Pathogenesis of Septoria nodorum (Berk) Berk. on wheat cultivars varying in resistance to glume blotch
by Mary Louise Straley

A thesis submitted in partial fulfillment of the requirements for the degree of DOCTOR OF PHILOSOPHY in Plant Pathology
Montana State University

Abstract:
Septoria nodorum, causal agent of glume blotch of wheat, was inoculated onto intact leaves of spring and winter wheats that exhibited varying degrees of resistance to the pathogen. Inoculated leaves were removed at 24 hour intervals up to 192 hours after inoculation for observations of nodorum germination, penetration, and disease development. Germination and penetration by the spores on the host tissue were the same regardless of the degree of host resistance with the exceptions of Fortuna and Manitou. Spore germination on Fortuna, a susceptible cultivar, was significantly higher than on the other cultivars. Spore germination on Manitou, a resistant cultivar, was significantly lower than on the other cultivars. Contrary to previous reports stomatal penetration was observed. Sto-matal penetration occurred either through open or closed stomata, but did not occur prior to 72 hours after inoculation. Trichome penetration, also was observed although infrequently. Stained thin-sections, fluorescent whole mounts, and scanning electron microscope examinations were used to monitor the development of the pathogen within the leaf. Such development occurred both intra- and inter-cellularly with the latter predominating. Hyphae could be distinguished within lesion areas in resistant cultivars and both within and outside of lesion areas in susceptible cultivars. The degree of intercellular ramification and cellular disintegration was correlated with host resistance. Wheat leaves were analyzed for the presence of fungal growth stimulators and inhibitors. No active compounds were detected with the experimental procedure used. The degree of water congestion in a cultivar was correlated with disease resistance and the amount of free intercellular water was important in this disease. Several reasons for the importance of this intercellular water were postulated.

PATHOGENESIS OF SEPTORIA NODORUM (BERK) BERK. ON WHEAT CULTIVARS VARYING IN RESISTANCE TO GLUME BLOTCH

by

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ABSTRACT

Septoria nodorum, causal agent of glume blotch of wheat, was inoculated onto intact leaves of spring and winter wheats that exhibited varying degrees of resistance to the pathogen. Inoculated leaves were removed at 24 hour intervals up to 192 hours after inoculation for observations of S. nodorum germination, penetration, and disease development. Germination and penetration by the spores on the host tissue were the same regardless of the degree of host resistance with the exceptions of Fortuna and Manitou. Spore germination on Fortuna, a susceptible cultivar, was significantly higher than on the other cultivars. Spore germination on Manitou, a resistant cultivar, was significantly lower than on the other cultivars. Contrary to previous reports stomatal penetration was observed. Stomatal penetration occurred either through open or closed stomata, but did not occur prior to 72 hours after inoculation. Trichome penetration also was observed although infrequently. Stained thin-sections, fluorescent whole mounts, and scanning electron microscope examinations were used to monitor the development of the pathogen within the leaf. Such development occurred both intra- and inter-cellularly with the latter predominating. Hyphae could be distinguished within lesion areas in resistant cultivars and both within and outside of lesion areas in susceptible cultivars. The degree of intercellular ramification and cellular disintegration was correlated with host resistance. Wheat leaves were analyzed for the presence of fungal growth stimulators and inhibitors. No active compounds were detected with the experimental procedure used. The degree of water congestion in a cultivar was correlated with disease resistance and the amount of free intercellular water was important in this disease. Several reasons for the importance of this intercellular water were postulated.

INTRODUCTION

Glume blotch of wheat (Triticum aestivum) caused by the fungus Leptosphaeria nodorum Müller, is an economically important disease throughout the wheat growing areas of the world. As more areas are converted to wheat production this disease could have an even greater impact on potential crops. The correct name for the fungus is Leptosphaeria nodorum Müller (after its perfect stage), although the pathogen is commonly referred to by its imperfect name, Septoria nodorum (Berk) Berk. The disease is commonly called Septoria glume blotch.

In recent years this disease has become increasingly prevalent due to the phenotypic, cultural, and genotypic vulnerability of the cultivars grown. Phenotypically shorter and leafier cultivars are more susceptible to the disease because of increased canopy moisture (90). Increased usage of fertilizers often combined with irrigation has also contributed to the increase in incidence of this disease. These same factors combine to increase the density of the canopy and hence increase canopy moisture. Until recently there has been available little genetic resistance to Septoria glume blotch, and most cultivars grown have been susceptible under proper environmental conditions. Genetically controlled disease resistance has been investigated extensively in recent years and sources of resistance have been found (75). In the case of glume blotch, disease resistance is

of a general types with no major dominant genes being found to date.

This study was undertaken to understand more fully the glume blotch resistance mechanisms functioning in wheat. Comparisons of the disease progression in resistant, intermediate, and susceptible cultivars of wheat were made. These comparisons involved monitoring the activity of the pathogen on the leaf surface as well as within the leaf tissue. It is hoped from this study that the mechanism(s) of resistance by wheat to S. nodorum may be more clearly understood and defined.

LITERATURE REVIEW

DISEASE ORGANISM

The initial identification of the pathogen causing glume blotch of wheat was by Berkley in 1845 (86). Subsequently, the disease and pathogen were reported from all major wheat growing areas of the world. It was not until 1898, however, that economic losses attributable to the disease were studied and published (80). Since then minor to severe epidemics have been reported in all wheat growing regions of the world with losses ranging from 5-50% (40).

The causal organism was described by Berkley and named Septoria nodorum in 1945 (80). Orginally Berkley suggested that the organism attacked only the nodes and internodes of the host plant. Later Passerinii (66) described a similar organism which appeared on the glumes of wheat. Passerinii named this organism Septoria glumarum. It was later determined by Grove (86) that S. nodorum and S. glumarum were the same organism and the earlier name, S. nodorum, was retained. Although perithecia were found associated with the fungus as early as 1904, it was not until 1952 that Müller described Leptosphaeria nodorum as the perfect stage of S. nodorum (80).

The mycelium of S. nodorum is typically branched, septate, and hyaline although later it may turn a dark olivaceous color (19). In artifical culture there is considerable variation in growth habit, color, sporulation, and pathogenicity (74). Pycnidia and pycnidiospores form readily either in culture or on host material. The

pycnidia are irregular in shape, subepidermal, and dark in color (74), although recently aerial pycnidia have been seen (73). Pycnidiospores, released through the ostiole are bacillary, 1-3 septate, hyaline, and average 3 x 26 u in size (63). Each cell within the spore contains one nucleus (79). The spores exude from the pycnidia in long pink serpentine strands (53, 86).

Perithecia, formed on host plant material, contain numerous club-shaped, eight-spored, bitunicate asci (53). The ascospores are straight to slightly curved, three-septate, fusoid, with the second cell from the apex enlarged. All of the cells contain one or two prominent guttules, are hyaline to pale-yellow brown, and average 5 x 27 u. Perithecia have been formed in culture on oat extract agar (53). Micropycnidia, producing microspores, have also been observed (77).

Septoria nodorum has been reported on plants in 17 genera (78, 82) including wheat, barley, oats, and various wild grasses. There is little evidence of strict host specificity (80). However, it has been reported that isolates from one host are usually less virulent on other hosts (44, 80).

THE DISEASE

On wheat the organism is pathogenic on all above ground plant parts (63) and can infect anytime from seed germination to plant

maturity (80). Septoria nodorum may be seed-borne and cause characteristic seedling infection (4, 14, 29, 87). Symptoms include stunted and deformed coleoptiles (4). Also, there may be brown streaks arising from the base of the coleoptiles. When browning is severe enough death of the seedling may result (71). Plants generally survive although yield is often decreased (80). Under high relative humidity, the infection on the coleoptiles has been reported to produce pycnidia which can act as an inoculum source later in the growing season (31). Other researchers could find no evidence of pycnidia in coleoptile lesions (14, 29). Infection results when subpericarpic mycelium emerges from under the seed-coat and penetrates the epidermal cells of the coleoptile (4).

The more typical disease cycle begins with infection of growing plants by pycnidiospores produced in plant debris (28, 33, 88). The cirrhi are produced during wet periods and the pycnidiospores are spread by splashing or wind-blown rain (71, 72). Spore germination and penetration are maximum between 15^{o} and 25^{o}C with a minimum of six hours of wetness (98% RH) necessary for good infection (90). Secondary infection takes place during periods of wet, windy weather and prolonged favorable environmental conditions may cause epidemics. The primary source of inoculum is from pycnidiospores produced on plant debris with secondary hosts and the perfect stage playing a

relatively minor role in this disease. Infected plant debris may produce several crops of pycnidiospores with alternate wet and dry periods (72), thus providing for an efficient continual source of primary inoculum.

Light green to yellowish pathces between veins on the leaf or leaf sheath are typically the first symptoms of S. nodorum infection (78). Later symptoms on infected leaves are yellow to brown elongate lesions which tend to be slightly darker in the center (5, 80). Lesions may appear 2-22 days after inoculation depending on environmental conditions (90). There is evidence for toxin production by the fungus as the necrosis extends well beyond the colonized cells (9, 48). The toxin produced by S. nodorum is non-specific in nature affecting both susceptible and resistant cultivars as well as non-host plants. When artifically inoculated onto plants this toxin produces characteristic yellow elongate elsions with dark brown centers. Pycnidia normally occur in the center of the lesions, although they may be scattered throughout the leaf tissue (19). On the culm, sheath, and rachis the lesions are generally dark and coalesce readily to form elongate brown to dark brown colored areas. Pycnidia may also form on these areas in 10-21 days after inoculation (80).

Yield loss from Septoria infections is due to improper filling of the seed, with a decrease in seed numbers playing a less important role (5, 51, 79). Very severe early infections may result in decreased

tiller numbers thus reducing yield (4). Ecnonmic losses from Septoria range from 1-2% for light infections and up to 50% for severe infections if infection occurs before heading (80, 90). Infection occuring after heading cause no decrease in yield. However, they may result in infected seed. Several methods have been developed for assessing the degree of infection and yield losses due to S. nodorum (51, 52, 55, 76).

The influence of age of plant tissue, temperature, light, relative humidity, and spore concentration on the incidence of S. nodorum have all been studied. Typical infections may occur in tissue of any age (70). However, more yield loss is associated with infections in older tissue (16, 17, 32, 46, 80). Infections in seedlings (resulting from infected seed) can cause death, although plants frequently outgrow the disease (3, 4). Invasions by the pathogen in plants of various growth stages result in infections which may or may not be damaging to yield of grain depending upon subsequent environmental conditions (16, 17, 22, 32, 61). Light is said to be necessary for pycnidiospores to germinate (61) and Baker and Smith (6) report that light is necessary for full development of symptoms subsequent to successful invasion by the pathogen. Little work has been done concerning the effects of different light quantities or qualitites on S. nodorum invasion and infection processes, although Benedict (7) studied this problem with the related S. tritici, and Cooke (15) studied the effect of near-ultraviolet

light on S. nodorum sporulation in culture.

Considerable more work has been done on the effect of dew or post-inoculation wetness periods on the disease development (11, 22, 32, 61, 71). It has been found that this is a critical controlling factor in the initiation of the disease (71) as well as the continued development of the disease within infected tissue (22, 32, 61). Even after successful invasion and colonization of host tissue by S. nodorum further progression of the disease is limited by the relative humidity surrounding the infected plant (32, 61). Differing dew period lengths can greatly influence the reaction of a cultivar to the disease (22, 75). Determining the proper length of dew period for successful infection is necessary when screening plants for disease resistance. It is necessary to increase the lengths of post-inoculation dew periods as resistance levels are increased in order to detect even higher levels of resistance in plant populations (75). In several other leaf spotting fungal diseases, resistance to the pathogen is directly correlated with post-inoculation dew periods (34, 35, 36, 37, 38). For some host-parasite interactions, the "expression of susceptibility or resistance to leaf spotting was associated with the duration of the wet period" (36).

Another important aspect of the disease etiology is the effect of spore concentration on disease development. There is general agreement that increasing spore concentrations increases disease

development (5, 12). Increasing the spore concentration will, however, have an unequal effect on resistant and suceptible cultivars (5, 12). The quantitative differences between reactions on the susceptible and resistant cultivars will become greater. With decreasing spore loads, it is possible to lower the concentration to a point where no reaction is elicited in resistant cultivars (5). From artifical inoculation studies the amount of surfactant or sticker has been shown to play an important role. The addition of a surfactant may allow a similar degree of symptom development to remain the same while the total spore concentration in the inoculum is lowered (45). The most frequently used concentrations are between 1×10^6 and 1×10^7 spores/ml and plants are generally sprayed with this suspension to "run-off" (11, 22, 46).

Some degree of control for this disease has been achieved by cultural practices (stubble and volunteer control), use of disease-free seed, seed treatment, and crop rotations (19, 90). Disease resistant cultivars are also currently being used, although none can be classified as being highly resistant (49, 74). Lines and cultivars having increased levels of disease resistance are being developed for future use (5, 75). The type of resistance being incorporated currently into wheat lines is described as general resistance rather than specific resistance.

DISEASE RESISTANCE

The terms general and specific resistance can be correlated with Van der Plank's (85) concepts of horizontal and vertical resistance respectively. As originally defined by Van der Plank the term horizontal is used when resistance is evenly spread against all races of the pathogen while vertical is when a variety is more resistant to some races of a pathogen than others and differential interactions are found (85). These types of resistance are differentiated from tolerance in that tolerance implies the ability of a plant to yield normally even while severly infected (69). Specific and general resistance imply some type of restriction of the pathogen's ability to either 1) successfully invade the host or 2) develop normal disease expression in the plant (57). In most disease resistance breeding either of the two "resistance" types are used in preference to tolerance as it is more difficult to measure the tolerance levels while resistant reactions are usually more dramatic (69). Also, it is the feeling of some researchers that tolerant varieties provide for a continuous source of inoculum which could then infect other cultivars (10, 84).

Specific resistance has often been equated with the presence of a single major gene which is effective against a particular race of pathogen. Once a gene for specific resistance has been incorporated

into the cultivar the particular race of the pathogen is no longer able to cause disease (10, 85, 86). This type of resistance is easy to follow genetically since it is simply inherited and is relatively insensitive to environmental changes (56, 85). A disadvantage of specific resistance is that genetic changes in the pathogen often result in a "new" physiological race which is then able to infect previously resistant plants (10, 56, 86).

General resistance is more difficult to work with since its effects do not fall into easily categorized classes, but rather show a lessening of lesion development, a lessening of spore production, or some other restriction placed on pathogen growth and reproduction (10, 86). Thus increases or transfers of this resistance often confer small changes which are difficult to measure and/or quantify. General resistance is usually more effective against all races (or pathogenic virulence types) of the pathogen (85). Also, it is considerably more stable in its reaction to genetic changes in the pathogen (10, 85, 86). The expression of general resistance, however, shows a high degree of sensitivity to environmental changes. Some researchers feel that general resistance is controlled by polygenic action with many "minor genes" contributing to the overall resistant reaction (10, 56).

