Tan spot of wheat, caused by *Pyrenophora tritici-repentis*: host reaction and pathogen variability, host pathogen interactions and involvement of toxin in disease.

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© Lakhdar Lamari

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FOREWORD

The materials, methods and results in this thesis are presented in the form of three manuscripts intended for publication in the Canadian Journal of Plant Pathology for sections 1 and 2 and in Phytopathology for section 3. The style as well as the preparation of tables and figures comply with the requirements of the journals. A general discussion and bibliography are included after the manuscripts.

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GENERAL ABSTRACT

Lamari L., 1988. Tan spot of wheat, caused by Pyrenophora tritici-repentis: host reaction and pathogen variability, host-pathogen interactions and involvement of toxin in disease. Major Professor C.C. Bernier.

Tan spot of wheat, caused by Pyrenophora tritici-repentis (Ptr), has become in recent years a major leaf spotting disease of wheat in Western Canada. A study was initiated to evaluate wheat reaction to the pathogen and to assess the variability for virulence in the population of the pathogen in Manitoba. A rating system based on lesion type was used to evaluate 695 wheat accessions for reaction to Ptr. High levels of resistance, characterized by small dark brown to black primary lesions with very little or no chlorosis and necrosis, were identified in cultivated as well as in wild wheat species. A previously undescribed symptom, consisting of small brown to black lesions and extensive chlorosis was observed in about 5 % of the accessions. Seedling reactions at the 2 leaf stage in the growth room were generally similar to adult plant reactions in the field. Tan necrosis and extensive chlorosis were intrinsic to individual wheat cultivars and their expression was the result of specific interactions with individual isolates of the pathogen. Three pathotypes were identified when 92 isolates of Ptr from Western Canada were inoculated on 11 differential wheat cultivars. Pathotype 1 induced both tan necrosis and extensive chlorosis (nec+ ch+), pathotype 2 induced tan necrosis only (nec+ ch-) whereas pathotype 3 induced extensive chlorosis only (nec- ch+). A cultivar-specific toxin(s) produced by the fungus in vitro was associated with the induction of tan necrosis, but not chlorosis. Sensitivity to the toxin and susceptibility to the fungus (tan necrosis) were found to be controlled by a single dominant gene. Toxin production in the pathogen was associated with tan necrosis inducing isolates. The toxin(s) of Ptr appears to be a pathogenicity factor.

GENERAL INTRODUCTION

In recent years, tan spot, caused by the ascomycete Pyrenophora tritici-repentis (Died.) Drechs. (anamorph Drechslera tritici-repentis (Died.) Shoem.), has become an important disease of wheat worldwide (Hosford, 1982). The disease has been reported in several countries including Australia (Valder and Shaw, 1952), Belgium (Maraite and Weyns, 1982), Brazil (Mehta and Almeida, 1977), Canada (Tekauz, 1976; Tekauz et al, 1982; Tekauz et al, 1983), India (Misra and Singh, 1972), Mexico (Gilchrist et al, 1984), and the U.S.A. (Hosford, 1971; Hosford and Bush, 1974; Watkins et al, 1978). The increase in disease incidence appears to have coincided with the introduction of soil conservation practices in the major wheat producing areas of the world. In Australia the shift from stubble burning to stubble retention and zero-tillage practices are believed to have largely contributed to increased incidence of the disease (Rees, 1982; Klein and Ellison, 1982). In Manitoba, the potential of the disease was first recognized in 1974 (Tekauz, 1976) and disease losses were subsequently assessed on winter wheat (Tekauz et al, 1983). Tan spot is currently found in all the major wheat growing areas of Manitoba and Saskatchewan.

Resistance to tan spot in wheat has been reported (Hosford, 1982; Nagle et al, 1982; Misra and Singh, 1972; Gilchrist et al, 1984; Lee and Gough, 1984; Raymond et al, 1985), and found to be dependant on the length of the postinoculation wetness period (Hosford, 1982). A leaf wetness duration of 24 h was found to be satisfactory for the expression of symptoms and separation between susceptible and resistant cultivars (Gilchrist et al, 1984). However, according to Hosford (1982), most cultivars, including those considered to be resistant, became "spotted" after 48 h of leaf wetness. Wheat resistance to P. tritici-repentis has been reported to be monogenically (Lee and Gough, 1984) or polygenically (Nagle et al, 1982; Cantrell et al, 1985) inherited. The

species Tritici-repentis is believed to have the widest host range in the genus Pyrenophora (Shoemaker, 1962) and at least 26 gramineous species can be infected (Krupinsky, 1982). Furthermore, variation in virulence has been reported in India (Misra and Singh, 1972), Mexico (Gilchrist et al, 1984) and the United States (Luz and Hosford, 1980; Krupinsky, 1987). In the study of Luz and Hosford (1980), 40 isolates were compared on six wheat and one barley cultivar, and 12 races were identified on the basis of disease severity and lesion size.

Control of tan spot with foliar fungicides is feasible (Tekauz et al, 1983; Watkins et al, 1982), but may be cost prohibitive. The incorporation of resistance into presently susceptible wheat cultivars through breeding is desirable. To achieve stable resistance, the variability in reaction of the host as well as the virulence of the pathogen must be known and utilized. Also, the understanding of the interactions between wheat and P. tritici-repentis is essential to the development of effective control measures, through genetic means.

The first section after the literature review deals with an evaluation of host reaction to P. tritici-repentis, a newly-developed procedure for the production of the pathogen inoculum and a comparison between host reactions obtained in controlled environment and field tests. The second section is concerned with variations in the virulence of the pathogen population and with host-pathogen interactions. The third section deals with toxin production by P. tritici-repentis, cultivar-specificity, inheritance of the toxin reaction in wheat and the role of the toxin in disease.

REVIEW OF LITERATURE

1. THE FUNGUS

1.1 Taxonomy

Tan spot of wheat is caused by the ascomycete Pyrenophora tritici-repentis Died. (syn. P. trichostoma (Fr.) Fckl.), anamorph: Drechslera tritici-repentis (Died.) Shoem. (syn. Helminthosporium tritici-repentis Died.). The pseudothecia of P. tritici-repentis are spherical and measure 400-500 µm in diameter. The ascocarp body is glabrous, but has a sparsely setose beak (Shoemaker, 1962). The asci are bitunicate, constricted at the base and 8 spored. The ascospores are typically 3 septate and have one longitudinal septum in the central cell (Shoemaker, 1962). The asexual stage sets P. tritici-repentis apart from other species in the genus. Conidia produced by D. tritici-repentis are cylindrical, rounded at the apex, and have a characteristic conical or "snake head-shaped" basal cell. Conidia measure (75)95-165(255) x (12)14-18(20) µm (Shoemaker, 1962).

P. tritici-repentis most resembles Pyrenophora bromi (Died.) Drechs. in gross morphology, and spore color. Because both can infect brome grass, they are often isolated together from this host species (Krupinsky, 1986). However, P. bromi has been found to be slow growing on sucrose proline agar (SPA) (Shoemaker, 1962; Krupinsky, 1986) and to sporulate better on lima bean agar than P. tritici-repentis (Krupinsky, 1986). The taxonomic significance of mycelial growth rate on SPA has not been resolved. At present, P. tritici-repentis is identified most reliably on the basis of the conidial morphology and the sexual reproductive structures when they are produced.

Color variation in P. tritici-repentis has not been extensively studied. Changes in colony color have been reported following subculturing (Hosford, 1971). It was also found that ascospores from the same ascus produced colonies segregating with regard to color (Hunger and Brown, 1987). Segregation for spore color has not been reported for

for P. tritici-repentis and spore colour mutants would facilitate genetic studies with the fungus.

1.2 Host range

P. tritici-repentis is believed to have the widest host range of all members of the genus (Shoemaker, 1962). Pathogenicity of the fungus to wheat and other Triticum and Agropyron species, and to brome grass and rye has been demonstrated (Hosford, 1971). Several studies have shown, however, that the fungus is only slightly pathogenic to barley (Luz and Hosford, 1980; Hosford, 1971). In an epidemiological study, P. tritici-repentis was found to be the main pathogen on a native prairie dominated by Agropyron spp. in Saskatchewan, Canada (Morrall and Howard, 1975; Howard and Morrall, 1975). At least 26 species of grasses were infected by P. tritici-repentis to some degree, when inoculated with four pathogen isolates (Krupinsky, 1982).

The wide host range of P. tritici-repentis is in contrast with that of other members within the genus, such as Pyrenophora teres Drechs., which only infects barley. The ability of P. tritici-repentis to colonize such a large number of grasses, most of which are perennial and grow in wheat producing areas, may facilitate overwintering and provide inoculum to initiate tan spot epidemics in farm fields (Krupinsky, 1982, 1986,1987).

2. EPIDEMIOLOGY

2.1 Initial Inoculum

P. tritici-repentis overwinters on infected wheat stubble and likely on straw and stubble of other grasses. Infections in the field were observed when wheat was at the 3 leaf stage (Hosford, 1971). The frequency of these infections was associated with the prevalence of ascostromata.

The involvement of ascospores in the early stages of epidemic development has been demonstrated directly or indirectly by several workers. Morrall and Howard (1975) trapped spores over a native prairie and found that the number of ascospores peaked before that of the conidia. An epidemiological study in Australia suggested that tan spot epidemics were mainly initiated by ascospores and possibly conidia from the stubble (Rees and Platz, 1980). However, large numbers of conidia were produced late in the season on old tissues, suggesting a secondary cycle in the development of the epidemics. Ascospores were found to be disseminated only over relatively short distances. In a study of disease gradient there were almost no infections at 200 m from the inoculum source early in the season (Rees and Platz, 1980).

The role of ascospores in the initiation of disease was clearly demonstrated in field studies by Raymond et al (1985). The researchers spread oat kernels, on which pseudothecia of P. tritici-repentis were produced, on the surface of their winter wheat plots in the fall and found that infections prior to the month of May in the following year were caused by ascospores. Thereafter, conidia were the only propagules released and caused all subsequent infections. Whether conidia are released in naturally infested fields, sufficiently early in the season to provide initial inoculum, remains an interesting epidemiological question to resolve. Further knowledge of the possible role of conidia, in the initiation of disease in the field would help to better understand the dynamics of the disease.

2.2 Conidial Sporulation

The conditions of conidial production in P. tritici-repentis have been studied to some extent. The fungus is a diurnal sporulator and needs a light period for conidiophore induction and a dark period for conidial production (Khan, 1971; Platt and Morrall, 1980a, 1980b). Odvody and Boosalis (1978) determined that conidiophore induction was

achieved at wavelengths of less than 350 nm over a temperature range of 10 to 31 C, with an optimum of 24-26 C. A minimum dark period of three hours was required for conidiation by isolates of P. tritici-repentis studied by Platt and Morrall (1980a). However, Odvody and Boosalis (1982) later demonstrated that no dark period was needed to produce conidia at temperatures between 10-15 C.

2.3 Spore liberation

Release of conidia was shown to be dependant on wind speed and relative humidity (RH) (Platt and Morrall, 1980b). Wind speeds of 3.3 $m.s^{-1}$ resulted in the liberation of 100 % of spores at all RH values. Even at a relatively low wind speed, 60 % spore liberation was obtained at 35 % RH. Liberation was greater with changing than with constant RH. The environmental conditions occurring in a western Canadian grassland, where the study was conducted, were found to satisfy all the requirements necessary for a 100 % release of conidia of P. tritici-repentis.

2.4 Leaf wetness duration

Leaf wetness duration is regarded as a major factor in tan spot disease initiation and development. According to Hosford (1982), wheat resistance to P. tritici-repentis and six other leaf spotting imperfect fungi was dependent on the length of the postinoculation leaf wetness period. The most susceptible lines and cultivars required only six hours whereas in more resistant ones, 48 h of leaf wetness were required for infection and lesion development. All wheat cultivars became "spotted" after 48-54 h (Hosford, 1982). In a study of the interaction between leaf wetness duration and temperature, Hosford et al (1987) found that increasing leaf wetness duration and/or temperature increased disease development in both resistant and susceptible cultivars.

3. INOCULUM PRODUCTION

In spite of a substantial body of information on the conidiation process in P. tritici-repentis, simple techniques for the large scale synchronous production of conidia are not available. In early studies, cultures of the fungus growing on agar medium were blended in water and used as inoculum in pathogenicity tests (Hosford, 1971). The fungus was also grown in liquid medium and the mycelial mats blended and used as the infective propagules (Lee and Gough, 1984). In more recent studies, conidial inoculum has been produced by transferring plugs from cultures grown on potato dextrose agar (PDA) to V8-agar and incubating under light (24-48 h) and dark (18 h) periods (Odvody and Boosalis, 1982). Subsequently, the conidia were harvested by transferring the plugs to flasks containing water and shaking these vigorously, or the plugs were simply blended in water and the resulting suspension containing conidia, conidiophores and mycelium was used (Luz and Hosford, 1980; Hosford, 1982; Cox and Hosford, 1987). A more efficient technique was recently developed by Raymond et al (1985). This method uses overlapping, though not mixed, PDA and V8-agar on the same plate. The fungus is placed on the centre of the PDA "pad" and allowed to grow onto the V8 medium. The mycelium is then wetted, "knocked down" and incubated for 12 h in light followed by 12 h of darkness.

4. INFECTION PROCESS

Very few studies have been concerned with the infection process in tan spot of wheat. The only two existing reports (Larez et al, 1986; Loughman and Deverall, 1986) indicate that the process is similar to that described for net blotch of barley caused by P. teres (Keon and Hargreaves, 1983). No differences were found in spore germination, the number of appressoria produced or the successful penetration of epidermal cells between resistant and susceptible wheats (Larez et al, 1986; Loughman and Deverall,

1986). Penetration usually occurred directly on the anticlinal wall of epidermal cells or occasionally through stomates. The fungus then produced a vesicle and subsequently colonized the entire epidermal cell. Hyphae from the vesicle entered the mesophyll and grew intercellularly, resulting in visible lesions (Larez et al, 1986; Loughman and Deverall, 1986). There was no visible evidence of any structures that might be responsible for resistance to P. tritici-repentis in wheat (Larez et al, 1986; Loughman and Deverall, 1986). The only difference found between resistant and susceptible wheats was the greater distance and faster speed of intercellular growth achieved by the pathogen in the mesophyll of susceptible cultivars. In cases were the fungus failed to penetrate, papillae were apparently involved. However, such papillae are not considered to play a major role in wheat resistance to tan spot (Larez et al, 1986).

5. HOST-PARASITE INTERACTIONS

Very little work has been reported on physiologic variation in P. tritici-repentis. The only study that involved a substantial number of isolates was that of Luz and Hosford (1980). They tested 40 isolates from a wide geographic area and differentiated 12 races on the basis of the amount of spotting each caused on a differential set of six wheat and one barley cultivar. Misra and Singh (1972) tested three isolates on 50 genotypes and found significant differences in the level of pathogenicity of the isolates. More recently Gilchrist and co-workers (1984) reported significant differences in lesions per plant, on the cultivar Morocco inoculated with eight isolates of P. tritici-repentis. Most of this work was carried out using rating systems based, at least in part, on some quantitative variables, such as percent infection or number of lesions per unit area. While such systems may be useful in distinguishing between levels of susceptibility in field trials, or the assessment of partial resistance, they are not sufficiently precise for use to identify pathogen races.

