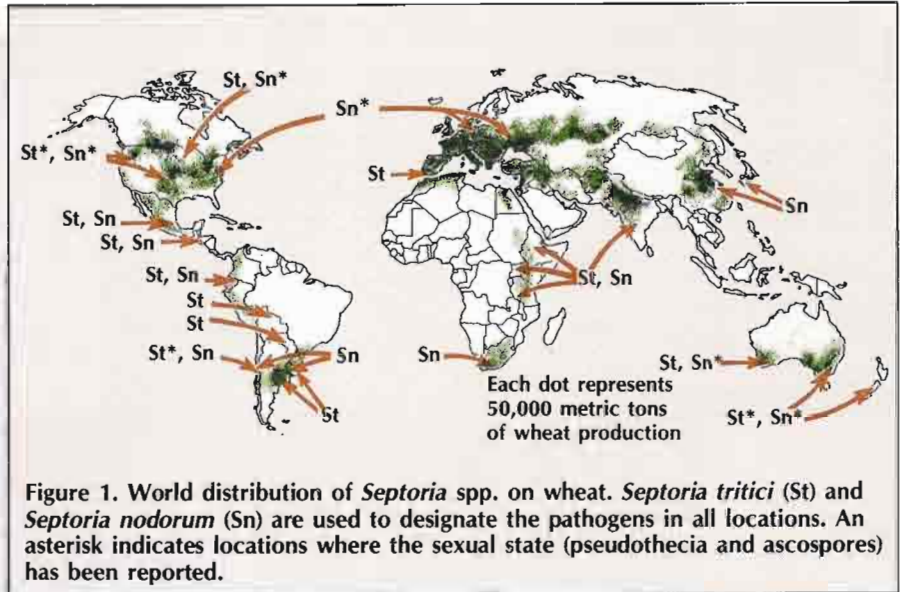
Figure 1. World distribution of *Septoria* spp. on wheat. *Septoria tritici* (St) and *Septoria nodorum* (Sn) are used to designate the pathogens in all locations. An asterisk indicates locations where the sexual state (pseudothecia and ascospores) has been reported.
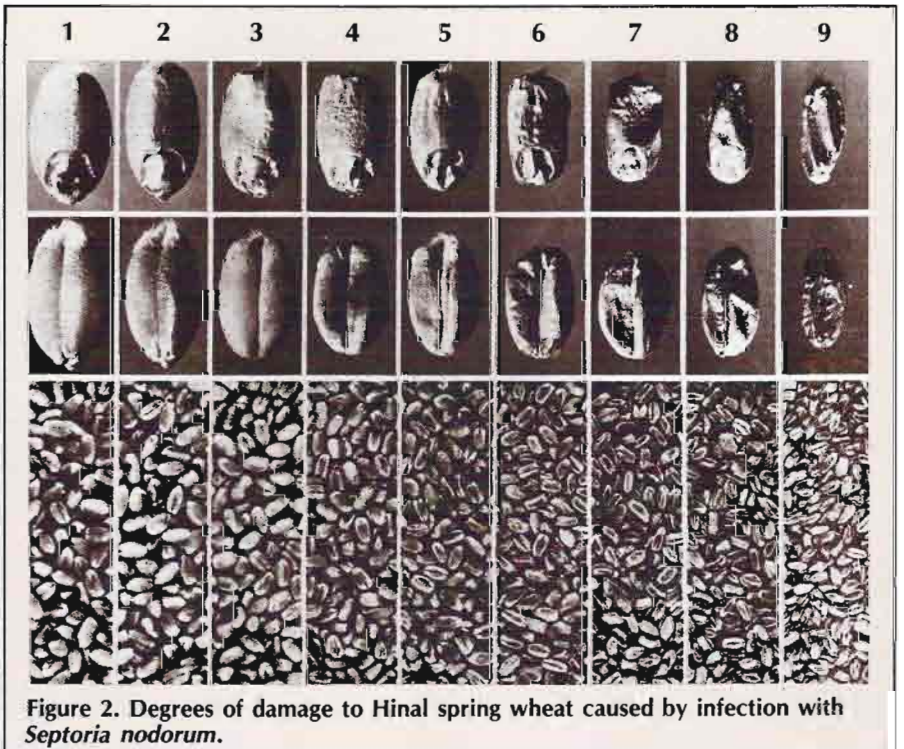
Figure 2. Degrees of damage to Hinal spring wheat caused by infection with *Septoria nodorum*.

Source: Bronnimann, A. (15)

Table 1. Classification and nomenclature of the sexual states of *S. tritici* and *S. nodorum*

EUMYCOPHYTA (True Fungi)
Class: Ascomycetae (Ascomycetes)
Subclass: Loculoascomycetes (asci bitunicate, perithecioid pseudothecium)

	S. tritici	*S. nodorum*
Order	Dothideales	Pleosporales
Family	Dothideaceae	Pleosporaceae
Genus	*Mycosphaerella*	*Leptosphaeria*
Species	*M. graminicola* [Fückel] Schroeter	*L. nodorum* Müller
Disease	Septoria tritici blotch	Septoria nodorum blotch

Nomenclature

Within the Fungi Imperfecti, fungi of the genus *Septoria* are classified among the order Sphaeropsidales, characterized by the production of conidia, termed pycnidiospores, which are produced in variously shaped, semiclosed fruiting bodies known as pycnidia. The sexual states of *S. tritici* and *S. nodorum* are associated with the class Ascomycetes (Table 1).

During the 2nd International Septoria Workshop (123), a motion was passed stating that "the taxonomic names of the fungi involved in the septoria disease complex would be based on their sexual state, namely, *Leptosphaeria nodorum* E. Müller, *Leptosphaeria avenaria* Weber f. sp. *triticea* T. Johnson, and *Mycosphaerella graminicola* (Fückel) Schroeter, and the common names of the diseases would be septoria nodorum blotch of wheat, septoria avenae blotch of wheat, and septoria tritici blotch of wheat,

Table 2. Descriptive comparison of the septoria wheat pathogens

Sexual state	Pseudothecium (μ)	Ascospore (μ)	Number of cells	Lesion
Mycosphaerella graminicola	70-100	10-15 x 2-3	2	Irregular to rectangular, elongated between veins
Leptosphaeria nodorum	120-200	23-32 x 4-6	4	Lens shaped, with chlorotic border
Asexual state	**Pycnidium (μ)**	**Pycnidiospore (μ)**	**Number of septa**	**Lesion**
Septoria tritici	60-200	35-98 x 1-3	3-5	Irregular to rectangular, elongated between veins
Septoria nodorum	160-210	15-32 x 2-4	0-3	Lens shaped, with chlorotic border

respectively. The lower case 's' will be used for septoria, septoria nodorum blotch, etc. which are not written in italics."

Leptosphaeria avenaria, which is not discussed in this manual, is the most recent *Septoria* species to be characterized on wheat and is probably of lesser importance than those previously mentioned. The intermediate size of the pycnidiospores often leads to confusion with *S. nodorum*.

Although the sexual state has been reported in several countries and will most likely be found elsewhere, it is the asexual state that causes most disease symptoms and associated yield losses. Therefore, throughout this text the pathogens will generally be designated by their asexual state.

The descriptive comparisons of *S. tritici* and *S. nodorum* are presented in Table 2 (123).

Identification

Symptoms vary according to cultivar, cultural practices, and geographic location (44). Under Mediterranean conditions, where spring wheats are grown during the cool and rainy winter months (November-May) of the year, *S. tritici* is most important. It is important to note that the sexual state has not been reported as yet in the literature from this region. Usually many pycnidia are produced making identification relatively simple. In the southeastern United States and northern Europe, *S. nodorum* is most common, usually producing an abundance of pycnidia allowing identification with ease; however, under certain environmental conditions, pycnidia of *S. nodorum* may not occur readily within the necrotic lesions. In many other wheat-growing areas, both *S. tritici* and *S. nodorum* occur, thus introducing some difficulties

in differentiation and identification. In the United Kingdom, northern U.S.A., Brazil, Uruguay, western Australia, and other areas, septoria tritici blotch and septoria nodorum blotch are often found together, many times with fruiting structures of both organisms on the same leaf. Moreover, other fungi that form similar

fruiting structures, spores, and other symptoms are often present to complicate identification (Figure 3). Thus, field identification without confirmation in the laboratory is often difficult if not impossible; however, with the preparation of a few slides and microscopic examination at 100x or 400x, identities of the pathogens can usually be confirmed.
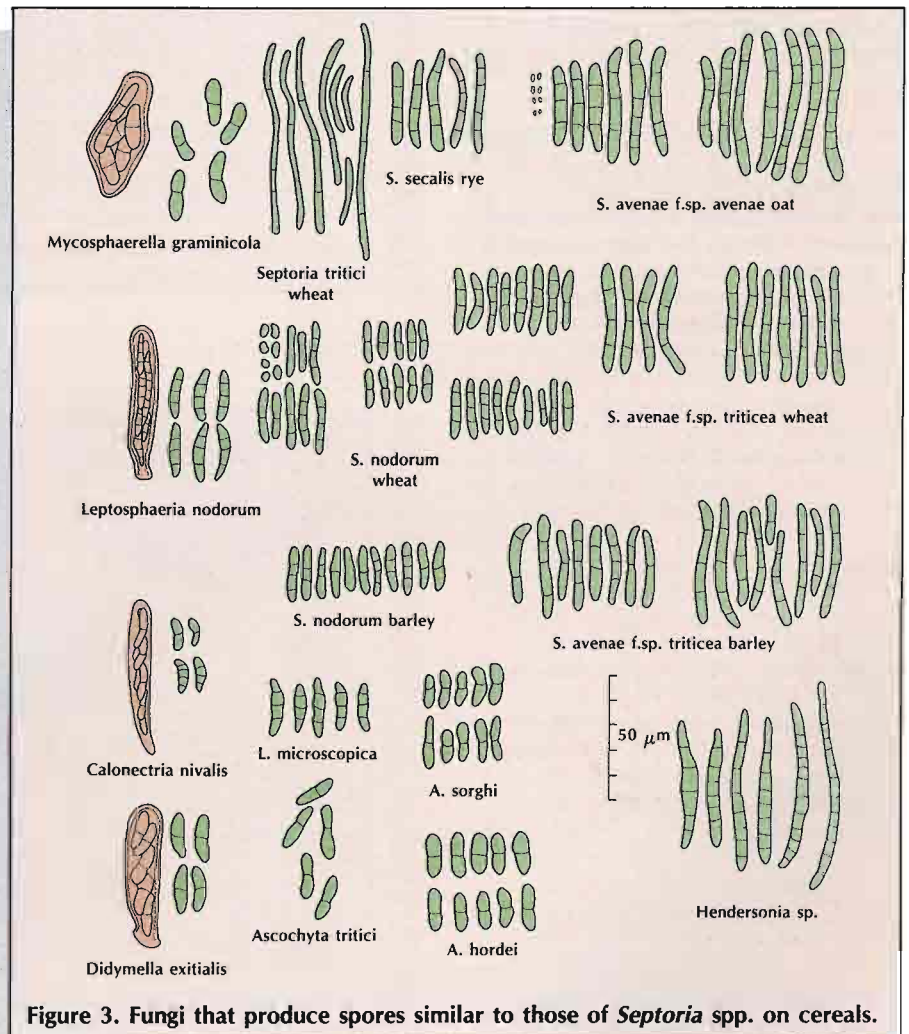
Figure 3. Fungi that produce spores similar to those of *Septoria* spp. on cereals.

Septoria tritici

Introduction

The asexual state of *M. graminicola*, namely *S. tritici* Rob. ex Desm., was found on wheat and described by Desmazieres in 1842 (133). The sexual form *M. graminicola* (Fückel) Schroeter was described by Sanderson in 1972 in New Zealand (113). The sexual state has also been identified in Australia, Brazil, the Netherlands, the United Kingdom, and the U.S.A. Pycnidia bearing pycnidiospores of the asexual state were found on plant specimens of wild emmer (*Triticum turgidum dicoccoides*) collected in Israel in 1906. Only occasional yield losses of economic impact were reported prior to the 1960s.

The increase in the economic importance of septoria tritici blotch was largely due to the widespread and rapid replacement of local wheat cultivars with early-maturing, semidwarf cultivars that were susceptible to the pathogen. Cultivars with adequate resistance are now replacing the original introductions. Changes in cultural practices have also significantly contributed to the increase in disease incidence. Severe outbreaks of septoria tritici blotch have occurred in high-rainfall areas such as South America. Epidemics also occur in semiarid countries along the Mediterranean Coast and in Australia.

Biology

A pseudothecium, asci, and ascospores of *M. graminicola* are presented in Figure 4 and Plate 2. A pycnidium and pycnidiospores of the asexual state, *S. tritici*, are presented in Figure 5. Ascospores of *M. graminicola* have two cells which are unequal in size. *Septoria tritici* forms slender, elongated pycnidiospores enclosed within a
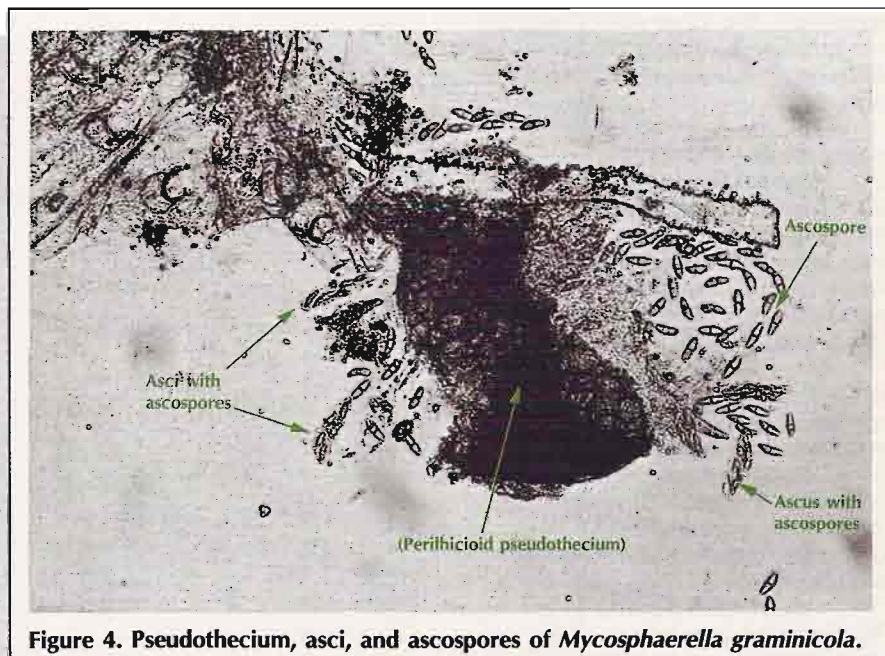
Figure 4. Pseudothecium, asci, and ascospores of *Mycosphaerella graminicola*.
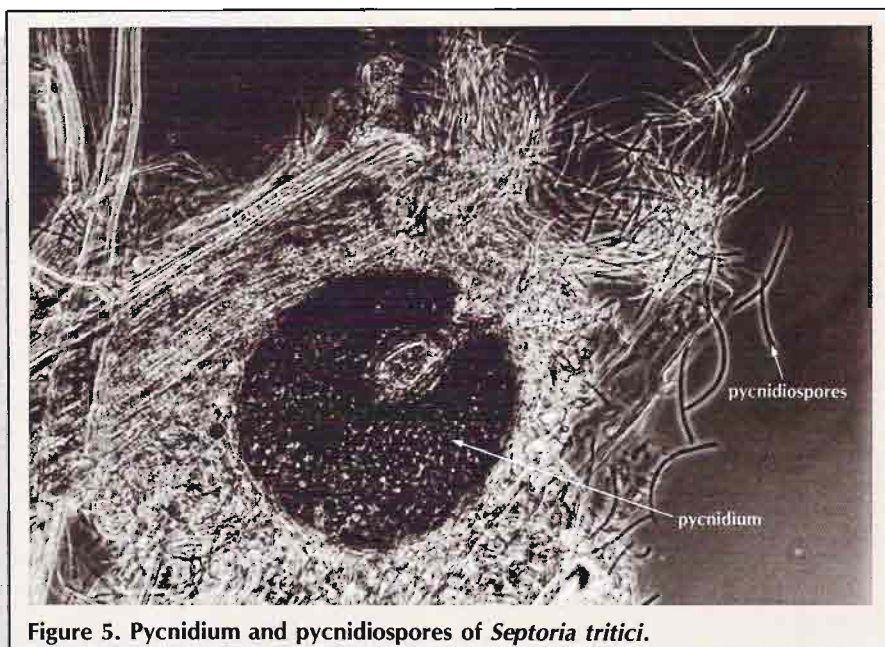
Figure 5. Pycnidium and pycnidiospores of *Septoria tritici*.

pycnidium. The pycnidia are embedded in the epidermal and mesophyl tissue on both sides of the leaf with an opening (ostiole) on top.

The pycnidiospores of *S. tritici* can be present in two forms within the pycnidium: macropycnidiospores (35-98 x 1-3 μm) with 3-5 septa (Plate 3) or micropycnidiospores (8-10.5 x 0.8-1 μm) without septa (114,137,138). Both spore forms are equally able to infect wheat (137).

Environmental conditions required for germination, penetration, and infection
Pycnidiospores germinate on a suitable substrate, following release from the pycnidium, when the plants are wet. Germination occurs either by elongation of the apical cell or by budding. In the laboratory, spores begin to germinate within 12 hours and leaf penetration occurs after 24 hours. The fungus may penetrate the leaf through the stomata or directly through the cell walls of the epidermis.

Moisture is required for all stages of infection: germination, penetration, development of the mycelium within the plant tissue, and subsequent pycnidial formation (21, 60, 130). Periods of 72 and 96 hours in a moisture chamber result in similar levels of disease, while only 48 hours may produce significantly less disease. A moist period of only 24 hours is generally insufficient to produce disease symptoms (57).