Recently Nelson (56) has disagreed with the terms horizontal,

vertical, major gene, and minor gene in relation to disease resistance. He says that vertical (major gene, specific) resistance may confer elements of horizontal (minor gene, general) resistant reactions to some races of the pathogen and that horizontal resistance factors are not necessarily spread evenly over all races of a pathogen. Also, that a single major gene in a different genetic background may act like a minor gene or the converse. Because of these factors Nelson (56) has proposed that the two types of resistance be called by new terms. He refers to horizontal resistance as a rate-reducing resistance and vertical resistance as a resistance that reduces the amount of effective initial inoculum. He contends that there are no major or minor genes for resistance but simply resistance genes which may act in a "horizontal" manner when combined together and/or in a "vertical" manner when appearing singly in the appropriate genetic background.

Resistance to S. nodorum has been sought by researchers in all wheat growing areas of the world (2, 5, 47, 49, 50, 59, 75). Varietal differences, although they occur, are influenced by environmental changes which makes utilizable heritable reactions hard to definitely establish (59). Among those tested, spring wheats are more susceptible than winter wheats and T. aestivum is more susceptible than some of the other Triticum spp (49). Various inoculation

and screening techniques have been tried in order to standardize conditions so that repeatable results will allow for the selection of plants which show true resistance (2, 11, 47, 49, 50). Greenhouse screening of seedlings at the three leaf stage (Feekes' Scale 1) has proven effective and correlates well with field results (49, 50, 75). This type of screening procedure gives more repeatable results because it is possible to duplicate the environmental conditions. It also reduces the number of plants that need to be screened in the field (49). Through various screening procedures disease tolerant or resistant cultivars have been detected and studies are presently being done to determine the type of gene action(s) and mode of inheritance they exhibit (5, 75). Tolerance to S. nodorum has been worked with extensively (5). Other workers have reported resistance that is due to dominant, partially dominant, or recessive gene action (5, 49, 80). Progeny of diallel crosses of resistant and susceptible lines have been studied (50, 73). Laubscher et al. (50) found there was a large number of resistant types in the progenies of the most resistant parent except when crossed with the most susceptible. Susceptible individuals comprised the majority of the progenies from the susceptible parent except when it was crossed to the most resistant. They concluded from their study there was workable heritable resistance to S. nodorum in wheat. Scharen (75) and Krupinsky (49) have

both shown that through a selection, crossing, and reselection program that levels of resistance may be increased in succeeding generations of wheat lines. None of these studies have shown evidence for major dominant gene action, but instead show a quantitiative type of inheritance for resistance to S. nodorum. Because of the resistance levels found in Triticum spp other than T. aestivum, efforts are being made to transfer resistance genes into T. aestivum lines. This is being done by the selection, crossing, selfing, reselection scheme mentioned above (75). Agronomic traits also are being evaluated under field conditions. Evidence for transgressive segregation in cultivars being studied (49, 73) suggests that this phenomenon may be of major importance in the development of resistant lines for the future.

HISTOLOGY

Several histological studies have been made on non-haustoria forming, non-obligate leaf spotting fungi (1, 8, 13, 30, 39, 58, 81, 83). Penetration was direct (through the cuticle), between adjacent epidermal cell walls, or through the stoma, and accompanied or not by the formation of appressoria depending upon the pathogen. The activities of pathogens on leaf surfaces have been monitored (1, 58, 83) and a variety of techniques have been developed to study intra- and intercellular fungal growth (18, 24, 60, 64, 65, 68). Also,

researchers have looked at the differential responses of susceptible and resistant cultivars to invasion and colonization by pathogens (23, 24, 26, 58, 81). These papers have described resistant reactions in main categories. The first type of resistance mechanism is the hypersense reaction in which the host cells die rapidly with successful fungal invasion limiting further spread (10). The second type of mechanism results in the slowing down of the pathogen's invasion, colonization, and sporulation processes in the resistant cultivar (10). There are instances where both types of resistance mechanisms are manifested in the same host-pathogen complex (89).

With Septoria, less histological work has been done. However, in 1957 Green and Dickson (23) published a thorough cytological study of S. passerinii, causal agent of Septoria leaf blotch of barley. They found, in contrast to previous reports (86), that S. passerinii rarely exhibited direct cuticle penetration and the primary mode of penetration was through stomata. They reported that the disease seemed to progress along a "stomatal gradient". Reactions to this organism by susceptible and resistant varieties were studied and it was found that the susceptible plants allowed the pathogen to grow freely through the leaf tissue. Host cell death did not affect the pathogen's ability to spread. In contrast, the host-parasite interaction in resistant plants was deleterious to both

the host and the pathogen. The intermediate reaction showed aspects of both susceptible and resistant reactions. Some of the hyphae were able to spread beyond the areas of cellular disruption, although not as frequently as in the susceptible reaction. Benedict (7) reported that S. tritici primarily penetrated through the stomata of wheat leaves, but did not study further development of the pathogen within host tissue.

The initial cytological study of S. nodorum infection of wheat was done by Weber (86). He found that Septoria entered the host by direct penetration of the cuticle in the depressions directly above adjacent epidermal cells. Shipton (80) and Brown (12) later indicated that stomatal entry had never been observed. After penetration the hyphae grew between the epidermal cells and did not branch until they had come in contact with the "parenchymatous cells" below the epidermis, Weber reported (86). The hyphae then grew parallel to the leaf surface and often occurred in the crevices formed by the epidermal cells running parallel with the vascular bundles. Host cells in the vicinity of the invading hyphae appeared normal until all the intercellular spaces had been filled with hyphae. He also found aggregations of hyphae in the substomatal cavities which initiated the formation of pycnidia. The pycnidia were formed so that their ostioles were directly beneath the opening of the stoma.

Baker and Smith (6) recently have completed a study on the development of resistant and susceptible reactions in wheat when inoculated with S. nodorum. They used light and scanning electron microscopy in interpreting the reactions elicited by the pathogen in the two wheat cultivars, Maris Ranger (susceptible) and Engelen (resistant). Engelen, however, has recently been found to be susceptible when screened against isolates used by Shcaren (73). Detached leaves were observed, by Baker and Smith, at specific intervals of time after inoculation for pathogen development and host cell response. They evaluated the effects of various spore concentrations, light regimes, and temperature regimes on disease progression. They found that lesion development was in direct correlation to the spore concentration in both varieties. A lower limit of spore concentration was eventually reached in the resistant cultivar. At all three temperatures studied (18, 20, 25^{o}C) lesions developed, but 20^{o}C was optimum for total numbers and speed of development. Incubation in darkness gave more restricted lesion development and was necessary for development of the discoloration reaction. The discoloration reaction occurred in both wheat lines although it occurred first and most intensely in the resistant cultivar. The major differences between the resistant and susceptible reactions noted are summerized in the following chart:

	Resistant	Susceptible
Spore germination	Same	Same
Spore penetration	Lesser	Greater
Necrosis	Noted first, but lesser in final degree	Eventually greater in degree and spread
Hyphal development	Lesser	Greater
Sporulation	Negative (for time period studied)	Positive

No differences in spore germination on the leaf surfaces were observed between the two cultivars, although penetration occurred more frequently on the susceptible leaf pieces. This was demonstrated by a differential degree of epidermal cell staining. Penetration, reportedly, took place directly beneath appressoria which formed at the junctions of cell walls.

Necrotic browning was noticed in the resistant variety as early as 72 hours after inoculation. The necrosis which did not affect epidermal cells was observed in the intercellular spaces of the sub-epidermal cells. This necrosis appeared to be in conjunction with the veins, although the vascular bundles themselves seemed to be normal. The necrosis in the susceptible leaf differed by involving whole cells, not just intercellular spaces, and within 96 hours after inoculation extended from the inoculated leaf surface to the opposite side. Intercellular hyphal development appeared less extensive in

the resistant reaction, although this was not quantified. Hyphae in the resistant reaction tended to ramify intercellularly along the length of the leaf axis. After 144 hours of incubation no hyphae could be detected within or surrounding the lesion. In contrast, hyphal development in the susceptible leaf occurred both inter- and intracellularly, was heavily branched, and could be detected in advance of susceptible lesions after 240 hours.

Recent histological work with four cultivars of wheat has shown no observable reactions to infection in the leaves of the susceptible cultivars (26). The researcher postulated that the fungus may rapidly disrupt the normal physiological/biochemical processes and thus stop the formation of a normal histological defense mechanism. Following penetration, colonization of the host was intercellular and typically was characterized by chlorosis and necrosis of colonized tissue. Tissue in advance of the zone of colonization also exhibited chlorotic and necrotic reactions. In contrast to previous reports, Harrower (26) did observe penetration through open or closed stomata.

Although resistance to S. nodorum has been found and utilized it was not known what exact mechanism of resistance was functioning. The progression of the disease in wheat cultivars of varying resistance was therefore studied to determine what these mechanisms were. The pathogen-host interaction was studied at both the leaf surface as

well as the internal level to determine exactly when and where the resistant reaction occurred. Often when specific defense mechanisms are defined and understood it is possible to make more efficient use of these mechanisms in developing disease resistant cultivars. Also knowledge gained about defense mechanisms operating in one host-parasite interaction may have implications in understanding other host parasite interactions. It was for these reasons that this research project was undertaken.

MATERIALS AND METHODS

GENERAL PROCEDURES

Eighteen wheat cultivars (Table 1) were grown in 7.5mm plastic pots filled with washed sand and watered with non-nutrient water until ten days after seeding (Feekes Scale 1). Plants were maintained in a growth chamber with a 12 hour day temperature of 15°C and night temperature of 23°C. Plants were watered from the bottom of the pot in order to minimize interference from other foliar pathogens. Seed lots were the same throughout the study. Eighteen cultivars were used as the base population and selected cultivars were used in individual experiments.

The S. nodorum culture used in this study was obtained from a single-spore isolate of a known virulent culture. The isolate was chosen for its ability to maintain sufficient, uniform spore production through repeated transfers. Reculturing from infected Fortuna (CI 13596) leaves was done occasionally to make sure that the continously transferred cultures remained the same as the original isolate. Yeast-malt agar plates were inoculated with S. nodorum by flooding with a suspension of pycnidiospores obtained from one week old cultures. These were maintained under 20 hours of light at 15°C for seven days prior to utilization. Inoculum was prepared by gently scraping the mycelia and pycnidia from the surface of seven-day old plates, mixing with 60ml distilled water for one minute in a Waring

Table 1. Cultivars of wheat with habit and disease reaction to *S. nodorum*

Cultivar	Triticum Species	C.I. Number	Disease 1/ Reaction	Habit 2/
Fortuna	aestivum	13596	S	Spr
Newana	aestivum	17430	I	Spr
Manitou	aestivum	13775	R	Spr
Redhart	aestivum	8898	R	W
Red Chief	aestivum	12109	R	W
CI 12373	aestivum		R	W
CI 14032	aestivum		R	W
Oasis	aestivum	15929	R	W
Arthus 71	aestivum	15282	R	W
Centurk	aestivum	15075	S	W
Michigan Amber	aestivum	11379	S	W
Hadden	aestivum	13488	I	W
Blueboy	aestivum	14031	I	W
Mt 7406	aestivum		I	W
Mt 74394	aestivum		I	W
CVLO 2204	aestivum	13554	R	W
Yamhill	aestivum		R	W
Zenati-Boutielle	durum		VR	Spr

1/ S = susceptible, I = intermediate, R = resistant, VR = very resistant

2/ Spr = spring, W = winter

Blender, and filtering through doubled cheesecloth. Spore counts were made with the aid of a haemocytometer and the final concentration was adjusted to 20 x 10^6 spores/ml (allowing ten mls per pot of wheat). One drop of surfactant (Ivory soap) was added to the spore suspension which was atomized into the plants.

Unless otherwise stated all plants were allowed a pre-inoculation dew period of four hours and a post-inoculation dew period of 48 hours. When possible plants were inoculated in place in the dew or mist chamber itself. It was necessary during the germination studies to remove the plants from the dew chamber before inoculating them. In this case all plants were moved as a unit with special attention being paid to cause as little of the dew to run off as possible. Disease ratings were made seven days after inoculation. In addition some material was kept for 25 days to study the production of pycnidia. In all cases appropriate control plants were used and assessed for disease reactions at the same time as inoculated plants.

SPECIFIC PROCEDURES

The optimum length of time for pre- and post-inoculation dew periods was determined for four cultivars (Table 2). Pre-inoculation dew periods were assessed by placing ten day old seedlings in a Percival Dew Chamber with a water bath temperature of 35°C and a wall temperature of 7°C (air temperature was 20°C). Plants were given

Table 2. Wheat cultivars used in pre- and post-inoculation dew period studies.

Cultivar	Triticum Species	C.I. Number	Disease Reaction	Habit
Fortuna	aestivum	13596	S	Spr
Manitou	aestivum	13775	R	Spr
CI 14032	aestivum		R	W
Blueboy	aestivum	14031	I	W

preinoculation dew periods of 0, 2, 4 hours, inoculated, and then samples were removed at 4, 12, and 24 hours after inoculation. Germination counts were made on the oldest leaf from each of three seedlings in each pot and replicated twice in a completely random design. Leaves were cut into 10mm pieces (tip and base ends were discarded), placed in a lactophenol-trypan blue solution (43) for four hours, and mounted in glycerine on microscope slides. All germinated and ungerminated spores were counted on five seperate areas on each leaf. Each area consisted of a section 0.516 mm across the width of the leaf blade.

Dew length periods after inoculation were studied using the same four cultivars and techniques as above. A four hour pre-inoculation dew period was used and samples were removed at 24, 48, and 72 hours of post-inoculation dew periods.

EXTENDED DEW PERIODS

Sixteen pots each of ten day old Fortuna, Newana, and Manitou seedlings (15 plants/pot) were subjected to extended pre- and post-inoculation dew periods in each of two replicated experiments in a completely random design. Pre-inoculation dew periods of 4, 12, 24, and 48 hours were used with pots removed 24, 48, 72, and 96 hours after inoculation. The plants were all inoculated at the same time with the standard inoculum previously described. Ten days after inoculation

all pots were scored for severity of disease symptoms on the primary leaf. Disease severity was recorded as average percent necrosis on ten primary leaves of each cultivar. Percent necrosis was recorded as the length of the necrotic region of each leaf/total length of the leaf x 100.

SPORE GERMINATION

All 18 cultivars of wheat were used in spore germination studies. Pre- and post-inoculation dew periods were achieved in a Percival Dew Chamber set as described above. Germination counts were made at 2, 4, 8, 12, 24, and 48 hours after inoculation and were replicated three times in a completely random design. During germination observations irregular germination, degree of germ tube elongation, branching, and formation of appressorial structures were all noted.