6. RESISTANCE TO TAN SPOT

The relatively low yields of wheat achieved in the major wheat growing areas of North America, in contrast to those achieved by intensive cultures in other regions, make the use of chemicals for disease control cost-prohibitive. Therefore, genetic resistance has been sought (Hosford, 1982; Gilchrist et al, 1984; Raymond et al, 1985).

Hosford (reported by Nagle et al, 1982) tested over 4000 wheat accessions for resistance to P. tritici-repentis. He found that resistance was dependent on the length of the leaf wetness period following inoculation. No cultivar was found to express a high level of resistance after a wet period greater than 48-54 h (Hosford,1982). Resistance has also been reported from Mexico in greenhouse and field tests conducted on wheat lines in the International Bread Wheat Screening Nursery (IBWSN) (Gilchrist et al, 1984). Nine lines were rated resistant in both the greenhouse and in the field. Cultivar BH1146 was found to be moderately susceptible in this study. The variability in the reaction of cultivar BH1146 may be due in part to differences in the virulence of the isolates used by various researchers, but may also be due to the use of different criteria for rating resistance. The absence of a universally acceptable rating system for tan spot reaction precludes any meaningful comparisons in the data reported by different workers.

7. RATING SYSTEMS

Several rating systems have been developed for the wheat/ P. tritici-repentis system. These reflect a lack of agreement and the confusion that exists in the conceptualization of resistance and susceptibility. Most rating systems reported in the literature include a quantitative measurement of disease, i.e. disease severity (Misra and Singh, 1972), number of lesions/leaf (Gilchrist et al, 1984), a combination of lesion size, percentage infection and leaf position (Raymond et al, 1985), lesion size only (Cox and Hosford,

1987) or a combination of lesion types and number of lesions per unit area (Gilchrist et al, 1984).

The assessment of tan spot under field conditions has usually been done using scales developed for wheat leaf spots in general, and most often for Septoria leaf blotch (Couture, 1980; Saari and Prescott, 1975). Such scales may be useful for epidemiological purposes, however, they may be inadequate for assessing cultivar resistance.

A system based on lesion types has been used successfully in screening for resistance to net blotch of barley caused by P. teres (Buchannon and McDonald, 1965). More recently the scale was refined to accommodate two pathotypes of the pathogen (Tekauz, 1985). The monitoring and detection of virulence is made considerably easier as reported for P. teres in Canada (Tekauz and Mills, 1974; Tekauz, 1976; Tekauz and Buchannon, 1977; Tekauz, 1978; Tekauz, 1986).

The development of a rating system based on lesion types is desirable to further tan spot research. Until such a system is developed, attempts to study host-parasite interactions and to breed for resistance will remain a difficult task.

8. TOXIN PRODUCTION AND PATHOGENESIS

8.1 Review

In the early stages of toxin research, some rather restrictive criteria were suggested for the assessment of the role of toxin in pathogenesis (Dimond and Waggoner, 1953; Wheeler and Luke, 1963). According to Wheeler and Luke (1963), a substance must satisfy the following criteria to qualify as a "pathotoxin" : 1) The toxin must induce all characteristic symptoms in the host 2) It must have the same specificity as the pathogen itself. 3) The toxin producing ability of the pathogen must be correlated with the ability to produce disease and, 4) One toxin should be involved.

The criterion that all symptoms of the disease should be induced by the toxin (Wheeler and Luke, 1963) has been questioned by Smedegaard-Petersen (1976). The existence of the net and the spot-net blotch of barley suggests a genetic control of symptom development that is independent of toxin action (Smedegaard-Petersen, 1976). Similarly there is no obvious reason why a pathogen should produce only one toxin. Other criteria have been reviewed and include host-specificity, production at a key step in disease development, and correlation of virulence with quantity of toxin produced in vitro (Yoder, 1980).

A broad definition of toxins has been recently formulated (Scheffer, 1982) and sets the following three criteria: toxins must a) " be produced by the pathogen", b) "cause obvious damage to plant tissues"; and c) " be known with confidence to be involved in disease development". Although there is abundant literature on the subject, only a small number of fungi have been identified, with confidence, to produce toxins that conform to the above criteria.

Toxins have been considered as primary or secondary factors of disease (Scheffer and Pringle, 1967). More recently, the terms "pathogenicity" and "virulence" factors have been used to classify the role of toxins in disease (Yoder, 1980). Pathogenicity is defined as the ability (of a pathogen) to cause disease whereas virulence meant the "amount or extent of disease", ie: expressing a quantitative phenomenon. The meaning of these two terms is therefore different from that used by rust researchers, where virulence is "intended to describe a specific relationship" between a host cultivar and a race of the pathogen (Watson, 1970; Green, 1975). Virulence is expressed when the host lacks resistance genes or when its resistance genes are "matched by virulence genes in the pathogen" (Green, 1975).

The strongest evidence for toxin involvement in disease has been obtained, so far, by genetic analyses of the host and/or the pathogen (Yoder, 1980). Crosses between

Helminthosporium victoriae Meechan & Murphy (oat pathogen) and Helminthosporium carbonum Ullstrup race 1 (maize pathogen) produced two parental and two recombinant types indicating the involvement of two loci (Scheffer et al, 1967). The recombinant types were as follows: i) Production of toxins against both maize and oats and ii) complete loss of toxicity to both maize and oats. Other pathogens are also known to produce host-specific toxins and to have lost pathogenicity (or virulence) with the loss of toxin production. These include Periconia circinata (Mangin) Sacc., the causal agent of the milo disease of sorghum (Odvody et al, 1977), and some members of the genus Alternaria (Nishimura and Kohmoto, 1982).

8.2 Toxin(s) involvement in tan spot of wheat

The involvement of a cultivar-specific toxin in the tan spot disease of wheat was recently reported (Tomas and Bockus, 1987). The toxin was found to induce "typical tan spot symptoms", when infiltrated in leaves using a "Hagborg" device (Hagborg, 1970). Compared to previously reported toxins, this did not seem to have much potency. This is in contrast to most host-specific toxins, which exhibit high potency, ie: produce an effect even at high dilutions. More information is needed to establish the role of the toxin(s) found in culture filtrates of P. tritici-repentis in disease syndrome.

9. CONTROL OF TAN SPOT OF WHEAT

In the absence of high levels of resistance in wheat, cultural practices consisting of rotations with non-host species have been attempted to control tan spot in Australia (Rees and Platz, 1979, 1980). However, since there is evidence that P. tritici-repentis infects several species of grasses which may provide inoculum in the absence of wheat stubble (Krupinsky, 1982, 1986), rotations may not be always effective. Fungicides have been used to effectively control tan spot in field experiments (Tekauz et al, 1983) and

ammonium nitrogen was found to decrease the disease severity (Huber et al, 1987), as based on lesion size. There are no published reports of the effects of plant type or canopy structure on disease development and severity. These parameters have been found to influence disease development of Septoria leaf blotch (Bahat et al, 1980; Scott et al, 1985).

RESULTS OF RESEARCH

1. Evaluation of wheat for reaction to tan spot caused by Pyrenophora tritici-repentis

ABSTRACT

A total of 695 wheat accessions were evaluated for reaction to P. tritici-repentis in the growth room and categorized, using a rating system based exclusively on lesion type. A range of reactions was observed in the di-, tetra-, hexa- and octoploid wheats, and high levels of resistance were identified in all ploidy groups. The wild wheat species had generally higher proportions of resistant genotypes than the cultivated tetra- and hexaploid species. Resistance was characterized by small dark brown to black lesions (primary lesions) without, or with slight amounts of, tan necrosis or chlorosis, and susceptibility by primary lesions surrounded by either tan necrosis or chlorosis. About 5 % of the entries in all the ploidy groups, except the octoploid, developed primary lesions and extensive chlorosis, that expanded to cover the entire leaf. Reactions obtained in tests at the 2 leaf stage were similar to those obtained at the 4-6 leaf stage in the growth room and on mature plants in the field. Cultivars with the lowest lesion type scores had the lowest disease severity rating in artificially inoculated field plots and in plots sown into infected wheat stubble.

INTRODUCTION

Tan spot of wheat caused by Pyrenophora tritici-repentis (Died.) Drechs. (syn. P. trichostoma (Fr.) Fckl.), anamorph Drechslera tritici-repentis (Died.) Shoem., is known to be a potentially destructive disease (Hosford and Bush, 1974; Watkins et al, 1978; Tekauz et al, 1983) and to occur world wide (Hosford, 1982). In 1974, tan spot was found to be the most important leaf spot disease in the Canadian Prairies (Tekauz, 1976) and severe infections at the seedling stage were observed for the first time in 1982 (Tekauz et al, 1982).

Recent increases in disease incidence have been attributed to changes in cultural practices (Rees, 1982; Rees and Platz, 1979). The shift from stubble burning to its retention, along with conservation and zero-tillage practices are believed to be the most important causes of the increase in disease incidence in Australia (Rees, 1982). Control of tan spot can be achieved with foliar fungicides (Tekauz et al, 1983; Watkins et al, 1982) but costs may be prohibitive. Recently, application of ammonium nitrogen was found to reduce disease severity (Huber et al, 1987).

P. tritici-repentis is known to infect many gramineous species (Morrall and Howard, 1975; Krupinsky, 1982), some of which are common to the major wheat growing areas of North America. The pathogen overwintering on these hosts may provide inoculum, in the spring, in the absence of wheat infested stubble (Krupinsky, 1982). Variation in virulence patterns has been demonstrated (Luz and Hosford, 1980; Misra and Singh, 1972) and differences in virulence were observed between isolates on a single host (Gilchrist et al, 1984).

The expression of wheat resistance to tan spot has been reported (Hosford, 1982; Cox and Hosford, 1987; Gilchrist et al, 1984; Raymond et al, 1985) and found to be influenced by the duration of the postinoculation leaf wetness period (Hosford, 1982). A leaf

wetness period of 24 h was found to be adequate for separation of susceptible and resistant wheat genotypes (Gilchrist et al, 1984; Hosford, 1982) whereas most wheats become "spotted" after 48-54 h of continuous leaf wetness (Hosford, 1982). Disease severity was also found to increase with increasing leaf wetness duration and/or temperature (Hosford et al, 1987). Resistance to tan spot has been reported to be polygenically (Nagle et al, 1982; Cantrell et al, 1985) or monogenically inherited (Lee and Gough, 1984).

There have been several rating systems used to describe host reactions to P. tritici-repentis. These include % infection (Nagle et al, 1982), lesion size and % infection (Luz and Hosford, 1980), number of lesions/cm^2 (Nagle et al, 1982), an index combining lesion size, % leaf area infected and leaf location (Raymond et al, 1985), lesion type and number of lesions (Gilchrist et al, 1984) and, most recently, lesion size alone (Cox and Hosford, 1987). In a preliminary test for this study, % leaf infection and a combination of lesion size and % leaf infection were used to characterize host reaction to tan spot and found to be unsatisfactory.

Despite a substantial body of information on the sporulation process in the pathogen, large scale production of inoculum remains tedious. Odvody and Boosalis (1982) found that large numbers of conidia could be obtained by first culturing the fungus on potato dextrose agar (PDA) and then transferring disks from the colony margins onto a modified V-8 agar medium (Platt et al, 1977). The cultures were subsequently exposed to a light source for 24 to 48 h followed by 18 h of continuous darkness. Recently, Raymond et al (1985) simplified this procedure by including sectors of both media in the same Petri plate.

A program was initiated in 1984 at the University of Manitoba to: i) evaluate wheat germ plasm for reaction to local isolates of P. tritici-repentis; 2) develop a rating system for the rapid and reliable identification of host reaction for use in studies of

host-pathogen interactions and for use in breeding for resistance; and 3) evaluate the variation in the pathogen population in Manitoba with regard to virulence.

This report deals with the identification of resistant genotypes, and the assessment of the range of symptoms induced in various wheat genotypes by P. tritici-repentis using a rating system based on lesion type. Modifications to existing techniques of inoculum production and germ plasm evaluation are described, and results of growth room and field evaluations are compared.

MATERIAL AND METHODS

Fungal isolation. Naturally infected leaf samples were cut into 0.5-1 cm pieces and placed in a Petri plate containing a wet filter paper to maintain high humidity. The plates were incubated under fluorescent light for 24 h at room temperature (20-25 C) to promote the production of conidiophores. The plates were then incubated for 18 to 24 h in the dark at 15 C to produce conidia. Single conidia were transferred to V8-PDA medium (described below) and incubated at 20 C in the dark until the colony reached 3-4 cm in diameter.

Inoculum production. The method of Raymond et al (1985) was modified as follows for use in all steps of inoculum production: i) the separate V8 and PDA media were combined into a single medium (V8-PDA), consisting of V8-juice (150 mL), Difco PDA (10g), $CaCO_3$ (3 g), agar (10 g) and distilled water (850 mL); and ii) the cultures were grown at 20 C under continuous darkness, instead of continuous light, to prevent conidiophore formation during mycelial growth. Small plugs, 0.5 cm in diameter, from a 4-8 day culture of P. tritici-repentis were transferred singly to 10 cm Petri plates, containing 30 mL of V8-PDA. Alternatively two plugs were placed on the same plate at ca. 3-4 cm of each other for faster colonization of the medium. The cultures were then incubated further in the dark until the colony had reached ca. 4 cm in diameter. The cultures were then flooded with sterile distilled water and the mycelium flattened with the bottom of a flamed test tube, essentially as described by Raymond et al (1985). After the water was decanted, the plates were subjected to the same light and dark regimes as described above for single spore isolation.

Conidia were harvested following the procedure used by A. Tekauz (Agriculture Canada, Winnipeg, personal communication) for P. teres and P. tritici-repentis. The

culture plates were flooded with sterile distilled water and the conidia dislodged with a wire loop by applying only slight pressure on the loop, so as not to remove conidiophores or pieces of the medium from the plate. Two or three additional water rinses were made to resuspend and recover the conidia that had settled. The spore suspension was left at 5 C for 10 to 15 min until the spores had settled. One half to 2/3 of the water was decanted, and the spore suspension adjusted with sterile distilled water to approximately 3000 conidia/mL using a cell counter (Hausser Scientific, Blue Bell, Pa.). Ten drops of Tween20 (Polyoxyethylene sorbitan monolaurate) were added per litre of conidial suspension, to reduce surface tension. If needed, the plates were subjected again to light and dark regimes for additional harvests.

Inoculation. The monoconidial isolate ASC1 of P. tritici-repentis was used as a source of inoculum throughout the present study. This isolate was recovered from leaves of the winter wheat cultivar Norstar, grown near Minto, Manitoba. The inoculation technique was similar to the method used by A. Tekauz (Agriculture Canada, Winnipeg, personal communication). Seedlings were sprayed with a conidial suspension until runoff using a DeVilbis sprayer connected to an air outlet and operated at a pressure of 10 psi. After inoculation the seedlings were placed in an incubation chamber that ensured continuous leaf wetness for the desired duration. During incubation, the temperature was maintained at 20 C and the photoperiod at 16 h. The incubation period always coincided with the beginning of the dark period. After inoculation, the pots were transferred to a growth room at 20/17 C (day/night) and 16 h photoperiod and rated for lesion morphology 6-8 days later.

Incubation chamber. An incubation chamber consisting of a polyvinyl chloride frame (2.5 m x 1 m x 1.4 m) and covered with a clear polyethylene sheet was assembled inside

a growth cabinet. Continuous leaf wetness was obtained by periodic application of distilled water using two ultrasonic humidifiers under microcomputer (Commodore Vic20) control. The wetness level was monitored using an impedance grid connected to the computer's analog-to-digital converter. A simple BASIC program measured the resistance of the grid and operated the humidifiers to maintain a user selected wetness level. At the end of the humidification period, two fans were actuated by the computer, under program control, to dry the plants for a predetermined period. The software and interface between the computer and the equipment were developed by the author (unpublished).