Cardinal temperatures reported for germination of *S. tritici* conidia are a minimum of 2-3°C and a maximum of 33-37°C, with an optimum of 20-25°C. Infection can be delayed in the field if the temperature falls below 7°C during 2 consecutive nights (129, 130). Low temperatures (4°C) affect spore germination, mycelial growth, and lesion and pycnidia development by lengthening the time required for each. Symptoms generally appear after 14-21 days. The time from infection to production of pycnidia depends, however, on environmental conditions (moisture, temperature, and light), the cultivar, and the septoria isolate. It appears that there is a compensation effect between moisture and temperature in susceptible wheats. Where the moist period is short, an increase of temperature up to 25°C may still result in severe levels of disease. With long moist periods and low temperatures, high disease levels are again observed (57). Spore germination and mycelial growth of *S. tritici* are optimum at 8-12,000 lux (8). Pycnidial formation is most rapid at 2,000 lux. It may be concluded that the infection processes occur best on rainy, cloudy days with temperatures between 20 and 25°C.

Symptom expression and disease development
The life cycle of *S. tritici* is shown in Figure 6. First symptoms of infection on wheat leaves are expressed as irregular chlorotic lesions that usually appear 5-6 days after inoculation. However, the time of first expression is highly dependent on the cultivar and environmental conditions during the infection process. Three to six days later, at 18-24°C and high relative humidity, necrotic (dead tissue) lesions develop at the chlorotic sites (Plates 4 and 5). The necrotic lesions appear sunken and grayish-green at first. By holding the leaf up against the light, the beginning of pycnidia formation (when occurring) can often be seen, usually after 15 days (Plates 6, 7, and 8). The pycnidia, ranging in color from light to dark brown, develop in the necrotic lesions. The pycnidia are scattered within the lesion, and can be on both the upper and lower
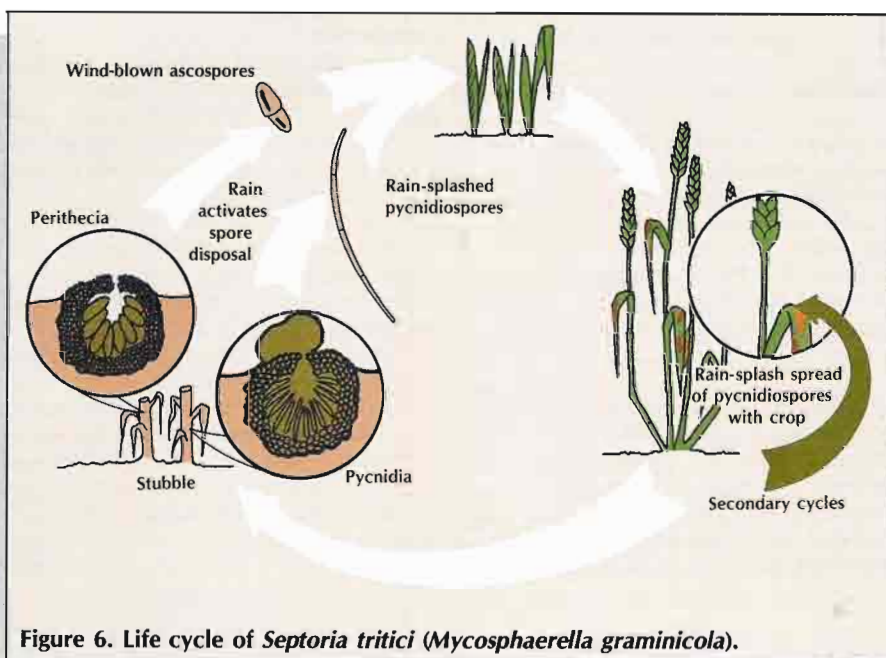
Figure 6. Life cycle of *Septoria tritici (Mycosphaerella graminicola)*.

surfaces of the leaf. The size of pycnidia may vary among cultivars and is also affected by the number of pycnidia present. As the number of pycnidia on the leaf increases, the pycnidia themselves may become smaller (37). The size of the pycnidia and pycnidiospores is not significantly affected by changes in the percentage of the leaf area covered by lesions bearing pycnidia, or by the isolate of S. tritici (133). Pycnidiospore production may be related to cultivar response, with lower pycnidiospore production occurring on the resistant cultivars (51). It is important to state that immunity in Triticum species is rare.

Pycnidiospores can remain viable in pycnidia on infested stubble for several months (58). Nevertheless, there are reported instances of epidemics of septoria tritici blotch developing in field plantings following several years of nonwheat cropping. The primary inoculum could possibly have arisen from windblown infested crop debris, airborne ascospores, volunteer wheat, other susceptible grass species, or from latent septoria mycelium in crop residues (though the last is not proven). Information on some of these parameters is scarce. The sexual state, M. graminicola, is a source of primary inoculum wherever it occurs. The morphological appearances of asexual pycnidia and sexual pseudothecia are quite similar. This may lead to the false conclusion that the pycnidiospores are the sole source of primary inoculum. As a result, the sexual forms can be and often are overlooked.

Pycnidiospores are released from pycnidia when the leaf has been wet for 30 minutes or more. The spores are produced in a thick, sticky matrix containing a high concentration of preserving sugars and proteins (48). This "preserving medium" or ooze permits the pycnidiospores to remain viable during periods of dry weather. An oozing drop, or cirrhus, containing pycnidiospores exudes through the ostiole at the top of the pycnidium following sufficient leaf wetting. After drying, part of the oozing drop may return into the pycnidium or remain on top of the ostiole for additional rewetting.

There are reports that S. tritici does not form new pycnidia on dead tissue, and that pycnidia are not capable of regenerating new pycnidiospores after each release of spores. Fewer pycnidiospores are released after each wetting, with the bulk of the spores released on the first wetting (34). However, in Tunisia, regeneration of pycnidiospores did occur when pycnidia that had been dried and emptied were moistened by autumn rains (32). This regeneration of pycnidiospores continued in a cyclic manner and formed the primary inoculum to infect autumn-sown wheat.

Septoria nodorum
Introduction
The asexual state of L. nodorum Müller, namely, S. nodorum (Berk.), was described by Berkeley in 1845 as a pathogen affecting mainly the glumes and nodes of wheat. Pseudothecia were found in cultures of S. nodorum as early as 1904. But it was not until 1952 that Müller described L. nodorum as the sexual state of the septoria nodorum blotch fungus. Septoria nodorum has been isolated from hosts in 17 genera, and it has recently been identified as a disease of barley in Britain, Ireland, and Scandinavia (47). Cross-inoculation studies have shown that wheat isolates are more harmful to wheat than barley. Both wheat and barley isolates are capable of infecting many grasses without causing obvious symptoms.

Septoria nodorum is especially important in warm, moist growing areas such as the southeastern U.S.A., Europe, and southern Brazil (122). It can occur and cause damage in relatively dry areas such as Montana, U.S.A., as well (75). In the Federal Republic of Germany and the German Democratic Republic, head infection was stated to be the main cause of yield reduction (70), yet foliar infection can be as detrimental to yield as head infection. In both cases, infection results in shrivelled seeds.

The highest reduction in number of heads/plant, number of kernels/head, and thousand kernel weight occurred with artificial inoculation of S. nodorum after emergence, followed by reinoculation when the second node was formed (49). Susceptibility is generally expressed at its maximum during heading-flowering-maturity (132).

Biology
A pseudothecium, asci, and ascospores of L. nodorum are presented in Plates 9 and 10. A pycnidium and pycnidiospores of the asexual state, S. nodorum, are shown in Figure 7.

Pseudothecia, formed on host tissue, contain numerous club-shaped asci holding eight ascospores (Plate 11). The ascospores are straight to slightly curved and have three septa (Plate 12). The second cell from the apex is the largest. These sexual spores are known to play an active role in over-seasoning. It is also a source of primary inoculum in many areas of the world. Its full role in the disease cycle is still not understood completely.

The mycelium of S. nodorum is usually branched, has dividing walls of tissue called septa, and is transparent. Later, however, it may turn dark in color. Pycnidia and pycnidiospores develop quickly in artificial culture (unlike S. tritici) and on host tissue. The pycnidia appear under the epidermal layer of cells

and are dark. Pycnidiospores released through the ostiole are cylindrical, transparent, with 0-3 septa, and 15-32 x 2-4 μm in size (114,137,138) (Plate 13). Each spore cell contains one nucleus (137). Infective micropycnidiospores (3-6 x 0.7-1 μm) may also be present (56). An atypical form of pycnidiospores (12-27 x 2-3 μm) with no or one septum has been isolated in Pennsylvania, U.S.A. (55).

The organism on wheat can attack all plant parts above ground. It can infect any time from seed germination to plant maturity. The mycelium of *S. nodorum* also can be seedborne and can cause seedling infection. Brown lesions on coleoptiles of wheat seedlings grown from infected seed were first described in 1945 in Canada (82).

Environmental conditions required for germination, penetration, and infection
Pycnidiospores germinate in a moist environment, usually free water, following exudation from the pycnidium on rainy and/or dewy days. They will germinate within a temperature range of 5-37°C with an optimum between 20-25°C. The pycnidiospores germinate in the laboratory within 2 hours after emerging from the pycnidium. Spore germination and penetration are greatest between 15-25°C, with a minimum of 6 hours of wetness (high relative humidity) necessary for good infection (121).

Infection of *S. nodorum* is best at 22-24°C, with symptoms appearing after 7-14 days. Infection in Wales occurred when relative humidity was greater than 63% (63). In addition, in the 24 hours that followed inoculation, at least 4 hours had to be at a temperature above 6°C and a relative humidity greater than 69%. The period from inoculation to the production of mature pycnidia (latent period) was as short as 6 days after inoculation was achieved at 22°C on plants kept in a continuously water-saturated atmosphere. The latent period extended to 10 days when plants were kept at 20°C under a regime of 12 hours of complete saturation alternating with 12 hours at 85-90% relative humidity (134). A dry period of 8 hours every 16 hours resulted in lower disease levels than with continuous wetting. A dry interruption of the wet period occurring within 24 hours of the application of spores may result in even less disease development (142). In the field, an increase in temperature, duration of leaf wetness, and high inoculum density cause a decrease in the latent period (135).

The pycnidiospores are spread by splashing or windblown rain. Dispersal of pycnidiospores in a droplet was found to occur when at least 5 mm of rainfall and a temperature greater than 10°C was followed by at least 10 mm more rain falling during a 48-hour period and reaching an intensity of 2 mm/hour (65, 66). Pycnidiospores of *S. nodorum* were dispersed by rain at a height up to 2 m and to a distance greater than 92 cm from infected plants (53, 150). Airborne *S. nodorum* spores were collected at a height of 40 cm at distances up to 10 m downwind of a target spore suspension on which simulated rain fell (12). Wind greatly increases the dispersal of smaller droplets and spores in the downwind direction.

Symptom expression and disease development
The life cycle of *S. nodorum* is shown in Figure 8. *Septoria nodorum* lesions are often lens-shaped with a yellow-green border surrounding the dead tissue area (Plates 14 and 15). Pycnidia may or may not appear within the center of the lens-shaped lesions on the leaves, but are more common on nodes and stems, leaf sheaths, and glumes (Plates 16 and 17). Whenever nodes are infected, it may cause distortion and bending of the straw
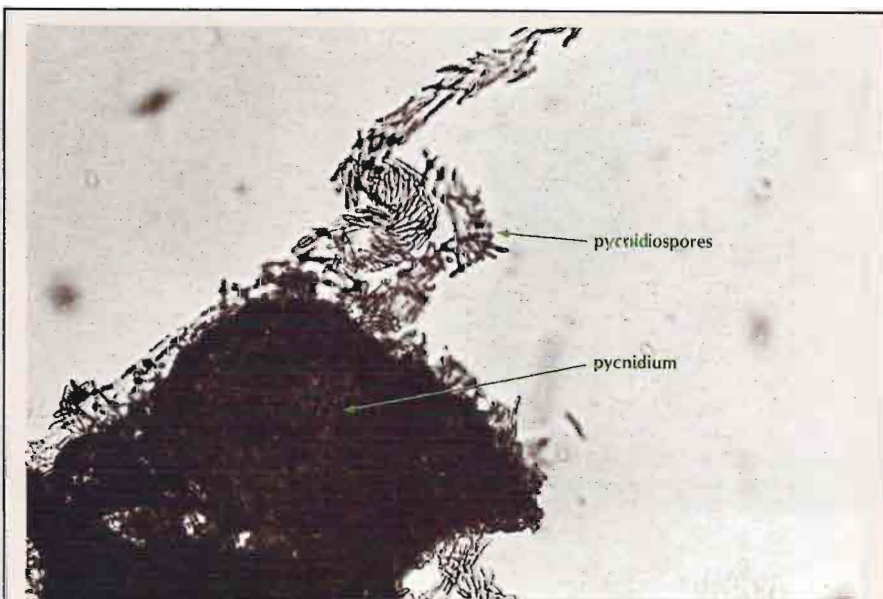
Figure 7. Pycnidium and pycnidiospores of *Septoria nodorum*.

with a possibility of lodging and breakage of the straw at the node with subsequent losses in yield. Cyclic regeneration of pycnidia and pycnidiospores in dead wheat tissue has been reported in *S. nodorum* (116). The pycnidia initiated new pycnidiospores in 10-33 days, depending upon the wheat cultivar.

Infected seed has been the primary source of septoria nodorum blotch inoculum in Germany (92). Seed infection ranging as high as 80% has been reported in Georgia, U.S.A. (26). Several authors have discussed the relationship of seed infection to symptoms on glumes (26, 53, 91). Just one infected seedling among 5,000 plants in a field may be enough to initiate an epidemic (54). The extent to which *S. nodorum* colonizes wheat seeds might be more important than the percentage of infected seeds (26). In the southeastern U.S.A., infected seeds of susceptible wheat cultivars often exceed 40-50%, even when septoria nodorum blotch is not severe. As the incidence of seed infection at planting increases from 1 to 40%, the intensity of subsequent disease increases (80). However, 10% seed infection can supply sufficient inoculum to cause a severe epidemic, and higher levels of seed infection only slightly increase the disease levels in the crop. Disease infection may occur in crops grown in areas where wheat has not been cultivated for a number of years if infected seed is used. This clearly demonstrates the role of seed as one of the potential sources of primary inoculum.

Septoria nodorum produces various phytotoxic compounds such as septorin and ochracin when grown in liquid culture (10, 11, 33). Some of them may play a role in symptom development (68). For example, septorin reduces seedling growth of the susceptible wheat cultivar, Etoile de Choisy. In mitochondria isolated from the same cultivar, septorin induced changes in respiratory activities similar to that of 2,4-D (10). Ochracin is a phytotoxin that inhibits photosynthesis and leads to a decrease in the opening of the stomata. It may affect stomatal behavior indirectly by inhibiting CO_2 assimilation (33).

Histological studies have shown that during mycelial invasion, the hyphal colonization in the leaf was both between and within the cells and the host cell walls seemed disorganized (5). On wheat leaves during the infection process, as well as in an artificial medium containing wheat cell walls, *S. nodorum* releases digestive enzymes that break down cell wall material (83).

Infection Process
Comparison of *Septoria tritici* and *Septoria nodorum*

The processes associated with infection of *S. tritici* and *S. nodorum* are summarized in Table 3.
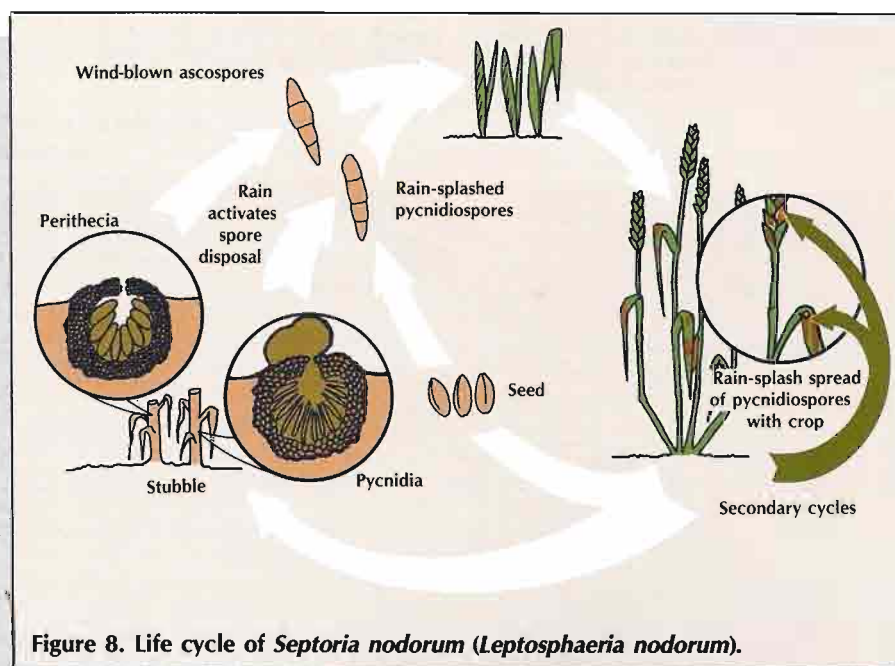
Figure 8. Life cycle of *Septoria nodorum* (*Leptosphaeria nodorum*).