HISTOLOGICAL EXAMINATIONS

For the histological experiments, which were replicated twice, a greenhouse bench mist chamber provded the pre- and post-inoculation dew periods. The mist chamber was constructed of a wooden frame surrounded by a covering of clear vinyl plastic. An air seal was achieved on three sides of the plastic-greenhouse bench junction by burying the end of the plastic in the sand. The remaining side of the plastic was loosely fastened. Mist was provided by three "Walton-type" cold water humidifiers. Leaf samples were removed from cultivars

listed in Table 3 at 24 hour intervals from 24 and 196 hours after inoculation these were cut into 5mm sections and immediately immersed in FAA fixing solution (41). Other samples were removed 25 days after inoculation to study formation of the pycnidia. Leaf pieces in FAA were vacuum-infiltrated to assure complete fixation. After fixing overnight, samples were taken through a t-butyl alcohol graded dehydration series (41), equlibrated overnight in a t-butyl alcohol-paraffin oil solution (50-50 v/v), infiltrated with Paraplast (four changes), and subsequently Paraplast embedded in Tissue-Tek plastic mounts. After initial trimming of the blocks, samples were allowed to soften for two weeks in a solution of glycerin-water (50-50 v/v) before sectioning on an A-O Model 820 rotary microtome. The ten u serial sections were affixed to slides with Haupt's adhesive (41), dried overnight on a warming tray (43^{o}C) and stained with one of the following 1) a Safranin-Fast Green combination; 2) Johansen's Quadruple Stain; or 3) Conants' Quadruple Stain (41). After staining cover slips were affixed with Permount and slides were examined with a Zeiss Binocular Research Microscope Model Standard 14 equipped with a 35mm camera and optivar magnification changer. Extachrome Tungsten 160 and Panatomic X film were used to record development of the pathogen within leaf tissue.

Table 3. Wheat cultivars used for histological studies.

Cultivar	Triticum Species	C.I. Number	Disease Reaction	Habit
Fortuna	aestivum	13596	S	Spr
Newana	aestivum	17430	I	Spr
Manitou	aestivum	13775	R	Spr
Centurk	aestivum	15075	S	W
Michigan Amber	aestivum	11379	S	W
Mt 7406	aestivum		I	W
Mt 74394	aestivum		I	W
Arthur 71	aestivum		R	W

SPECIAL STUDIES

VOLATILE INHIBITORS

Inoculated and uninoculated leaf pieces were placed in close proximity to the S. nodorum seeded agar surface of a 55mm petri dish in order to test for production of volatile inhibitors. Leaf sections (35mm) were removed from leaves of the different wheat cultivars and were taped with double sticky tape to the lid of the petri dish in the form of an X. The petri dishes were sealed with Parafilm and allowed to incubate upside down at 15°C with a 12 hour photoperiod. At 96 and 196 hours after sealing, the plates were examined for any differences in S. nodorum growth patterns although the Parafilm wrapping was not removed until after 196 hours.

WHOLE LEAF MOUNTS

Various whole leaf mount analyses were done to demonstrate the processes occurring during infection of wheat by S. nodorum. Leaf pieces (5mm) from Fortuna (S), Newana (I), and Manitou (R) spring wheats were used in these studies. Plants were grown and inoculated under standard conditions and samples were removed at 24 hour intervals for 120 hours.

Leaf pieces were fixed in GAA-ETOH solution overnight, cleared overnight in lactophenol, and stained for one to 24 hours in 0.1% acid fuchsin in lactophenol (w/v) (54). Excess stain was removed by careful blotting and pieces were counter stained in fast green for

for five minutes. This procedure allowed fungal structures on the leaf surface to stain green while those in the interior of the leaf stained red (54).

In a seperate set of studies leaf pieces were fixed in either GAA-ETOH, FAA, or lactophenol-ETOH and then stained with the Periodic Schiff Acid staining procedure (60), lactophenol cotton blue (12), or acetic aniline blue (6).

A technique using Calcofluor was also attempted (65). An epi-fluorescent attachment for the Zeiss microscope was utilized. The filter set in the attachment consisted of Exciter filter BP 450-490, Beam splitter FT 510, and Barrier Filter LP 520. This filter set did not permit the fluorescence of Calcofluor to be expressed and therefore it was modified by replacement of the Exciter filter (BP 450-490) by a BG-3 filter mounted in an accessory filter holder. The Barrier filter and the Beam splitter were not removed.

SECONDARY SPORE PRODUCTION

Fortuna, Newana, and Manitou were inoculated with a standard spore suspension in the usual manner and were then allowed to grow under greenhouse conditions for 25 days. Four 25mm leaf segments were removed from inoculated leaves. One end of the leaves was placed in a small metal clip which was suspended in a tightly stoppered jar. One inch of deionized distilled water was placed in the

jar and a filter paper cylinder was inserted so it rested on the bottom and around the inside perimeter of the jar to act as a wick. Prior to placement in the jar the leaf pieces were dipped in deionized distilled water to wet the pycnidia. All three of the wheat cultivars could be placed in the same jar insuring uniform conditions. The jar was placed in an incubator at 15^{o}C with a 12 hour photoperiod. Spores were harvested at 48 hours in a manner similar to that described by Gough (24). After removal of the spores the number of pycnidia in the leaf segments were determined by observation of the leaf pieces under a dissecting microscope. The number of pycnidiospores per pycnidium was determined for the three different cultivars.

STOMATA

The number of stomata per cm^{2} was determined in ten day old Fortuna, Newana, and Manitou seedlings. Stomatal condition (open or closed) was determined during different dew periods. Epidermal leaf peelings were made of the upper surfaces of the three wheat cultivars after exposure to 24 and 48 hours of mist and were mounted in lactophenol cotton blue. These epidermal leaf peels were removed from plants of similar age and similar leaf expansion. In order to determine the exact stomatal condition, the peelings were immediately fixed in 100% ETOH that contained 0.1% cotton blue (62). Peelings were examined microscopically and stomatal numbers and condition were

recorded. Two replications of this completely random designed experiment were made.

WATER CONGESTION

Six pots each of Fortuna, Newana, Manitou, Frondoso, Klein Toledo, and T. timopheevi were grown to ten day old seedling stage (15 plants/pot). Fronodoso, Klein Toledo, and T. timopheevi all show an extremely high degree of resistance to infection by S. nodorum. T. timopheevi is considered to be the most resistant of all Triticum lines (49). One-half the pots were sprayed with a standard inoculum dose and one-half were left as healthy controls. One pot of each cultivar was removed from the mist chamber at 48, 72, and 96 hours after inoculation. Leaf samples were removed from both the inoculated and uninoculated pots and were assayed for degree of water congestion (42). Plants were read for disease symptoms seven days after inoculation. In addition four plants in each pot were tagged and the leaf blades were pulled through a thumb and forefinger which had been dipped in water or chloroform. The water and chloroform treatments were used with the intention of disrupting the wheat leaf trichomes and/or the waxy cuticle of the leaf. This two structures are possible barriers to infection by fungi so the effect of their disruption was studied. These four leaves were then assayed for any differences that this treatment made in degree of infection or degree of water congestion.

SCANNING ELECTRON MICROSCOPE

Two pots each of Fortuna, Frondoso, Klein Toledo, and T. timopheevi were grown and inoculated under standard conditions. Pots contained 15 plants and a completely random experimental design was used. Leaf samples (7mm) were removed at 24, 48, and 96 hours after inoculation; fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer pH 7.0; and dehydrated in a graded ethanol series. Dehydrated leaf pieces were gradually infiltrated with liquid Freon, critical point dired in a Bomar Model SPC-900 critical point drying apparatus, and coated with fold-palladium in a SPI Sputter-Triode Coater. An ETEC Autoscan Scanning Electron Microscope was used for examining the coated leaf pieces; positive and negative images were recorded simultaneously by means of an attached Polaroid camera.

RESULTS

PRE- AND POST-INOCULATION DEW PERIODS

Results from the pre- and post-inoculation dew period experiments (Tables 4 and 5) indicated that a four hour pre-inoculation and a 48 hour post-inoculation dew period allowed the expression of a suitable range of symptoms on cultivars studied. In this study, and in work by other researchers with different organisms, both pre-inoculation and post-inoculation dew periods have an effect on disease expression (22, 37). The longer the dew period, primarily post-inoculation, the greater the disease reaction on cultivars of all reaction types.

Results from extended dew periods are given in Table 6. Percent necrosis increases in Fortuna and Newana during the first 24 hours of the pre-inoculation dew period. In contrast, the percent necrosis in Manitou remains fairly constant until after 48 hours of the pre-inoculation dew period is reached.

The post-inoculation dew period is of major importance in development of the disease. With short (24 hours) post-inoculation dew periods there is little disease development on resistant cultivars, in contrast to the disease development on susceptible lines. When post-inoculation dew periods were extended beyond 48 hours the resistant cultivars appeared more susceptible (necrosis greater than 50%). Other researchers (22) have found that the most resistant cultivars continue to show resistance until after 96 hours of dew chamber

Table 4. Effect of preinoculation dew periods on germination of Septoria nodorum pycnidiospores on wheat cultivars varying in resistance.

Cultivar	Samples 1/ (hours after inoculation)	Germination percentages 2/ Hours of preinoculation dew period 0	2	4
Fortuna	4	8	7	20
	12	14	22	47
	24	20	45	57
Manitou	4	5	10	17
	12	9	20	38
	24	15	31	41
CI 14032	4	8	10	21
	12	10	17	40
	24	23	25	39
Blueboy	4	11	9	20
	12	15	27	31
	24	19	33	43

1/ Based on two replications of three leaves per replication.

2/ No statistically significant differences between treatments were found.

Table 5. Effect of post-inoculation dew periods on germination of Septoria nodorum pycnidiospores on wheat cultivars varying in resistance.

Cultivar	Germination percentages 1/ Hours of post-inoculation dew period 24	48	72
	2/		
Fortuna	51a	60a	64a
Blueboy	41b	50ab	60a
CI 14032	35b	39bc	48b
Manitou	30c	35c	43b

1/ Based on two replications of three leaves per replication

2/ Column numbers followed by different letters are significantly different (Duncan's Multiple Range Test; P = 0.05)

Table 6. Effect of extended dew periods on expression of symptoms in three wheat cultivars varying in Septoria nodorum resistance.

Cultivar	Disease severity (percent necrosis) 1/ 2/ (four hour pre-inoculation dew period) Hours of post-inoculation dew period 24	48	72	96
Fortuna	55a 3/	75a	70a	90a
Newana	30b	45b	60ab	90b
Manitou	10c	10c	50b	70b

Cultivar	Disease severity (percent necrosis) (twelve hour pre-inoculation dew period) Hours of post-inoculation dew period 24	48	72	96
Fortuna	70a	65a	90a	90a
Newana	50b	70a	85a	90a
Manitou	10c	15b	55b	75b

Cultivar	Disease severity (percent necrosis) (twenty-four hour pre-inoculation dew period) Hours of post-inoculation dew period 24	48	72	96
Fortuna	75a	80a	90a	90a
Newana	55b	80a	90a	90a
Manitou	20c	10b	55b	90a

Cultivar	Disease severity (percent necrosis) (forty-eight hour pre-inoculation dew period) Hours of post-inoculation dew period 24	48	72	96
Fortuna	75a	90a	90a	90a
Newana	65a	80a	90a	90a
Manitou	45b	40b	75a	90a

1/ Based on percent necrosis 10 days after inoculation. Inoculum concentration of 2×10^7 spores/ml with 10 mls of inoculum per pot of 10 plants.

2/ Based on two replications of three leaves per replication.

3/ Column numbers followed by different letters are significantly different. (Duncan's Multiple Range Test; P = 0.05)

conditions at which time resistance starts to break down (22). The effect of longer dew periods may influence disease development by providing 1) a more favorable environmental condition for maximum germination and penetration by S. nodorum and 2) longer periods of free moisture on leaves which places the plant under a stress condition. This condition may lead to water-congestion and increased disease development. Resistance to S. nodorum thus depends on environmental conditions and the physiological condition of the host during inoculation and incubation.

SPORE GERMINATION AND PENETRATION

Spore germination results are given in Table 7. There are no differences 48 hours after inoculation in germination of S. nodorum pycnidiospores on leaf surfaces among the 18 cultivars with the exceptions of Fortuna and Manitou. Germination of spores on Fortuna was significantly higher 48 hours after inoculation than on other cultivars while germination on Manitou was significantly lower. Other researchers working with S. nodorum (6) and other leaf spotting fungi (23, 58) have not found differences in germination percentages on leaf surfaces.

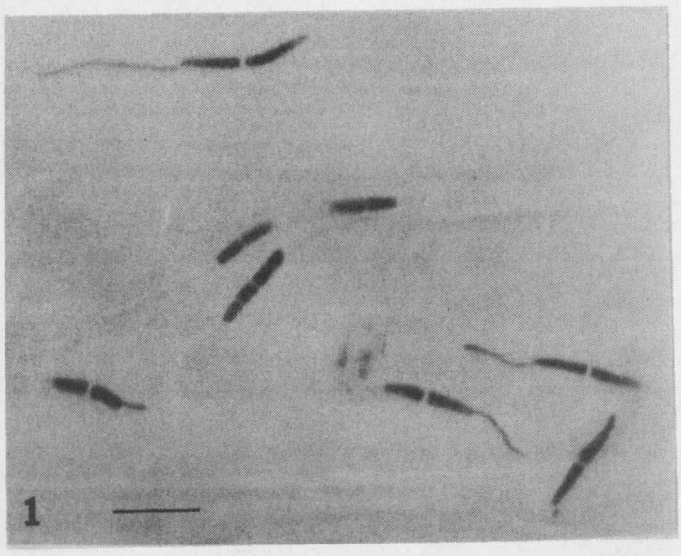
There were no differences among the 18 cultivars in development and morphology of germination hyphae. Typically, at 48 hours, the spores would be germinated out of one or both ends (Fig 1.) although

Table 7. Germination of Septoria nodorum pycnidiospores on leaf surfaces of 18 cultivars of wheat varying in their disease resistance.

Cultivar	Germination percentages (hours after inoculation) 1/ 2	4	8	12	24	48
Fortuna	5	28	43	61	68a 2/	77a
Manitou	2	18	34	35	38b	37c
Newana	4	20	36	40	51c	54b
Redhart	5	23	36	36	44c	48b
Red Chief	3	21	38	39	39b	48b
CI 12373	2	27	40	43	40b	43b
CI 14032	8	19	36	40	40b	43b
Oasis	5	21	35	38	43c	51b
Arthur 71	4	25	37	36	48c	47b
Centurk	6	21	39	50	49c	53b
Michigan Amber	5	18	37	43	44c	51b
Zenati-Boutielle	4	19	41	41	40b	46b
Hadden	6	24	38	44	50c	57b
Blueboy	8	22	40	48	50c	61b
Mt 7406	6	28	36	49	59c	58b
Mt 74394	4	26	39	42	54c	51b
CVLO 2204	8	25	39	39	47c	50b
Yamhill	3	22	38	48	51c	59b

1/ Based on three replications of three leaves per replication.

2/ Column numbers followed by different letters are significantly different (Duncan's Multiple Range Test; P = 0.05).