Accessions. A total of 695 lines and cultivars from the wheat collection of the University of Manitoba (U of M) were evaluated for their reactions to isolate ASC1 of P. tritici-repentis. These included cultivated as well as wild wheat species from different ploidy groups (Table 1) and from diverse geographic origin.

Growth room evaluation. In a first experiment, plants were inoculated at the 4 to 6 leaf stage and incubated for 36 h under continuous leaf wetness. Accessions were evaluated in groups of 80-90 entries by seeding six seeds of each of three accessions as three clumps in 15 cm diameter clay pots, filled with commercial soil mix (Metro-Mix 220, W.R. Grace and Co., Ajax, Ontario). In a subsequent experiment, plants from 116 accessions were inoculated at the 2 leaf stage and incubated for 24 h. Each pot was sown with six seeds from a single accession. When sufficient seed was available, each accession was replicated 3 times. The susceptible cultivars Glenlea (hexaploid) and Coulter (tetraploid) were included in each test as controls.

Field evaluation. A total of 156 lines and cultivars, including 81 from the initial 695 accessions were tested in the field in 1 m rows arranged in plots of 12 rows in a 1 m x 3 m space. Fifty to sixty seeds were sown by hand in each row. The susceptible cultivars Columbus (hexaploid) and Coulter (tetraploid) were included in each plot. Evaluation was conducted at three locations: at U of M plots in Winnipeg and Portage-la-Prairie, on winter wheat stubble known to be infested with Cochliobolus sativus (Ito & Kurib.) Drechs. ex Dastur., Septoria spp. and P. tritici-repentis, and on fallow land at the U of M (Winnipeg). Most lines were replicated two to three times depending on seed availability.

Plants were inoculated when approximately 50 % of the lines were at growth stage 10-10.5 on the Feekes scale (Large, 1954). Each plot received 500 mL of inoculum prepared as described above. Inoculum was delivered with a commercial backpack sprayer (Cooper-Pegler & Co., Burgess Hill, Sussex,England).

To favour continuous leaf wetness for at least a 12 h period, plants were inoculated late in the day and the soil between the rows was watered prior to inoculation. Wooden frames were placed over the plots and covered with a clear polyethylene sheet immediately after inoculation. The polyethylene cover was opened early in the morning to prevent excessive heat buildup. Late in the evening, the plots were sprayed with water until runoff and covered for a second time. The frames and the polyethylene cover were removed the next morning. Plots were rated for disease reaction 12 days after inoculation and every 10 days thereafter.

Rating. A rating system based on lesion type was developed to categorize host reactions to P. tritici-repentis. This system is based on lesion types recognized previously (Hosford, 1971; Gilchrist et al, 1984), but excludes lesion frequency or % leaf

area infected. The newest fully expanded leaf at the time of inoculation was rated numerically as follows:

1: Small dark brown to black lesions without any surrounding chlorosis or tan necrosis (Resistant).

2 : Small dark brown to black lesions with very little chlorosis or tan necrosis (Moderately resistant).

3 : Small dark brown to black lesions completely surrounded by a distinct chlorotic or tan necrotic ring; lesions are generally not coalescing (Moderately resistant to moderately susceptible).

4 : Small dark brown or black lesions completely surrounded with chlorotic or tan necrotic zones. Some of the lesions are coalescing (Moderately susceptible).

5 : The dark brown or black centres may or may not be distinguishable. Most lesions consist of coalescing chlorotic or tan necrotic zones (Susceptible).

In the field, artificially inoculated plants were rated for lesion type on the above scale of 1 to 5 and for disease severity on a scale of 0 to 9 as described by Couture (1980). Plants sown in stubble were rated for disease severity only.

RESULTS AND DISCUSSION

Lesion type varied greatly amongst the 695 wheat accessions tested at the 4 to 6 leaf stage in the growth room. The lesions all had small brown to black centers, referred to hereafter as primary lesions, but differed in the amount of chlorotic or tan-necrotic tissue surrounding the dark centre as shown in Fig. 1 and 2. Individual accessions could be effectively differentiated using a rating scale, based on lesion type (Fig. 1).

The number of accessions in each reaction class is presented in Table 1 and a list of the accessions is included in Appendix 1. The relatively large proportion of resistant and moderately resistant (scores 1-2) accessions in the tetraploid and in the hexaploid wheats may have been due the presence of a large number of "wild" wheat species including Triticum dicoccoides Koern, T. dicoccum Schrank, T. persicum Vav., T. timopheevii (Zhuk.) Zhuk., and T. zukhovskii Men. & Er., most of which were resistant.

Although over 50 % of the accessions in the tetra- and hexaploid wheats were moderately susceptible or susceptible (scores 4-5), approximately 20 % of the total were resistant to moderately resistant. These could provide a substantial amount of germ plasm for further evaluation and use in breeding programs. The proportion of resistant and moderately resistant accessions was significantly greater in the diploid wheats than in the tetra- and hexaploid wheats (Chi square significance test, Appendix 2), but the utilization of this resistance in tetra- and hexaploid wheats may be more difficult to achieve, because of gene-transfer problems (Heyne and Smith, 1967).

Reactions of 116 of the above accessions inoculated at the 2 leaf stage were generally similar to those observed previously at the 4-6 leaf stage. However, twelve accessions were rated resistant (scores 1-2) at the 4-6 leaf stage and susceptible (scores 4-5) in subsequent tests at the 2 leaf stage (Appendix 3). Since these accessions were susceptible in one test, it is more likely that the reduced score was more due to an escape from infection and not to a reversal in the disease reaction. In view of the general

Figure 1. A range of lesion types induced by a virulent isolate of Pyrenophora tritici-repentis on various wheat accessions.

Leaves from left (1) to right (9):
1,2: small dark brown to black primary lesion with no or slight amounts of tan necrosis or chlorosis (types 1 and 2). 3: primary lesion surrounded by a thin ring of chlorosis, but no coalescing (type 3); 4,5: primary lesion totally surrounded by chlorosis. Some coalescing is visible (type 4); 6: same as leaves 4 and 5 above but primary lesion surrounded by tan necrosis (type 4); 7,8,9: primary lesion surrounded by large amounts of tan necrosis (type 5). Note the coalescing of lesions.
All leaves are from plants inoculated at the 2 leaf stage with a conidial suspension (3000 conidia/mL) and incubated for 24 hours under continuous leaf wetness. The photograph was taken 8 days after inoculation.

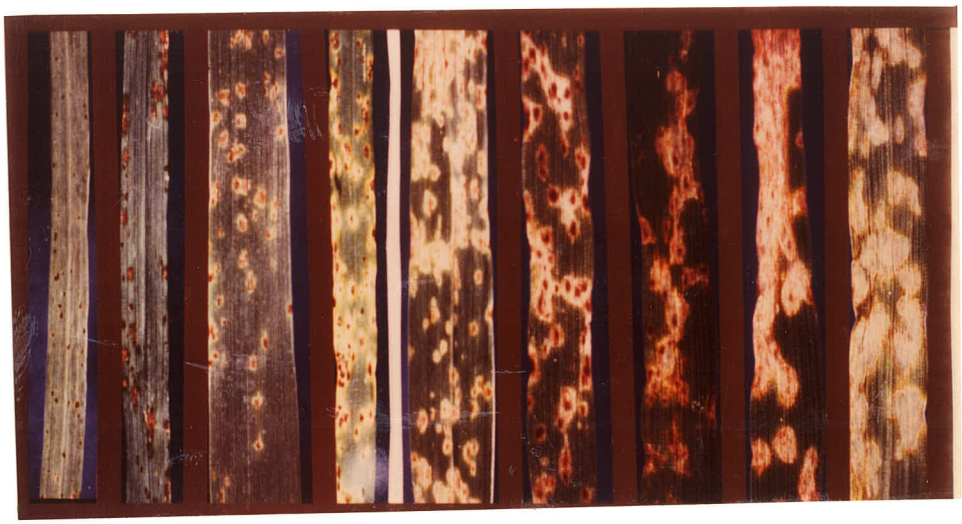


Figure 2. Symptoms of tan necrosis induced by isolate ASC1 of Pyrenophora tritici-repentis on susceptible wheat cultivar Glenlea.

Note the well defined lesions.

TABLE 1. SUMMARY OF THE REACTIONS OF 695 WHEAT ACCESSIONS TO ISOLATE ASC1 OF *PYRENOPHORA TRITICI-REPENTIS*.

PLOIDY CLASS	REACTIONS[&]					
	1	2	3	4	5	TOTAL
DIPLOID	8	11	14	5	3	41
TETRAPLOID	23	34	78	83	70	288
HEXAPLOID	18	34	68	101	108	329
OCTOPLOID	1	4	2	1	0	8
UNKNOWN	0	1	4	7	17	29
TOTAL	50	84	166	197	198	695

Values represent number of accessions.

& Rating scale of 1 to 5, where 1=small brown lesions without tan necrosis and chlorosis (primary lesion); 2= primary lesion with minute amounts of tan necrosis or chlorosis; 3= primary lesion surrounded by a thin ring of tan necrosis or chlorosis; 4= primary lesion surrounded by a definite ring of tan necrosis or chlorosis, sometimes coalescing; and 5= most lesions consist of coalescing tan necrotic or chlorotic tissues.

agreement between the results obtained at the two growth stages, testing at the 2 leaf stage rather than at the 4-6 leaf stage as in previous studies (Nagle et al, 1982; Larez an Hostford, 1985) may be preferable, as it requires less space, time and inoculum. Moreover, at the 4-6 leaf stage, senescent leaves may be present and these can interfere with the rating.

The reactions of a selection of cultivars representing a range of lesion types, including cultivars first found to be resistant in this study as well as some previously reported to be resistant, are presented in Table 2. All cultivars currently recommended for commercial production in Manitoba were found to be susceptible (score 4-5). The resistance reported for cultivars BH1146 (Luz and Hosford, 1980; Larez et al, 1986; Loughman and Deverall, 1986; Nagle et al, 1982), Carifen 12 (Lee and Gough, 1984; Cox and Hosford, 1987), and Norstar (Cox and Hosford, 1987) was not confirmed in this study. Resistance was also not confirmed for cultivar BH1146 tested in Mexico which was found to be moderately susceptible (Gilchrist et al, 1984) and cultivar Norstar which was found to be susceptible under field conditions in Manitoba (Tekauz et al, 1983). Although the discrepancies in the reactions of the cultivars can be explained, in part, by possible differences in the virulence of the isolates used, the different rating scales used by the various workers may also have contributed. For instance, the reaction of BH1146, described as resistant by Larez et al (1985), would be considered moderately susceptible (rating 3-4) in our system. Furthermore, the % leaf area infected measured on cultivars BH1146, Glenlea and Coulter using video image analysis (Lamari and Bernier, 1987) showed that, notwithstanding its moderately susceptible reaction, BH1146 was damaged considerably less by disease (20 %) than were the fully susceptible cultivars Glenlea (39 %) and Coulter (38 %) (Lamari, unpublished). The magnitude of the damage (ca. 20 %) sustained by BH1146 was similar to that reported previously (Hosford et al, 1987), suggesting that the reaction of BH1146 is stable and may be useful in studies on components of partial resistance.

TABLE 2. REACTIONS OF 19 SELECTED WHEAT LINES AND CULTIVARS TO ISOLATE ASC1 OF PYRENOPHORA TRITICI-REPENTIS.

CULTIVAR	PLOIDY[$]	ORIGIN	REACTION[&] AT LEAF STAGE	
			2	4-6
ARCOLA	4B	CANADA	5	5
BH1146	6B	BRAZIL	3-4	4
CARIFEN 12	6B	CHILE	3	3
COLUMBUS	6B	CANADA	4-5	5
COULTER	4B	CANADA	5	5
ERIK	6B	USA	2	1-2
GLENLEA	6B	CANADA	5	5
KATEPWA	6B	CANADA	5	5
NEEPAWA	6B	CANADA	5	5
NORSTAR	6B	CANADA	5	5
SALAMOUNI	6B	LEBANON	1-2	1-2
T.p. x T.m.	6B	UNKNOWN	1	1
4B 233	4B	LEBANON	1-2	1-2
4B 242	4B	USA	1	1-2
4B 1149	6B	MEXICO	1	1

$ 2B= diploid, 4B= tetraploid and 6B=hexaploid wheats.

& Rating scale of 1-5, where 1=small brown lesion without tan necrosis and chlorosis (primary lesion); 2= primary lesion with minute amounts of tan necrosis or chlorosis; 3= primary lesion surrounded by a thin ring of tan necrosis or chlorosis; 4= primary lesion surrounded by a definite ring of tan necrosis or chlorosis, sometimes coalescing; and 5= most lesions consisting of coalescing tan necrotic or chlorotic tissues.

A type of symptom consisting of minute dark brown to black primary lesions surrounded by extensive chlorosis was observed in about 5 % of the accessions tested. The chlorosis was usually not visible until the 4 th day after inoculation, when it rapidly expanded to cover the entire leaf (Fig. 3). By day 7, the leaves turned bright yellow and eventually collapsed. This type of reaction, which does not seem to have been reported previously, was referred to as "extensive chlorosis" and denoted as CH^+.

To confirm that the development of extensive chlorosis in wheat was an unique response to P. tritici-repentis, lines 6B365 and 6B780 (both CH^+) were inoculated with three other leaf pathogens: i) a mixture of races of Puccinia graminis f.sp. tritici Eriks. & E. Henn., ii) an isolate of Pyrenophora teres Drechs. and iii) an isolate of Cochliobolus sativus. The extensive chlorosis syndrome was not observed to occur with any of these pathogens (Table 3), suggesting that this type of symptom is the result of the specific interaction between P. tritici-repentis and wheat hosts.

It would appear from the present study that susceptibility to P. tritici-repentis is expressed either by tan necrosis as in BH1146 and Glenlea, or by chlorosis that can be extensive, as shown in lines 6B365 and 6B780. Resistance can therefore be characterized by an absence of chlorosis or tan necrosis or by very slight amounts of either. Similarly virulence in the pathogen is defined as the ability to cause tan necrosis and/or chlorosis. The meaning of the term "virulence" is analogous to that used by rust workers whereby a cultivar is resistant to a particular race of the rust, which is then considered to be "avirulent", but may be susceptible to another race said to be "virulent" (Green, 1975; Watson, 1970).

The rating scale used in the present study, which was based exclusively on lesion type, allowed for rapid and reliable evaluation of a large number of wheat accessions for reaction to P. tritici-repentis. However, while rating infected plants, it was observed that lesion size, but not lesion type, within each category could vary. Whether this variation was an artifact of experimentation was not determined.

Figure 3. Extensive chlorosis induced by isolate ASC1 of Pyrenophora tritici-repentis on wheat line 6B365.

From left to right: 1) non-inoculated; 2) symptoms at 4 days after inoculation; 3) symptoms at 6 days after inoculation; and 4) symptoms at 8 days after inoculation.
Note the absence of definite margins around the small dark brown to black primary lesions.

TABLE 3. REACTION OF TWO EXTENSIVE CHLOROTIC WHEAT LINES TO THREE LEAF PATHOGENS.