Table 3. Comparison of the processes associated with infection by *S. tritici* and *S. nodorum*

Causal agent	Septoria tritici blotch	Septoria nodorum blotch
Asexual state	*Septoria tritici* Rob. ex Desm.	*Septoria nodorum* (Berk.)
Class	Deuteromycetes (Fungi Imperfecti)	Deuteromycetes (Fungi Imperfecti)
Order	Sphaeropsidales	Sphaeropsidales
Fruiting body	Pycnidium	Pycnidium
Pycnidiospore	Filiform	Cylindrical
Sexual state	*Mycosphaerella graminicola* (Fückel) Schroeter	*Leptosphaeria nodorum* Müller
Class	Ascomycetes	Ascomycetes
Fruiting body	Perithecioid pseudothecium	Perithecioid pseudothecium
Spore	8 ascospores in bitunicate ascus, 2-celled, cells of unequal size	8 ascospores in bitunicate ascus, 4-celled, with second cell from tip enlarged
Symptoms	Rectangular lesions (numerous lessions may merge); pycnidia may or may not appear in lesion	Lens-shaped lesions; pycnidia may or may not appear in lesion
Pycnidia found on:	Leaves, sheaths, culms, glumes, awns	Leaves, nodes, sheaths, glumes, awns, seeds
Epidemiology Primary source of inoculum	Infected debris	Infected debris, seed
Spore dissemination	Splashing of pycnidiospores, mechanical transmission, wind-blown ascospores	Splashing of pycnidiospores, mechanical transmission, wind-blown ascospores
Infection requirements	Prolonged, high relative humidity, temperatures higher than 7°C. No desiccation during process.	Prolonged, high relative humidity, temperatures higher than 7°C. No desiccation during process.
Symptom appearance (days after inoculation)	15-21 days at 20-24°C	7-14 days at 22-24°C

The following methods deal with collection and handling of infected plant material, isolation of the fungi, maintenance of septoria cultures, production of inoculum, inoculation procedures, and disease assessment.

Collection and Handling of Infected Plant Material

These procedures are identical for both *S. tritici* and *S. nodorum*. Pycnidia of the two septoria pathogens occur on leaves, sheaths, glumes, and awns of green and dried plant material. Collection of infected material is intended for two purposes: 1) for isolation of *Septoria* spp. for future use in inoculating plants in the greenhouse or field plots, to measure pathogenicity patterns, and for other research goals (genetic and physiologic studies, etc.); and 2) for inoculation with infected straw for which large quantities of infected plant material, either green or dried plants after harvest, are needed.

The investigator's goals determine the sampling and collecting strategies. When *Septoria* spp. isolates are used in germplasm evaluation, isolates should represent as wide a spectrum as possible of the fungus population. Samples can be taken from commercial wheat fields, from different geographic regions, and/or from specific cultivars within nurseries. Whenever possible, the cultivar from which the sample is taken should be specified, and each collection sample should represent an independent item within a location and be kept separate from other samples.

Collected green leaves with pycnidia of septoria should be placed in paper envelopes. Do not use plastic bags because they trap moisture within the bag which permits growth of secondary contaminant organisms. Each collection envelope should specify the following information: date, disease, crop, cultivar, location, previous crop (if known), and the name of the collector. The paper collection envelope containing the

specimen is air dried at room temperature for about 1 week. The specimen is later placed in a refrigerator at 5°C for future use or kept as a pressed herbarium specimen. The paper envelopes containing the dried leaves should be sealed inside dry plastic bags to prevent readsorption of moisture from the rather high humidity in the refrigerator, a condition that can result in a loss of pycnidiospore viability. Pycnidia, bearing pycnidiospores stored under proper conditions, may remain viable for several years, but most likely only for 1 year or less.

If the septoria worker has chosen to maintain large quantities of infected plant material for future inoculation, the collected material should be kept in a dry place. Dry plant material can be kept in aerated sacks or as bales.

Isolation of the Fungi

Both *S. tritici* and *S. nodorum* produce pycnidia on green plant parts. Under high humidity (usually free water) an ooze that contains pycnidiospores will emerge from the opening of the pycnidium (ostiole) and form a drop (cirrhus) on top of the dark pycnidium. This can be observed with the aid of a magnifying hand lens (x10) or under a stereoscope (x40). To grow cultures from leaf samples, isolation methods utilize this oozing process.

Isolation of *Septoria tritici*
Direct method—Leaf segments are attached to glass slides with tape that is resistant to moisture. The pycnidium opening must be facing up. Check this with a magnifying glass. The number of the collection is marked on the slide if more accurate studies are planned with a particular collection (Figure 9). Each slide is placed in a petri dish fitted on the bottom with filter paper saturated with sterile water. Replace the petri dish cover to provide a moist environment. The wetting period necessary for oozing to

begin depends on the degree of leaf dehydration and how quickly the leaf becomes moist. Dry, dead leaves require several hours; dry, green leaves require 1-2 hours. Temperature is also important; best results are obtained at 24°C. The leaf segment must be checked periodically to see whether oozing drops are formed on top of the pycnidia. This is done by observing the petri dish, with its cover still on, under the stereoscopic microscope with illumination from above. The oozing drop can be clear or cloudy. Cloudiness indicates that many pycnidiospores are present within the drop.

Transfer procedures require a microbe-free environment. Depressurized isolation hoods, UV sterile chambers, laminar-flow clean air cabinets, isolation chambers, or similar devices may provide this sterile environment. Whenever the oozing drops are ready to be transferred, the petri dishes are placed within a microbe-free environment. All necessary tools (needles, media plates, stereoscopic microscope, etc.) are also placed there before actual isolation. The closed petri dishes are observed under the stereoscopic microscope and oozing pycnidia are located. With the help of a fine-pointed needle, sterilized in a flame and cooled briefly, the oozing drop is quickly transferred to water agar or potato dextrose agar (PDA) (39 g of PDA in 1,000 ml of water), containing any of the following antibiotics: 250 mg/liter chloramphenicol succinate, 50 mg/liter streptomycin, 0.13 mg/liter kanamycin sulfate, 10 mg/liter aureomycin, or 10 mg/liter of gentamycin sulfate. The antibiotics gentamycin and kanamycin can be autoclaved (30-40 minutes at 1.5 kg/cm^2 pressure and 126°C), and thus added with other ingredients prior to autoclaving. All other antibiotics must be added to warm medium (approximately 50°C or less) after autoclaving and before pouring it into petri dishes in a microbe-free environment. If the antibiotics are added in a liquid form, then it is important that water, syringe, and/or pipettes be sterilized before the liquid antibiotic is prepared or added.

If the pycnidia do not ooze after several hours, they should be kept longer and checked for oozing later in the day. Do not allow the leaves to remain in the moist petri dish for an extended period (more than 8 hours), because secondary organisms (*Alternaria*, etc.) may grow on the leaf surface. This will interfere with the isolation procedure since the antibiotics will exclude many bacteria but not other fungi. If oozing does not occur within the day, open the cover of the petri dish and let it dry overnight. Rewet and repeat the process the following day or days. Often, this wetting-drying process will initiate oozing in difficult specimens. If oozing does not occur, repeat the entire procedure with other leaf samples. Whenever pycnidia do not produce oozing drops after repeated wetting and drying, it is possible to transfer the

I. Collection of leaf samples

II. Mounting leaf on glass slide with pycnidia facing up

III. Incubation of leaf with pycnidia in moist environment

IV. Transfer of ooze to media with antibiotic

V. Transfer of septoria colony to slant and storage at 5°C

VI. Growth of *S. nodorum* colony on YMA under light at 20°C

VII. Agitation of *S. tritici* culture in liquid medium at 18-20°C

VIII. Observation of conidial suspension and spore counting

IX. Spraying of conidial spore suspension in field on rainy days or dewy nights

Figure 9. Sequence of events from sample collection to artificial inoculation of septoria field trials (direct method).

content of a pycnidium, that is the pycnidiospores, directly from wetted leaves. This is done by digging with a sterile needle inside the pycnidium and transferring the contents to a medium containing antibiotics. The chances of transferring pycnidiospores by this method are smaller, yet the technique is much simpler.

The inoculated petri plates are kept at 18-20°C for 7-10 days. Following this, the small, pinkish-orange colonies that develop are transferred to PDA or yeast malt agar (YMA) without antibiotics.

Yeast-malt agar (YMA):

Yeast extract	4 g
Malt extract	4 g
Sucrose	4 g
Agar	15 g
Distilled water	1000 ml
	(1 liter)

The success of isolation depends on: 1) the condition of the leaves, 2) keeping the environment sterile, and 3) procedures and methods used during the isolation.

Indirect method—A different method for isolating bulk *S. tritici* isolates may also be used (46). Active leaf lesions (green leaves with pycnidia) caused by *S. tritici* are washed for 1 hour in running tap water, then immersed in 5% sodium hypochlorite for 2-3 minutes, and blotted dry on sterile filter paper. The leaf pieces containing pycnidia are moved across the surface of an agar plate (PDA + 50 mg/liter Rose Bengal + 125 mg/liter streptomycin). Where pycnidiospores ooze out onto the agar surface, small colonies develop.

Isolation of *Septoria nodorum*
Direct method—The pathogen is isolated after surface sterilizing of the infected plant material, leaves or kernels. The following surface sterilizing solution has been used: 0.5% sodium hypochlorite plus 5.0% ethanol (95%) in 100 ml of distilled water. One or two drops of a

surfactant (Ivory Liquid, Tween 20, glycerine) are added to the suspension in order to reduce the surface tension. Plant material is completely immersed for 3 minutes. Then the leaves or kernels are put on water agar plates containing one or more of the antibiotics mentioned above for *S. tritici* to avoid bacterial contamination. The plates are kept at 19-20°C, about 10-15 cm below a cool-white fluorescent tube and, if possible, in an incubator. After 1 week, single or mass spore transfers are made by removing the cirrhi with a needle from pycnidia formed on the leaf or kernels onto YMA, PDA, oatmeal agar, or Czapek Dox V-8 agar (23). All the above procedures should be performed using a stereoscopic microscope under microbe-free conditions.

From symptomless leaves—A method of detecting *S. nodorum* in symptomless leaves of wheat is described as follows (7). The medium used contains 20 mg paraquat, 200 mg chloramphenicol, 200 mg fentin hydroxide, and 5 g agar in 1,000 ml of distilled water. Paraquat, chloramphenicol, and fentin hydroxide are added to the agar after autoclaving. Leaves from the field are surface-sterilized with 0.5% sodium hypochlorite for 1 minute and washed three times in distilled water to remove any excess sodium hypochlorite. Leaf segments are then placed in contact with the special medium in the plastic petri dishes. The lower surface must be in contact with the agar. The segments are inoculated and then are kept under 12 hours darkness and 12 hours near-ultraviolet (NUV) irradiation at 18-20°C. Pycnidia first appear after 6 days.

From seeds—Seeds are plated on a medium (10 g dextrose, 10 g peptone, 15 g oxgall, and 20 g agar in 1,000 ml of distilled water) in 9-cm petri dishes, 10 seeds per dish, and incubated for 6 days at 20°C under 12-hour alternating cycles

of NUV light and darkness. The light is supplied by two black light tubes (Philips TL 40W/80) mounted 20 cm apart and 40 cm from the dishes. Keep the dishes with the covers facing up for the first 3 days. On the remaining days, turn them upside down. Fluorescence of the *S. nodorum* colonies may be observed after several days' incubation (85).

A modification of this fluorescence test is described as follows (69). A double thickness of filter paper is moistened with sterile water and placed in plastic trays. Seeds are placed equidistant on each paper pad. The samples are enclosed in polyethylene bags to prevent drying out and are incubated at 20°C in darkness for 3 days to permit imbibition and initial germination. They are then transferred to a deep freeze at -20°C for 3 hours to kill the seedlings and are then removed and incubated in darkness at 28°C for 4 days. The trays are removed from the polyethylene bags and the seeds are examined under a 100-watt NUV light at 360 nm.

A modified blotter test for checking seeds infected with *S. nodorum* involves pretreating the seed in sodium hypochlorite on moist blotters in 9-cm petri dishes which are kept at 20°C for 1 day to allow imbibition. The samples are transferred to a deep freeze at -20°C for 1 day and then incubated for 5 days in cycles of 12 hours darkness and 12 hours NUV light at 350 nm. Seeds are observed under the stereoscopic microscope (x25-50) for production of pycnidia (103).

Single-spore Method
If cultures derived from single pycnidiospores are desired, this can be achieved by attaching a surface-sterilized wet leaf segment with pycnidia on the interior surface of a petri plate should contain 1.0% water agar (10 g agar per liter of water) with or without the recommended antibiotics. Oozing cirrhi will fall onto the agar surface. After about

24 hours, view with a stereoscopic microscope, pick up single pycnidiospores with a sterile needle under microbe-free conditions, and transfer to PDA containing antibiotics. Be sure to transfer approximately 10 spores to each petri plate. The success rate is usually low. If the water agar petri plates are left for longer than about 24 hours, colonies will start growing which may have been derived from a single pycnidiospore. The mycelium then can be transferred to PDA.

Summary and recommendations
The easiest and most effective method to isolate both *S. tritici* and *S. nodorum* is the direct method, in which pycnidiospores are directly transferred to an appropriate artificial medium. When very specific studies are to be carried out, the single-spore method may be necessary to ensure absolute uniformity of the inoculum source.

Maintenance of Septoria Cultures
Several methods have been suggested for maintaining *Septoria* spp. isolates for short or long periods. Isolates can be preserved either in the pycnidial or in the conidial forms.

Short-term maintenance
Pycnidial form—Short-term maintenance of isolates of both *S. tritici* and *S. nodorum* can be achieved by storing green leaves with pycnidia which were separately inoculated with the specific isolates. The leaves are placed in a marked paper envelope (isolate, cultivar, date, etc.) for drying during several days at room temperature. Then the envelopes are placed in a sealed plastic bag in the refrigerator at 5-10°C. The pycnidia remain viable for several months and often up to 1 year if kept dry and cold. This method is useful if the pathogenicity of the fungal cultures on artificial media

becomes attenuated. Then reisolation of the culture from pycnidia will be required to recover pathogenicity.

Pycnidia of *S. tritici* and *S. nodorum* on solid media may be obtained on a modified Czapek Dox V-8 medium: 200 ml V-8 juice, 10 g agar, 800 ml deionized water (24). Irradiation is supplied with a black light (NUV) tube (Philips TL40 W/80) mounted approximately 45 cm above the petri plates inside an enclosed cabinet that is kept at 20°C. The inner walls of the cabinet are covered with aluminum foil to give a more uniform radiation. Sporulation of *S. nodorum* may be induced with high relative humidity in a cabinet fitted with a water bath, a vent, and an air fan (59).

Septoria nodorum may also produce pycnidia directly on YMA (75, 76). When at regular intervals only spores are transferred, pathogenicity is maintained.

Conidial form—*Septoria tritici*. The production of slanted *S. tritici* cultures is as follows: 3-5 ml of medium (PDA or YMA) in liquid form is placed into test tubes. These are closed with plastic caps or cotton plugs. Immediately following autoclaving, the test tubes are placed at an angle and the medium allowed to solidify. Thus, so-called "slants" are obtained. When the slants are cool, *S. tritici* spores can be transferred to them under microbe-free conditions. *Septoria tritici* grows well on such slants, which can be easily handled.

On artificial medium, *S. tritici* reproduces mainly by the production of conidia through budding. Such cultures of *S. tritici* usually remain pathogenic following repeated monthly transfers of spores over several years. Their relative ability to cause infection may decline somewhat, although they continue to grow well on slants. Therefore, the cultures should be renewed periodically by reisolating

pycnidiospores from newly infected seedling leaves of a susceptible cultivar. At some laboratories, this procedure is being followed every 4-6 months. For routine laboratory work, reculturing on agar slants is performed at 14- to 21-day intervals. When a fungal culture is used to inoculate a liquid medium, fresh 5- to 10-day old cultures should be used.

Cultures of certain isolates may form a mycelial mat (usually dark) in the slant as they become older. The cultures vary greatly in their sporulating (budding) or mycelial formation characteristics. A culture which tends to form mycelium after a rather short period requires more frequent transfers. By increasing the frequency of transfers, conidial production is maintained. The cultures should be transferred in their conidial form if they are to be used for inoculations. This is especially true if sprayers with fine nozzles are used to apply inoculum in the field since the mycelium may block the apparatus.

Conidial form—*Septoria nodorum*.
Septoria nodorum is maintained on agar slants or petri plates with appropriate artificial medium on which it usually forms pycnidia. Cirrhi on top of pycnidia may be directly used for transfer of spores from the original medium to a fresh medium. Alternatively, the following procedure may be employed, which allows the collection of a larger number of spores. Sterile water (2-5 ml) is transferred with a sterile Pasteur pipette in a microbe-free environment to the slant or petri plate containing the fungal culture. Cirrhi with pycnidiospores are mixed with the water on the surface of the medium by gently rubbing with a glass rod

previously sterilized in ethyl alcohol and flamed. A sterile Pasteur pipette is then used to transfer the suspension of pycnidiospores to a fresh medium.

Summary and recommendations—The simplest short-term maintenance method for either fungus is proper storage of leaves infected with pycnidia as described in the "Pycnidial form" section above. If the fungi are to be maintained on artificial media, the respective methods described in the "Conidial form" section above are preferred.

Long-term maintenance
Soil—*Septoria tritici* can be increased on Elliot V-8 juice agar (133). Five-gram samples of a coarse sandy loam soil at 1% moisture are placed in bottles. The bottles are autoclaved twice (20 minutes at a 12-hour interval). Conidial suspensions (2 ml) are transferred to the bottles. The inoculated soil-spore bottles are sealed, thoroughly shaken to evenly distribute the spores throughout the soil, and immediately stored in the dark at 4°C. Soil from soil-spore preparations is suspended in 2 ml of sterile deionized water and spread on the surface of nutrient agar for conidial increase.

Lyophilization—Both freezing and lyophilization have been studied as methods for long-term storage. Freezing results in loss of pathogenicity. However, lyophilization proved very successful (109, Ubels, personal communication).

Procedures for lyophilization of *S. tritici* conidia and *S. nodorum* pycnidiospores are as follows: Pyrex test tubes (10- x 0.6-cm) and Pasteur pipettes should be sterilized in an autoclave or in an oven (48 hours at 90°C). A skimmed-milk suspension (12%) is steamed for 15 minutes, three times, preferably during 3 separate days in an autoclave without pressure buildup. Spores of *S. tritici* and

S. nodorum grown in liquid shake cultures (*S. tritici*) or on solid media (*S. tritici* and *S. nodorum*) are transferred to test tubes to which 2.5 ml of the skimmed milk suspension was previously added. A sterile paper label with an isolate identification code and the date is placed in each test tube. Cotton plugs are inserted and pressed down the tube above the spore suspension and the tube label. The test tubes are then freeze dried at -20°C in a dry ice-acetone bath for several minutes. After the contents are frozen (this only takes a few minutes), the tubes are then placed into a vacuum chamber, and subjected to 20 mm Hg vacuum for about 4-5 hours (Ubels, personal communication).