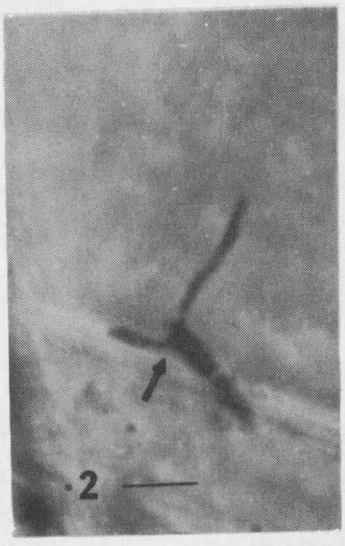


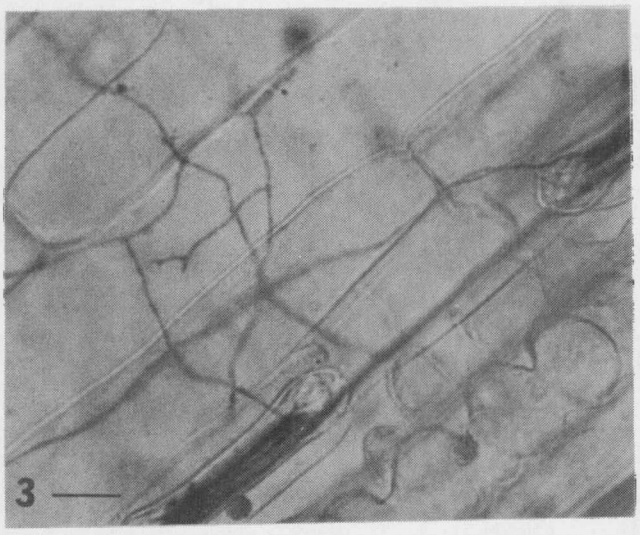
Figure 1. Septoria nodorum pycnidiospores exhibiting early germination patterns on inoculated wheat leaves. Spores are typically germinating from one end. Bar represents 15 microns.

Figure 2. Septoria nodorum pycnidiospore exhibiting germination from one end and from the side of the spore (arrow). Bar represents nine microns.

germ tubes would occasionally arise from the side of the spore (Fig. 2). The majority of spores lodged in depressions made by the joining of two epidermal cells and germ tubes extended along these depressions. the germ tubes from spores located outside these depressions grew at random over the leaf surface with occasional branching (Fig. 3).

Small, dark staining, enlarged areas of the germination hyphae were formed frequently in the junctions between two epidermal cells. These structures have been referred to by other researchers as appressoria (6, 86). Appressorial structures are formed infrequently during the first 24 hours, but were much in evidence by 48 hours after inoculation. There were no differences in appressorium formation either in time of initiation or total numbers produced among the 18 cultivars studied.

Similar to results of Green and Dickson (23) there was frequent evidence of germination hyphae extending beyond appressoria (Fig. 4). Attempts were made to show that penetration took place via a penetration peg beneath these appressoria, but even in vaccum stained whole mounts there was a lack of epidermal cells staining as described by Baker and Smith (6). Since positive hyphal penetration could not be demonstrated it was sought in the thin section staining experiments. Penetration of stomatal openings occurred 72 hours after inoculation (Fig. 5). This was similar to work done by Green and Dickson with S. passerinii (23).



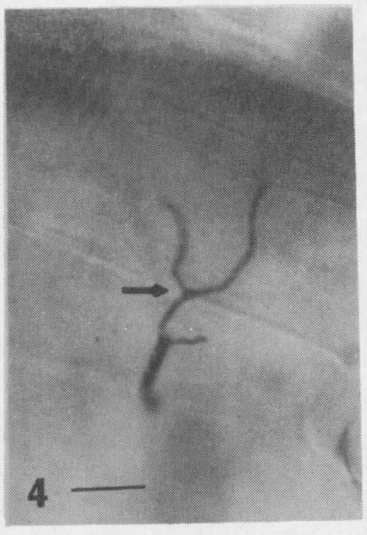


Figure 3. Branching of germination hypha of Septoria nodorum on wheat leaf surface. Bar represents 50 microns.

Figure 4. Germinating Septoria nodorum pycnidiospore showing the formation of an appressorium in an epidermal crack (arrow) and hypha extending beyond the appressorium. Bar represents 25 microns.

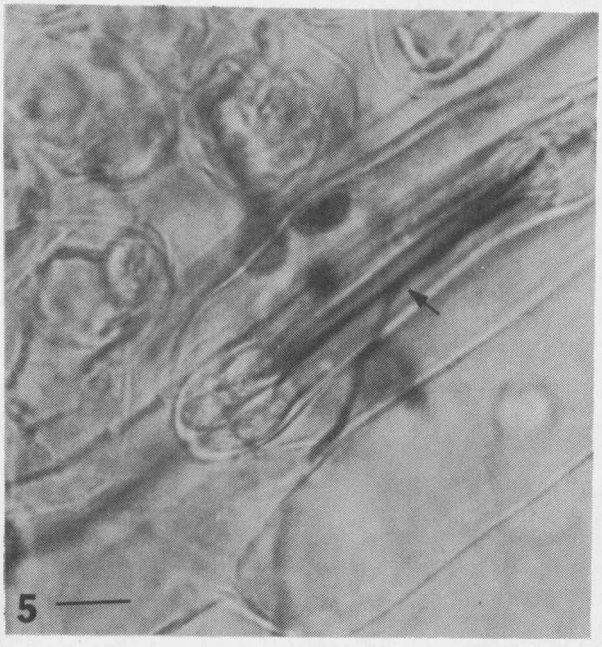


Figure 5. Stomatal penetration (arrow) by the hypha of Septoria nodorum without the formation of an appressorium. Bar represents ten microns.

WHOLE LEAF MOUNTS

Whole leaf samples of Fortuna, Newana, and Manitou were examined using a variety of staining procedures (6, 12, 54, 60). Samples were removed at 24 hour intervals until 144 hours after inoculation to study fungal development within leaf tissue. The staining procedures were modified by utilizing vacuum infiltration, longer staining times, or stronger solutions in order to obtain stained fungal tissue within cleared leaf segments. All such procedures and their modifications gave negative results and the only fungal structures that stained were the spores and germination hyphae on the leaf surface.

A technique described using Calcofluor, an optical brightner (65), was also attempted. The spores on the leaf surface fluoresced weakly, but were often obscured by the fluorescence of the leaf material itself. Attempts made to combine the Calcofluor with standard staining procedures in order to quench the fluorescence of the leaf material allowed for a greater differentiation of fungal hyphae, but only surface fungal structures were visible. Development of disease progression therefore had to be monitored by thin-section histological examination rather than on cleared whole leaf mounts.

VOLATILE INHIBITORS

There were no apparent volatile pre-formed or inducible inhibitors present in the wheat tissues. The mycelial growth of S. nodorum and subsequent pycnidia formation were not visably affected in the area

immediately opposite the leaves. There was no change in growth patterns or total amount of growth of S. nodorum on the sealed plates that contained leaves as opposed to those plates that didn't contain leaves. Other workers have reported antifungal compounds in wheat to be active against S. nodorum, but these compounds were 1) in minute quantities, 2) not correlated with resistant and susceptible varieties and/or 3) not present in mature or field grown plants (5, 6).

HISTOLOGICAL

Disease development was similar among the eight cultivars studied, although there were occasional histological differences. For ease of description the histological reactions will be grouped into susceptible, intermediate, and resistant classes. The only reaction difference between winter and spring wheat types was a matter of degree of infection rather than a difference in specific defense reactions. Resistant, intermediate and susceptible winter wheat cultivars typically had less infection than the respective spring wheat lines under the same environmental conditions and inoculum concentrations.

12 HOURS

Twelve hours after inoculation none of the cultivars showed evidence of reaction to the presence of the pathogen on the surface. Occasional spores were noticed on the surface, but no penetration

structures were seen. There were no differences between the healthy checks and inoculated controls in cell staining reactions and cellular integrity. In the safranin-fast green staining method the cell walls, chloroplasts, and cytoplasm stained with fast green, while the fungal spores and the vessel element walls stained with safranin. No cellular changes were noted in tissue stained with Johnsen's Quadruple and Conant's Quadruple staining procedures.

24 HOURS

Twenty-four hours after inoculation there was little change in cellular morphology among the wheat lines. No apparent penetration structures were noted on any of the slides examined, athough there was an occasional cellular reaction in the susceptible lines. The cell walls of the mesophyll cells lining some of the substomatal cavities stained a dark blue (Fig. 6). This was accompanied by a granular appearance in these cells. This reaction occurred only in Michigan Amber, Centurk, and Fortuna. Uninoculated controls and inoculated plants showed this reaction indicating that it was due to an environmentally induced physiological response rather than a disease reaction. In the other wheat cultivars there was no evidence of any type of cellular reaction to either the pathogen or the environment.

48 HOURS

In susceptible cultivars there was an increase in the disruption

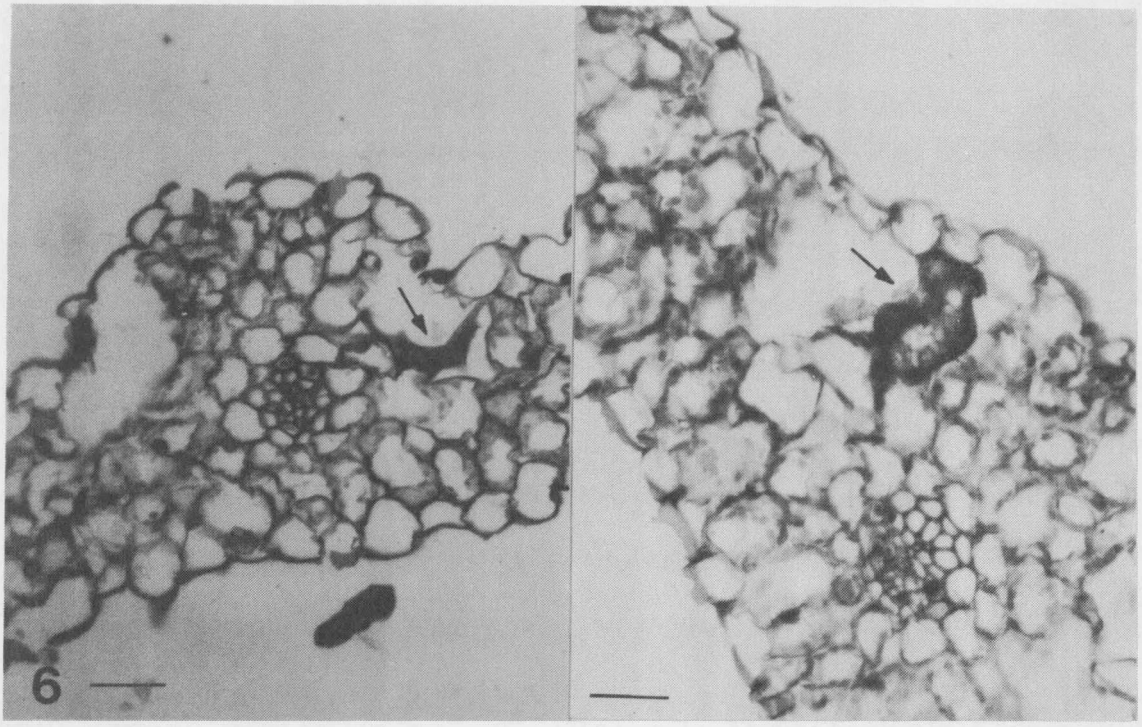


Figure 6. Photomicrographs of the initial stage of disease development in a susceptible wheat cultivar. Mesophyll cells lining the substomatal cavity show typical accumulation of blue granulation. Reaction occurred 24 hours after inoculation. Bar represents 30 microns.

of cells lining substomatal cavities. There was more granulation and increasing evidence of cellular collapse (Fig. 7). Mesophyll cells were affected and epidermal cells did not appear to be affected at this stage. This is consistent with results reported by other researchers (6, 85). There was no evidence of cellular disruption in areas away from the substomatal cavities and no hyphae could be discerned within the affected tissue. No penetration structures were evident in any of the sections examined. The healthy controls of the susceptible class, although still showing slight cellular disruption did not have the same degree of reaction as did the inoculated plants. There was a granulation of the mesophyll cells surrounding the substomatal cavity; to a lesser extent in the healthy controls and to a greater extent in the inoculated plants. There was no visible reaction to the disease or environment in the resistant class of cultivars. Although spores and germination tubes could be seen on the surface there was no evidence of penetration in the intermediate and resistant classes.

72 HOURS

By 72 hours after inoculation there was considerable damage in the susceptible class of wheat cultivars. In addition to the granulation of the mesophyll cells there was also cellular collapse (Fig. 8), the presence of inter- and intra-cellular hyphae (Fig. 9), and a large amount of safranin staining material deposited on the cell walls (Fig.

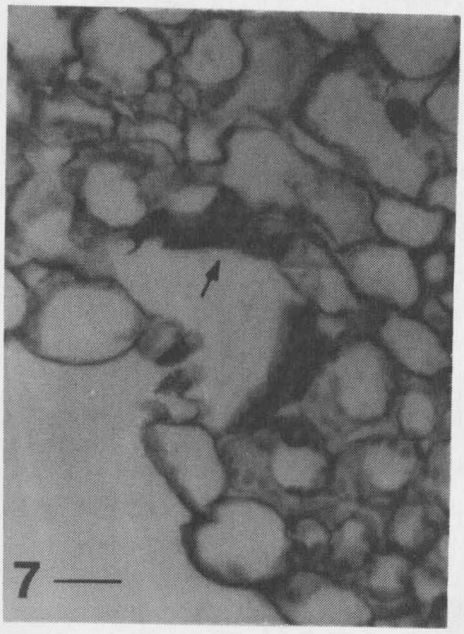
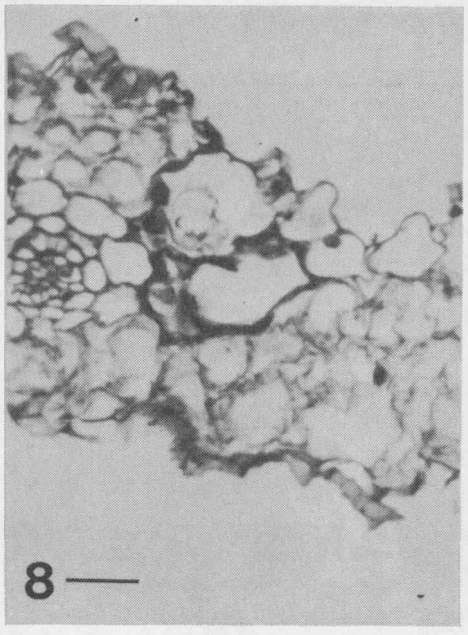


Figure 7. Photomicrograph of increasing disease development in a susceptible cultivar. Cells beneath granulated cells (arrow) are beginning to show disease effects. Bar represents 15 microns.

Figure 8. Photomicrograph of disease development in a susceptible cultivar 72 hours after inoculation. Internal cell destruction has occurred. Bar represents 30 microns.