	REACTION*		
LINES	PT[1]	PGT[2]	CS[3]
6B365	CH- (R)	CH-(S)	CH-(S)
6B780	CH- (R)	NT[&]	CH-(R)

1. Pyrenophora teres 2. Puccinia graminis f.sp. tritici
3. Cochliobolus sativus.
* Reaction: R= resistant; S= susceptible;
CH- : absence of extensive chlorosis.
& NT= not tested

In the field, considerable disease developed on plants grown in both inoculated plots and on plots sown into wheat stubble. A list of all the accessions tested in the field is included in Appendix 4. In inoculated plants, small lesions were generally observed 3 to 4 days after inoculation but their development was much slower, taking about twice the time observed under growth room conditions. Lesion development from naturally occurring infections, observed in the stubble planted lines, was even slower than in inoculated lines. It took approximately three weeks for small, dark brown lesions on susceptible cultivars to develop into susceptible-type lesions, similar to those observed in the growth room.

Even though there was a slight tendency towards lower scores in the field, most cultivars had similar reactions when tested in the growth room and the field. Furthermore, the correlation between results of field and growth room tests, computed on scores of 55 accessions (Table 4), was highly significant. Agreement between wheat reactions in field and greenhouse tests was also found in other studies (Gilchrist et al, 1984; Raymond et al, 1985; Cox and Hosford, 1987).

The resulting disease levels, as measured using the scale of Couture (1980), were generally higher in inoculated than in stubble infected plots. This was expected, since the former plots were sprayed with a considerable amount of inoculum and were incubated over two consecutive nights to promote conditions of high relative humidity. Generally, however, resistant cultivars, identified in greenhouse tests, had the lowest scores under both conditions, whereas moderately and fully susceptible cultivars were more severely affected in inoculated than in stubble infected plots.

Despite the high magnitude and the significance of the correlation ($r=0.73$) observed between disease severity ratings in inoculated and stubble sown plots (Table 4), the relevance of the relationship should be interpreted with caution, because of the presence of other leaf spotting pathogens in the stubble. A sampling of lesions from infected leaves of 33 cultivars grown in stubble revealed the presence of Septoria spp. on 90.6

TABLE 4. CORRELATION BETWEEN GROWTH ROOM AND FIELD REACTIONS OF WHEAT ACCESSIONS TO PYRENOPHORA TRITICI-REPENTIS.

	FIELD REACTIONS		
	ARTIFICIAL INOCULATION		STUBBLE
	LESION TYPE	DISEASE SEVERITY	DISEASE SEVERITY
Greenhouse Inoculation			
Lesion Types	0.630** (55)	0.664** (55)	0.519** (55)
Field Inoculation			
Lesion Types		0.824** (106)	0.580** (106)
Disease severity			0.730** (106)

** Significant at 1% level.

Numbers between parenthesis represent the number of lines/ cultivars used to compute the correlation coefficient.
$ Lesion type and disease severity scores were taken on plants of lines artificially inoculated in the field.

% of leaves, P. tritici-repentis on 65.6 % and C. sativus on 40.6 %. The presence of other leaf pathogens and/or of undetermined flora may interfere with the infection process of P. tritici-repentis and confound results. It has been reported that C. sativus, a common wheat pathogen, was somewhat antagonistic to P. tritici-repentis (Luz and Bergstrom, 1987).

A necrotic condition ascribed to physiological necrosis was observed to develop on plants of the following durum wheat accessions in non-inoculated field plots: Golden Ball, Juljuli, Karalicik # 1133, Medora, Mourisco, Orgaz, PI 136575, Russo, Triticum turgidum I36-45, 227056 and 4B146. On cultivars Mourisco and Orgaz, the symptoms were observed for three consecutive years, indicating that physiological necrosis was a stable trait. Physiological necrosis, also referred to as splotching (Sallans and Tinline, 1962), can be easily confused with fully susceptible tan spot lesions showing chlorosis and/or tan necrosis (Fig. 4).

The production of large quantities of conidia by existing methods (Odvody and Boosalis, 1982; Raymond et al, 1985) is tedious and requires two culture media. The modified method of Raymond et al (1985), developed for the present study was found to be simpler and more efficient for large scale synchronous spore production, with the advantage of using a single medium.

The results of the present study indicate that large amounts of wheat germ plasm can be effectively and reliably evaluated for reaction to P. tritici-repentis in the growth room by inoculating seedlings at the two leaf stage. This reduces the number of entries which require testing in the field at later growth stages. It is preferable that field tests be conducted subsequent to growth room evaluation, and this is practical if the number of entries has been reduced to a manageable size and the seed of individual cultivars sufficiently increased.

The procedures used in the present study were effective and accurate in characterizing host reactions as well as in identifying wheat cultivars with high levels

Figure 4. Symptom of physiological necrosis on the durum wheat line Mourisco in pathogen-free field plots.

of resistance to tan spot. Although the results obtained in the growth room using a single isolate of the pathogen were generally reproduced in the field, further testing of the accessions to additional isolates of P. tritici-repentis with possible different virulence would be desirable, in order to insure the development of stable resistance to the entire local pathogen population. Also, it would be desirable to assess the effect of the various lesion types and the extensive chlorosis syndrome on yields of wheat in inoculated field plots.

2. Pathogenic variation and host-pathogen interactions in tan spot of wheat, caused by pyrenophora tritici-repentis.

Abstract

Ninety two isolates of Pyrenophora tritici-repentis from Western Canada were tested for virulence on 11 wheat cultivars and grouped into three pathotypes. Pathotype 1, the most prevalent, selectively induced either tan necrosis or extensive chlorosis on the differential cultivars and was denoted as (nec+ ch+). Pathotype 2 induced tan necrosis only (nec+ ch-) whereas pathotype 3 induced extensive chlorosis only (nec- ch+). The development of tan necrosis as well as of extensive chlorosis was the result of specific interactions between wheat cultivars and individual isolates of P. tritici-repentis. Cytological observations of compatible and incompatible interactions in cultivar BH1146 indicated that host resistance was not expressed until hyphae were established in the intercellular space of the mesophyll. Differences between compatible and incompatible reactions were not observed until after 48 h when tan necrosis appeared in the compatible reaction. In line 6B365, which develops extensive chlorosis, a dense intercellular network of hyphae was associated with the appearance of extensive chlorosis. Penetration of cells in the mesophyll was not observed throughout the study.

INTRODUCTION

Tan spot, caused by the ascomycete Pyrenophora tritici-repentis (Died.) Drechs. (anamorph Drechslera tritici-repentis (Died.) Shoem.), has become in recent years an important disease of wheat. The disease has been reported in several countries including Australia (Valder and Shaw, 1952), Belgium (Maraite and Weyns, 1982), Brazil (Mehta and Almeida, 1977), Canada (Tekauz, 1976; Tekauz et al, 1982), India (Misra and Singh, 1972), Mexico (Gilchrist et al, 1984), and the U.S.A. (Hosford, 1971; Hosford and Bush, 1974; Watkins et al, 1978). In the genus Pyrenophora, the species tritici-repentis is believed to have the widest host range (Shoemaker, 1962) and has been reported to infect many gramineous species (Morrall and Howard, 1975; Krupinsky, 1982).

Wheat resistance to P. tritici-repentis has been reported (Luz and Hosford, 1980; Hosford, 1982; Gilchrist et al, 1984; Raymond et al, 1985) and was found to be dependant on the length of the post-inoculation leaf wetness period (Hosford, 1982). Hosford et al (1987) found that an increase in the temperature and/or the wetness duration increased disease severity in susceptible and, to a lesser extent, in resistant cultivars.

Cultivars considered to be resistant in specific studies (Hosford, 1982; Luz and Hosford, 1980; Lee and Gough, 1984; Loughman and Deverall, 1986) were found to be more susceptible in others (Gilchrist et al, 1984; Lamari, paper 1). The discrepancy may be due, in part, to differences in the methods of rating host reactions amongst the various workers (Lamari, paper 1), as well as to possible differences in the virulence of the isolates of P. tritici-repentis used at each location. Although isolates have been reported to differ in virulence in studies in India (Misra and Singh, 1972), Mexico (Gilchrist et al, 1984) and the USA (Luz and Hosford, 1980), the latter study was the most comprehensive: 40 isolates from the Central Great Plains area were compared on six wheat and one barley cultivar and differentiated into 12 races on the basis of lesion size and % leaf area infected. More recently krupinsky (1987) tested 27 isolates of P.

tritici-repentis from smooth bromegrass on wheat and smoothbrome grass, and found that these isolates varied in their ability to cause disease in wheat, as measured by lesion size and % leaf area infected.

The inheritance of wheat reaction to single isolates of P. tritici-repentis has been found to be both polygenic (Nagle et al, 1982; Cantrell et al, 1985) and monogenic (Lee and Gough, 1984). However, little is known about the genetics of the pathogen, although several potential genetic markers have been identified (Hunger and Brown, 1987) and a technique for inducing the sexual reproductive structures has been recently improved (Pfender and Wootke, 1987).

A study to evaluate wheat reaction to tan spot was initiated at the University of Manitoba and resistant lines were identified in diploid, tetraploid, and hexaploid wheats, by using a rating system based exclusively on lesion type (Lamari, paper 1). Resistance was characterized by the presence of small brown to black primary lesion with very little or no chlorosis and tan necrosis, and susceptibility by the presence of either. In some cultivars, susceptibility was expressed by particularly extensive chlorosis.

The development of cultivars with effective and stable resistance requires an understanding of the nature of host-pathogen interactions, and of the extent of variation for virulence in the population of the pathogen. This paper reports on the study of interactions between wheat cultivars,
representing a range of reactions to P. tritici-repentis (Lamari, paper 1) and specific isolates of the fungus, collected in western Canada.

MATERIALS AND METHODS

Eighty four monoconidial isolates of P. tritici-repentis were recovered from naturally infected wheat leaves as described previously (Lamari, paper 1). The samples were either collected in Manitoba by the author or received from cooperators in Saskatchewan and Alberta. Eight of the isolates were recovered from wheat leaf samples supplied by Dr. A. Tekauz (Agriculture Canada, Winnipeg) from collections made during a 1985 survey of western Canada.

To evaluate the variability in virulence of the pathogen, a differential set of 11 wheats (Table 5) was selected on the basis of reactions to isolate ASC1 of P. tritici-repentis from a previous study (Lamari, paper 1). Six seeds of each of two differential cultivars were sown as two clumps into 10 cm diameter pots. Inoculum was prepared and seedlings inoculated at the 2 leaf stage as described previously (Lamari, paper 1). The isolates were tested in groups of eight. Isolate ASC1 and a treatment consisting of water to which 10 drops/L of Tween 20 were included in each test. All the treatments (isolates) were replicated twice. When an isolate with a virulence pattern other than that of the standard isolate was found, it was retested at least three times to insure the reproducibility of the reactions. Upon confirmation, the new pathotype was included in all subsequent tests as an additional standard.

Seedlings were rated for lesion type on a scale of 1-5 as described previously (Lamari, paper 1). Lesion types 1-2 refer to small dark brown to black lesions (primary lesions) with none or very little chlorosis or tan necrosis. Lesion type 3 has a definite ring of chlorotic or tan necrotic tissue but no coalescing. Types 4-5 refer to lesions with increasingly undefined boundaries, resulting in rapid coalescing and leaf collapse. The induced chlorotic reactions on lines 2B13 and 6B365 were rated as CH+ and CH- for the development of extensive and no chlorosis, respectively.

Infected leaf tissues of cultivar BH1146, inoculated with isolates ASC1 and HY331-6 of P. tritici-repentis, were sampled for cytological observations at 6, 12, 24, 48 and 72 h after inoculation. Samples were also taken from line 6B365, inoculated with isolate ASC1, at 6, 12, and 24 h and daily thereafter until the sixth day post-inoculation. Four cm pieces from the middle of each leaf were cut into 1 cm pieces and cleared by boiling in a mixture of 95 % ethanol (3 parts) and lactophenol (1 part) for 5 to 6 min. The leaf pieces were stained overnight in a clearing-staining solution made of 95 % ethanol (300 mL), chloroform (150 mL), lactic acid (125 mL), phenol (150 g), chloral hydrate (450 g), and aniline blue (0.6 g) (Bruzzese and Hasan, 1983). Clearing and staining was also achieved in one step by immersing the leaf pieces directly in the above clearing-staining solution for 48 h and destaining in saturated chloral hydrate for at least 12 h. Leaf pieces were mounted in clear lactophenol and observed for fungal structure using a compound microscope (Wild, Heebrugg, Switzerland) fitted with a 36 mm camera (Pentax 1000).

RESULTS

Virulence of isolates. The 92 isolates of P. tritici-repentis evaluated could be grouped into three pathotypes on the basis of lesion type or the induction of extensive chlorosis on the 11 differential cultivars (Table 5). Pathotype 1, as represented by isolate (ASC1), comprised 90.1 % of all isolates tested. It is characterised by its ability to induce chlorosis on lines 6B365 (Fig. 5a) and 2B13 as well as tan necrosis on Glenlea, BH1146 (Fig. 6a) and Coulter. Cultivars Salamouni, Erik, 4B242 and 4B1149 developed small dark brown to black lesions and were considered to be resistant to this pathotype. Isolates belonging to this group originated from winter and spring wheats collected throughout western Canada. Isolates with this virulence pattern are denoted (nec+ ch+) because of their ability to cause both necrosis (nec+) and chlorosis (ch+).

Pathotype 2 differed from pathotype 1 only by its inability to induce extensive chlorosis on lines 6B365 (Fig. 5b) and 2B13. It was comprised of only two isolates, one (86-124) was recovered on cultivar BH1146 planted in a "trap nursery" at Portage--la-Prairie, Manitoba and the other (HY331-3) from a heavily infected durum wheat field near Newton, Manitoba. Isolates from this pathotype are denoted (nec+ ch-).

Pathotype 3 represented by isolate HY331-6, was similar to pathotype 1 in its ability to induce extensive chlorosis on lines 2B13 and 6B365 and in its avirulence to cultivars Erik, Salamouni, 4B242 and 4B1149. However, it differed from pathotypes 1 and 2 by its avirulence to cultivar BH1146 (Fig. 6b) and its inability to induce tan necrosis on Glenlea (avirulent) and Columbus (virulent). Instead, Pathotype 3 induced the development of a chlorotic ring surrounding the primary lesion in these cultivars. The halo was restricted in size in cultivar Glenlea but not in Columbus. On cultivar Coulter, however, pathotype 3 induced large and coalescing lesions, often without dark centres. All nine isolates of this pathotype were recovered from the same field near Newton, Manitoba. Isolates of this pathotype are referred to as (nec- ch+).

TABLE 5. SUMMARY OF THE VIRULENCE OF 92 ISOLATES OF PYRENOPHORA TRITICI-REPENTIS ON 11 DIFFERENTIAL WHEAT CULTIVARS.

CULTIVAR	PLOIDY[$]	PATHOTYPES[&] 1	2	3
COLUMBUS	6B	V	V	V
COULTER	4B	V	V	V
BH1146	6B	V	V	A
GLENLEA	6B	V	V	A
6B365	6B	V(CH+)	A(CH-)	V(CH+)
2B13	2B	V(CH+)	A(CH-)	V(CH+)
ERIK	6B	A	A	A
SALAMOUNI	6B	A	A	A
Tp. x Tm.	6B	A	A	A
4B242	4B	A	A	A
4B1149	4B	A	A	A
Number of isolates		81	2	9

Wheat seedlings were inoculated at the 2 leaf stage with a spore suspension (3000 conidia/ml), incubated 24 h under continuous leaf wetness, and rated for lesion morphology 6 to 8 days after inoculation.