The freezing and vacuuming are not done under sterile conditions. Sterile conditions should be maintained before and while the cotton plugs are inserted into the tube. Some investigators prefer to flame-seal the test tubes. This requires a set up in which the test tubes are attached to a vinyl or rubber hose capable of withstanding the vacuum. Then the tube is sealed under vacuum with an oxygen/gas torch. If test tube sealing is not performed, the following procedures should be used: after 2 hours, the dry-ice acetone bath is removed and the drying is continued at room temperature. As long as the tubes are still evaporating, they will feel cool to the touch. After they reach room temperature let them dry for another hour. The tubes maintain sterility due to previous sterile conditions and the cotton plug. Both the sealed and the unsealed tubes should be kept at 4°C in a refrigerator. Whenever cultures are withdrawn from cold storage, the following procedures are necessary for sealed and unsealed tubes:

• Sealed tubes are opened at room temperature by scoring the tube with a file and breaking it open near the center of the cotton plug. The whole

contents of the test tube (milk, conidia, powder, plug, and label) are transferred to an agar plate with antibiotics under microbe-free conditions.

• Unsealed tubes should be marked with a file under the cotton plug, flamed, and broken in half. Add 0.2 ml of sterile water to each test tube with a sterile pipette under microbe-free conditions to resuspend the milk and the spores and then transfer the contents to an agar plate.

When high spore concentrations, 1 x 10^6 spores/ml or higher, are used, germination of spores and pathogenicity are indistinguishable from "fresh" fungal cultures. Especially for *S. nodorum*, a slightly modified method of lyophilization has been published (109).

Cold storage—Cultures of septoria on PDA or YMA slants can be kept in cold storage (4°C) or in regular refrigerators. The test tubes should be carefully sealed, especially if cotton or foam rubber stoppers are being used. Cultures stored in this manner tend to dry up but keep viability for several months. This method is useful in providing a backup storage of specific isolates under study. It also provides the needed backup if cultures in use get contaminated or lost for some reason.

Summary and recommendations—The lyophilization method is recommended for well-equipped laboratories that conduct long-term studies on virulence or other studies where the original characteristics of the isolates need to be maintained. However, the short-term maintenance method recommended for the pycnidial form, in which infected

leaves are stored under dry conditions in a refrigerator, is often applicable as well for long-term maintenance. In that case, as an additional precaution, spores should be obtained from the stored material every 6 months, multiplied, and used for inoculation of new seedlings. Thus, freshly infected leaves are available for continued storage.

Production of Inoculum

Artificially cultured conidia of *S. tritici* and pycnidiospores of *S. nodorum* are often used for greenhouse and field trials (102, 122). These types of trials call for a high concentration of live spores per volume (ml). High concentrations of *S. tritici* pycnidiospores can be produced in either solid or liquid media. *Septoria nodorum* can be increased on solid medium or on kernels.

Solid media

Solid media on which *S. tritici* and *S. nodorum* grow well and develop many spores (PDA + 4-5% of yeast extract, or YMA) are good for inoculum increase. Large numbers of petri plates containing medium are inoculated with either fungi by streaking the spores from a 5- to 10-day-old slant or petri plate across the surface. They can also be inoculated by transferring a spore suspension to the fresh plates in microbe-free conditions. These suspensions are obtained by adding 2-5 ml sterile water with a sterile Pasteur pipette to 5- to 10-day-old slants or petri dishes containing the fungus. The surface of the culture is then scraped with a glass rod in order to suspend the spores into the surface water. Then 2 ml of the cloudy suspension are transferred to petri plates with the help of a sterile pipette. A spore suspension of *S. tritici* can also be obtained from liquid shake cultures where an aliquot of 1-2 ml is transferred to the

solid medium plates. The petri plate is rotated to ensure that the suspension is distributed evenly. The plates are incubated at 18-22°C in growth chambers or on the laboratory bench with illumination. After 5-10 days, pinkish reproductive spores or pycnidia (*S. nodorum*) should occur. The petri plates are flooded with sterile water (or tap water if deionized sterile water is not available) and scraped lightly with a glass slide or other utensil without damaging the surface of the agar. To avoid clogging the inoculation equipment with agar or fungus mycelium, filter the suspension through 2-3 layers of cheesecloth or other coarse cloth.

Liquid media

This method is applicable only to *S. tritici* since *S. nodorum* cannot be produced on liquid shake culture. Small amounts of fresh reproductive agar cultures are scraped from the petri plate or slant and transferred to liquid medium. The following liquid media can be used (in order of preference):

a) Yeast sucrose liquid medium

Sucrose	10.0 g
Yeast extract	10.0 g
Distilled water	1000 ml
	(1 liter)

b) Modified Fries liquid medium (146)

NH_4 tartarate	5.0 g
NH_4NO_3	1.0 g
$MgSO_4.7H_2O$	0.5 g
KH_2PO_4	11.3 g
K_2HPO_4	2.6 g
Glucose	20.0 g
Yeast extract	5.0 g
Distilled water	1000 ml
	(1 liter)

c) Potato dextrose yeast liquid medium

Decant from cooked potatoes (15 minutes in steamer or 20 minutes in autoclave)	200 g
Dextrose	200 g
Yeast extract	20 g
Distilled water	1000 ml
	(1 liter)

All liquid media are prepared in large Erlenmyer flasks or beakers, 2 liters or larger if needed. The liquid medium is transferred to smaller Erlenmyer flasks and then autoclaved. For greenhouse seedling inoculations, usually involving only a small number of plants, about 100-125 ml of medium is placed in a 250-ml Erlenmyer flask. This ratio of 1:2.5 for medium volume to flask volume is also kept for flasks of other sizes.

The flasks are shaken on a shaker (wrist, rotary, horizontal movement, etc.) for 5-10 days at 20°C, depending on the cultures. Some cultures grow fast and need less shaking time (5 days). Others grow slowly and need more shaking time (7-10 days). When shaking is done either by wrist or rotary movement, the shaking speed should not be too fast. Slower shaking prevents the flask plugs from getting wet with media. If they do get wet, contamination, especially by bacteria, may follow. At the end of the shaking period, the inoculum is filtered through several (2-3) layers of cheesecloth to remove any mycelia. Counting chambers, usually a hemacytometer, are used to determine the spore concentration. Cloudy liquid cultures might have a spore concentration ranging from 1×10^5 to 1×10^7 spores/ml. If concentration is important, it should be checked and counted for each isolate in suspension. For inoculum increase, each isolate should be grown in several flasks.

This assures that if growth is poor in one flask, other flasks of the same isolate can serve as substitutes.

For germplasm evaluation of field trials, grow each *S. tritici* isolate in a separate flask instead of growing the isolates in mixed cultures. Just before inoculation, the separately grown isolates are mixed together.

Kernel media
This method has been most successfully applied to *S. nodorum*. A culture of *S. nodorum* grown on V-8 juice/Czapek Dox agar or YMA incubated at 17°C under NUV light is flooded with sterile distilled water. The surface of the culture is scraped to remove air bubbles and allow the water to reach the pycnidia. The pycnidiospores are then discharged into the water. After 30 minutes, about 3 ml of the resulting spore suspension are transferred with a sterile Pasteur pipette to a 250-ml flask containing sterile wheat kernels (6). Prior to transfer, these flasks are prepared by autoclaving 25 g of wheat seed and 30 ml of water for 20 minutes at 1.5 kg/cm^2 pressure and 126°C. During this time, all free water is taken up by the seed. The inoculated flasks are incubated in the dark at 5°C for about 4 months. More than one flask is prepared for each isolate, so substitutes are available in case of contamination or poor growth.

To prepare inoculum for a field trial, flood each flask with 150 ml distilled water. This breaks up the mat of infected grain in the bottom of the flask. The pycnidia are then allowed to discharge their spores over a 30-minute period. The spore suspension is filtered through cheesecloth to remove fragments of fungus, pycnidia, and grain. The spore

concentration of each isolate is determined with a counting chamber and adjusted to 1 x 10^6 spores/ml.

Summary and recommendations
For *S. tritici* the liquid media method is recommended for large-scale production of spores. Although the kernel method for *S. nodorum* is very successful, it requires a lot of time and thus is less flexible. Therefore, when large-scale increase is requested on short notice, the solid media method is used for *S. nodorum*.

Inoculation Procedures
Greenhouse inoculation
Seedlings can be inoculated with a spore suspension by using quantitative or nonquantitative methods. The method used depends on the objectives of the study. Seedlings can be inoculated by gently rubbing the leaves with cotton swabs that have been soaked in a spore suspension. One drop of a surfactant is a helpful additive since it reduces surface tension and increases the creation of a uniform suspension. This method does not provide good control of the various steps involved in the inoculation process, such as the number of spores reaching the leaves. But if other more quantitative methods are difficult to use, the results from rub inoculation can serve as a preliminary evaluation method. Quantitative inoculation methods allow the researcher to determine the number of spores/ml, and the volume of spore suspension sprayed onto the plants. Special techniques, such as the use of a turntable or a settling tower, can control the delivery of a known number of spores per volume during a given time.

Revolving inoculation technique—A method using rotary motion (a turntable) devised by Eyal and Scharen (38) has successfully been used for evaluating

seedling-host response to both *S. tritici* and *S. nodorum* (38, 40, 148).

The increase of inoculum for this method was described in the section on production of inoculum. Inoculum is prepared from 5- to 7-day old septoria cultures. A 15-ml spore suspension (1 x 10^6 to 1 x 10^7 spores/ml) is sufficient to inoculate about 200 10- to 12-day old seedlings. Ten to twenty seedlings should be used per cultivar when host response is to be evaluated. Seedlings are grown in rows in a square container. The container is placed on a turntable and, while rotating at 45 rpm, seedlings are spray inoculated with a 15-ml spore suspension per container during about a 2-minute period (Plate 18). A drop of a surfactant should be added to the spore suspension. After inoculation, the container with seedlings is placed into an incubation chamber with a saturated atmosphere for 48-72 hours at 18-22°C (Plate 19). A saturated atmosphere can be made by putting very fine tap water mist nozzles in the chamber which is enclosed with clear plastic film. It can also be made by creating high relative humidity within the chamber with water pans, wet cloth, etc. (122). At the end of the incubation period, the plants in the seedling containers are left to air dry. They should not be removed while wet because the inoculum might be spread or mixed by contact with other containers. They are then transferred to a greenhouse bench or to controlled environment chambers. *Septoria tritici* trials are kept there from 14 to 30 days (usually 21) at 22°C before recording disease infection. After 10-15 days (usually 14), infection of *Septoria nodorum* can be evaluated. Symptom development may be poor at high-temperature, high-irradiation, and low-humidity conditions (summer).

Intact leaf technique—Intact wheat leaves can be tested for their reaction to *Septoria* spp. while still functioning as parts of living plants (147). Several leaves are partially inserted into a plastic "humidity box" above water placed on the bottom. They are then inoculated with a drop of spore suspension. Subsequently, the lid is closed. Thus, while still part of normal plants, the leaves are enclosed in a humid chamber conducive to disease infection.

Detached leaf technique—To test host response to *S. tritici*, the cut ends of seedling first leaf segments may be placed in a benzimidazole solution (96, 128). The leaves are sprayed with a fresh reproductive suspension of *S. tritici* in a 0.5% gelatin solution and kept moist for 4 days. The greatest amount of difference in resistance is obtained at 40 mg/liter benzimidazole concentration, at 21°C, and with a 12-hour day or 24 hours under weak illumination. Uninoculated leaves are green and vigorous for about 20 days under these conditions. Loss of green coloring of susceptible cultivars appears about 6 days after inoculation. Sporulation of the pathogen occurs in about 12 days.

Agar containing benzimidazole has also been used for *S. nodorum*. Leaf sections are placed on the medium, inoculated, incubated, and subsequently evaluated for infection (5, 9, 18, 67). This method shows a fairly good correlation with field assessments (9, 67).

Adult plant technique—It may be desirable to evaluate germplasm beyond the seedling stage in the greenhouse. Wheat plants have been inoculated at different growth stages, from jointing to medium milk, by spraying the spore suspension onto plants on a greenhouse bench (131). The plants should be sprayed as uniformly as possible from all directions. After inoculation, enclose the plants in a moist chamber consisting of wet cloth hung on a frame around the plants and covered with clear plastic. This reduces solar radiation and will keep the chamber at 100% relative humidity for the required 7 days. During the first 3 nights, keep the leaves wet by spraying them with water. Following the 7-day wetting period, place the plants on an open greenhouse bench. Disease can be assessed after about 14-21 days.

Summary and recommendation—The choice of inoculation method will depend on the degree of accuracy required in the experiment and on equipment availability. Where quantitative inoculations are required, the revolving inoculation technique method, widely used for both pathogens, is recommended.

Field inoculation
Infested crop debris—Nurseries, yield trials, and chemical control studies, etc., can be readily inoculated with the two septoria pathogens. After seedlings emerge, usually about 2-3 weeks after planting, they are covered with pycnidia-bearing straw. There is a danger that seeds remain present in loose or baled straw and thus render genetic studies useless. Therefore, straw spread over the plants should have all seeds removed and be finely chopped. Chopped straw can be spread throughout the season. Do not spread straw on a windy day. Infected straw is most effective as a primary inoculum source in the evenings when dew forms. Infected straw with viable pycnidia or pseudothecia should be collected and stored in a dry place immediately after harvest for the next year's trials.

Spore suspension—Spore suspensions can originate from liquid media (*S. tritici*), solid media (*S. tritici* and *S. nodorum*), and/or kernel media (*S. nodorum*). These spore suspensions can be used for artificial inoculation. Septoria isolates of different origins are grown separately, filtered, and mixed just before inoculation. Inoculations done under favorable weather conditions achieve the best results (rainy days, temperatures not less than 8-10°C nor higher than 28°C, low velocity winds, etc.). The location and condition of the field, transportation, and equipment are important in deciding when to mix cultures for field inoculation. Evidence shows that bench life of cold stored spores after mixing with water is short, not longer than 12 hours. Bacteria or other microorganisms might contaminate mixed cultures and reduce the life of the spore suspension. If conditions are not good for inoculation, it might be better to continue to grow the cultures for a few more days before mixing and inoculating. It is best to prepare several batches of inoculum at regular intervals. That way, fresh inoculum will always be available. If sprinkler or nozzle irrigation is available for use in the field, inoculation can proceed even on days without rain, if other conditions are favorable. The spore suspension is mixed with a few drops of a surfactant (Tween 20, 0.5% gelatin, or a mild soap such as Ivory Liquid) and sprayed with low-volume, low-pressure sprayers during high-humidity days (light rain or irrigation) (Plate 20) or on dewy nights, once or twice a week during the inoculation period.

The inoculation should begin at the tillering growth stage and may continue until the later maturing cultivars reach the post-flowering growth stage. Establishing septoria epidemics in the field requires repeated inoculations (usually at least 3-4) throughout the inoculation period under proper conditions (rain, temperature). These efforts will reduce escape and facilitate the proper selection of resistant germplasm. Loss-severity trials also require adequate and uniform levels of disease. However, when inoculum is artificially applied from the top (spray inoculated) after head emergence, the upward spread of the disease is limited making selection for genetic factors difficult or impossible.

Inoculated trials in semiarid countries need special attention. If possible, disease progress during the season should be promoted by 15-30 minutes of sprinkler or nozzle irrigation over the crop once or twice a day. Irrigation should be done in the mornings before the dew dries to extend the dew period or in the evenings after the dew forms to promote splashing of the oozing pycnidiospores. A wet period of at least 24-48 hours following inoculation is best to ensure infection in the field.

An increase in humidity can be achieved by wetting the soil prior to inoculation. Also, portable plastic humidity chambers can be placed over the plants immediately after inoculation with a spore suspension. If pressurized water is available, increased relative humidity can also be obtained by fitting the chamber with a dewspraying nozzle. Inoculation of a limited number of entries (crossing block, segregating populations, etc.) can be carried out in a nethouse or in other permanent housing covered with

transparent plastic or fitted with dew-spraying water nozzles connected to a timer.

Summary and recommendations—
Spreading infected straw, collected at the right time in the previous season and stored in a dry place, is the simplest field inoculation procedure, but adequate levels of disease for selection can not always be guaranteed. Repeated inoculations using spore suspensions will ensure good infection in most situations. However, if inoculation is continued into the adult plant stage, certain resistance components that, under natural infection, would limit the upward spread of the disease from lower to upper leaves may become difficult, if not impossible, to select for. It may be advisable to inoculate repeatedly only during the tillering stage, and no longer once stem elongation commences.

Disease Assessment

Assessment of disease infection is essential for evaluating germplasm response to pathogens in genetic and epidemiological studies and for studying other aspects of the interaction of the hosts and pathogens.

Septoria diseases of wheat are usually evaluated on the basis of plant tissue affected by the pathogen. Estimates of disease severity are made two ways: 1) evaluating how dense the pycnidia are, and 2) determining the area of dead tissue on the affected plant, the nongreen leaf area or the remaining green leaf area. The former method estimates the total presence and direct manifestation of the pathogen. The latter takes into account interactions between the host and the pathogen. This interaction is not always

directly related to the effect of the disease. The host does not always show obvious loss of quality because of the presence of the disease. In these cases, a combination of both approaches may be necessary. The presence of disease may also be evaluated by quantifying mycelial or spore production.

Although research workers usually evaluate the presence of disease, the nonaffected area or the absolute green leaf area is likely to be more closely related to yield potential than the disease index (the sum of the percentage of nongreen leaf area on the top four leaves of diseased plants minus the sum for healthy plants) (50).

Host response may be greatly influenced by the growth stage of the host. Several investigators studied the relationships between host growth development and disease severity (35, 124, 126). These relationships are strongly affected by host genotype and phenotype. It is thus of great importance to record the growth stage of the host at the time of disease assessment.