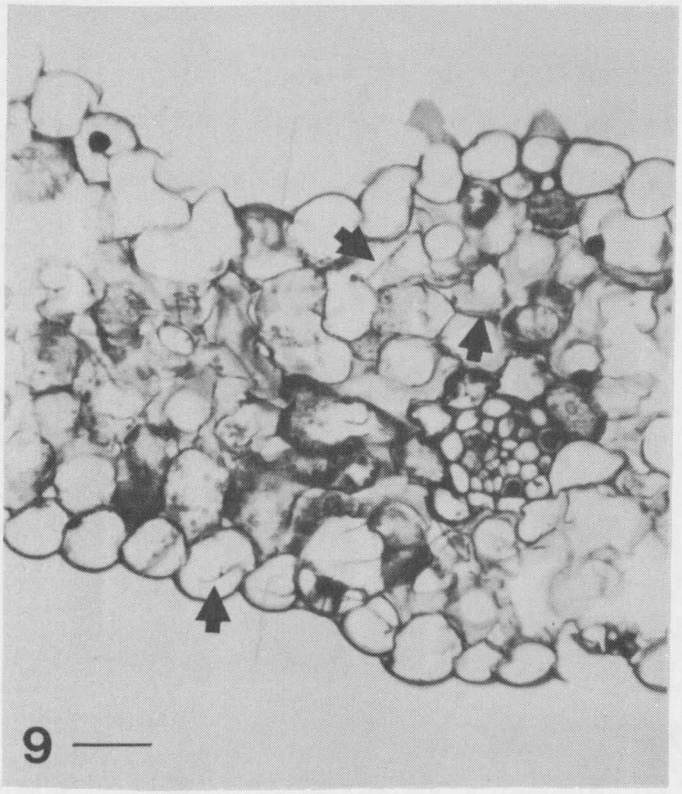


Figure 9. Photomicrograph of inter- and intra-cellular hyphae (arrows) of Septoria nodorum within a wheat leaf. Susceptible cultivar of wheat with hyphae both within and outside of lesion area. Bar represents 30 microns.

10) and in the intercellular spaces (Fig. 11). The hyphae could only be seen clearly in tissues where cellular disruption was minimal. Hyphae of S. nodorum stained primarily with safranin and the safranin staining material in conjunction with the cell walls and in the intercellular spaces often masked the presence of the hyphae. This masking occurred wherever there was moderate cellular disintegration. In addition to the involvement of the mesophyll cells, the epidermal cells were affected and occasionally lesions extended through the entire leaf to the opposite side (Fig. 12). Since serial sections were used throughout this study, it was possible to follow the lesions in the long axis of the leaf. The lesions parallel to the leaf vein, extended from stoma to stoma. The lesions began to extend laterally at this stage, but not to the degree of longitudinal spread. No penetration stuctures indicating direct penetration of the cuticle were seen. However, stomatal penetration was observed (Fig. 13) as well as ocassional penetration of leaf trichomes (Fig. 14). The intermediate reactions intensified and at 48 and 72 hours after inoculation resembled the susceptible reaction at 24 and 48 hours respectively. The resistant class started to show a reaction to the pathogen at this time. The reaction was confined to the cells lining the substomatal cavity, but in the resistant reaction there was no granulation of the cells and the reaction was typified by an accumulation of a safranin

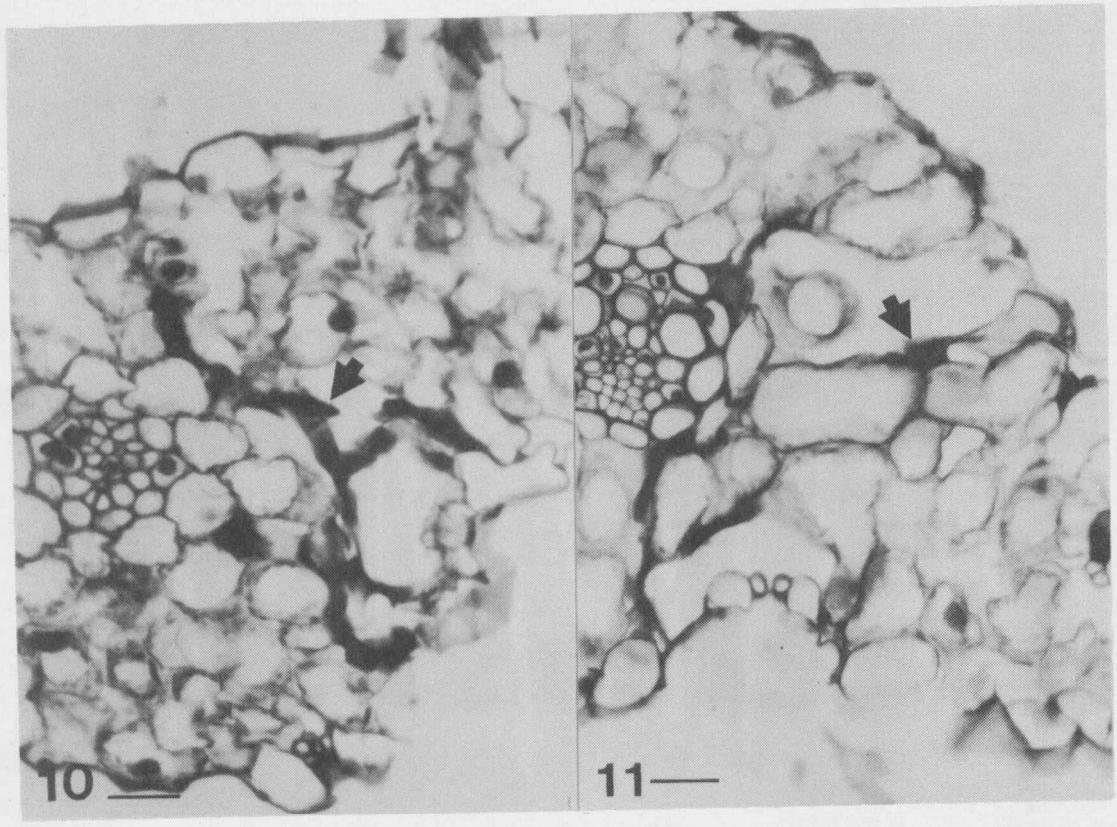


Figure 10. Photomicrograph of safranin-stained material accumulated on mesophyll cell walls (arrow) of a wheat leaf in response to invasion by Septoria nodorum. Bar represents 25 microns.

Figure 11. Photomicrograph of safranin-stained material accumulated in the intercellular spaces of a wheat leaf in response to invasion by Septoria nodorum. Bar represents 20 microns.

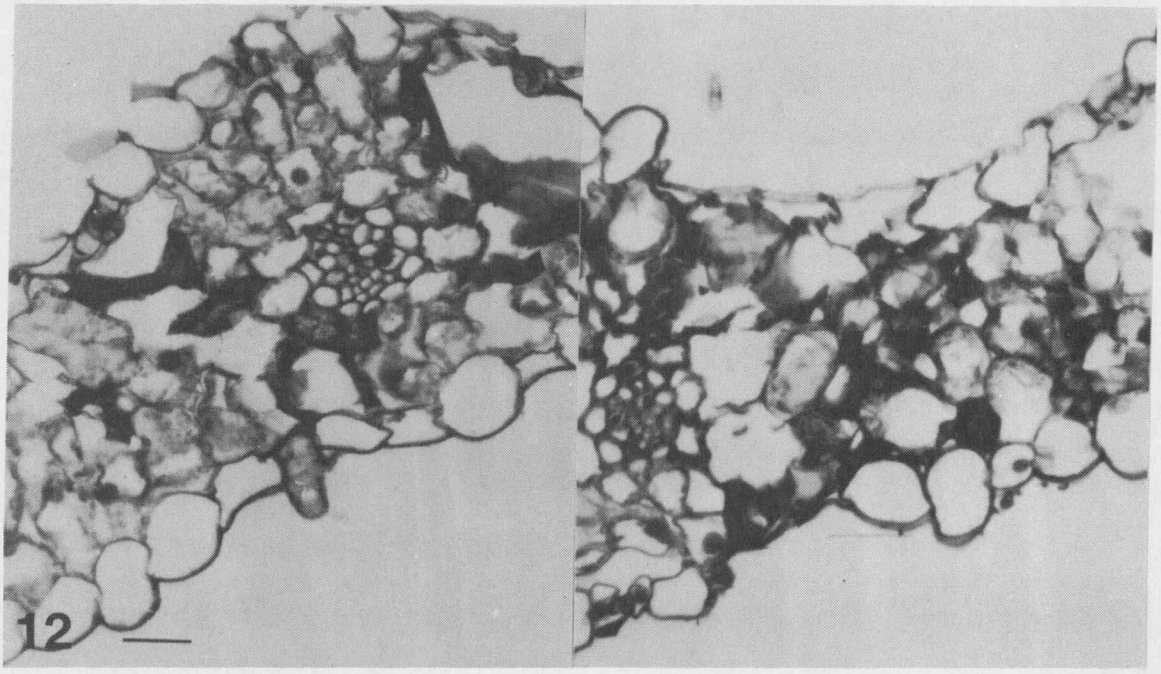


Figure 12. Photomicrographs of lesions on a susceptible wheat cultivar extending to the opposite epidermal surface. Large amounts of the blue granulation reaction and safranin-stained material are present. Bar represents 30 microns.

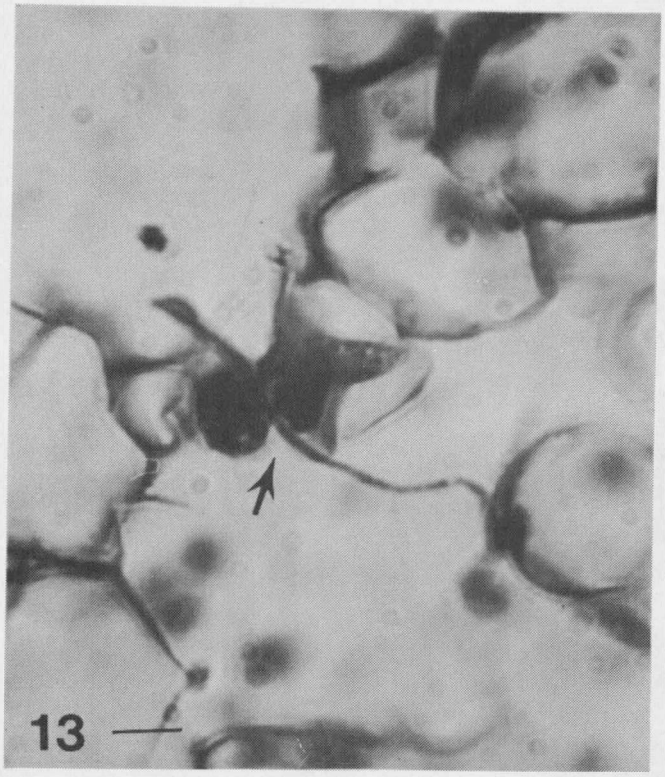


Figure 13. Photomicrograph of stomatal penetration by a germinating pycnidiospore of Septoria nodorum. Bar represents 6 microns.

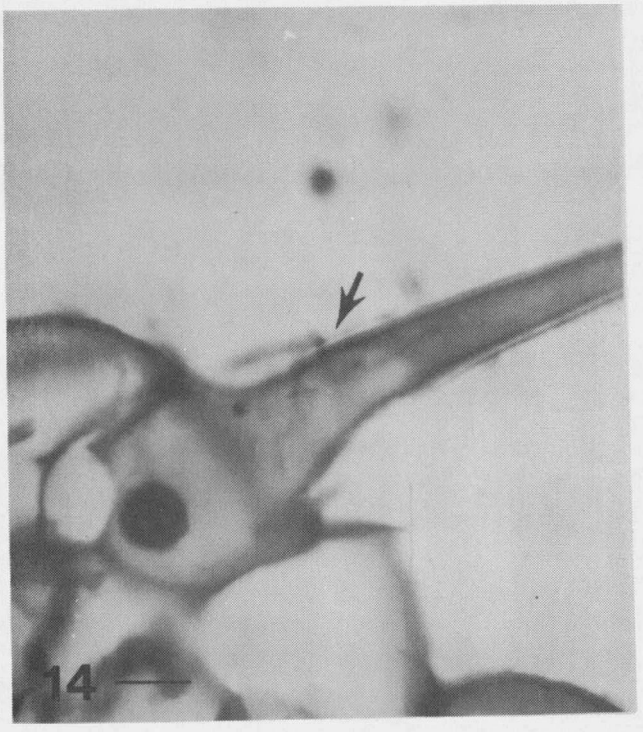


Figure 14. Photomicrograph of trichome penetration (arrow) by a germinating pycnidiospore of Septoria nodorum. Bar represents 10 microns.

staining substance deposited on the cell walls. No hyphae could be seen in the resistant class of wheat cultivars either in the affected area or in adjacent cells. There was a lack of the blue staining material in the resistant reaction at this stage.

96 HOURS

Ninety-six hours after inoculation the reaction in all three classes of wheat intensified. In the susceptible class lesions extended through the width of the leaf and had moved laterally to involve all the tissue between vascular bundles. The lesions were normally confined to the areas bwtween the vascular bundles (Fig. 15), although this phenomenon depended on the size of the bundle. If bundles were small and sclerenchyma tissue was minimal the pathogen seemed able to spread around the vascular bundle and into the next interveinal area (Fig. 16). However, if the sclerenchyma tissue was well defined there was no movement by the pathogen around the bundle. This is in agreement with Green and Dickson's findings in their work with S. passerinii (23). Cellular collapse at this stage included the epidermal cells (Fig. 17) and there was a greater deposition of the safranin stained-material in and around the cells. The intermediate reaction also increased in intensity and after 96 hours no discernable differences in the appearance of individual lesions between the intermediate class and the susceptible class could be determined. There was a visual

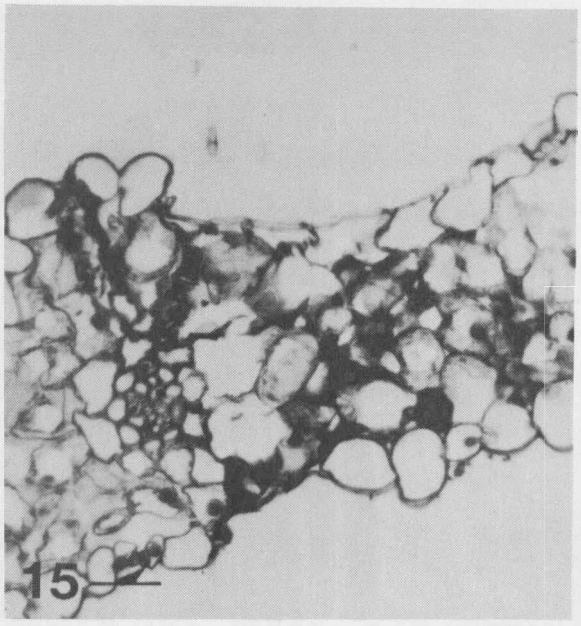
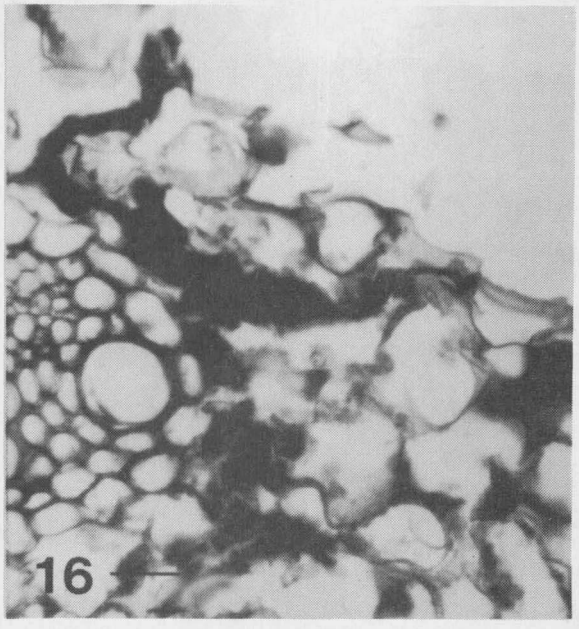


Figure 15. Photomicrograph of a typical Septoria nodorum lesion contained by a vascular bundle. Bar represents 35 microns.