& V= virulent and A= avirulent. Virulence refers to the ability of the pathogen to induce tan necrosis or chlorosis on the specific host cultivar, and avirulence to its inability to cause either. Isolates which cause only minute amounts of tan necrosis or chlorosis are also considered to be avirulent. CH+,CH- : presence and absence of extensive chlorosis respectively.

$ Ploidy groups: 2B= diploid, 4B=tetraploid, 6B= hexaploid.

Figure 5. Symptoms of extensive chlorosis induced on wheat line 6B365 by Pyrenophora tritici-repentis:

a) Left (first leaf): extensive chlorosis induced by isolate ASC1 (nec+ ch+). Note the yellow discoloration and the absence of well defined lesions.
b) Second and 3rd leaf: symptoms induced by non-chlorosis inducing isolate 86-124 (nec+ ch-). Note the absence of extensive chlorosis.
c) Fourth leaf: non-inoculated.

Leaves are from plants inoculated at the 2 leaf stage with a conidial suspension (3000 conidia/mL) and incubated for 24 hours under continuous leaf wetness.
Photograph taken 8 days after inoculation.

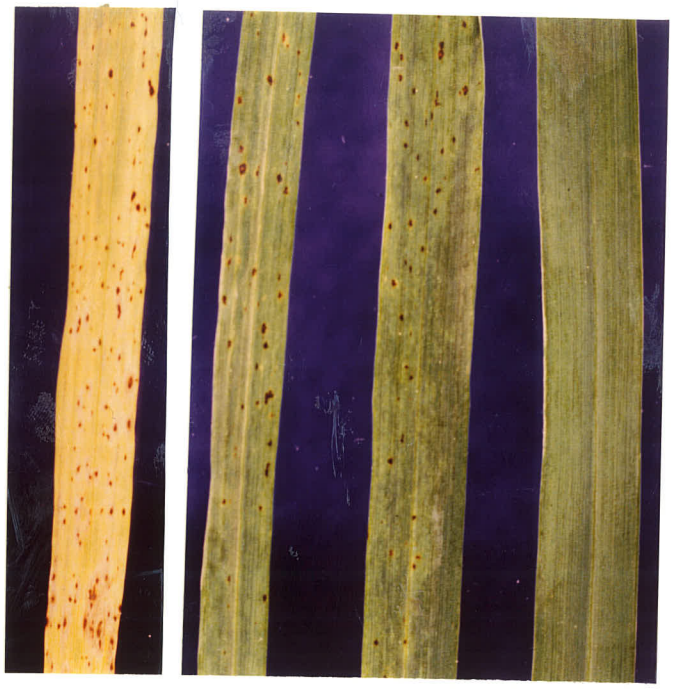
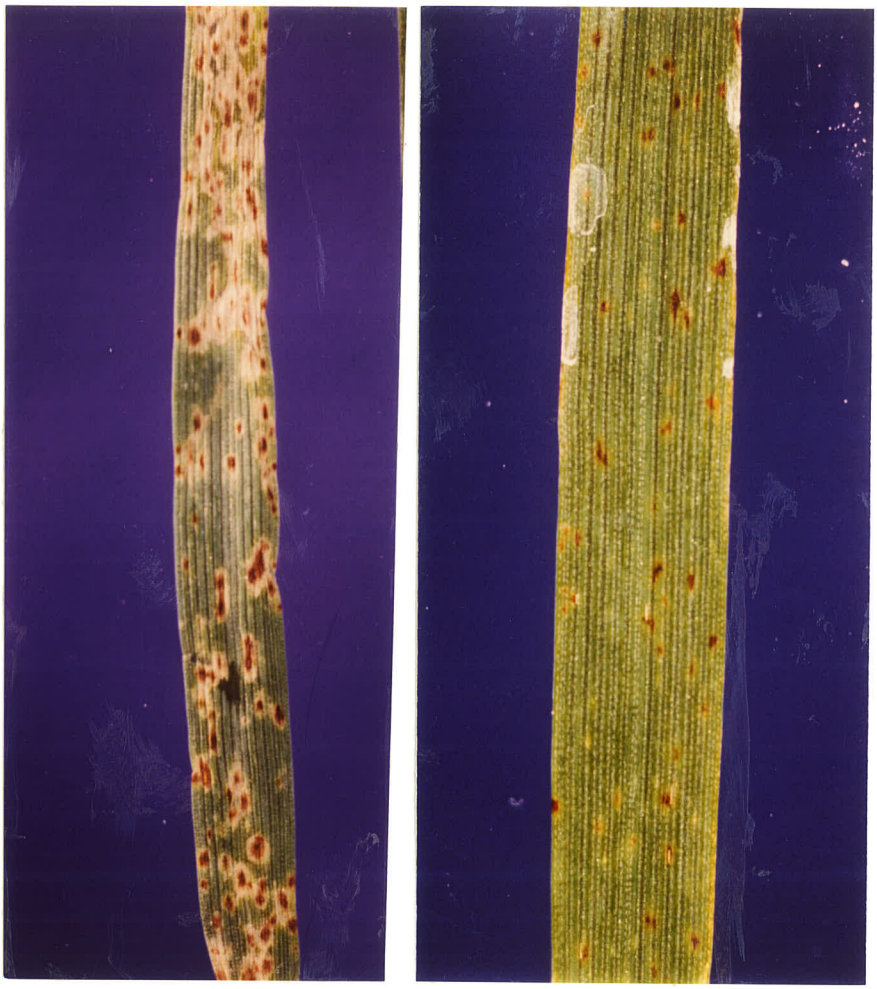


Figure 6. Symptoms of tan necrosis induced on wheat cultivar BH1146 by Pyrenophora tritici-repentis:

a) Left: tan necrosis induced by isolate ASC1 (nec+ ch+). Note the tan necrosis around the primary lesions (small dark brown lesions).

b) right: symptoms induced by necrosis non producing isolate HY331-6 (nec- ch+). Note the absence of tan necrosis around the primary lesions.

Leaves are from plants inoculated at the 2 leaf stage with a conidial suspension (3000 conidia/mL) and incubated for 24 hours under continuous leaf wetness.
Photograph taken 8 days after inoculation.



Isolates belonging to each of the three pathotypes were found to occur within the same field (Table 6). Although disease severity was not recorded, isolates of pathotype 3 produced generally larger and somewhat paler lesions on Coulter than did isolates of pathotypes 1 and 2. However, cultivar Coulter was susceptible to all three pathotypes.

The identity of isolate ASC1 (pathotype 1), 86-124 (pathotype 2) and D308 (pathotype 3) was confirmed by R.A. Shoemaker, of the Biosystematics Research Institute, Ottawa.

Fungal development in compatible and incompatible interactions. No major cytological differences were observed when cultivar BH1146 was inoculated with isolate ASC1, which induces lesions of type 4 (compatible), and with isolate HY331-6 which induces lesion type 1 (incompatible). Germination of conidia, formation of appressoria, penetration of epidermal cells and vesicle formation were similar. Penetration of the intercellular space of the mesophyll was seen at 6 h and hyphae extended intercellularly 6 to 8 cells beyond the infection site by 12 h, in both compatible and incompatible reactions (Fig 7). At this stage all penetrated epidermal cells stained deep blue, indicating that major physiological changes had taken place, whereas mesophyll cells, including those in contact with the growing hyphae, remained unchanged. By 24 h, a zone of deeply stained mesophyll cells was apparent near the infection site in both interactions. This zone turned very dark by 48 h, and generally corresponded to the appearance of small dark brown to black primary lesions in the host. Hyphae were occasionally seen outside the stained area in both interactions, but these extended to only a distance of one to two cells beyond. At 72 h, however, differences between the compatible and incompatible reactions were clearly evident as the deeply stained area of lesions caused by isolate ASC1 (compatible) had expanded while that caused by isolate HY331-6 (incompatible) did not. This expansion corresponded to the thin ring of tan necrosis that develops and surrounds the primary lesion in the compatible reaction. Penetration

TABLE 6. VIRULENCE OF 13 ISOLATES OF PYRENOPHORA TRITICI-REPENTIS RECOVERD FROM A DURUM WHEAT FIELD ON TWO WHEAT CULTIVARS, AND THEIR PATHOTYPE DESIGNATION.

	REACTION&		
ISOLATE*	BH1146	6B365	PATHOTYPE
HY331-1	A	V(CH+)	3
HY331-2	A	V(CH+)	3
HY331-3	V	A(CH-)	2
HY331-4	V	V(CH+)	1
HY331-5	A	V(CH+)	3
HY331-6	A	V(CH+)	3
HY331-7	A	V(CH+)	3
HY331-8	V	V(CH+)	1
HY331-9	A	V(CH+)	3
HY331-10	A	V(CH+)	3
HY331-11	V	V(CH+)	1
HY331-12	A	V(CH+)	3
D308	A	V(CH+)	3

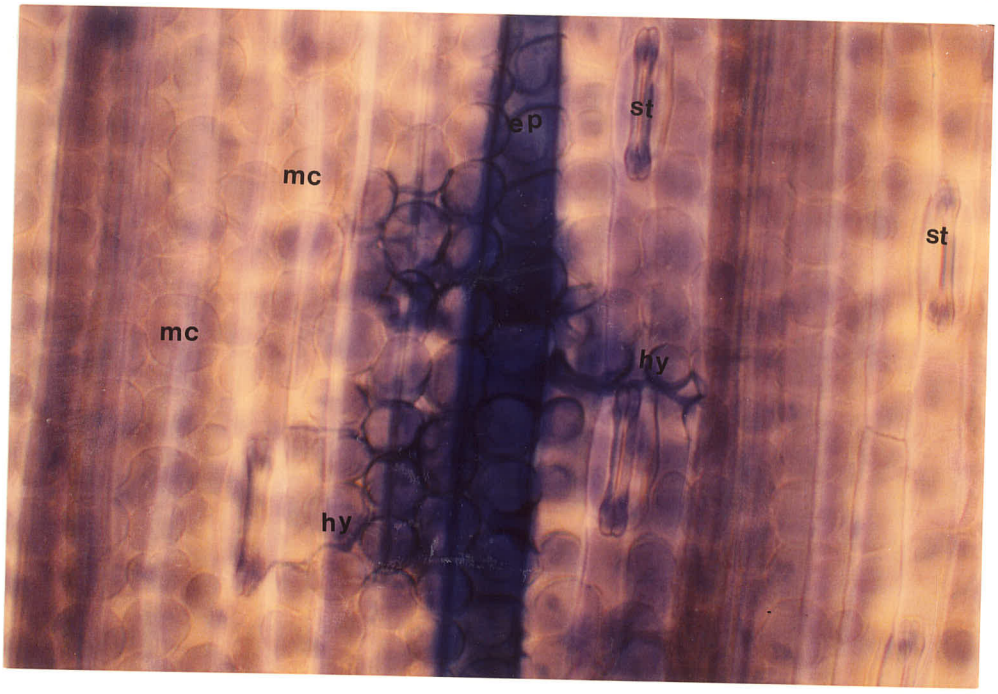
* All isolates were obtained from the same durum wheat field near Newton, Manitoba.

& V= virulent and A= avirulent. Virulence refers to the ability of the pathogen to induce tan necrosis or chlorosis on the specific host cultivar, and avirulence to its inability to cause either. Isolates which cause only minute amounts of tan necrosis or chlorosis are also considered to be avirulent.

CH+,CH- : presence and absence of extensive chlorosis respectively.

Figure 7. Colonization of an epidermal cell and invasion of the mesophyll of necrotic cultivar BH1146 by hyphae of avirulent isolate HY331-6 (nec- ch+) of Pyrenophora tritici-repentis.

Photograph taken 12 hours after inoculation at 250x magnification. Note the intercellular growth of the fungus in the mesophyll. Legend: ep : epidermal cell, hy : intercellular fungal hyphae, mc: mesophyll cell, st: stomate.

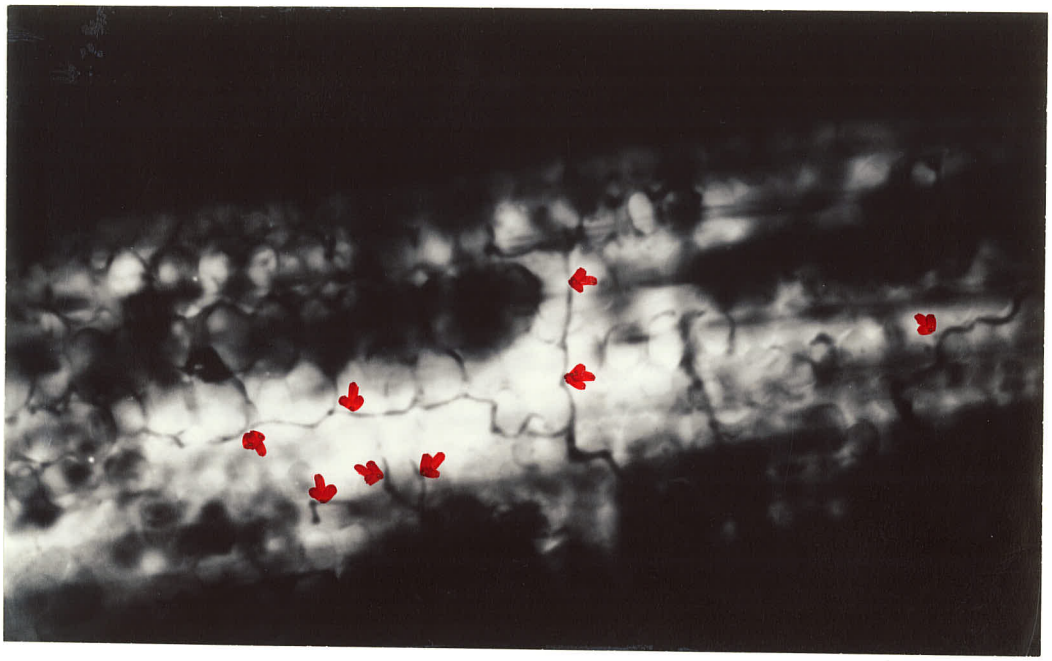

ep
st
mc
st
mc
hy
hy

of mesophyll cells was not observed at any time in either the compatible or incompatible interactions.

All the initial stages of infection in wheat line 6B365 inoculated with isolate ASC1, which induces extensive chlorosis, were similar to those observed in the necrotic system described above. The deeply stained area did not expand between 48 and 72 h, and hyphae generally did not extend beyond the deeply stained zone. However, after 4 or 5 days, a dense network of intercellular hyphae was evident in leaf sections with extensive chlorosis, taken from leaves which had not yet collapsed (Fig. 8). Despite the massive amounts of mycelium present in the mesophyll, no cell penetration was observed.

Figure 8. Intercellular hyphal network caused by isolate ASC1 (nec+ ch+) of Pyrenophora tritici-repentis on wheat line 6B365 with extensive chlorosis.

Photograph taken 5 days after inoculation at 250x magnification.
Legend: red arrows indicate intercellular hyphae.