The decimal code of Zadoks et al (154), which was developed from the Feekes growth stage scale (78), is used by many cereal workers (Figure 10). It applies to all small grain cereal species growing in a wide range of environments.

When the effects of infection on yield are studied, disease evaluations are usually made between medium milk (growth stage 75) and late milk (growth stage 77). This is the period when the kernels are accumulating dry matter most rapidly and compensation for diseased plants by adjacent healthier plants is least likely to

Plate 1. *Septoria tritici* on durum wheat.

Plate 2. Pseudothecium, asci, and ascospores of *Mycosphaerella graminicola*.
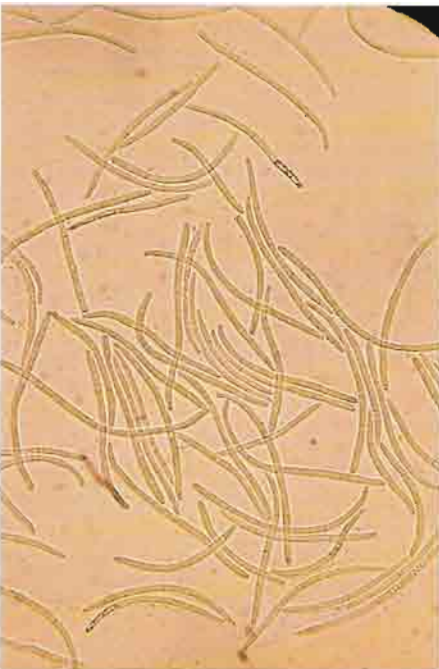
Plate 3. Macropycnidiospores of *S. tritici*.

Plate 4. Typical symptoms of septoria tritici blotch.

Plate 5. Necrotic and chlorotic lesions of *S. tritici*.

Plate 6. Advanced symptoms of septoria tritici blotch on bread wheat.

Plate 7. Pycnidia formation of septoria tritici blotch.

Plate 8. Mature pycnidia of septoria tritici blotch.
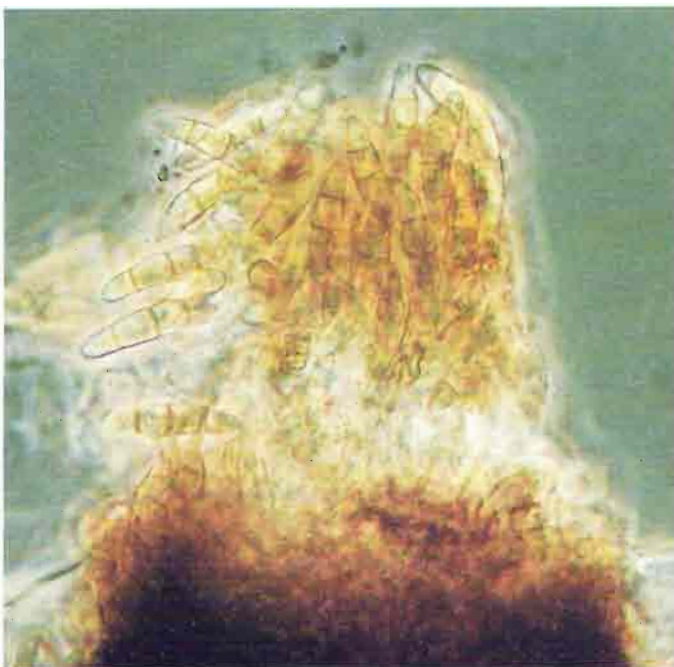
Plate 9. Pseudothecium, asci, and ascospores of *Leptosphaeria nodorum*.
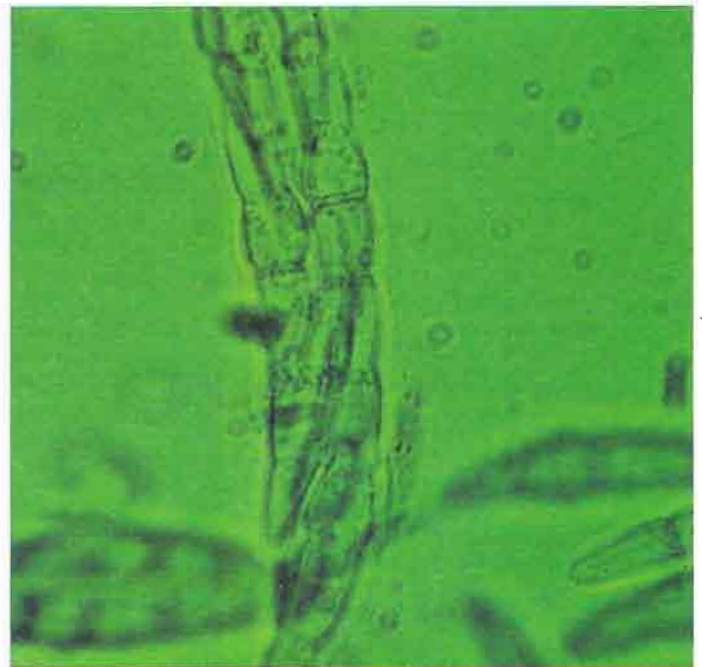
Plate 10. Asci and ascospores of *L. nodorum*.

Plate 11. Mature pseudothecia of *L. nodorum*.

Plate 12. Ascospores of *L. nodorum* are straight to slightly curved, and have 3 septa.
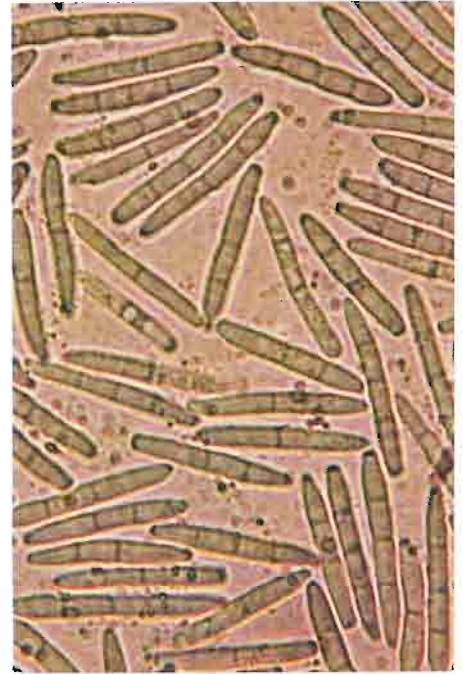
Plate 13. Pycnidiospores of *S. nodorum* are cylindrical and transparent, with 0-3 septa.

Plate 14. Leaf symptoms of septoria nodorum blotch.

Plate 15. *Septoria nodorum* lesions are often lens-shaped, with a yellow-green border surrounding the necrotic area.

Plate 16. Symptoms of septoria nodorum blotch on a bread wheat glume.

Plate 17. Head infection of septoria nodorum blotch.

Plate 18. Revolving inoculation technique for evaluating seedling-host reponse to *S. tritici* and *S. nodorum*.

Plate 19. Mist chamber for incubating inoculated seedlings.

Plate 20. Spraying of conidial spore suspension in the field.

Key to figure 10. Descriptions of the principal and secondary growth stages of the Zadoks scale, as modified by Tottman and Makepeace (143).

Code	Stage	Code	Stage	Code	Stage
0	**Germination**	**3**	**Stem elongation**	**7**	**Milk development**
00	Dry seed	30	Pseudostem erection	71	Kernel water ripe
01	Start of imbibition		(winter cereals only)	73	Early milk
03	Imbibition complete	31	1st node detectable	75	Medium milk
05	Radicle emerged from seed	32	2nd node detectable	77	Late milk
07	Coleoptile emerged from seed	33	3rd node detectable		
09	Leaf just at coleoptile tip	34	4th node detectable	**8**	**Dough development**
		35	5th node detectable	83	Early dough
1	**Seedling growth**	36	6th node detectable	85	Soft dough (fingernail
10	First leaf through coleoptile	37	Flag leaf just visible		impression not held)
11	First leaf unfolded	39	Flag leaf ligule just visible	87	Hard dough (fingernail
12	2 leaves unfolded				impression held; head
13	3 leaves unfolded	**4**	**Booting**		losing chlorophyll)
14	4 leaves unfolded	41	Flag leaf sheath extending		
15	5 leaves unfolded	43	Boots just visible swollen	**9**	**Ripening**
16	6 leaves unfolded	45	Boots swollen	91	Kernel hard (difficult to
17	7 leaves unfolded	47	Flag leaf sheath opening		divide by thumbnail)
18	8 leaves unfolded	49	First awns visible	92	Kernel hard (can no longer
19	9 or more leaves unfolded				be dented by thumbnail)
		5	**Ear emergence**	93	Kernel loosening in
2	**Tillering**	51	First spikelet of ear just visible		daytime
20	Main shoot only	53	One-fourth of ear emerged	94	Overripe; straw dead and
21	Main shoot and 1 tiller	55	One-half of ear emerged		collapsing
22	Main shoot and 2 tillers	57	Three-fourths of ear emerged	95	Seed dormant
23	Main shoot and 3 tillers	59	Emergence of ear complete	96	Viable seed giving 50
24	Main shoot and 4 tillers				percent germination
25	Main shoot and 5 tillers	**6**	**Flowering**	97	Seed not dormant
26	Main shoot and 6 tillers	61	Beginning of flowering	98	Secondary dormancy
27	Main shoot and 7 tillers	65	Flowering halfway complete		induced
28	Main shoot and 8 tillers	69	Flowering complete	99	Secondary dormancy lost
29	Main shoot and 9 or more tillers				
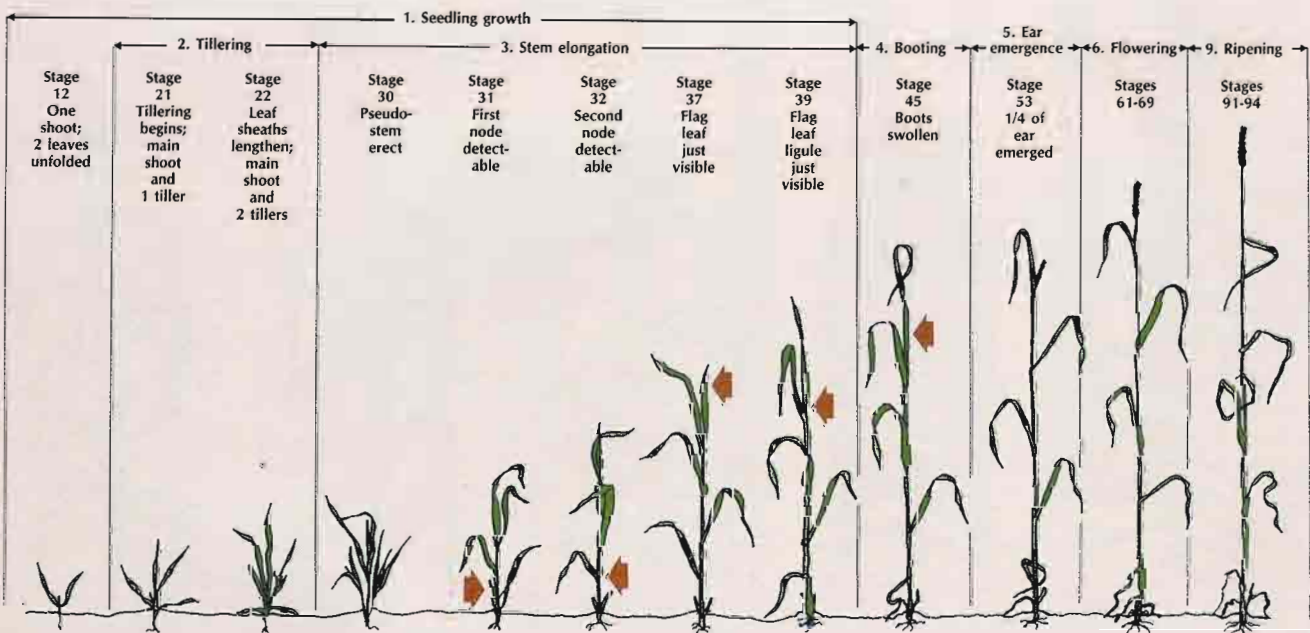
Figure 10. Zadoks scale of cereal growth stages.

occur (70). Yet, in many cases, disease assessment is conducted throughout the growing season starting with the onset of the disease. Evaluation of disease progress with time may provide some explanations as to relationships between disease and plant development and its reflection on yield. Moreover, various types of disease protection (slow disease progress, etc.) can only be evaluated by following disease development over time.

Several methods used to assess disease for each of the pathogens will be presented and discussed. There is not a single uniform assessment method accepted by all septoria workers for either controlled studies in the greenhouse or for field evaluation.

Saari-Prescott 0-9 scale,
double digit 00-99 scale
The Saari-Prescott 0-9 scoring scale (110) for evaluating the intensity of foliar

diseases other than rusts in wheat, triticale, and barley is most commonly used for both septoria diseases when taking notes in the field (Figure 11).

The method was recently improved by using two digits, representing the vertical disease progress and an estimate of severity (Figure 12). The *first* digit gives the relative height of the disease using the original 0-9 Saari-Prescott scale as a measure. The *second* digit shows the disease severity as a percentage but in terms of 0-9. Because it is difficult to evaluate diseases on dead leaves, disease notes should be taken when at least four leaves are still alive and green (soft to mid-dough growth stage). Then visually evaluate the average percentage severity on only those leaves of the uppermost four that are infected (Figure 12). In practice, the percent severity is estimated by looking at 10-20 plants and deciding

on an overall score. The following format is used for scoring severity:

10% coverage = 1   60% coverage = 6
20% coverage = 2   70% coverage = 7
30% coverage = 3   80% coverage = 8
40% coverage = 4   90% coverage = 9
50% coverage = 5
The score of 10 is not used.

For example, a certain line of wheat is infected by *S. tritici*. If the height of the disease is at about the mid-point of the plant, the score on the 0-9 Saari-Prescott scale for relative height is 5. The average coverage with *S. tritici* on only those leaves of the uppermost four that are infected, that is, those at and below the midpoint, is 10%. Then the numerical disease description is 51 (Figure 12). This scale is called the double-digit 00-99 scale and can be used for many foliar diseases that "climb up" the plant, including the septoria blotches, but *should not be used to evaluate the rusts*.
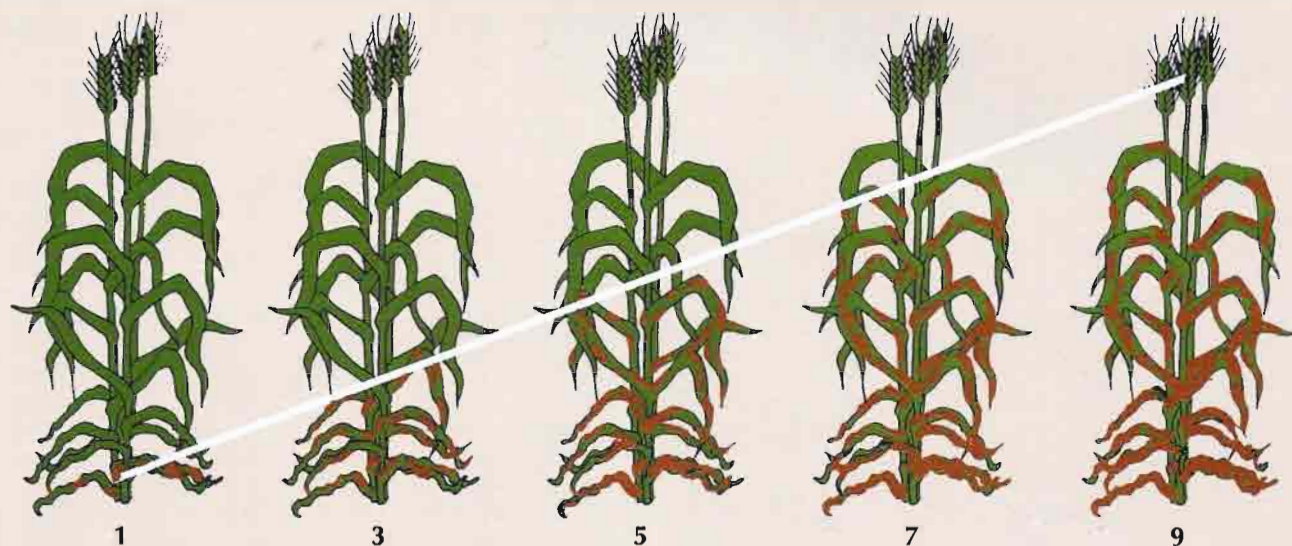
Figure 11. Saari-Prescott (0-9) scale for appraising the intensity of foliar diseases in wheat and barley.

Bronnimann's *Septoria nodorum* leaf and head evaluation scale

Septoria nodorum blotch is usually evaluated by estimating dead leaf tissue or loss of color, and by the amount of glume infection if that symptom occurs (15) (Figure 13). Pycnidia are almost always present in lesions when the disease is severe, but they are not considered separately from the other symptoms (26, 122).

Rosielle's *Septoria tritici* scale

Rosielle developed a six-point scale for *S. tritici* (105):

0 - Immune (Imm) - No pycnidial formation, no symptoms or occasional hypersensitive fleck.

1 - Highly Resistant (HR) - No or only occasional isolated pycnidia formed, particularly in older leaf tissue, hypersensitive flecking in younger leaf tissue.

51
1) relative disease height = 5
2) 10% of infected leaves is diseased = 1

59
1) relative disease height = 5
2) 90% of infected leaves is diseased = 9

81
1) relative disease height = 8
2) 10% of infected leaves is diseased = 1

89
1) relative disease height = 8
2) 90% of infected leaves is diseased = 9

Figure 12. Double-digit (00-99) scale representing the vertical disease progress (first digit) and severity estimate (second digit).

0   1   5   10   25   50   75   % 100

Figure 13. Percentage of wheat leaf or head area affected by *Septoria nodorum*.