Figure 16. Photomicrograph of lesion caused by Septoria nodorum that is beginning to surround a small vascular bundle. Bar represents 25 microns.



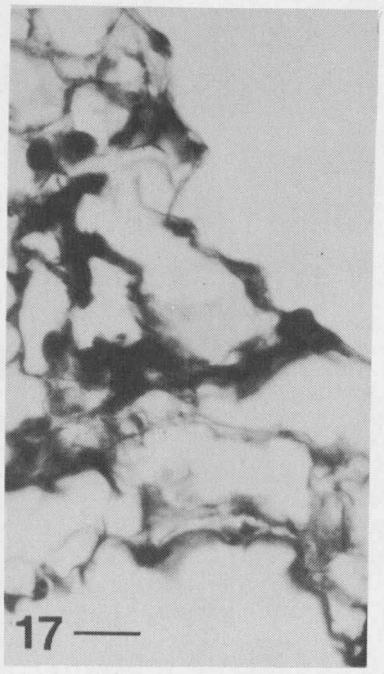


Figure 17. Photomicrograph showing total cellular collapse in a susceptible wheat cultivar. Bar represents 12 microns.

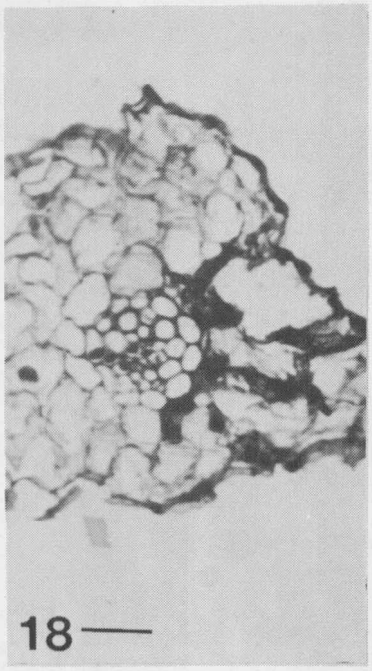


Figure 18. Photomicrograph showing total cellular collapse in a resistant cultivar. Bar represents 50 microns.

difference in the total amount of leaf tissue affected between the two classes although this was not quantified. The resistant class also reacted more intensely to the pathogen although cellular granulation of the mesophyll cells was not apparent. Safranin-positive materials around cell walls accumulated to significant levels. The percentage of tissue involved in the resistant class was less, although individual lesions could progress to total cell collapse as in the susceptible reaction (Fig. 18).

120 HOURS

By 120 hours after inoculation there was little if any difference in appearance of individual lesions in all three classes of cultivars. All showed densely stained areas of cellular disintegration and disruption. Individual hyphae could not be distinquished within the areas of cell collapse. The differences between the three classes of wheat cultivars were in the percentage of leaf tissue involved.

Formation of pycnidia was the same in the various cultivars. Hyphae accumulated in the area below the substomatal cavity and eventually gave rise to pycnidia (Fig. 19). The formation and enlargement of the pycnidial mass caused further cell disruption and disintegration from pressure applied to adjacent cells.

Tissue stained with the safranin-fast green staining method fluoresced with the epi-fluorescent equipment previously mentioned

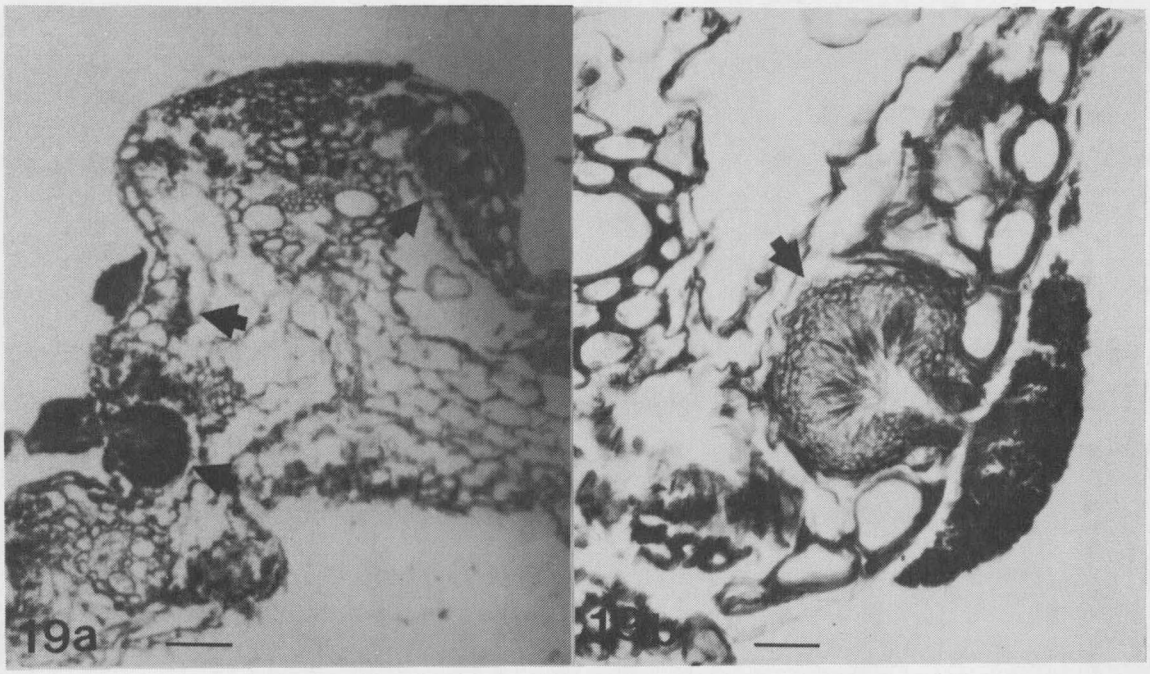


Figure 19. Photomicrograph showing the morphology and orientation of Septoria nodorum pycnidia (arrows) in a susceptible wheat cultivar. Bar represents 150 microns in Figure 19a and 50 microns in 19b.

(standard filter set). The epidermal cell walls fluoresced a dark purple color, chloroplasts a dull red, vessel element walls a bright yellow-red, fungal structures bright red, and cells in the immediate proximity of fungal tissue were white. Cells ringed with a whitish fluorescence could be observed before any evidence of plant response with a normal light source (Fig. 20 and 21). White fluorescence often appeared between two epidermal cells (Fig. 22) indirectly indicating pathogen penetration at this point. Examination of these areas under oil immersion and phase contrast did not indicate definable fungal structures. As lesions enlarged, surrounding adjacent cells were ringed with a white fluorescence although they appeared healthy under normal lighting. The first cells to show evidence of a reaction were the mesophyll cells lining the substomatal cavity. Uninoculated susceptible controls also evidenced a fluorescence in the substomatal cavities, but not to the extent of the inoculated plants. This reaction did not occur in the resistant plants.

SECONDARY SPORE PRODUCTION

The results of the secondary spore production experiments are given in Table 8. There was a significant reduction in the number and size of pycnidia formed in the resistant leaf material. As a consequence of the size reduction there was also a concurrent reduction in the number of spores/pycnidium. Individual pycnidiospores sizes

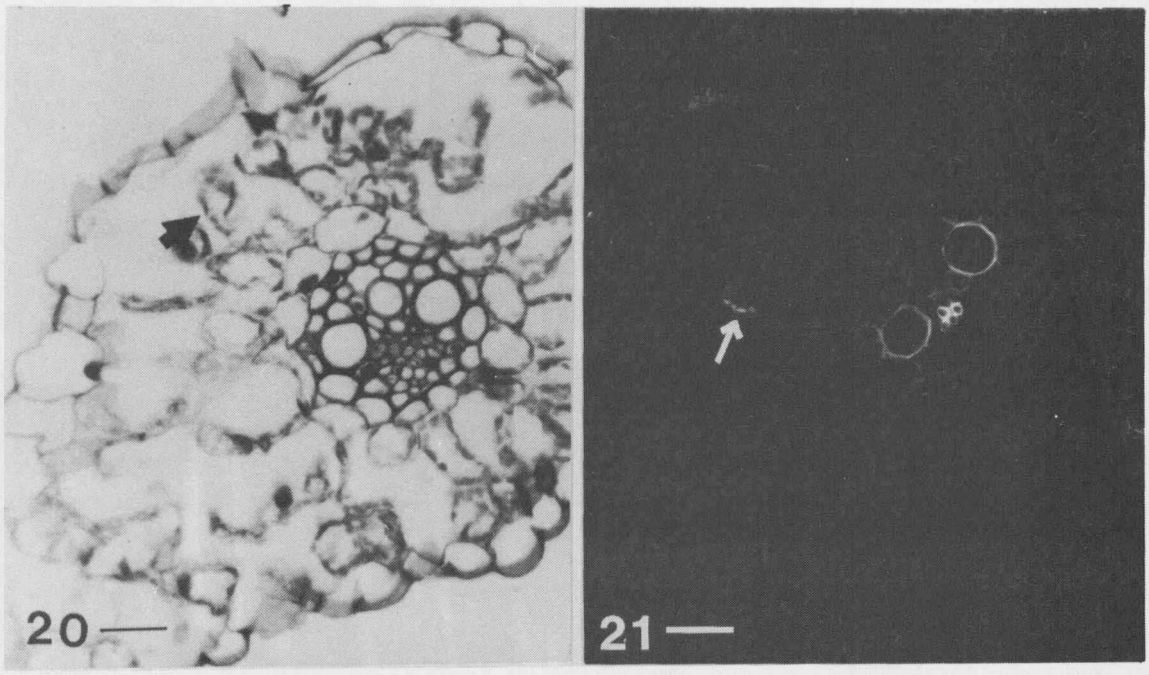


Figure 20. Photomicrograph of an inoculated susceptible wheat cultivar showing no evidence of pathogen invasion (arrow) Bar represents 25 microns.

Figure 21. Fluorescent photomicrograph of the leaf tissue in Figure 20 showing evidence of pathogen invasion (arrow). Bar represents 25 microns.

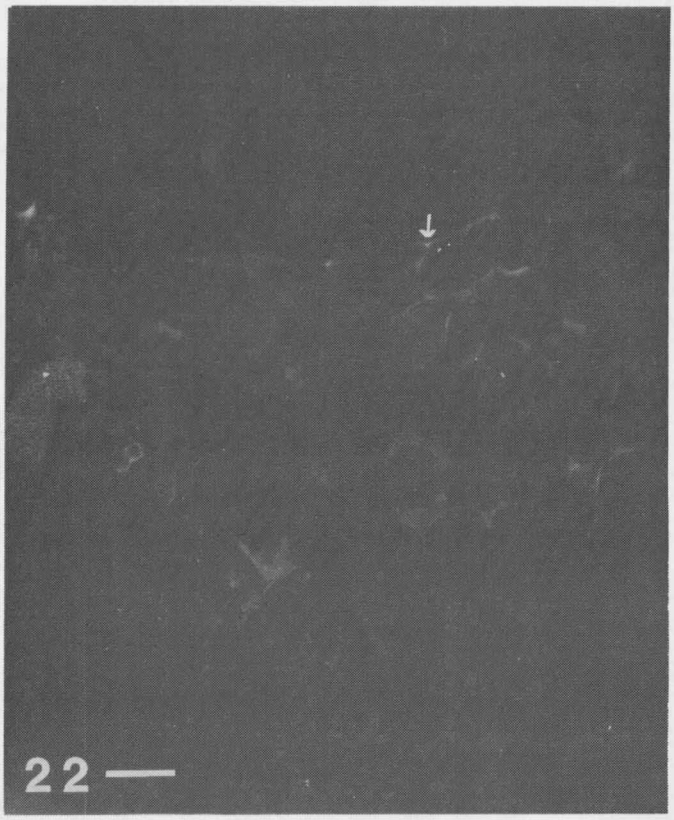


Figure 22. Fluorescent photomicrograph showing fluorescence between epidermal cells walls (arrow) indicating pathogen penetration. Bar represents 25 microns.

Table 8. Secondary spore production in three cultivars of wheat varying in disease resistance.

Cultivar	Average number of pycnidia produced/cm^2 1/	Average size 2/ of pycnidia	Average number of pycnidiospores/ pycnidium x 10^3 1/
Fortuna	41a 3/	204 x 163 u	6.1a
Newana	30ab	179 x 140 u	4.2ab
Manitou	24b	171 x 134 u	2.2b

1/ Based on three subsamples with two replications.

2/ Based on 50 dry pycnidia chosen at random from three subsamples with two replications.

3/ Column numbers followed by different letter are significantly different (Duncan's Multiple Range Test; P = 0.05).

remained the same, regardless of the disease reaction of the cultivar. Although, not quantified, there was a trend for pycnidia to initiate formation sooner (1-2 days) in the resistant plant. These results agree with work by Gough (24) and Eyal (21) in studying the effects of host resistance on pycnidia and pycnidiospore formation by S. tritici. Gough (24) found that between 60-80% of total pycnidiospores within a pycnidium are released within the first wetness period and alternate drying and rehydration periods will cause the release of more spores. Some of the spores are never discharged from the pycnidium. Tertiary pycnidia of S. nodorum are formed with alternating periods of wetness and dryness. It has been estimated that a minimum of 25,000 spores/cm^2 of leaf tissue may be released (72).

STOMATA

Number of stomata varied extensively within and between cultivars and therefore could not be correlated with cultivar disease reaction. The numbers of stomata ranged from 3000/cm^2 to 5400/cm^2. The susceptible cultivar appeared to have a greater percentage of open stomata at the end of 48 hours in the mist chamber, but the number was not significantly different from resistant cultivars (Table 9). Ninety-six percent of the stomata were open in the susceptible cultivar as opposed to 92% and 93% in the intermediate and resistant cultivars respectively.

Table 9. Numbers of stomata on adaxial leaf surfaces of three cultivars of ten day old wheat seedlings grown under identical environments.

Cultivar	Stomata/cm^2 1/	Percent stomata open	
		24 hours	48 hours
Fortuna	4800 2/	95	96
Newana	3000	93	92
Manitou	5400	93	93

1/ Based on two replications.

2/ No significant differences were found among cultivars.