DISCUSSION

Virulence in P. tritici-repentis was found to be associated with the ability of individual isolates to induce tan necrosis and/or chlorosis in wheat cultivars. However, tan necrosis or chlorosis are expressed as a result of specific interactions between isolates of the pathogen and appropriate host genotypes. Thus, the cultivars BH1146 and Glenlea which are normally "necrotic" to (nec+ ch+) and (nec+ ch-) isolates fail to develop tan necrosis if matched with (nec- ch+) isolates. Similarly, lines 2B13 and 6B365 will not produce extensive chlorosis unless matched with a (nec+ ch+) or (nec- ch+) isolate. Even though a large number of cultivars and lines were included in the wheat differential set, only two lines, such as BH1146 (necrosis) and 6B365 (extensive chlorosis), were needed to differentiate between the three pathotypes. However, it is desirable to maintain the present differential set for further testing to more isolates of P. tritici-repentis from different host species and geographic locations. There is no simple explanation for the very low frequencies of pathotypes 2 and 3 (Table 5). The fact that the isolates used in this study were all from wheat may have reduced the probability of detecting more variability. Nevertheless, the virulence patterns observed may provide some insight into the evolution of virulence in this pathogen. That pathotypes evolved toward greater virulence is suggested by the fact that pathotype 1, with the broadest virulence, was the most prevalent, and also by the absence of an "avirulent" type (nec- ch-). The latter pathotype, if it exists, would be at a selective disadvantage, making its recovery from lesions highly unlikely. The co-habitation of isolates of all three pathotypes in the same durum wheat field (Table 6) likewise suggests a common ancestry. The genetic materials of the host and the pathogen, identified in the present study, provide a future opportunity to study the genetics of virulence in the pathogen, by deriving pathotypes 2 or 3 from the wild type (nec+ ch+)

by mutagenesis and/or synthesis of the wild type by mating pathotype 2 (nec+ ch-) and pathotype 3 (nec- ch+).

Cultivar BH1146 was included in this study because it had been found previously to be both resistant (Luz and Hosford, 1980; Nagle et al, 1982; Larez et al, 1986; Loughman and Deverall, 1986; Luz and Bergstrom, 1987; Hosford et al, 1987) and moderately susceptible to P. tritici-repentis (Gilchrist et al, 1984; Lamari, paper 1). The identification, in the present study, of isolates that are virulent (nec+) and avirulent (nec-) on BH1146 helps to reconcile those conflicting reports. The existence of isolates virulent and avirulent to BH1146 had been reported previously (Luz and Hosford, 1980), however, that virulence was based on the quantitative variables of lesion size and % leaf area infected. This implied a continuum of reactions which can be separated from each other only by statistical analysis, as opposed to the qualitative nature of the variation in the pathogen observed in this study.

Cytological observations of infected host tissues failed to detect any morphologically distinct structure that could be associated with compatibility or incompatibility. This result is in agreement with previous studies (Larez et al, 1986; Loughman and Deverall, 1986), which were based on the reaction of a single isolate of P. tritici-repentis on resistant and susceptible wheat cultivars. The results suggest that the specific host reaction is expressed only after the fungus has grown intercellularly in the mesophyll. The association of a dense intercellular mycelial network with the appearance of extensive chlorosis has not been reported previously and its significance should be investigated.

The ability to easily characterize pathotypes in P. tritici-repentis on a qualitative basis should be of direct benefit to breeding programs aimed at incorporating resistance to this pathogen. The identification of isolates capable of inducing extensive chlorosis and/or necrosis should prove useful in genetic studies of the fungus.

3. Toxin of Pyrenophora tritici-repentis: host-specificity, role in disease and inheritance of host reaction.

ABSTRACT

Dialyzed culture filtrates from isolates of Pyrenophora tritici-repentis contained a heat-labile (121 C for 20 min) toxin(s), which induced necrosis on susceptible cultivars but not on species outside the genus Triticum. Cultivars resistant to the fungus were insensitive to the toxin, whereas susceptible cultivars which develop chlorosis, were insensitive. Segregation of F_2 populations from four different crosses between resistant and susceptible (necrotic) cultivars indicated that susceptibility to the fungus and sensitivity to the toxin were controlled by the same dominant gene. Toxin production by the pathogen was associated with the ability of individual isolates to induce necrosis (nec+) in the necrosis producing cultivars. Isolates which induced extensive chlorosis but not necrosis (nec- ch+) did not produce toxin in vitro. The toxin differentiated two near-isogenic lines from the cultivar Columbus. When inoculated with a (nec+) isolate, only the toxin sensitive line (Col+) developed necrosis. The toxin(s) of P. tritici-repentis is cultivar-specific, involved in the induction of necrosis in the host, and appears to be a pathogenicity factor.

INTRODUCTION

Pyrenophora tritici-repentis (Died.) Drechs., anamorph Drechslera tritici-repentis (Died.) Shoem. (syn. Helminthosporium tritici-repentis Died.) causes leaf spots on several gramineous hosts (Hosford, 1971; Krupinsky, 1982; Morrall and Howard, 1975), including wheat, where the disease is known as tan spot. In recent years tan spot has become a potentially destructive disease of wheat worldwide (Dubin, 1983; Gough and Johnston, 1982; Rees et al, 1982; Rees, 1982). In 1974, tan spot was observed to be the most important leaf spot disease in the Canadian Prairies (Tekauz, 1976) and in 1982, severe infections on seedlings were observed in Manitoba and Saskatchewan for the first time (Tekauz et al, 1982). The disease was subsequently found to be damaging to winter wheat (Tekauz et al, 1983).

The reaction of wheat germ plasm to P. tritici-repentis was evaluated using a rating scale based on lesion type, and resistant cultivars were identified (Lamari, paper 1). Susceptibility to P. tritici-repentis was expressed by the presence of either tan necrosis or chlorosis, and resistance was characterized by the absence of, or the presence of slight amounts of either of tan necrosis or chlorosis. Some genotypes developed small brown to black primary lesions and extensive chlorosis that covered most the leaf.

Variation for virulence in P. tritici-repentis has been reported (Misra and Singh, 1972; Luz and Hosford, 1980; Gilchrist et al, 1984) and three pathotypes were identified in a population of the pathogen from western Canada (Lamari, paper 2). Pathotypes were classified on the basis of their ability to induce both tan necrosis and extensive chlorosis (nec+ ch+), extensive chlorosis only (nec- ch+), and tan necrosis only (nec+ ch-) on the appropriate susceptible cultivars (Lamari, paper 2). The development of each reaction (tan necrosis or chlorosis) was found to depend on the individual wheat cultivar. Furthermore, the expression of tan necrosis as well as extensive chlorosis

was found to be the result of specific interactions between individual host genotypes and pathotypes of the pathogen.

Several lines of evidence suggest the involvement of toxin(s) in tan spot of wheat. These include: i) the extensive chlorosis or necrosis observed in some wheat cultivars in response to infection by certain isolates (Lamari, paper 1,2), and ii) the consensus from all reported cytological studies that hyphae of P. tritici-repentis grow intercellularly without penetrating the mesophyll cells (Larez et al, 1986; Loughman and Deverall, 1986; Lamari, paper 2).

The search for toxins in host-pathogen systems is stimulated, not only by an interest to better understand the pathogenic process, but also for their potential use in identifying resistant cells or protoplasts in tissue cultures, or in screening and breeding for disease resistance. There is presently one report on toxin production by isolates of P. tritici-repentis (Tomas and Bockus, 1987). The toxin(s) in culture filtrates of the fungus was reported to be cultivar-specific and to mimic the symptoms caused by the fungus. However, the potency of the toxin was very low, compared to that of the host-specific toxins involved in other host-pathogen systems (Luke and Wheeler, 1955; Yoder, 1973; Comstock et al, 1973; Scheffer and Pringle, 1961). Two low molecular weight toxins were isolated and purified from culture filtrates of Pyrenophora teres Drechs., and also from barley leaves infected by isolates of this pathogen (Smedegaard-Petersen, 1977). Toxin A was found to produce symptoms at 25 ug/ml and to be more potent than toxin B. Both toxins were specific to barley, but were not cultivar-specific. The toxins in P. teres were found to be associated with virulence in individual isolates rather than with pathogenicity and the most virulent isolates produced the most toxin (Smedegaard-Petersen, 1977).

The presence of toxic metabolites in fungal culture filtrates has been reported for many host-pathogen systems, but in only a few studies, has their relevance to the disease been convincingly demonstrated (Scheffer, 1982). Toxins have been categorized

as primary or secondary determinants of disease (Scheffer and Pringle, 1967) and as factors in virulence or pathogenicity (Yoder, 1980). Criteria for establishing the significance of toxins in disease syndrome have been reviewed (Yoder, 1980). According to Yoder (1980) and Scheffer (1982), genetic analyses of the host and/or pathogen, so far, have provided the strongest evidence of toxin involvement in disease.

This report deals with the isolation and partial characterization of toxin(s) in the culture filtrates of certain isolates of P. tritici-repentis, the inheritance of host reaction to the toxin, and the determination of the role of the toxin in disease.

MATERIALS AND METHODS

Production of culture filtrates. One ml of a conidial suspension (ca. 10^4 spores/ml) of the pathogen was transferred to 250 ml Erlenmeyer flasks containing 50 ml of Fries medium amended with 0.1% yeast extract (medium # 66 in Dhingra and Sinclair, 1985, Appendix 5) and incubated at 20 C in the dark without agitation for three weeks. The cultures were then filtered through Whatman #1 paper, passed through a 0.45 µm millipore membrane and stored at 4 C or kept in a freezer at -19 C until processed further.

Partial purification and stability. Initially, attempts were made to isolate and purify the toxin from culture filtrates of P. tritici-repentis using the methods of Smedegaard-Petersen (1977), however, these were not successful. Subsequently, a different approach was tried and culture filtrates were desalted by overnight dialysis against distilled water in 5 ml batches at 4 C, using a dialysis membrane (Spectrapor, Spectrum Medical Industries Inc., Los Angeles, CA) with a cutoff at 8000 m.w.. The stability of toxin from P. tritici-repentis was assessed in crude and desalted filtrates, previously adjusted to pH 6.5, that were subjected to autoclaving at 121 C for 20 min and filtered through a 0.45 um millipore membrane to remove the precipitates.

Bioassay. Seedlings were infiltrated with ca. 150 ul of a toxin dilution at the 2 to 4 leaf stage, using a Hagborg device (Hagborg, 1970). Sterile distilled water and sterile Fries medium were used as controls throughout the study. Seedlings were examined daily for three days and classified as (+) if necrosis was present and (-) if no symptoms were evident.

Toxin specificity. Host-specificity was assessed by infiltrating crude and desalted filtrates of isolates ASC1 and 86-124 of P. tritici-repentis into seedlings from eleven species outside the genus Triticum as well as four wheat cultivars previously identified to be susceptible to the fungus (Table 7) (Lamari, paper 1,2). Dilutions of 1:1, 1:10, 1:100, 1:500 and 1:1000 were used. The test species and wheat cultivars were inoculated with isolate ASC1 of the pathogen.

To avoid confusion in terminology, resistance and susceptibility are used hereafter to describe the reaction of wheat cultivars to the fungus, whereas sensitivity and insensitivity are used to describe the reaction to the toxin. The term "necrosis" refers to the tan area of dead tissue, usually visible 3 to 4 days after inoculation, surrounding a small, brown to black lesion (primary lesion). Chlorosis refers to the zone surrounding the primary lesion that consists of tissue exhibiting a gradual yellow discoloration. Chlorosis may be restricted, as a halo, or may cover most of the leaf (extensive); in the latter case it becomes necrotic within 7-8 days of inoculation.

Twelve wheat cultivars previously characterized for their reactions to the fungus (Lamari, paper 2), were used in a first test to assess cultivar-specificity of the culture filtrates. These are listed in Table 8. To further evaluate the cultivar specificity and assess the relationship between the reaction to the toxin and to the fungus, a second test was conducted. Seedlings from 92 resistant and moderately resistant and from 69 susceptible lines and cultivars from a previous study (Lamari, paper 1) were inoculated at the 2 leaf stage with isolate ASC1 of the P. tritici-repentis. Desalted culture filtrates, at a dilution of 1:50, were infiltrated into the third leaf 6 days after inoculation and reaction to the fungus and to the toxin were recorded 24-48 h after toxin infiltration.

The relationship between virulence and toxin production by the pathogen was assessed using 11 isolates previously tested for virulence to a differential wheat set (Lamari, paper 2). Isolates ASC1, HY331-11, TK85-243 and TK85-245 were characterized by their

ability to induce necrosis and chlorosis (nec+ ch+), isolates 86-124 and HY331-3 induced necrosis only (nec+ ch-) whereas the remaining isolates induced chlorosis only (nec- ch+) (Table 9). Desalted culture filtrates from each isolate were infiltrated into leaves of 30 wheat cultivars, selected on the basis of their reaction to isolate ASC1 and its toxin.

Inheritance of wheat reaction to the toxin. Two hexaploid and one tetraploid wheat cultivars previously identified as resistant to P. tritici-repentis (Lamari, paper 1) were crossed to the susceptible hexaploid cultivars Celtic and Columbus (toxin sensitive selection), and the tetraploid cultivar Coulter respectively. F_1 and F_2 and backcross progenies were tested for reaction to isolate ASC1 of P. tritici-repentis and its toxin (1:50 dilution).

Fungal inoculation. Seedlings were inoculated at the two leaf stage using procedures described previously (Lamari, paper 1). Seedlings at the 2 leaf stage were sprayed until run-off with a conidial suspension, previously adjusted to 3000 conidia/ml, using a DeVilbis sprayer fitted to a compressed air outlet and operated at 10 psi. Ten drops of Tween20 (Polyoxyethylene sorbitan monolaurate) were added per litre of spore suspension prior to inoculation. The seedlings were then incubated under continuous leaf wetness for 24 h at 20 C and 16 h photoperiod. Seedling reactions were recorded at 6 to 8 days post-inoculation, using an 1 to 5 scale, based on lesion type, where 1 denotes resistance and 5 susceptibility (Lamari, paper 1). When extensive chlorosis developed on seedlings from the "extensive chlorotic" lines ie: 6B365 and 2B13, the symbol CH+ was given to the reaction. Absence of extensive chlorosis was denoted as CH-.

RESULTS

Toxin production. Crude filtrate from isolates ASC1 and 86-124 consistently contained metabolite(s) that induced a severe necrosis on cultivars Glenlea, Celtic, Coulter and BH1146 (Table 7) at dilutions of 1:100. Dilutions of 1:500 and occasionally of 1:1000 also induced necrosis within 48-72 h of infiltration. Non Triticum species were not affected by the metabolites contained in the culture filtrates at any dilution and developed only minute necrotic or chlorotic flecks in response to inoculation with P. tritici-repentis (Table 7). Water and non-inoculated Fries medium did not induce any symptom when infiltrated into the above test species.

Although a toxin was present in the filtrates from cultures grown in the Fries medium by the first week, high concentrations of toxin, causing symptoms at 1:500-1:1000 dilutions, were more consistently obtained when cultures were incubated 2 to 3 weeks. Toxin levels from isolates ASC1 and 86-124 grown in the low sugar content medium used by Tomas and Bockus (1987) were at least 10 times lower than when grown in the regular Fries medium. However, symptoms and host specificity of the toxin(s) produced in either medium were identical.

Cultivar specificity. In the first test involving 12 wheat lines and cultivars, all the lines resistant to the fungus and those that developed extensive chlorosis were insensitive to the toxin. In contrast, all lines which developed necrosis to nec+ isolates were toxin-sensitive (Table 8). The cultivar Columbus segregated for toxin reaction and 10 to 15 % of the seedlings within the cultivar tested were found to be insensitive to the toxin. These are referred to as Col- whereas their sensitive sister-lines are referred to as Col+

In the second test, all lines and cultivars resistant or moderately resistant to the fungus were insensitive to the toxin whereas susceptible lines segregated into two

TABLE 7. REACTION OF 12 PLANT SPECIES TO TWO ISOLATES OF PYRENOPHORA TRITICI-REPENTIS. AND THEIR DESALTED CULTURE FILTRATES.