Source: Bronnimann, A. (15)

2 - Resistant (R) - Very light pycnidial formation. Some coalescing of lesions mainly toward the leaf tip and in older leaf tissue.

3 - Intermediate (I) - Light pycnidial formation. Coalescing of lesions normally noticeable towards the leaf tip and elsewhere on the leaf.

4 - Susceptible (S) - Moderate pycnidial formation, lesions coalescing considerably.

Figure 14. Septoria Progress Coefficient (SPC). SPC = Disease height (cm)/Plant height (cm). Disease height = the maximum height (cm) above ground level at which the pycnidia of S. tritici could be found on green plant tissue.

5 - Very Susceptible (VS) - Large, abundant pycnidia, lesions coalescing extensively.

Eyal's Septoria tritici disease evaluation methods
Septoria progress coefficient—To overcome some of the difficulties associated with plant growth habit (maturity and height) and the expression of symptoms, Eyal and Ziv (43) have used the Septoria Progress Coefficient (SPC) together with an evaluation of disease severity (Figure 14). Plant and disease height (cm) are determined. Disease height is the maximum height (cm) from the ground where pycnidia of the pathogen are found on the plant.

$$SPC = \text{Disease height (cm)/Plant height (cm)}$$

The coefficient indicates the position of pycnidia relative to plant height regardless of pycnidial coverage. It allows the comparison of infection placement on cultivars with different plant stature. Despite plant stature, the vertical progress of the pathogen from the ground level might be the same. Variation in how high

the pathogen is on the plant might be due to the characteristics of the plant and how these relate to the spread of the disease. This variation might also be due to genetic factors that determine the upward progress of the disease over time. The spread of disease cannot be measured by only looking at the uppermost leaf (flag leaf). If this were done, taller plants would generally show less susceptibility to disease and vertical disease spread would not be taken into account.

Diagrammatic scale—Disease severity can be evaluated according to the Eyal and Brown diagrammatic scale (37), which is used to evaluate the actual pycnidial density per unit leaf area (Figure 15).

Disease severity classes—In the screening and evaluation of germplasm for breeding programs, disease severity classes, based on infection of the four uppermost leaves, have been made as follows:

VR - Very Resistant - Average pycnidial density of 0-5%.

R - Resistant - Average pycnidial density of 5-15%.
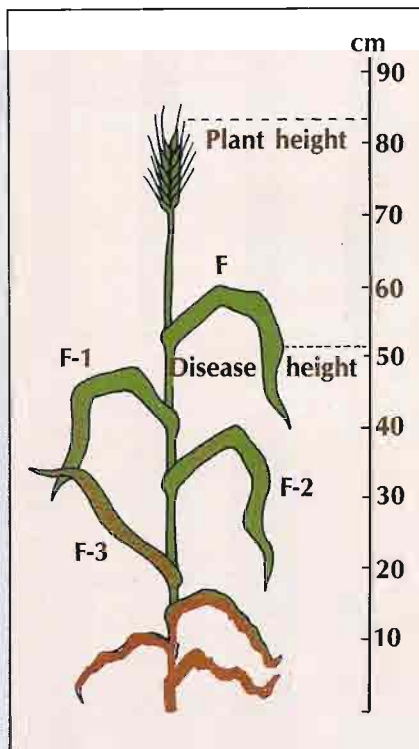
| A | 2.74 | 5.72 | 11.44 | 17.15 | 19.89 |
| B | 12 | 25 | 50 | 75 | 87 |
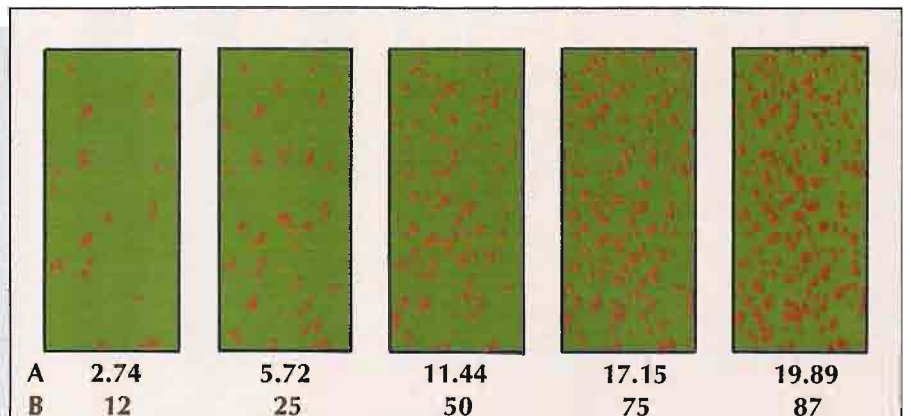
Figure 15. The Eyal-Brown scale estimating coverage of wheat leaves by pycnidia of Septoria tritici. A = actual observed pycnidial coverage (%). B = scaled possible pycnidial coverage (%).

Source: Eyal, Z. and M.B. Brown (37)

MR - Moderately Resistant - Average pycnidial density coverage of 15-30%.

MS - Moderately Susceptible - Average pycnidial density coverage of 30-40%.

S - Susceptible - Pycnidial density greater than 40%.

The septoria infection classes (VR, R, MR, MS, S) are strongly affected by the overall disease level in the trial. The level of disease in the trial can be shown by including wheat cultivars of known and varying host response (susceptible, moderately resistant), plant stature, and maturity.

PCD/SPC—Eyal et al (42) categorized the relationships between the percent coverage of disease (PCD) or coverage of pycnidia (Figure 16) on the four

uppermost leaves and the vertical disease placement or Septoria Progress Coefficient (SPC) into four distinct cultivar response classes:

Class A...... PCD $\leq$ 15% / SPC$\leq$0.40

Class B...... PCD $\leq$ 15% / SPC = 0.40-0.65

Class C...... PCD = 15-40% / SPC = 0.40-0.70

Class D...... PCD $\geq$ 40% / SPC $>$ 0.70

James' septoria foliar keys
James' key (62) is an illustrated series of evaluation keys for plant leaf diseases, their preparation, and usage. The standard area diagrams were accurately prepared with an electronic scanner (Figure 17).

Gough's pycnidiospore production method
Other methods have used pycnidiospore or mycelial production to evaluate host response. A method based on pycnidiospore production is presented. Gough (51) has reported on a method to evaluate cultivar response to *S. tritici* based on pycnidiospore production. Leaf segments (1-3 cm long) with thick pycnidia coverage are removed from wheat cultivars and soaked in deionized distilled water for about 15 seconds to wet them and the pycnidia. They are then mounted in petri dishes containing filter paper moistened with deionized distilled water. The petri dishes are kept at 18-25°C. One-half milliliter (about 4 drops) of deionized distilled water is deposited into spot glass depressions. Spores are then harvested after 24-26

Figure 16. The Ziv-Eyal rough scale for estimating pycnidial coverage of *Septoria tritici*.

Figure 17. James' key for assessing the intensity of symptoms of septoria tritici blotch and septoria nodorum blotch of wheat.

Source: James, W.C. (62)

hours by dipping each leaf segment 10 times into the distilled water and counted using a hemacytometer. After the first harvest, the dishes are left for 30 hours. Then they are rewetted. After another 24-26 hours, a second spore harvest and counting take place. The total number of spores produced is determined from the two hemacytometer counts.
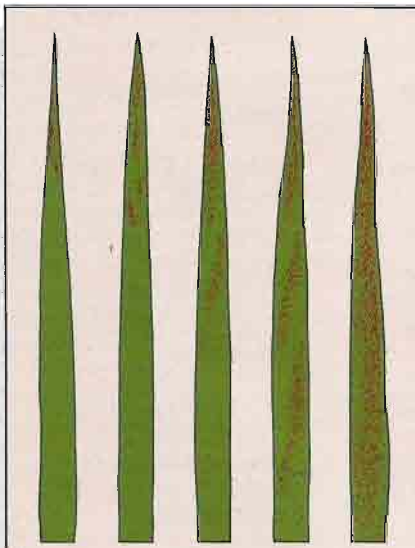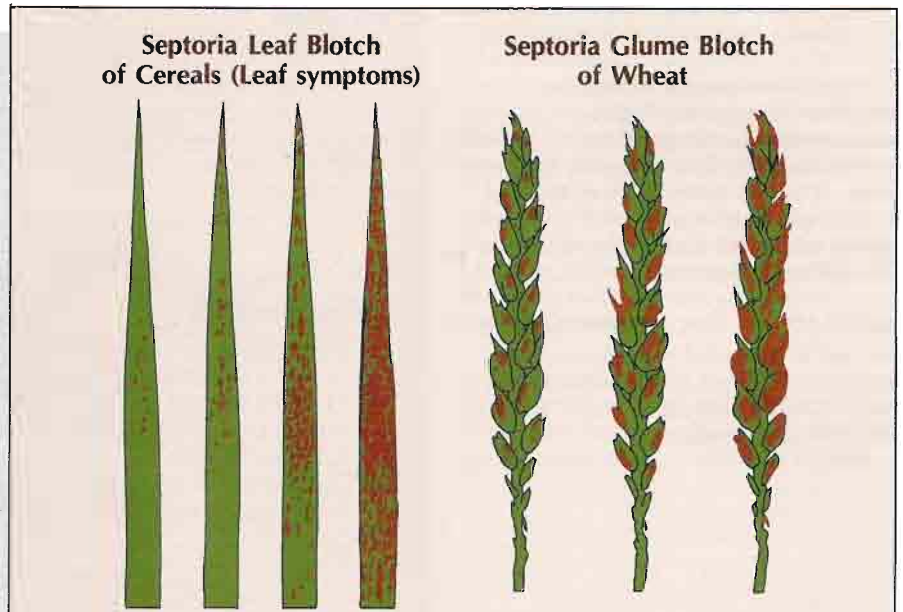
Summary and recommendations
The nine assessment methods include two distinct approaches:

1) One is designed to evaluate germplasm response on a comparative or relative basis, thus allowing the large collection of cultivars usually sown in disease evaluation nurseries to be evaluated in a relatively short time. Examples are the Saari-Prescott 0-9 scale and its modification, the double-digit 00-99 scale. These are widely used by plant breeders and pathologists. The inclusion of the disease severity assessment of the area of the plant affected adds a quantitative parameter to the method.

2) When more precise evaluation of germplasm is required, then the quantitative scales designed by Bronnimann (15), Eyal et al (42), Eyal and Brown (37), and James (62) can be used or other quantitative assessment methods may be employed, such as quantification of pycnidiospore production (51).

Summary of Recommendations
For both *S. tritici* and *S. nodorum*, the procedures outlined in this methodology chapter are summarized in Table 4 in the form of recommendations.

Table 4. Summary of recommended methodologies discussed in this chapter

	Septoria tritici	*Septoria nodorum*
Isolation	Pycnidiospore transfer from leaf (**direct method**)[1]	Pycnidiospore transfer from leaf or kernel (**direct method**)
Maintenance Short-term	Storage of infected leaves (**pycnidial form**)	Storage of infected leaves or kernels (**pycnidial form**)
	On yeast-malt agar (YMA) (**conidial form**)	On yeast-malt agar (YMA) (**conidial form**)
Long-term	Storage of infected leaves (**pycnidial form**)	Storage of infected leaves or kernels (**pycnidial form**)
	Freeze dry (**lyophilization**)	Freeze dry (**lyophilization**)
Inoculum production	In suspension (**liquid media**)	On agar media (**solid media**)
Inoculation Greenhouse	Quantitative method (**revolving inoculation technique**)	Quantitative method (**revolving inoculation technique**)
Field	Straw (**infested crop debris**)	Straw (**infested crop debris**)
	Spraying spores (**spore suspension**)	Spraying spores (**spore suspension**)
Disease assessment Greenhouse	Coverage of disease (necrosis) or presence of pycnidia (**disease assessment**)	Coverage of disease (necrosis) or presence of pycnidia (**disease assessment**)
Field	Combination of relative disease height and severity (**Saari-Prescott 0-9 scale, double digit 00-99 scale**)	Combination of relative disease height and severity (**Saari-Prescott 0-9 scale, double digit 00-99 scale**)

[1] Boldface terms in parentheses refer to sections in the Methodology chapter.

Epidemiology and
Cultural Practices

Epidemics of septoria tritici blotch and septoria nodorum blotch of wheat are associated with favorable weather conditions (frequent rains and moderate temperatures), specific cultural practices, availability of inoculum, and the presence of susceptible wheat cultivars.

The splashing dispersal mechanism affected by rain limits distances to which pycnidiospores can be spread. The usual vertical progress of septoria from lower to upper leaves is affected by the distance between consecutive leaves—the "ladder effect." The distances between the first emerging three to four leaves are similar for short and tall cultivars. On tall varieties, the distance between each leaf is greater toward the flag leaf. In the dwarf cultivars (70-90 cm), the closeness of the upper leaves to the lower leaves facilitates contact between newly emerging leaves and splashed pycnidiospores. Movement of the pathogen from infected lower leaves is thereby made simpler. As a result, pycnidia often appear earlier on upper plant parts of dwarf cultivars than they do on leaves of taller cultivars. Thus both resistance- and morphology-related genetic factors influence disease spread and resulting severity. Under severe epidemics, the differences in plant architecture and stature of susceptible cultivars are of no importance to the pathogen. In moderate to light epidemics, however, upper plant parts of dwarf cultivars are more receptive to the pathogen than taller wheats as they are nearer to inoculum sources (34). In wheat-growing regions where septoria pathogens are a potential danger, plant architecture, especially leaf placement, should be taken into account when new wheat cultivars are to be released.

Because of the splashing dispersal mechanism, exposed plants are often infected to a higher degree than plants closely surrounded. Therefore, observing disease levels on plants on field borders usually indicates the greatest infection level at a particular time during plant growth. Open areas within the field that result from skips during machine sowing are also good areas to observe disease occurrence. In areas facing the rain, the splashing effect is increased because the penetration of drops is undisturbed.

Septoria tritici

In countries where *M. graminicola* has not been found, it is still assumed that pycnidiospores of *S. tritici* serve as the primary inoculum. It is probable, however, that *M. graminicola* will be found in other wheat-growing areas and countries as more effort is devoted to systematically searching for the pseudothecia and ascospores.

The primary inoculum for initiating epidemics of septoria tritici blotch in New Zealand, Australia, and the United Kingdom is wind-blown ascospores of *M. graminicola*. Early seedling infection by ascospores was reported to have a greater effect on yield in New Zealand than later infection by pycnidiospores on upper plant parts. This phenomenon is called a two-staged epidemic cycle.

Cultural practices in New Zealand leave the wheat plants after harvest as standing stubble during wet periods, whereas in many other places the wheat residue is left as debris on the soil surface or incorporated into the soil (114). This difference in wheat residue management is considered the main factor for the development of the sexual fruiting bodies when the environmental conditions are

favorable (summer rains). Because the standing stubble is predominantly dry and when wetted dries out rapidly, it is not subjected to rapid breakdown by saprophytic microorganisms. Standing stubble, therefore, is in a much better physical position to produce pseudothecia and release ascospores. During milder autumn and winter conditions, pseudothecia and ascospores have been found in Australia, Europe, New Zealand, and the United States. Where the absence of summer rains and high temperatures makes conditions unfavorable for development of the sexual state and leaf debris remains relatively untouched on the soil surface for long periods, pycnidia of the asexual state are most likely the main primary source of inoculum. Crop residues that remain in direct contact with the soil surface are, however, very vulnerable to decay, as are incorporated crop residues.

Soil management practices that leave large amounts of wheat stubble and debris on the soil surface increase the chance of septoria epidemics under favorable climatic conditions. Cultural practices that reduce wheat residue through plowing, burning, removal for feeding, crop rotation, etc., help remove the major source of primary inoculum. Crop rotation with wheat cropping intervals of 3-5 years has decreased septoria tritici blotch incidences in Israel. However, spores themselves may survive in soil up to 20 months and remain pathogenic (136).

Unlike pycnidiospores, ascospores have the potential to travel long distances by air currents from the source of origin and threaten new crops, in addition to their

ability to introduce new virulence combinations. The horizontal spread of septoria tritici blotch from an infected center is associated with the upward spread of the disease in infected plants. The vertical and horizontal spread is slow under unfavorable conditions, such as low temperatures and lack of rainfall. The spread is faster when the minimum temperatures rise to 8-10°C during the nights, provided that rainfall is adequate. The horizontal spread increases in less dense fields because splashing raindrops penetrate better to infected lower plant parts.

Long rainless intervals with high temperatures often occur in Mediterranean environments towards the end of the growing season. These intervals interrupt septoria tritici blotch progress from lower infected leaves to upper plant parts.

Septoria nodorum

Septoria nodorum epidemics can start from infected seeds, especially in wetter years (26). In the southeastern U.S.A., seed infection by S. nodorum was chronic and varied from 40 to more than 50% (80). One infected seedling in 5,000 was enough to initiate a septoria nodorum blotch epidemic in the field (53).

Besides infected seed, crop debris is an important source of primary inoculum. After 1 year, wheat straw still contains pycnidia able to produce viable and infective pycnidiospores (115). Crop rotations out of wheat for 1 or 2 years did not lead to lower disease levels in the subsequent wheat crop if infected seed was used for planting. Even in conjunction with fungicide treatment (benomyl) of the seed, a 1-year rotation did not reduce infection in the crop.

However, when the seed was treated and a 2-year rotation was observed, then the amount of disease was greatly reduced, but nevertheless still present (81). It appears therefore that infected crop debris on the soil may function as a source of primary inoculum for a number of years. A combination of seed treatment or the use of clean, certified seed, plus at least 2 years of rotation, seems desirable if high levels of disease are to be avoided. A confounding factor may nevertheless be the survival of S. nodorum as potentially pathogenic spores in the soil up to 20 months (136).

Septoria nodorum spores are mostly dispersed over short distances within crops causing localized disease spread. Although most spore-carrying rain droplets are 200-400 μm in diameter, some are smaller and often become airborne in moving air (13). Septoria nodorum spores may be carried in such small droplets, and can be dispersed over considerable distances (44, 150). Most S. nodorum spores, however, are dispersed less than 2 m in the large "ballistic" splash droplets. Wind greatly increases the dispersal of smaller droplets and spores in the downwind direction (12, 13).