WATER CONGESTION

Water congestion is defined by Johnson (42) as "the accumulation of excessive water in the intercellular spaces as the result of internal water pressures." This water congestion may be induced by certain mist chamber conditions or may occur naturally under field conditions (excessive periods of high relative humidity accompanied by cool, cloudy days). Water congestion may also occur in conjunction with wind-blown rain. This type of water congestion may be accompanied by wounding of the epidermal layer of cells which facilitates invasion by pathogens. Differences in water congestion among the cultivars after 48 hours in the mist chamber were apparent (Fig. 23 and 24). The degree of water congestion was greater in the susceptible cultivars than in the resistant cultivars. T. timopheevi appeared essentially immune to water congestion under the same conditions (Fig. 25). Water congestion initially occured in the intercellular spaces of the cells surrounding the substomatal cavity, but rapidly spread along the leaf axis in the areas between the vascular bundles. When plants were removed from the mist chamber most of the water congestion disappeared within six hours. In uninoculated Fortuna, however, there was some cellular damage in the substomatal cavity area. This was evidenced by a browning reaction in these cells after all water congestion had disappeared. Inoculated plants showed more cellular disruption and small

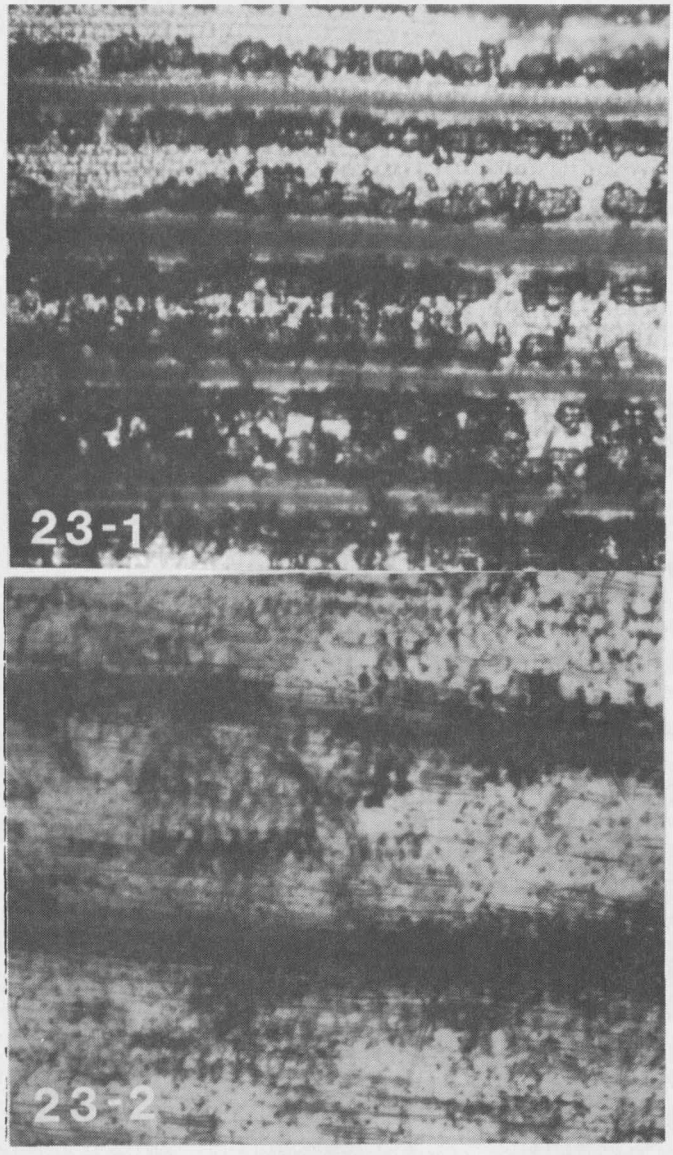


Figure 23. Photomicrograph showing water congestion in a susceptible cultivar of wheat after 48 hours in the mist chamber. 23-1 a non-stained leaf photographed with transmitted light (clear areas are water congested). 23-2 a leaf treated with rose bengal dye (dark areas are water congested).

Figure 24. Photomicrograph showing water congestion in a resistant cultivar after 48 hours in the mist chamber. 24-1 a non-stained leaf photographed with transmitted light (clear areas are water congested). 24-2 a leaf treated with rose bengal (dark areas are water congested).

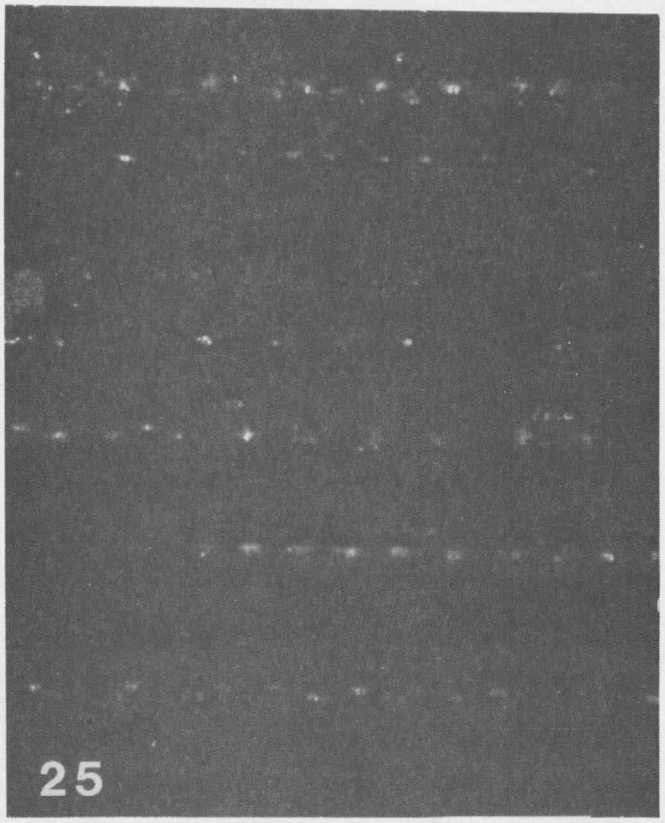


Figure 25. Photomicrograph showing water congestion in a very resistant cultivar of wheat (T. timopheevi) after 48 hours in the mist chamber. The non-stained leaf was photographed with transmitted light (clear areas are water congested).

necrotic areas at that time. The resistant plants and T. timopheevi evidenced no reaction to water congestion on a cellular level. Leaves that were pulled through the thumb and forefinger dipped in water or chloroform had increased incidence of disease when compared to controls (measured as percent necrosis) (Table 10). The chloroform treated leaves were more severely affected than water treated ones. A portion of the increase in incidence of disease may be attributed to mechanical wounding of the epidermal layer allowing for a greater invasion by S. nodorum. The treated plants also had a greater degree of water congestion than controls especially in the case of T. timopheevi. This increase in water congestion may also be due to the wounding or removal of epidermal hairs. T. timopheevi has a large number of long trichomes which efficiently keeps drop of dew (or spore suspensions) off the surface of the leaf. When the surface hairs of T. timopheevi are removed mechanically an increased number of water drops or spore suspension drops rest on the surface. This increases the incidence of both water congestion and pathogen invasion. The chloroform treatment may increase both water congestion and infection in the cultivars studied by dissolving portions of the cuticle and removing some the plants natural defense against water absorbtion.

SCANNING ELECTRON MICROSCOPE

Scanning electron micrographs of S. nodorum on the leaf surface

Table 10. Effect of disruption of leaf surface structure on expression of disease resistance to Septoria nodorum.

Cultivar	Disease severity (as percent necrosis) 1/ 2/		
	Control	Water Treatment	Chloroform Treatment
Fortuna	70	90	90
Newana	40	70	90
Manitou	10	60	70
Frondoso	5	60	70
Klein Toledo	0	70	80
T. timopheevi	0	40	50

1/ Based on percent necrosis 10 days after inoculation.

2/ Based on three replication of two leaves per replication.

showed the presence of various penetration structures: appressoria, penetration pegs, and infection hyphae. Appressoria were highly variable in morphology (Fig. 26 and 27). The morphology seemed to be correlated with the position of the appressorium. If the appressorium was in the center of an epidermal cell it tended to be rounded and distinct while if it occurred in the epidermal cracks or stomatal openings it was more irregular and had many "pods" or projections associated with it. Penetration pegs arose from appressoria, directly from a spore, or from the germination hyphae (Fig. 26 and 28). Penetration pegs were approximately 1/10 the size of appressoria (3-5 u) and 1/5 the size of a germination hypha (2 u). Infection hyphae were observed emerging directly from the spore (Fig. 29) or from the base of an appressorium (Fig. 30). These infection hyphae were approximately 1/5 of the size of the normal germination hyphae (2 u).

Within the leaf tissue hyphae could be distinquished. Ninety-six hours after inoculation the hyphae within lesions in resistant cultivars started to disintegrate (Fig. 31) while the hyphae within a susceptible cultivar remained intact (Fig. 32). This was the only difference noted between S. nodorum infection on resistant and susceptible cultivars by scanning electron microscope examination.

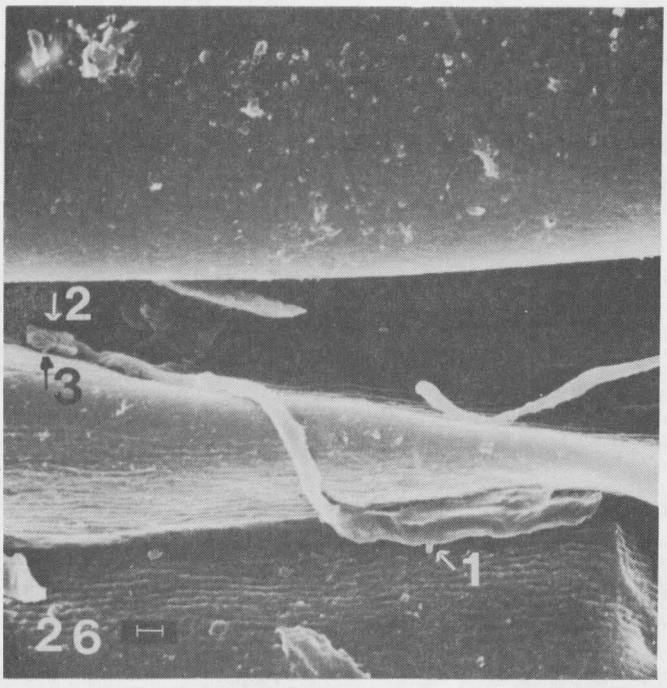


Figure 26. Scanning electron micrograph of a germinating pycnidiospore of Septoria nodorum with a penetration peg emerging directly from the spore (arrow 1), a podical appressorium (arrow 2), and another penetration peg (arrow 3). Bar represents 1.0 micron.

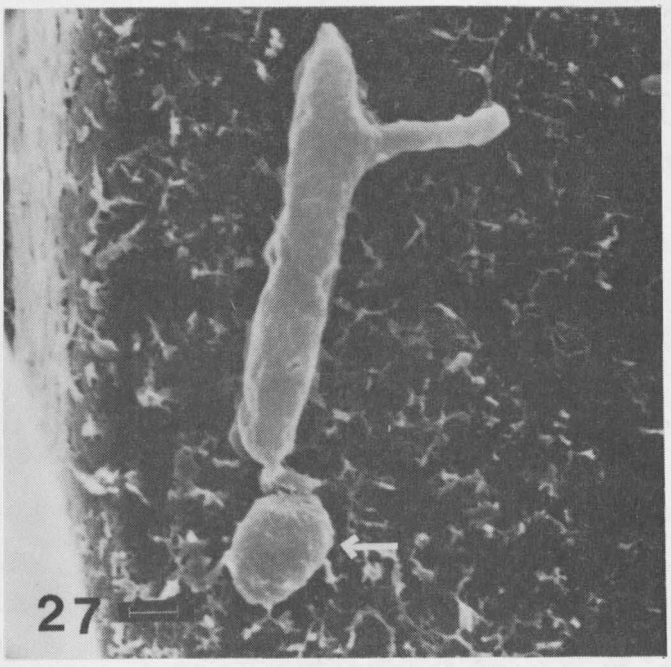


Figure 27. Scanning electron micrograph of a germinating pycnidiospore of Septoria nodorum showing a typical germination hypha (emerging from the side) and a rounded distinct appressorium (arrow). Bar represents 1.0 micron.

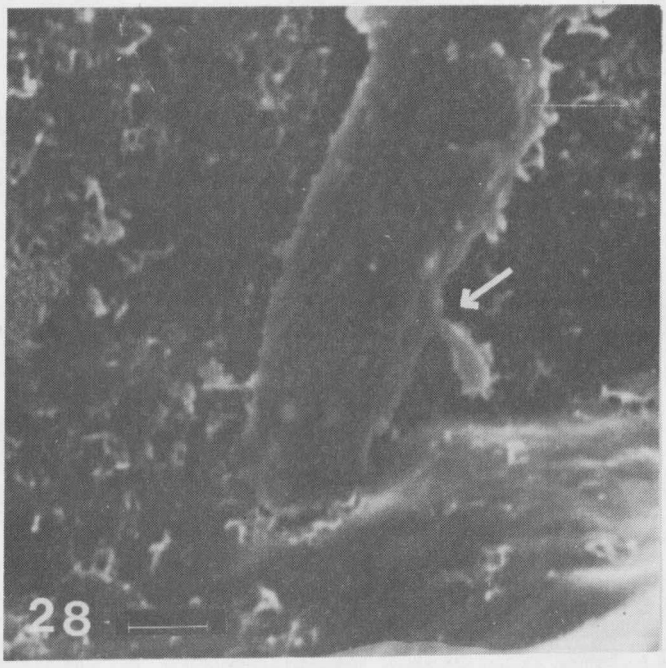


Figure 28. Scanning electron micrograph of a penetration peg (arrow) of Septoria nodorum emerging from the side of a germination hypha. Bar represents 1.0 micron.

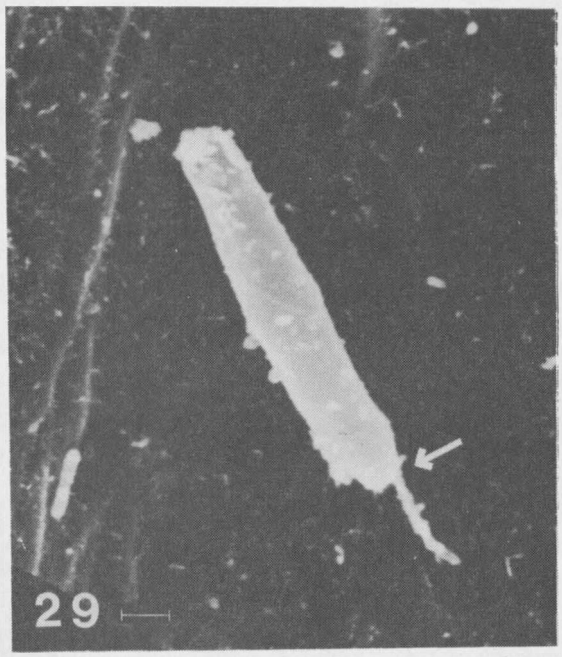


Figure 29. Scanning electron micrograph of an infection hypha of Septoria nodorum emerging directly from a pycnidiospore (arrow). Bar represents 1.0 micron.

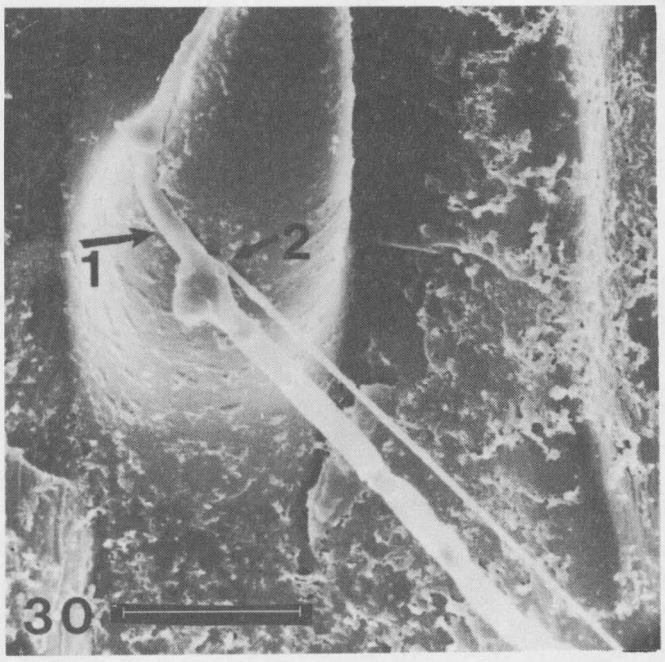


Figure 30. Scanning electron micrograph of a germination hypha (arrow 1) of Septoria nodorum growing around a wheat leaf trichome. An appressorium has formed and an infection hypha has emerged from its base (arrow 2). Bar represents 10.0 microns.