SPECIES	REACTION*	
	FUNGUS	TOXIN
MONOCOTS:		
Agropyron repens	R	-
Avena fatua	R	-
Avena sativa	R	-
Bromus inermis	R	-
Hordeum vulgare	R	-
Setaria viridis	R	-
Triticum sp.		
BH1146	S	+
Celtic	S	+
Coulter	S	+
Glenlea	S	+
Zea mays	nt	-
DICOTS:		
Fagopyrum sagittatum	R	-
Helianthus annuus	nt	-
Nicotiana tabacum	nt	-
Vicia faba	nt	-

*: Reactions to the fungus and to the toxin were similar for both isolates.
nt= not tested.
R : Resistant S: Susceptible to the fungus
+ and - : Respectively sensitive and insensitive to the toxin.
Filtrate dilution used: 1:100.

TABLE 8. REACTION OF 12 WHEAT CULTIVARS TO ISOLATE ASC1 OF *PYRENOPHORA TRITICI-REPENTIS* AND ITS TOXIN.

LINE OR CULTIVAR	ORIGIN	REACTION[&] FUNGUS	REACTION[&] TOXIN
HEXAPLOID			
BH1146	BRAZIL	3-4	+
CELTIC	USA	4	+
COLUMBUS	CANADA	4	SEG (+,-)[$]
ERIK	USA	1-2	-
GLENLEA	CANADA	5	+
SALAMOUNI	LEBANON	1-2	-
T.p. x T.m.	UNKNOWN	1	-
6B365	LEBANON	CH+	-
TETRAPLOID			
COULTER	CANADA	5	+
4B242	USA	1-2	-
4B1149	MEXICO	1	-
DIPLOID			
2B13	GERMANY	CH+	-

& Rating scale of 1-5, where 1=small brown lesion without tan necrosis and chlorosis (primary lesion); 2= primary lesion with minute amounts of tan necrosis or chlorosis; 3= primary lesion surrounded by a thin ring of tan necrosis or chlorosis; 4= primary lesion surrounded by a definite ring of tan necrosis or chlorosis, sometimes coalescing; and 5= most lesions consisting of coalescing tan necrotic or chlorotic tissues.

CH+,CH- = presence and absence of extensive chlorosis respectively.

Toxin reaction : + (sensitive) and - (insensitive).

$ SEG= Segregating for toxin reaction.

Leaves were infiltrated with ca. 150 ul of a 1:100 dilution of desalted culture filtrate.

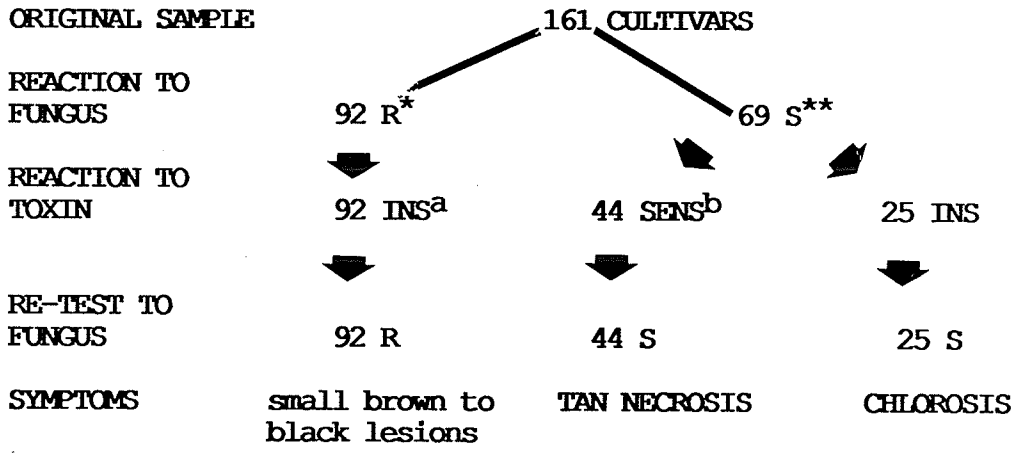
groups (Fig. 9). Of the 69 susceptible lines, 44 were sensitive to the toxin and 25 were insensitive. To confirm this finding, the 161 lines were inoculated once more with isolate ASC1 and checked daily to record their reaction type (Fig 9). The 92 resistant lines developed dark brown to black primary lesions with very little or no chlorosis. All 44 lines previously found to be toxin-sensitive developed necrosis within three to four days of inoculation whereas the 25 toxin-insensitive lines developed extensive chlorosis or chlorotic halos that became necrotic after 7 to 8 days.

Production of toxin by different isolates. All isolates with known ability to induce necrosis on cultivars BH1146 and Glenlea produced toxin in vitro (Table 9). These included wild type (nec+ ch+) isolates as well as isolates which did not induce extensive chlorosis (nec+ ch-). All isolates lacking the ability to cause necrosis (nec- ch+) failed to produce detectable levels of toxin in the culture filtrates (Table 9). The toxin had the same cultivar-specificity when infiltrated in leaves of 30 cultivars, and produced the identical symptoms, irrespective of the isolate from which it originated.

Dialysis and inactivation of the toxin. The toxic metabolite(s) from the culture filtrates were retained by the dialysis membrane and no detectable loss of activity was observed in the bioassay. Also, infiltration of the diffusate failed to cause any symptoms on seedlings of toxin sensitive cultivars Glenlea and BH1146, and on insensitive cultivars Erik, Salamouni, 4B1149 and 6B365. The crude and dialyzed culture filtrates from all the isolates listed in Table 9 completely lost their ability to induce symptoms (i.e. toxicity) after autoclaving.

Inheritance of toxin reaction. All F_1 progenies from each of four crosses between toxin sensitive/fungus susceptible, and toxin insensitive/fungus resistant lines were sensitive to the toxin and developed necrosis when inoculated with the fungus,

FIGURE 9. SUMMARY OF REACTIONS OF 161 WHEAT LINES AND CULTIVARS TO ISOLATE ASC1 OF PYRENOPHORA TRITICI-REPENTIS AND ITS DESALTED CULTURE FILTRATE.



Plants were inoculated with isolate ASC1 (nec+ ch+) at the 2 leaf stage and the third leaf was infiltrated on day 6 with ca. 150 uL of a 1:50 dilution of desalted culture filtrates of isolate ASC1. Reactions to both the fungus and the toxin were recorded 24 to 48 hours after infiltration of the culture filtrate.

*: R= Resistant; **: S= susceptible to the fungus
a: INS = insensitive; b: SENS= sensitive to the toxin

TABLE 9. RELATIONSHIP BETWEEN THE VIRULENCE PATTERNS OF 11 ISOLATES OF PYRENOPHORA TRITICI-REPENTIS ON TWO WHEAT CULTIVARS AND THEIR ABILITY TO PRODUCE TOXIN IN VITRO.

	REACTION		
ISOLATE	EXTENSIVE CHLOROSIS[&] ON 6B365	TAN NECROSIS[&] ON BH1146	TOXIN [$] PRODUCTION
86-124	-	+	+
HY331-3	-	+	+
ASC1	+	+	+
HY331-11	+	+	+
TK85-243	+	+	+
TK85-245	+	+	+
D308	+	-	-
HY331-1	+	-	-
HY331-2	+	-	-
HY331-6	+	-	-
HY331-7	+	-	-

& +,- = symptom expressed and not expressed, respectively.

$ +,- = toxin produced and not produced in-vitro, respectively, as revealed by the induction of visible symptoms on toxin-sensitive cultivar Glenlea.

indicating that sensitivity and susceptibility were dominant. The F_2 and backcross progenies segregated into toxin sensitive and insensitive plants (Table 10). The toxin-sensitive plants developed necrosis (to the fungus), whereas the toxin-insensitive plants were resistant to the fungus (no necrosis). The F_2 populations from all the crosses segregated in a ratio of 1 insensitive:3 sensitive, indicating that sensitivity to the toxin (and susceptibility to the fungus) was dominant over insensitivity (resistance to the fungus). Progeny from the backcross to Erik (insensitive) segregated in a ratio of 1 insensitive:1 sensitive, confirming that sensitivity was dominant over insensitivity. The segregation ratios of the backcross and the three F_2 populations are consistent with the hypothesis that a single gene is involved.

Comparison of Columbus selections for production of tan necrosis. The Columbus cultivar (pedigree RL4137 x Neepawa[6]) was found to be a mixture of two morphologically identical lines, but with opposite reactions to the toxin. The toxin sensitive (Col+) and the insensitive (Col-) lines were both susceptible to the fungus. When inoculated with a toxin-producing isolate, the Col+ line consistently produced tan necrosis 3 to 4 days after inoculation whereas the Col- line developed chlorosis instead (Table 11, Fig. 10). Inoculation with a toxin non-producing isolate failed to induce the development of necrotic lesions in both lines (Table 11).

TABLE 10. SEGREGATION FOR REACTION TO ISOLATE ASC1 OF PYRENOPHORA TRITICI-REPENTIS AND ITS TOXIN IN THREE F2 AND TWO BACKCROSS WHEAT POPULATIONS.

TYPE&	CROSS	RATIO OBSERVED* R : S	RATIO EXPECTED R : S	CHI^2	Prob.
BC_1	(ERIKxCOLUMBUS) X ERIK	34 : 42	1 : 1	0.4675	0.50
BC_1	(ERIKxCELTIC) X ERIK	30 : 22	1 : 1	0.9423	0.50-0.30
F_2	SALAMOUNI X COLUMBUS	48 : 131	1 : 3	0.2253	0.70-0.50
F_2	SALAMOUNI X COLUMBUS	98 : 271	1 : 3	0.4750	0.50-0.30
F_2	4B242 X COULTER	68 : 218	1 : 3	0.2141	0.70-0.50

Plants were inoculated with isolate ASC1 (nec+ ch+) at the 2 leaf stage and the third leaf was infiltrated on day 6 with ca. 150 uL of a 1:50 dilution of desalted culture filtrates of isolate ASC1. Reactions to both the fungus and the toxin were recorded 24 to 48 h after toxin infiltration.

& : All crosses are reciprocal, except 4B242 x Coulter. Erik, Salamouni and 4B242 are used as toxin insensitive/ fungus resistant cultivars, and Celtic, Columbus and Coulter as toxin sensitive/fungus susceptible cultivars. BC1 = F_1 plants were backcrossed once to the recessive parent.
F_2 = F_1 plants were selfed to produce F_2 seeds.

* : R= resistant to the fungus and insensitive to the toxin; S= susceptible to the fungus and sensitive to the toxin.

TABLE 11. REACTION OF TWO COLUMBUS WHEAT SELECTIONS TO TOXIN+ AND TOXIN- ISOLATES OF *PYRENOPHORA TRITICI-REPENTIS*.

	HOST REACTION	
ISOLATE	COL+[a]	COL-
ASC1 (TOX+)[b]	+[c]	-
HY331-6 (TOX-)	-	-

Seedlings were inoculated at the 2 leaf stage with a spore suspension adjusted to 3000 conidia/ml and incubated for 24 h under continuous leaf wetness.

a. Col+ and Col- are respectively toxin sensitive and insensitive Columbus selections.
b. Tox+ and Tox- : respectively toxin producer and non producer isolate.
c. + and - : tan necrosis induced and not induced by the fungus, respectively.

Figure 10. Symptoms induced by isolate ASC1 (nec+ ch+) of Pyrenophora tritici-repentis on toxin-sensitive Col+ (left) and toxin-insensitive Col- (right) wheat lines.

Leaves were sampled from plants inoculated with a conidial suspension (3000 conidia/ml) and incubated for 24 hours under continuous leaf wetness. The photograph was taken 8 days after inoculation. Note the chlorotic halos in Col- (right) by contrast to the well delimited, tan necrotic, lesions of Col+ (left).

DISCUSSION

The toxin(s) present in the culture filtrates of P. tritici-repentis appears to be restricted to the wheat species. However, the specificity of the toxin within the wheat cultivars corresponded to that of necrosis producing isolates, which are characterized by their ability to induce tan necrosis on susceptible cultivars, such as BH1146 and Glenlea (Lamari, paper2). Cultivars which develop chlorosis (line Col-) or extensive chlorosis (lines 6B365, 2B13) in response to fungal infection were found to be insensitive to the toxin, suggesting that the induction of chlorosis and extensive chlorosis may involve a second, unrecovered, toxin or different mechanism(s) than the one operating in the necrotic reaction.

The association between toxin sensitivity and production of necrosis by the fungus in the host is supported by several lines of evidence : i) the correlation between toxin sensitivity and production of necrosis in tests involving 161 cultivars (Fig. 9); ii) the control of both sensitivity to the toxin and production of necrosis in response to fungal infection by a single dominant gene, in all crosses made (Table 10); iii) the correlation between the ability of nec+ isolates to induce necrosis in susceptible cultivars and to produce toxin in vitro (Table 9) ; iv) the correlation between the inability of nec- isolates to induce necrosis in susceptible cultivars and their inability to produce toxin in-vitro (Table 9); and v) the association of toxin sensitivity and the production of necrosis in Col+ lines and of toxin insensitivity and absence of necrosis in Col- lines (Table 11).

The procedure for culturing the fungus and bioassaying the culture filtrates in the present study was not substantially different from that of Tomas and Bockus (1987), however, the levels of toxin produced under our conditions seem to be considerably higher. Their use of a different medium does not in itself explain the differences in activity observed. This suggests that there may be differences between the respective

isolates of P. tritici-repentis used with regard to toxin production, as has been reported in other pathogens (Smedegaard-Petersen, 1977; Smedegaard-Petersen and Nelson, 1969). The process of concentrating the toxin by evaporation at 45 C used by Tomas and Bockus (1987) may have partially inactivated the toxin. It was shown in this study that the toxin produced by P. tritici-repentis is totally inactivated by autoclaving for 20 min. at 121 C.

The ability of toxin-containing culture filtrates to reproduce all the symptoms of tan spot, as was reported by Tomas and Bockus (1987) was not confirmed in this study. The symptoms that developed under our conditions consisted mainly of necrotic components. Occasionally however, at very high dilutions of 1:1000, symptoms of chlorosis did appear at 30 to 48 h after infiltration, but by 4 to 6 days these subsequently became necrotic. The use, in this study, of cultivars such as 6B365 and 2B13, which support the development of extensive chlorosis, should have revealed the presence of any toxin(s) able to induce the chlorosis.

The toxin produced by P. tritici-repentis seems to have a much larger molecular weight than the toxins reported for P. teres (Smedegaard-Petersen, 1977) and other host-specific toxins (Macko, 1983). The dialysis process used in this study suggests a molecular weight larger than 8000.

The toxin produced by some isolates of P. tritici-repentis is not required for host penetration. This is supported by the fact that isolate HY331-6 (nec- ch+), avirulent on BH1146, has the ability to penetrate the epidermal cell and to invade the intercellular space in the mesophyll, although its growth is eventually restricted (Lamari, paper2). Furthermore, the penetration of the epidermal cell, the formation of vesicles and the initial invasion of the intercellular space of the mesophyll do not seem to be cultivar nor isolate-specific processes in tan spot of wheat (Larez et al, 1986; Loughman and Deverall, 1986); Lamari, paper 2). Pathogenesis of P. tritici-repentis seems to differ from that of other necrotrophic pathogens, where resistance was expressed at the

penetration stage (Nishimura and Kohmoto, 1982) or at the time the fungus moves from the epidermis to the mesophyll (Comstock and Scheffer, 1973), when the presence of a host-specific toxin is required for the continuation of the infection process. It is desirable that additional studies be conducted to determine toxin release in host tissues, as toxin production by P. tritici-repentis has been demonstrated in vitro only.