Tall cultivars often show lower levels of infection with S. nodorum than short ones. The dispersal of S. nodorum from the base to the top of the plant occurs less readily when the distance to be travelled is greater (127). The canopy of a taller cultivar might generate a microclimate that is less conducive to the development of S. nodorum than that of a short cultivar, which may be denser and closer to the soil. Leaf wetness may be less and its duration may be shorter than that in some short cultivars that have denser canopies.

Pathogenic Specialization

Wheat cultivars reported to be resistant in one country may sometimes succumb to attack by septoria populations in another country. Some sources of resistance were overcome by the pathogens after they were incorporated into agronomically suitable wheats and submitted to national trials. Knowledge of the virulence spectra of the septoria pathogens would be useful in establishing a reliable resistance breeding program (88). Specific host-pathogen interactions have been reported for both *S. tritici* and *S. nodorum*, but their generality remains unproven.

Septoria tritici

There are conflicting reports on the issue of physiologic specialization in *S. tritici*. Cultures of *S. tritici* isolated in Israel have behaved as races in the conventional connotation on both *Triticum aestivum* and *T. durum* (36, 153). Their parasitic characters have remained stable through successive host passages and repeated transfers on nutrient media. Physiologic specialization has been reported in the U.S.A. (94), Australia (6), and Uruguay (30). Isolates secured from *T. aestivum* are in general avirulent on *T. durum* with several exceptions (36). In Tunisia, there appears to be a lack of resistance in most durum wheats while several bread wheats are highly resistant to the local *S. tritici* population (31).

Isolates and cultivars may differ significantly with respect to the incubation period, percentage of leaf area infected, and the number of pycnidia produced (30). The interaction between cultivars x isolates may also be significant for the above parameters. In that case, this suggests the existence of races. Virulence patterns were evaluated for 97 isolates from 22 countries on seedlings of 35 wheat and triticale cultivars (39).

Significant cultivar x isolate interaction indicated the presence of specific virulence genes among isolates. The geographical regions and countries varied considerably in their relative virulence frequencies. The virulence frequencies of *S. tritici* were the highest in Latin America, with Uruguay and Mexico having the most virulent populations.

The cultivar x isolate interaction was minute when the reaction of 13 durum wheats to 34 isolates from seven countries was evaluated. Comparison of genetic effects among these cultivars also suggests that the presence of classical races is unlikely (148, 149). It seems that certain *S. tritici* isolates are better able to infect bread wheats than durum wheats, and vice versa. Isolates may differ in the infection levels they can cause within a species, either bread wheat or durum wheat. In the absence of differential interaction between cultivars and isolates, such differences are due to varying levels of aggressiveness among the isolates (84, 148, 149).

Septoria occurring naturally in common chickweed (*Stellaria media*) is pathogenic on wheat. Wheat was inoculated with this isolate and spores were collected from the resulting pycnidia. Upon reinoculation of new wheat plants, the level of virulence had increased. With repeated passages through wheat, the virulence on this crop kept increasing (95).

Inoculation with certain combinations of *S. tritici* isolates grown together in mixtures or grown separately and mixed prior to inoculation may result in a marked reduction in the level of symptoms compared to the level on plants inoculated separately with the individual components of the mixture.

Symptom expression may be dependent on the ratio of each of the isolates in the mixture (155).

Septoria nodorum

The presence of classical races for *S. nodorum* also remains unclear. Researchers have found 282 isolates of *S. nodorum* from the principal wheat-growing areas in northern Florida to have distinct resistance patterns. Despite differential interactions, this did not permit conventional race differentiation (1). Nine isolates of *S. nodorum* of diverse origin on four winter wheat cultivars were found to have significant cultivar x isolate interactions that indicate specific resistance (107, 108).

Cultivar x isolate interactions together with continuous variation in host response were reported among 14 different cultures of *S. nodorum* on 10 winter and spring wheats (119).

Virulence frequencies of 33 isolates of *S. nodorum* from eight countries were evaluated on 38 wheat and triticale cultivars. Assuming a gene-for-gene relationship, 21 different genes were determined operative among the cultivars. Isolates from Brazil, Chile, and Ecuador expressed high relative virulence (120).

It appears that, in *S. nodorum*, terms such as "race," "cultivar," and "isolate" might not be meaningful outside a specific experimental situation (54).

Barley isolates of *S. nodorum* exhibited increased virulence to wheat after two passages through wheat, but no change occurred during passage of wheat isolates through barley (47). Isolates of *S. nodorum* from wheat were characteristically virulent to wheat and avirulent to barley. However, a biotype

pathogenic on barley has been recovered from wheat isolates after various numbers of passages through barley. The biotype of *S. nodorum* on barley which occurs in the southern U.S.A. appears to be largely restricted to barley (25). *Septoria nodorum* isolates of barley and wheat were highly virulent to their original host but nevertheless weakly virulent to the opposite crop in reciprocal inoculations (27). Isolates from wheat and barley with differing characters might therefore be considered biotypes of *S. nodorum.*

Septoria nodorum may infect several forage grass species (74). Three of the isolates studied were still pathogenic on wheat after passage through the grass hosts.

Summary

For both *Septoria* spp., there are reports supporting and arguing against the presence of classical races operative in the host-pathogen system. There is a need to evaluate the diversity of the two pathogens in relation to their hosts. The implications of differential interaction, if shown to be widely applicable, would be great for growers, breeders, and pathologists alike.

Exact knowledge of the host-isolate responses will aid in the identification of distinct resistance sources and in the selection of resistant germplasm. Consequently, it will enable the design of more effective breeding and disease control strategies.

Breeding for
Disease Resistance

Most of the high-yielding wheat cultivars grown today are susceptible to septoria tritici blotch and septoria nodorum blotch. Therefore, resistance is a high-priority breeding goal. Host resistance is "the main pillar of defense against disease" (21, 122). But not enough is known about the types of resistance, their mode of action, inheritance, manipulation, and accumulation. These aspects, together with the possibility (35, 107, 108, 119) that *S. tritici* and *S. nodorum* are able to adapt their virulence or aggressiveness, are difficulties faced by the programs that breed for resistance to these pathogens.

Favorable environmental conditions, lack of resistant cultivars, chronic seed infection (*S. nodorum*), and improper cultural practices are the major factors that contribute to severe septoria outbreaks in certain parts of the world. A yield loss of 1% for each 1% increment in severity on the flag leaf and a loss of about 0.6% for each 1% increment on the leaf below the flag leaf has been recorded (71).

Evaluating the relationship between disease severity and losses in yield or yield components (17, 43, 145) in advanced cultivars of septoria-infected vs. fungicide-protected trials should provide information on the vulnerability of these lines to the pathogen. It should also permit agriculturists to design proper protective measures (chemical control, limited varietal distribution, improved breeding approaches, etc.). Resistance to septoria can be evaluated in field nurseries, which are naturally or artificially infected. Low infection levels are often associated with late maturity and tall plant stature. In countries where rains stop early in the season and/or temperatures increase rapidly, there is a greater chance of escaping infection.

In order to evaluate host response to both pathogens, disease epidemics of a uniform and quite high level should be established in the nurseries. Artificial inoculation of the nursery assures infection. Host response can then be evaluated. Evaluation is restricted to the pathogenicity spectrum of the selected isolates. Differences in aggressiveness among isolates can shift the initial virulence spectrum of the isolate mixture. This may result in an unbalanced virulence spectrum. Artificial inoculation of screening nurseries should be performed several times throughout the season, ending when later-maturing wheats reach anthesis. Such methods might introduce difficulties in evaluation if slow disease progress is sought or plant growth stage affects host receptivity (132). These difficulties might be partially overcome if early maturing wheats and later maturing wheats are divided into separate subnurseries. In these nurseries, accessions could be compared with the same check cultivars representing wheats of various growth habits. One may also prefer to inoculate heavily only in the tillering stage and subsequently allow natural development of the epidemic fueled by autoinfection.

Resistances to septoria tritici blotch and septoria nodorum blotch appear to be more widely distributed among bread wheat (*Triticum aestivum*) cultivars with winter growth habit than among those with spring growth habit. Resistance has also been reported in several wild relatives of wheat (14, 151, 152).

Dominant, partially dominant, recessive, and additive gene actions were found to condition resistance to both septoria tritici blotch and septoria nodorum blotch (20, 31, 72, 73, 75, 76, 89, 90, 98, 100, 104, 106, 107, 110, 116, 122, 128, 139, 148, 149, 152). The additional presence of genes that modify the expression of dominant genes for resistance might explain in part the lack of success in transferring adequate protection from the cultivars in certain crosses.

Resistance to both *Septoria* spp. did not often reside in the same line when 43 varieties resistant to *S. tritici* were evaluated for their reaction to isolates of *S. nodorum* collected in Montana, U.S.A. (118). However, when a similar group of cultivars was tested for resistance to a large number of isolates of both pathogens collected from eight different countries, a very high correlation was found between host responses to the two pathogens (120). This stresses the need to study the diversity in the two pathogens and their dissimilarities.

Plant height and growth habit (photoperiod and vernalization requirements) interact with specific genetic factors that control disease expression. This interaction makes evaluation of germplasm to septoria diseases difficult (29, 35, 125, 140).

Tolerance to septoria pathogens (that quality enabling a susceptible cultivar to endure severe attack by a pathogen without sustaining severe losses in yield) has been identified in certain high-yielding wheat cultivars (16, 156). The tolerant cultivars yielded well and produced heavy, unshrivelled kernels under severe septoria epidemics when compared to fungicide-protected plots and nontolerant wheat cultivars. In the future, tolerance could be combined with resistance expressed by low disease severity. This would provide the endurance together with a recognizable resistance.

Septoria tritici

Of 22 *T. monococcum boeoticum* lines (genome AA), only two were susceptible to a wide virulence spectrum of *S. tritici* in Israel (153). Of 47 wild emmer (*T. turgidum dicoccoides*) lines, 25 were resistant to all seven *S. tritici* isolates used in the experiment. A high level of resistance to *S. tritici* has been detected among populations and accessions of *T. longissimum*, *T. speltoides*, and *T. tauschii (Aegilops squarrosa)* no. 33. Resistance to *S. tritici* has been transferred to bread wheat from *Agropyron elongatum* (52).

In many countries (31, 35, 118, 119), durum wheats and triticales have a higher frequency of resistance to *S. tritici* than spring bread wheats. However, in Tunisia several bread wheat lines and cultivars were highly resistant to *S. tritici* whereas very few durum wheat cultivars showed good resistance (31). This condition might result from the fact that durum wheats are widely grown in Tunisia, thus producing directed selection pressure on the pathogen to adapt to durum wheats rather than bread wheats, which are grown on a much smaller scale.

Resistance to septoria tritici blotch from winter wheat germplasm (Aurora, Bezostaya 1, Kavkaz, and others), available in agronomically suitable, resistant semidwarf cultivars developed by the International Maize and Wheat Improvement Center (CIMMYT) in Mexico and released by national programs, although not universal, is effective against a rather wide spectrum of pathogenicity patterns. The inheritance of resistance of Bezostaya 1 and Bezostaya 1-derived winter wheats (Aurora, Kavkaz, and Trakia) to two distinct *S. tritici* isolates under controlled field trials indicated that the resistance of the four winter wheats to the isolate ISR398 (ATCC 48507) is controlled by one or two dominant genes. There was no indication for maternal effect on the expression of disease coverage. The two *S. tritici* isolates (ISR398 and ISR8036) possess at least two different genes for virulence. Low correlations were expressed between heading date and plant height and pycnidial coverage of septoria tritici blotch (28). A gene that modifies the expression of the dominant effect of Bezostaya 1 to *S. tritici* has been reported (29). Additive effects in the inheritance of resistance to *S. tritici* have been shown to be of prime importance, although dominance effects have been also often present. Epistasis seemed negligible in the durum wheat material studied (148, 149).

The dwarfing gene Rht2 has only a slight effect on resistance to *S. tritici* (125, 127). Therefore the relationship between height and resistance appears to be determined chiefly by genes other than Rht2. Resistance to *S. tritici* in some winter wheat cultivars is expressed by low pycnidial density which has been successfully transferred to early-maturing, short-statured wheats (29).

Septoria nodorum

Resistance to *S. nodorum* was successfully transferred from *T. tauschii (Aegilops squarrosa)* no. 33 to winter wheat (144).

In moderately resistant cultivars, resistance may be controlled by additive action of several genes, whereas in highly resistant cultivars, resistance may be governed by major resistance genes (119). There is some evidence that resistance at the seedling stage is conferred by one or more dominant genes. Available experimental analyses indicate, however, that the resistance of wheat to *S. nodorum* is mainly under polygenic control and involves several genes (79, 87, 89, 90, 120). General combining ability (GCA) effects are highly significant, but specific combining ability (SCA) effects have been observed as well, indicating nonadditive gene action for some specific crosses (89). In advanced generations, transgressive segregation may occur (117). One or more genes modify the expression of resistance of the dominant gene of Atlas 66 to *S. nodorum*.

Resistance in wheat to *S. nodorum* may be of a nonrace-specific or "horizontal" type and, while reasonably durable, relies on several individual partial resistance components (64). These components can be subdivided into resistance to infection, resistance to colonization, and resistance to reproduction (93). If all the components are acting together, disease will be reduced and yield increased. Four principal components of partial resistance have been determined which may represent genuine physiological processes under genetic control that may possibly be separable: 1) infection frequency; 2) latent period; 3) size, shape, and rate of growth of lesions; and 4) spore production and its mode of increase. Significant differences between lines under severe attack by *S. nodorum* were observed in the incubation time and in the rate of symptom expression, which explain the differences in epidemic development and the slowing down of disease progress (99). The durability of partial resistance to *S. nodorum* of the cultivars Razon and R82 can apparently be overcome only if a biotype with new aggressiveness is present in the pathogen population at the beginning of the epidemic (101).

There appears to be a connection between resistance to *S. nodorum* and plant height. This association was suggested to be due to chance association between shortness and susceptibility in parental lines, genetic linkage, or pleiotropy (128). Results indicate that these characters may not be associated by chance, but at least partly by pleiotropy or linkage (126). The same association is apparent, but less consistent, between resistance to *S. nodorum* and lateness. Resistance in the crosses studied is not determined by individually identifiable genes of large effect. Resistance may be determined by certain genes of small effect, possibly many in number. Pleiotropy may be the most probable cause of the association between height, heading, and resistance to *S. nodorum* in the material studied. The genetic variation in resistance to *S. nodorum* in the cultivars examined can be partitioned into height-dependent and height-independent components (127). The height-dependent component reflects at least, in part, pleiotropic inheritance of height and resistance. Microclimate effects of the canopy structure may play an important role in accounting for the pleiotropic relationship. The dwarfing gene Rht2 had little effect on resistance to *S. nodorum* or yield. Other genes than Rht2 seem to govern the relationship between height and resistance.

Numerous genetic studies indicate that tolerance to septoria nodorum blotch is additively and polygenically inherited with a relatively high heritability value (17). Most progress in breeding for septoria tolerance may arrive through a combination of tolerance with the "slow septoring" or slow disease development effect.

Summary
A uniform and moderately high level of disease is required in breeding nurseries so that there is sufficient disease pressure on the material for selection. Artificial inoculation will assure this. Positive selection will then be possible without the risk of escapes. In both pathogens, dominant, partially dominant, and recessive genes that condition resistance have been found. Additive gene action, polygenically inherited, appears to be of major importance. Resistance may also be available in wild relatives of wheat. Linkage between height and susceptibility does not seem to be strong. The relationship rather appears to be one of pleiotropy for some genes, mainly expressed in an altered plant architecture affecting disease spread and severity. Tolerance has been insufficiently explored.

Chemical Control

Fungicide protection has been used either as a stop-gap measure, or as an integral part of the crop management system. Its purpose has been to secure the high yields of susceptible cultivars (23). The design of an economical chemical control program for protection from the septoria pathogens of wheat depends upon several crop management considerations. Prior to applying chemicals, wheat growers and/or researchers must decide whether to resort to chemical control of the specific wheat field if necessary. The considerations are as follows: 1) early assessment of yield potential and economics of the specific wheat field; 2) vulnerability of the wheat cultivar to septoria and/or other diseases; 3) history of wheat cropping and septoria epidemics in the specific field; 4) disease levels in the specific field; 5) cultural practices before sowing (burying of refuse, deep plowing, etc.) that might reduce the amount of primary inoculum; 6) early detection of the diseases and assessment of their progress; 7) weather conditions; 8) cost of fungicide protection relative to other investments in the crop; and 9) projected yields and losses.

An effective chemical control program for septoria diseases should be accompanied by an extensive disease surveying system. Some countries routinely conduct disease surveys and disease forecasting and a few others incorporate computer-generated recommendations based on data collected in the field. This system provides for the early detection of diseases, evaluation of disease distribution, and evaluation of disease development. Success in decreasing the effect of these diseases on yield potential depends on the integration of all components into a disease management scheme that is part of the regular crop management system. These components include epidemiology, cultural practices, genetic protection, chemical control, biological control, and extension.

Foliar Applications

Protectants

Dithiocarbamates (maneb, manzate, mancozeb, zineb) have proved effective in controlling septoria diseases (31, 41). However, these protectant fungicides require repeated application at 10- to 14-day intervals. A chemical control program of 3-4 maneb applications, where the upper plant parts responsible for grain filling are protected, can be effective in reducing the impact of the pathogens. It is also economically justified when yield potential is high.

If the spray program begins before full emergence of the flag leaf, the use of mancozeb (Dithane M-45 or Manzate 200) fungicide to control septoria nodorum blotch on the flag leaf and head is profitable for wheat growers in Florida (77). When the spray program is begun at growth stage 32 (second node detectable) or growth stage 37 (flag leaf just visible), S. nodorum infection is reduced on the lower part of the plant. Because only three applications of mancozeb on wheat are legal in the U.S.A. and because residues of this fungicide decline with time, chemical control programs for septoria nodorum blotch are not recommended prior to growth stage 32.