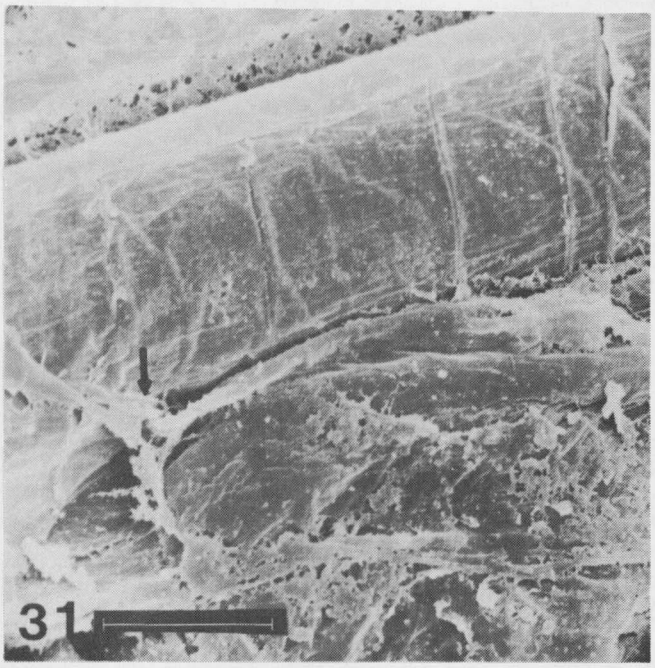


Figure 31. Scanning electron micrograph of a disintegrating Septoria nodorum hypha (arrow) in a resistant cultivar of wheat 96 hours after inoculation. Hyphae are located within a lesion area. Bar represents 10 microns.

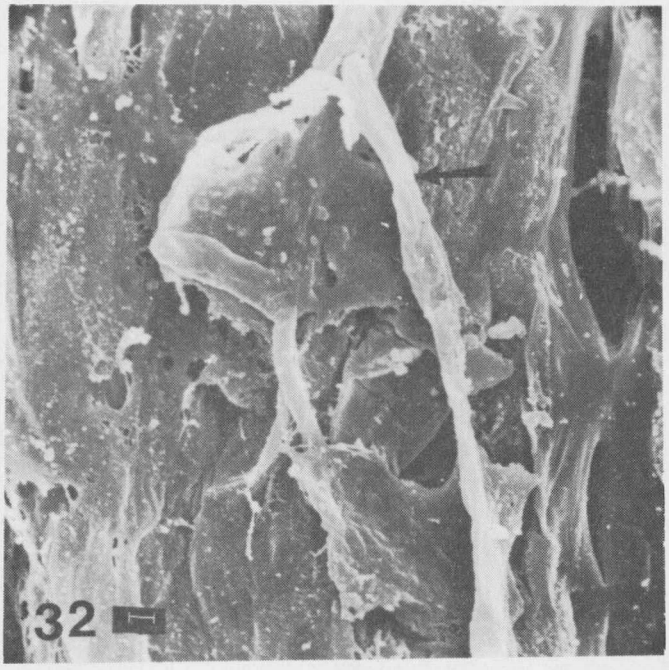


Figure 32. Scanning electron micrograph of an intact Septoria nodorum hypha (arrow) in a susceptible cultivar of wheat 96 hours after inoculation. Hyphae are located within a lesion area. Bar represents 1.0 micron.

DISCUSSION

Significant differences in germination of pycnidiospores occurred on leaves of Fortuna and Manitou. No explanation for this reaction was observed, but the possible presence of volatile fungal spore germination inhibitors was studied. No volatile inhibitors or stimulators were detected with the procedure used as spore germination and mycelial growth were not affected by close proximity to leaf material. Germination hyphae development and formation of appressoria were not affected by disease reaction of the cultivar. Spores frequently lodged in cracks between adjacent epidermal cell walls with their hyphae extending longitudinally along these cracks. Hyphae branched on leaf surfaces in contrast to Weber's findings (86). This branching occurred most frequently when the germination hyphae did not lie in epidermal cracks.

Twenty-four hours after inoculation, appressorial structures were seen in all cultivars. Formation of appressorial structures could not be correlated with any specific anatomical features of the leaf surface, although they occurred most frequently in the epidermal cracks. Frequently hyphae extended beyond these structures and there was occasional branching at the appressorial structure itself. Evidence for epidermal cell penetration under these appressorial structures although lacking with conventional light microscope examinations,

was confirmed with scanning electron microscope examinations. Forty-eight hours after inoculation there was no further increase in the formation of appressoria beyond what had been present at 24 hours. Stomatal penetration was readily observed 72 hours after inoculation. Stomata were penetrated either in the open or closed state with or without the formation of appressoria, as also seen by Harrower (26).

One of the resistance mechanisms possessed by the cultivar Manitou is a significant reduction in spore germination and penetration of leaves. All other cultivars, whether resistant or susceptible, did not exhibit reduction in spore germination on the leaf surface.

Scanning electron micrographs demonstrated the presence of various penetration and infection structures. Penetration pegs arose from germination hyphae, appressoria, and directly from the spore itself. In addition to the penetration pegs, the presence of infection hyphae was observed. These hyphae, which had been assumed to occur, had not previously been photographed due to their small size. These hyphae, approximately 1/5 the size of normal germination hyphae and 1/10 the size of an appressorium, are reported to enter between two epidermal cells. After penetration of the host tissue they enlarge to approximately 2 u in width (86). Infection hyphae were observed originating from appressoria or directly from the spore itself. The infection hyphae could not be seen with conventional light microscope

techniques due to the small size and "masking" by adjacent cellular staining reactions. Infection hyphae were seen by Nomarsky interference-constrast optic examinations although they were not sufficiently clear to permit photographs. Wherever S. nodorum penetrated the leaf tissue there was a fluorescent reaction in adjacent cells. Scanning electron microscope observations of fungal penetration structures and epidermal holes showed that direct cuticle penetration occurred most frequently 24 to 48 hours after inoculation. Direct cuticle penetration was further substantiated by the observation of infection hyphae with Nomarsky interference-contrast optic examination and typical cellular staining and fluroescent reactions in cells that were in direct contact with S. nodorum hyphae. Hyphae were also seen within the leaf tissue prior to observation of stomatal penetration.

Once the pathogen was inside the leaf tissue, the progression of events in formation of individual lesions was similar in all cultivars. The major difference between a susceptible and a resistant reaction, as noted in the histological staining, was a blue granulation in cells of the susceptible host in addition to the safranin staining reaction of the cells walls that occurred in both reaction types. The susceptible plant often apeared to have less safranin stained material and more of the blue granulation. The safranin stained material was associated with lignified and suberized cell walls and was assumed to be phenolic in character. Certain phenols function in the formation of

of cell protective tissue (lignin, suberin, etc) (67). Suberized tissues are a barrier to the spread of most fungi. The blue granulation appeared to be a clumping of the cellular contents and an accumulation of cellulose rather than an elaboration of chemical compounds. Hyphae could be distinquished in all cultivar classes, although in the resistant host no hyphae could be found outside the lesion area itself. Hyphae could be found frequently in advance of the lesion area in susceptible cultivars and to a moderate extent in the intermediate classes. Under scanning electron microscope examination, hyphae in the resistant cultivar began to show disintegration within the lesion area 96 hours after inoculation. Hyphae in the susceptible cultivar did not disintegrate. This, evidence, plus the staining reactions, indicates that there may be a chemical defense mechanism in the resistant cultivars studied which is detrimental to S. nodorum and restricts the fungus to more defined lesion areas. The individual lesions all begin the the cells lining the substomatal cavities and progress inward through the leaf to the other epidermal surface. Eventually the entire area between vascular bundles collapses. Chlorotic areas, perhaps due to toxin activity, did extend from one interveinal area to the next. Previous researchers have reported the presence of non-specific toxin production by S. nodorum (9, 48). The toxin may function in disease development or in affecting host cellular defense,

but the actual role of this toxin has not been determined. Under normal conditions lesions progressed longitudinally along the interveinal area. Several adjacent lesion areas often combined to resemble one lesion, but most frequently there was no evidence of hyphae surrounding the vascular bundle.

Formation of pycnidia was initiated within 25 days of inoculation under the experimental conditions. There were no morphological differences between cultivars in pycnidia development. Hyphae accumulated in the substomatal area, followed by development of a pycnidium. Pycnidia were oriented with their ostioles pointing toward the stomatal opening. Secondary spore production from these pycnidia was shown to be highly correlated with disease resistance. The resistant cultivars had significantly fewer pycnidia and pycnidiospores which would greatly influence the secondary spread of the pathogen under usual field conditions. The resistant cultivars are able not only to restrict the extent of lesion development, but also are able to restrict the quantity of secondary inoculum.

Resistance to water congestion of leaves was correlated with disease susceptibility or resistance. Cultivars that water congested easily also were susceptible to infection by S. nodorum while cultivars resistant to water congestion also were disease resistant. When the surface cuticle layer of resistant cultivars was mechanically

disrupted in order to increase their susceptibility to water congestion, their susceptibility to the disease also was increased. When non-treated resistant plants were left in the mist chamber for extended periods of time both the degree of water congestion and disease severity increased. Water congestion has been implicated as a predisposing factor in other diseases (27, 42).

A number of leaf-spotting diseases of wheat depend on the length of post-inoculation dew periods for infection (35, 36, 37, 38). Heggestad (27) has shown that there is a varietal response to degree of water congestion and that breeding for increased resistance to water congestion is feasible. A portion of the resistance of wheat cultivars to infection by S. nodorum may be due to their inherent ability to withstand water congestion. The presence of free intercellular water within the host tissue may alter the metabolism of the host sufficiently to allow successful colonization by the pathogen. Furthermore, when an infected susceptible plant is placed in an atmosphere not conducive to the maintenance of free intercellular water the progression of the disease stops (22). When the same plant is placed under conditions which allow for some free intercellular water (high relative humidity) the pathogen will start to spread again. The presence, therefore, of free intercellular water plays an important role in the host's response to invasion and colonization by S. nodorum. Since

varietal differences in susceptiblity to water congestion are known it seems likely that at least a portion of resistance to S. nodorum is due to resistance to water congestion. Free intercellular water may influence disease development in a number of different ways.

In the resistant cultivars disintegration of hyphae occurs starting approximately 96 hours after inoculation. This does not occur in the susceptible cultivars. This may be due to the accumulation of plant produced substances such as phenols or other antifungal compounds. It is impossible to state what these might be, if present, since they were not specifically analyzed. Water congestion may play a role here as any antifungal substance produced by stressed cells may be diluted away in the susceptible cultivar so that they are not as effective as in the resistant cultivar where they remain concentrated in a localized area and are able to inhibit fungal growth.

Water congestion may also play a direct role in stressing host plant cells thus weakening them which allows for increased pathogen colonization. Septoria nodorum is a very efficient colonizer of weak, dying, or dead tissue, but does not appear to be able to invade healthy, actively metabolizing tissue to any significant level (86). Water congestion would change osmotic conditions within the plant allowing for the leaching of metabolites and ions out of the affected cells and inhibiting those reactions which require aerobic conditions.

Water congestion essentially places the environment within the leaf tissue in an anaerobic state which would greatly affect the metabolic activities of associated cells. These stressed cells would thus be more efficiently colonized by the pathogen.

In addition to the stress factor, the leakage of host cell metabolites and ions would have to be considered as these would be beneficial for increased growth of S. nodorum. Septoria nodorum is able to germinate on water agar, but it is not able to sustain normal growth without nutrients from its surrounding environment. The leakage of materials from host cells into the intercellular water is the main source of nutrients for S. nodorum since this fungus does not produce a haustorum for nutrient extraction.

Septoria nodorum produces a non-specific toxin and it may be the role of this toxin to kill or severely stress the plant cells so that they are more readily colonized. This toxin is non-specific with regard to resistant and susceptible cultivars (48) and will cause typical chlorosis when concentrated fractions are artifically applied. In the highly water-congested susceptible cultivars, the intercellular water may act as a transporting substance for the toxin, allowing it to spread through the tissue. The increased number of toxin affected cells would allow for increased tissue colonization by S. nodorum. In the resistant cultivars (non-water congested), or in susceptible

cultivars under non-water congesting conditions, there is little intercellular water and thus the effects of the toxin are limited to small areas surrounding fungal hyphae. If the fungus is unable to colonize healthy, unstressed host tissue, then lesions must be self-limiting. With a subsequent period of high relative humidity causing an increase in intercellular water in the susceptible cultivar, there would again be a spread of the toxin and an increase in the extent of the tissue colonized. Those cultivars which take prolonged periods of time under extremely high levels of relative humidity to show water congestion would thus show little or no effects with the same conditions that allow disease progression in susceptible cultivars.

SUMMARY

The main differences found in this study with regard to the defense mechanisms in wheat to invasion and colonization by S. nodorum were: 1) a difference in germination of spores on the leaf surfaces of Fortuna and Manitou and the rest of the cultivars, and 2) differences among the three disease-resistant classes of cultivars as to the internal progression of the disease.

The germination difference is not explained by volatile compounds emitted from the leaves since germination was neither inhibited or stimulated under in vitro conditions. Septoria nodorum spores germinate well on water agar or on membranes. Therefore, the difference does not appear to be nutritional unless metabolites were present on the leaves of Fortuna and Manitou which stimulated or inhibited metabolic processes necessary for germination (ie. feed-back inhibition type mechanisms). It is probable that inhibitory or stimulatory compounds were present in the two cultivars that exhibited significant differences, but they were not concentrated enough to be evident in this experiment or else they were not volatile in nature. The majority of cultivars did not influence spore germination percentages. Spore germination on Manitou never reaches the level found on Fortuna, but disease severity may be as great when plants are held in the mist chamber for extended periods of time.

The major defense mechanism in the wheat cultivars studied was

related to the compatibility of the pathogen with the internal environment of the host. Here, the degree of water congestion played a significant role in disease progression. Water congestion may function in 1) the spreading of a toxic substance produced by the fungus; 2) placing plant cells in an unfavorable metabolic environment for adequate disease resistance; 3) the leaching of nutritional substances from the host tissue; or 4) combining one or more of the above factors.

The primary defense mechanism observed in this study was the effect of water congestion on the progression of this disease. Three possible effects of this water congestion on disease progression have been postulated although it is likely that they act in conjunction with one another and not separately. Varietal differences in water congestion have been noted by others (27) and incorporation of water congestion resistance into a S. nodorum resistance breeding program may be of value.

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