For the control of septoria nodorum blotch, especially in the wheat heads, captafol is the most widely used fungicide in Germany. It is usually applied at heading when 75% or more of the heads have emerged. Captafol, like other protectants, requires critical timing and has not controlled attacks of the leaves by the Septoria spp. (45). When captafol is applied with triadimefon at three successive stages, i.e., prior to flag leaf emergence (growth stages 32-37), preboot (growth stages 37-39), and at heading (growth stages 51-59), it is quite effective in controlling septoria nodorum blotch.

Systemics

Systemic fungicides with curative properties and longer protective action against several leaf organisms may be more beneficial than protectants. This is especially true when the action threshold is misjudged or the chemical protection program improperly executed. The systemic fungicides benomyl (Benlate), prochloraz (Sportak), triadimefon (Bayleton), and propiconazole (Tilt) have proved effective in controlling septoria tritici blotch and septoria nodorum blotch in several countries. Other new-generation systemic fungicides, such as HWG 1608, fenpropimorph (Corbel), and myclobutanil (RH 3866), have also been found to be effective. Combining protectant and systemic fungicides to control septoria disease might provide an alternative route, since tolerance to carbendazim was reported in S. nodorum (61). The systemic fungicides can lengthen the protection effect, counteracting outbreaks and timing difficulties. The protectant fungicide reduces the selection pressure on the pathogen exerted by the systemic fungicides and expands the control spectrum and longevity of the control program.

Methyl benzimidazole carbamate (MBC) group—Under the normal commercial situation in New Zealand, fungicide is applied toward the end of the winter when the plants are at the 4-5 leaf stage. At that time, the natural dispersal of ascospores has ceased, but no symptoms are yet visible. A single spray of benomyl at 0.25 kg active ingredient/ha is then adequate to control septoria tritici blotch (112).

When disease levels on the wheat heads due to S. nodorum are moderate to severe, chemical control with MBC-type fungicides has proven profitable in Europe. In West Germany, toxicological considerations have, however, led to the withdrawal of the official use of MBC-type fungicides (45).

Isolates of *S. tritici* resistant to benzimidazole have been reported in the U.K. The minimum inhibitory concentration was 0.2-0.4 ppm for benomyl-sensitive isolates and greater than 1,000 ppm for benomyl-resistant isolates. An *S. tritici* culture resistant to 4,000 ppm benomyl was recovered in Israel. The culture did not differ from the wild type in its virulence spectrum (155). The benomyl-resistant isolates secured in the U.K. were resistant to carbendazim, thiabendazole, and thiophanate-methyl, but not to 11 other fungicides including captafol, chlorothalonil, iprodione, maneb, prochloraz, propiconazole, triadimefon, and triadimenol (46). Poor control of *S. tritici* following five sprays of carbendazim has been associated with a high proportion of benzimidazole-resistant strains in the pathogen population (86).

Fungicides of the MBC group (e.g., benomyl) in combination with dithiocarbamates (e.g., maneb) are used in some countries in northwestern Europe to control septoria nodorum blotch (91, 92).

Ergosterol-biosynthesis inhibitors—The introduction of ergosterol-biosynthesis inhibitors such as prochloraz (Sportak), propiconazole (Tilt), and triadimefon (Bayleton) has, to a certain extent, overcome the deficiencies of the protectants. The new fungicides offer more flexibility in time of application, and they are broad-spectrum fungicides that control rusts and, in some cases, powdery mildew (*Erysiphe graminis*), in addition to the septoria diseases. The mean infection frequency of *S. nodorum* is greatly reduced by three fungicides (captafol, prochloraz, and propiconazole) on certain spring wheat cultivars, but less on others (64). The latent period often becomes longer following fungicide treatment. Propiconazole and prochloraz inhibit pycnidial production, while captafol markedly reduces pycnidial production in some cultivars.

In Germany, two applications of propiconazole (Tilt) several days apart were found to be more suppressive on septoria nodorum blotch development and more effective in increasing yield than a single treatment. Treatment at the early boot (growth stages 38-40) or boot (growth stage 45) stages is most likely to be economical if attack on the lower leaves is heavy and moderate to slight on the higher leaves (45).

In a fungicide trial conducted in Israel to control septoria tritici blotch, the most effective treatment was two early successive applications of either propiconazole (Tilt) or benomyl. The fungicides were applied at growth stages 40 and 47 when infection had reached 5% on the first or second leaf below the flag leaf (the action threshold). This resulted in slower disease progress, low pycnidia coverage, and significantly higher yields and kernel weights than the untreated, inoculated controls (22). A single application of propiconazole at the action threshold just mentioned was more effective in controlling the pathogen and securing high yields, than when applied at a later date, but less effective than the two successive early applications. Under high disease levels, the curative effect of propiconazole was less obvious than under low to moderate disease levels. Repeated applications of the protectant maneb were less effective than the systemic fungicides, especially if the action threshold was misjudged. When the protectant was applied after the disease severity was more than the recommended action threshold, an attempt to use propiconazole to correct the earlier misjudgement was not effective. A chemical control program may require an earlier action threshold if very short, susceptible cultivars are grown.

Application of triadimefon (Bayleton) + Manzate 200 gave good control of septoria nodorum blotch, leaf rust (*Puccinia recondita*), and powdery mildew in Louisiana, U.S.A. (3). Combinations of systemic + protectant fungicides (Bayleton + Dithane M45, Tilt + Dithane M45, Bayleton + Difolatan, Prochloraz + Dithane M45) may cause significant reductions in foliar symptoms and increase yields.

When applied as leaf sprays, the fungicides triadimefon (Bayleton), RH 2161, chlorothalonil (Bravo 500), carbendazim, and benomyl all reduced the severity of *S. tritici* in New Zealand. In addition, significant yield responses were obtained in field plots (141). A single application of prochloraz 5 days prior to artificial inoculation with *S. nodorum* was less effective than curative treatments applied 1 week after inoculation (45).

Seed Treatments
The economic effectiveness of seed dressing in controlling septoria tritici blotch is questionable and supportive information is lacking. Bimodal disease progress curves are characteristic of epidemics in Australia and New Zealand, in which *M. graminicola* ascospores are a primary inoculum source for septoria tritici blotch for 2-3 months after seedling emergence (19). As an alternative to foliar applications, seed treatment has been investigated. Seed treatment with systemic fungicides reduced pycnidiospore production in Victoria, Australia, for up to 3 months after sowing, though without a measurable increase in yield. The most effective chemicals for seed treatment were: thiabendazole (1.5 g/kg seed), triadimenol (0.3 g/kg seed), and nuarimol (0.2 g/kg seed), which reduced the number of plants infected with *S. tritici* by 62, 52, and 36%, respectively, but without improving yield.

When dealing with septoria nodorum blotch, seed dressing with suitable systemic fungicides can be effective in reducing the primary infections from seedborne inoculum. The frequency of current septoria nodorum blotch epidemics from infected seeds treated with seed-dressing fungicides is uncertain. Protecting the head with fungicides in seed production fields increases yield. It also can reduce the percentage of infected seed. Furthermore, seed treatment with fungicides can lessen the degree of infection by *S. nodorum*. Sanitary measures, such as decreasing seedborne inoculum, might also delay the start of an epidemic (26). Effective seed treatments combined with cultural practices that eliminate exposure to infested crop debris can further reduce infection of seedlings.

Summary

A comparison of different effective chemical control programs against septoria tritici blotch and septoria nodorum blotch is presented in Table 5. Table 6 lists the fungicides currently used in the chemical control of *S. tritici* and *S. nodorum*.

Table 5. Fungicides, rates, number of applications, thresholds, and application intervals of currently recommended chemical control programs against septoria tritici blotch (*Septoria tritici*) and septoria nodorum blotch (*Septoria nodorum*)

Fungicide	Rate (g/ha) (a.i.)	Number of applications	Threshold growth stage[1,2]	Application intervals (days)	Country
For *S. tritici*					
Maneb	2000	3-4	37-40	10-14	Israel
Mancozeb	1500	3	23	10-14	New Zealand
Chlorothalonil	166	3	23	10-14	New Zealand
RH2161	250	3	23	10-14	New Zealand
Benomyl	250	1-3	23	10-14	New Zealand
Benomyl	400	2	37-40	14-18	Israel
Benomyl	250-300	2	32-39		The Netherlands
Propiconazole	125	1-2	37-40	14-18	Israel
Propiconazole	125	2	32-39 +56-58	21-28	Fed. Rep. Germany
Triadimefon	125	3	23	10-14	New Zealand
Triadimefon	125	2	37-40	14-18	Israel
For *S. nodorum*					
Mancozeb	2250	3	32-39	10-14	U.S.A. (Florida)
Captafol	1600	1	56-58		Fed. Rep. Germany
Benomyl + Maneb	1600		56-58		Belgium, France, Fed. Rep. Germany
Propiconazole	250	2	43-45	30	U.S.A. (Texas)
Propiconazole		2	37-39	15	Fed. Rep. Germany
Prochloraz		1	37-39		Fed. Rep. Germany

1 Action threshold combined growth stage and 5% pycnidial coverage of *S. tritici* on flag leaf minus 3 or flag leaf minus 2 depending on cultivar height and vulnerability.
2 Growth stages according to Zadoks et al (154). See Figure 10.

Table 6. Fungicides used in chemical control of septoria tritici blotch and septoria nodorum blotch of wheat (chemical name, common name(s), and chemical composition)

Foliar applications

  Protectants

  Mancozeb (Dithane M-45, Fore, Manzate 200, etc.)
    (coordination complex of 16% manganese, 2% zinc, and 62% ethylenebisdithiocarbamate)

  Maneb (GR5, GX-101, Manex 4F, RM5, WB5, etc.)
    (manganous ethylenebisdithiocarbamate)

  Chlorothalonil (Bravo, Daconil, etc.)
    (tetrachloroisophthalonitrile)

  Captafol (Difolatan, Ortho Difolatan SK, etc.)
    (N-(1,1,2,2,-tetrachloroethylthio)-4-cyclohexene-1,2-dicarboximide)

  Systemics

  Benomyl (Benlate, Tersan 1991)
    (methyl-1-(butycarbamoyl)-2-benzimidazolecarbamate)

  Prochloraz (Sportak, BTS 40542, etc.)
    (N-propyl-N-(2-(2,4,6-trichlorophenoxy)ethyl)-imidazole-1-carboxamide)

  Propiconazole (Tilt, Banner, etc.)
    (1-(2-(2,4-dichlorophenyl)-4-propyl-1,3-dioxolan-2-ylmethyl)-1H-1,2,4-triazole)

  Triadimefon (Bayleton, etc.)
    (1-(4-chlorophenoxy)-3,3-dimethyl-1-(1H-1,2,4- triazole 1-yl)-2-butanone)

New chemicals

  Fenpropimorph (Corbel, etc.)
    (4-(3-(4-(1,1-dimethyl-ethyl)phenyl)-2-methyl) propyl-2,6-cisdimethylmorpholine)

  HWG 1608

  Myclobutanil (RH3866)
    (butyl-4-chlorophenyl-1H-1,2,4-triazole-1- propanenitrile)

Seed treatment of *S. nodorum*

  Triadimenol (Baytan, Summit, BAY KWG 0519, etc.)
    (β-(4-chlorophenoxy)-α -(1,1-dimethylethyl)-1H- 1,2,4-triazole-1-ethanol)

  Thiabendazole (Mertect, etc.)
    (2-(4-thiazolyl) benzimidazole)

  Nuarimol (Trimidal, EL-228, TF-3635, TF-3645, etc.)
    (α -(2-chlorophenyl-α -(4-fluorophenyl)-5-pyrimidinemethanol)

  Vitaflo 280 (carbathiin 14.9% + thiram 13.2%)

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Glossary

Agar—A gelatin-like material obtained from seaweed and used to prepare culture media on which microorganisms are grown.

Aggressiveness—A measure of the rate at which a virulent isolate produces a given amount of disease.

Apex—The tip or top.

Ascomycetes—A group of fungi producing their sexual spores (ascospores) within a sack—ascus.

Ascospore—A sexually produced spore borne in an ascus.

Ascus—A sack-like hypha usually containing 8 ascospores (pl. asci).

Asexual reproduction—Any type of reproduction not involving the union of gametes or meiosis.

Attenuate—To decrease in pathogenic activity.

Blotch—A disease characterized by large and irregularly shaped spots or blots on leaves, sheaths, stems, or glumes.

Boot—Sheath or portion of leaves enclosing the inflorescence.

Budding—A method of vegetative propagation of conidia from the mother cell as with *S. tritici* grown in liquid shake culture.

Caryopsis—Seed.

Chlorosis—Yellowing of normally green tissue due to chlorophyll destruction. The first type of symptoms prior to necrosis and pycnidial formation following infection with *Septoria* spp.

Cirrhus—A ribbon-like group of spores discharged through the ostiole.

Coleoptile—Protective sheath surrounding the primary leaves.

Conidium—An asexual fungal spore formed within an asexual fruiting body or on artificial culture medium.

Cultivar—Cultivated variety.

Culture medium—The prepared food material on which microorganisms are cultured.

Desiccation—Drying up.

Disease—Any disturbance of a plant that interferes with its normal structure, function, or economic value.

Disease cycle—The chain of events involved in disease development, including the stages of development of the pathogen and the effect of the disease on the host.

Dispersal—The movement of fungal units from the place where they are formed to the place where they may be active, e.g., rain-dispersed pycnidiospores and air-dispersed ascospores.

Epidemiology—The science of disease in populations, study of the development and spread of disease and of the factors affecting these processes.

Epistasis—Interaction between genes at different loci.

Exudate—Liquid or gel-like discharge from diseased or healthy plant tissue or the actual discharging of this liquid.

Fluorescence—Emission of light.

Fruiting body—A complex fungal structure containing spores (pycnidium, pseudothecium).

Fungicide—A compound toxic to fungi.

Fungus—An undifferentiated plant lacking chlorophyll and conductive tissues.

Gene—A material substance in the chromosome which determines or conditions one or more hereditary characters. The smallest functioning unit of the genetic material.

Genotype—The genetic constitution of an organism especially as distinguished from its appearance or responses.

Germination—The process in which a dispersal unit (pycnidiospore, ascospore), under specific environmental conditions, assumes increased metabolic activity, resulting in the production of new structures, most often the germ tube.

Haploid—A cell or an organism whose nuclei have a single complete set of chromosomes.

Hemacytometer—Special glass slide used to count spores (counting chamber).

Host—A living organism on or in which a parasite lives and from which the parasite obtains its sustenance (e.g. wheat plant).

Host range—The various kinds of host plants that may be attacked by a parasite.

Hypersensitivity—Excessive sensitivity of plant tissues to certain pathogens or isolates. Affected cells are killed quickly, blocking the advance of obligate parasites.

Imbibition—Absorption of water.

Immune—Free from infection by a given pathogen.

Incubation period—Period of time between penetration of a host by a pathogen and the first appearance of symptoms on the host.

Infect—To establish a pathogenic relationship with a host plant.

Infest—To introduce a pathogen into the environment of a host.

Inoculate—To introduce pathogen propagules on or into a host for the purpose of producing infection for testing susceptibility to infection.

Inoculum—A collection of pathogen propagules capable of initiating disease or introduced for that purpose.

Isolate—A single spore or pure culture and the subcultures derived from it.

Latent period—The time elapsed from arrival of pathogen propagules at a susceptible plant surface until the first formation of the next generation of dispersal units (spores).

Lesion—A discoloration of the host tissue around the point of invasion.

Life cycle—The sequence of stages between a spore form and its recurrence.

Ligule—Thin outgrowth at junction of leaf sheath and leaf blade.

Linkage—Association of genes because they are located on the same chromosome.

Lyophilization—Freeze drying.

Micron (μ)—A unit of length equal to 1/1000 of a millimeter

Millimicron (μm)—A unit of length equal to 1/1000 of a micron.

Mesophyll—The leaf tissue cells between epidermal layers.

Mycelium—The hypha or mass of hyphae that make up the body of a fungus.

Necrotic—Dead and discolored.

Ostiole—Opening in pycnidium through which pycnidiospores exudate from the fruiting body.

Parasite—An organism living on or in another living organism (host) and obtaining its food from the latter.

Pathogen—An organism able to cause disease.

Pathogenicity—The relative capability of a pathogen to cause disease.

Perithecioid pseudothecium—The ascocarp of the Loculoascomycetes, perithecioid in shape with an opening at the top.

Phenotype—The physical makeup of an individual resulting from the interaction of genotypic characters and environment.

Physiologic race—One of a group of forms that are alike in morphology but unlike in certain cultural, physiological, biochemical, pathological, or other characteristics.

Pleiotropy—Multiple effects of a single gene influencing more than one character.

Protectant—A substance that protects an organism against infection by a pathogen.

Pycnidiospore—Asexual spore borne in a pycnidium.

Pycnidium—An asexual, spherical, or flask-shaped fruiting body in which pycnidiospores are produced.

Resistance—The ability of a host to overcome, completely or in some degree, the effect of a pathogen or damaging factor.

Resistant—Possessing qualities that hinder the development of a given pathogen.

Septum—A cross wall in a hypha or spore.

Sexual state—The state of the life cycle in which sexual spores (ascospores) are formed after nuclear fusion or by parthenogenesis.

Surfactant—Compound which reduces surface tension of liquids.

Susceptible—Lacking the inherent ability to resist disease or an attack by a given pathogen.

Symptom—The external and internal reactions or alterations of a plant as a result of a disease.

Systemic—A chemical substance absorbed into the plant through roots or foliage.

Tolerance—The ability of a plant to endure (sustain) the effect of a disease without showing severe reduction in economic yield.

Vernalization—Exposure to a period of cold to initiate flowering.

Virulence—The degree or measure of pathogenicity.

Virulent—Capable of causing a severe disease; strongly pathogenic.