The characterization of the toxin produced by P. tritici-repentis as a virulence or a pathogenicity factor depends on one's definition of "disease" for tan spot of wheat. If "disease" (or susceptibility) is defined as the presence of tan necrosis or chlorosis and resistance as the presence of small brown to black necrotic primary lesions with little or no necrosis or chlorosis (Lamari, paper 1) then the toxin can be regarded as a pathogenicity factor, sensu Yoder (1980).

The toxin produced by P. tritici-repentis has potential use in breeding and screening wheat for resistance to tan spot. However, its use would be limited to susceptibility expressed by necrosis. Caution would have to be exercised when using the toxin in screening germ plasm for resistance, because fungus-susceptible, chlorotic lines, cannot be differentiated from resistant lines. The increasing use of tissue culture techniques in wheat may make the toxin of P. tritici-repentis useful in the early selection of resistant cells or protoplasts. Several questions regarding the mechanism(s) involved in the induction of chlorosis and extensive chlorosis in wheat cultivars remain to be resolved before a general model for pathogenesis in tan spot can be formulated.

GENERAL DISCUSSION

The wide range of wheat reactions observed in response to inoculation with P. tritici-repentis reflects the genetic diversity and the wide geographic origin of the accessions. The use of accessions from wild wheat species in the diploid, tetraploid and hexaploid groups helped to differentiate between resistant genotypes and those with moderate levels of resistance. Although some cultivars in the tetraploid and hexaploid groups were resistant, it is the wild species that constituted most of the pool of resistant germ plasm identified in this study (paper 1).

Results obtained with seedlings in the growth room were generally reproduced in the field (paper 1). However, further tests will be necessary to fully assess the superiority of resistant cultivars in providing protection against various isolates of P. tritici-repentis, in the growth room and in the field. It is desirable to assess the influence of the various lesion types and of extensive chlorosis on yield, by comparing inoculated and disease-free (fungicide treated) field plots. The real test of the value of resistance is ultimately the reduction or elimination of yield loss to disease.

The length of the post-inoculation wet period has been reported to determine the level of resistance to tan spot (Hosford, 1982). However, increasing the length of the wet period from 6 to 12 h resulted in significant increases in lesion size and disease severity, although further increases did not (Hosford et al, 1987). The same result was found by Gilchrist et al (1984) who failed to show any significant differences in disease severity between wet periods ranging from 24 to 72 h. The fact that disease severity increases with longer leaf wetness periods does not necessarily imply a breaking down of resistance, but may be an expression of factors, such as receptivity and lesion size, which are known to influence disease severity in partially resistant cultivars. This was demonstrated by Nutter and Pederson (1985) who found that receptivity and lesion size in five barley cultivars, inoculated with P. teres, were affected by the length of the

wetness period and accounted for increases in "disease proportion" (i.e. % leaf area infected). In view of the fact that cultivar BH1146 was considered to be moderately susceptible in our study (papers 1, 2, 3), it is not surprising that wheat cultivars, such as BH1146, could appear "severely spotted" under wetness durations longer than 48 h (Hosford, 1982). Whether resistance, as defined in this study, would break down (eg. lesion types 1 and 2 expanding into 4 or 5) with increasing leaf wetness duration, was not demonstrated. It would be desirable to test the most promising lines identified in the present study to longer wetness periods. In such tests, the inoculum concentration would likely have to be reduced as the number of penetrations might increase substantially due to an increased appressorial formation (Hosford et al, 1987) as well as receptivity (Nutter and Pederson, 1985) after the longer wetness periods.

The rating system developed for this study was found to be satisfactory in describing the reactions encountered. However, the existence of extensive chlorosis was not anticipated, nor was the finding that chlorosis and necrosis were separate traits and intrinsic to individual wheat cultivars. The rating scale in its present form does not differentiate between necrotic and chlorotic lesions (paper 1) and therefore, requires revision if these aspects are to be incorporated into the scale. This is particularly desirable in genetic studies involving the use of toxin produced by P. tritici-repentis (paper 3). Previous studies (Gilchrist et al, 1984; Hosford, 1982; Luz and Hosford, 1980; Nagle et al, 1982; Raymond et al, 1985) used a global approach to the evaluation of resistance, one that attempted to describe the reaction of the host as well as measure the effect of the reaction on the host (% leaf area infected) or, in some cases, assess components of partial resistance such as receptivity (lesions/unit area) and lesion size. This approach has merit in describing resistance in a broad sense, but is limited for use in breeding programs or in studies of host-pathogen interactions.

The rating scale used here, not only classified host reaction adequately, but also proved to be compatible for use with later findings about host-pathogen interactions

(paper 2), toxin involvement in disease (paper 3), and the inheritance of host reaction to both the toxin and the fungus (paper 3). The concept of lesion type used in this study closely resembles that of infection types in the cereal rusts (Stakman et al, 1962). A rating scale based on lesion type has been, and is still used to assess net blotch of barley (Buchannon and McDonald, 1965; Tekauz, 1985).

The existence of necrosis and chlorosis as separate traits in the symptomatology of tan spot (paper 1,2,3), suggests that different mechanism(s) may be involved in their development. The development of necrosis was found to be associated with a pathogen produced toxin (paper 3), but the mechanism(s) involved in the production of chlorosis and extensive chlorosis remains unknown. However, cytological observations in line 6B365, infected by a (nec+ ch+) isolate, linked the presence of an extensive network of intercellular hyphae to the appearance of extensive chlorosis (paper 2). Further studies are needed to determine whether the extensive chlorosis was the cause or the effect of the wide-spread hyphal growth observed.

The results of this study (paper 1,2,3) suggest that chlorosis and extensive chlorosis, as shown in line 6B365 infected with chlorosis-inducing isolates, are part of the same phenomenon, and comprise a continuum of chlorotic reactions. The insensitivity of all cultivars with extensive chlorotic reaction to the toxin of P. tritici-repentis strongly supports such a hypothesis. Similarly, necrosis can also be regarded as a continuum of reactions, encompassing intermediate (BH1146) and extensive (Glenlea) types, and characterized by susceptibility to the toxin (paper 2,3).

The development of the necrotic reaction was found to be associated with a single dominant gene in the four different crosses made between resistant/toxin-insensitive, and susceptible/toxin-sensitive cultivars. However, F_2 progenies of a reciprocal cross between Salamouni (resistant) and 6B365 (susceptible, with extensive chlorosis) were all non-chlorotic (Lamari, unpublished), indicating susceptibility was recessive in contrast to the results described above. Lee and Gough (1984) reported that in a cross between

wheat cultivars Carifen 12 (resistant) and Tam W-101 (susceptible), susceptibility was dominant in some F_3 families and recessive in others, and could find no obvious explanation. If chlorosis and extensive chlorosis were part of the same phenomenon as suggested above, the problem encountered in Lee and Gough's study might be resolved by assuming that the susceptible parent used in their cross was a mixture of toxin-sensitive and toxin-insensitive near-isogenic lines, such as found in cultivar Columbus (paper 3). Susceptibility would then appear dominant in those crosses involving the toxin-sensitive line (as in paper 3) and recessive in those involving the toxin-insensitive line (as in Salamouni x 6B365, above). It is therefore imperative that the relationship between chlorosis and necrosis be investigated further at both the physiological and genetic level.

Three virulence patterns were characterized in P. tritici-repentis on the basis of specific interactions between wheat cultivars and pathogen isolates. The three pathotypes identified amongst 92 isolates tested in the present study appear to be disproportionate when compared to the 12 "races" identified in 40 isolates by Luz and Hosford (1980). However, they identified the races on the basis of % leaf area infected plus lesion size i.e. separated on a quantitative basis. Another factor that may account for the disparity is that the wheat differential set used in the study of Luz and Hosford (1980) did not include highly resistant cultivars. This suggests that their 12 races were separated on the basis of aggressiveness and not virulence as defined in the present study (paper 1,2). In view of the increasing interest in tan spot, standardization of the methods used to evaluate host reaction to the fungus would be desirable.

The variation in pathogen virulence described in the present study is based on testing isolates sampled from wheat exclusively. Sampling of the pathogen from alternative hosts (Krupinsky, 1982) as well as from more wide-spread geographic locations is needed to advance our knowledge about the virulence in this fungus. The testing of isolates of P. teres from different continents disclosed differences in virulence between isolates

from the United States and the Mediterranean region (Karki and Sharp, 1986). A thorough knowledge of virulence patterns relating to pathogenesis, provides an insight into the evolution of pathogen virulence, and is also essential to the development of effective and stable resistance.

Toxin(s) involvement in tan spot of wheat was demonstrated in this study (paper 3). The toxin was associated with the development of necrosis in the host, whereas toxin production was associated with the ability of individual isolates to induce necrosis in sensitive cultivars. However, evidence of toxin production by the pathogen has only been demonstrated in vitro. Additional studies are needed to demonstrate the presence of the toxin in host tissues.

Knowledge of the inheritance of toxin production and of virulence in the pathogen is important for an understanding of the evolution of virulence in the pathogen. The materials identified in this study offer the opportunity to carry out such studies.

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APPENDIX 1. List of accessions inoculated with isolate ASC1 of Pyrenophora tritici-repentis.

	Ploidy*	Number	R1$	R2&
1	6B	.	4.50000	.
2	2B	1	1.00000	.
3	2B	2	1.00000	.
4	2B	3	3.00000	.
5	2B	4	2.00000	.
6	2B	5	2.00000	.
7	2B	6	2.00000	1
8	2B	7	4.00000	.
9	2B	8	3.00000	.
10	2B	9	3.00000	.
11	2B	10	2.00000	1
12	2B	11	.	.
13	2B	12	3.00000	.
14	2B	13	1.00000	5
15	2B	14	3.00000	.
16	2B	20	1.00000	.
17	2B	21	.	.
18	2B	22	3.00000	.
19	2B	23	2.00000	.
20	2B	25	3.00000	.
21	2B	26	2.00000	1
22	2B	27	4.00000	1
23	2B	29	5.00000	1
24	2B	30	1.00000	1
25	2B	31	3.00000	.
26	2B	32	3.00000	.
27	2B	33	2.00000	.
28	2B	34	2.00000	.
29	2B	35	2.00000	.
30	2B	36	3.00000	.
31	2B	37	5.00000	.
32	2B	42	3.00000	.
33	2B	43	3.00000	.
34	2B	44	2.00000	1
35	2B	45	3.00000	1
36	2B	46	3.00000	.
37	2B	48	4.00000	.
38	2B	50	1.00000	1
39	2B	53	1.00000	1
40	4B	54	1.00000	2
41	4B	55	3.00000	.
42	4B	56	1.00000	1
43	4B	57	1.00000	1
44	4B	58	4.00000	.
45	4B	59	4.00000	.
46	4B	60	4.00000	.
47	4B	61	1.00000	1
48	4B	63	4.00000	.
49	4B	64	1.00000	1
50	4B	67	2.33333	3
51	4B	68	3.00000	.
52	4B	69	3.00000	.
53	4B	71	1.00000	.
54	4B	72	4.00000	.
55	4B	73	3.00000	2

* 2B = diploid, 4B=tetraploid, 6B=hexaploid, 8B=octoploid, 0B = unknown.

$ R1 = score in test at the 4-6 leaf stage.

& R2 = score in tests at the 2 leaf stage.

Rating scale of 1 to 5 where 1=resistant and 5= susceptible

56	4B	74	2.0	4
57	4B	75	3.0	.
58	4B	76	.	.
59	4B	77	4.0	.
60	4B	78	4.0	.
61	4B	79	.	.
62	4B	80	1.0	.
63	4B	81	2.0	2
64	4B	82	3.0	.
65	4B	83	4.0	.
66	4B	84	3.0	.
67	4B	85	1.0	2
68	4B	86	.	.
69	4B	87	3.0	.
70	4B	88	4.0	.
71	4B	89	.	.
72	4B	90	2.0	4
73	4B	91	3.0	.
74	4B	92	3.0	.
75	4B	93	3.0	.
76	4B	94	2.0	.
77	4B	95	4.0	.
78	4B	96	1.0	1
79	4B	97	4.0	.
80	4B	98	4.0	.
81	4B	100	1.0	1
82	4B	108	2.0	3
83	4B	109	2.0	2
84	4B	110	2.0	4
85	4B	111	2.0	.
86	4B	112	5.0	.
87	4B	113	3.0	.
88	4B	114	3.0	.
89	4B	115	3.0	.
90	4B	116	2.0	3
91	4B	117	3.0	.
92	4B	119	3.0	.
93	4B	121	3.0	.
94	4B	122	5.0	.
95	4B	123	2.0	.
96	4B	125	4.5	.
97	4B	127	5.0	.
98	4B	128	.	.
99	4B	132	5.0	.
100	4B	140	5.0	5
101	4B	144	3.0	.
102	4B	145	3.0	.
103	4B	146	4.0	5
104	4B	147	3.0	5
105	4B	148	3.0	.
106	4B	149	2.0	.
107	4B	150	4.0	.
108	4B	151	3.0	.
109	4B	152	3.0	4
110	4B	153	3.0	.

111	4B	154	3	.
112	4B	155	3	.
113	4B	156	2	4
114	4B	157	3	.
115	4B	158	.	.
116	4B	159	3	3
117	4B	160	3	4
118	4B	161	3	4
119	4B	164	5	4
120	4B	165	3	.
121	4B	173	5	.
122	4B	175	2	1
123	4B	176	4	.
124	4B	177	3	5
125	4B	178	4	.
126	4B	179	3	.
127	4B	180	2	.
128	4B	181	4	.
129	4B	183	5	.
130	4B	184	5	.
131	4B	185	5	.
132	4B	186	.	.
133	4B	187	4	.
134	4B	188	3	2
135	4B	189	3	.
136	4B	190	3	3
137	4B	191	3	3
138	4B	192	3	.
139	4B	193	2	3
140	4B	194	3	.
141	4B	195	3	.
142	4B	196	5	5
143	4B	197	4	.
144	4B	198	4	.
145	4B	199	5	.
146	4B	200	5	.
147	4B	201	5	.
148	4B	202	5	.
149	4B	203	5	.
150	4B	204	4	.
151	4B	205	3	4
152	4B	206	3	.
153	4B	207	4	.
154	4B	208	4	.
155	4B	209	4	.
156	4B	210	4	.
157	4B	211	.	.
158	4B	212	2	5
159	4B	213	5	.
160	4B	214	5	.
161	4B	215	5	.
162	4B	216	5	5
163	4B	217	4	.
164	4B	218	5	5
165	4B	219	3	.

166	4B	220	4.0	.
167	4B	221	4.0	.
168	4B	222	4.0	.
169	4B	223	4.0	.
170	4B	224	4.0	.
171	4B	225	5.0	.
172	4B	226	5.0	.
173	4B	227	3.0	.
174	4B	228	5.0	.
175	4B	229	5.0	.
176	4B	230	5.0	.
177	4B	231	3.0	.
178	4B	232	5.0	.
179	4B	233	1.0	1
180	4B	234	5.0	.
181	4B	235	5.0	.
182	4B	236	2.0	.
183	4B	237	4.0	.
184	4B	238	5.0	.
185	4B	239	4.0	.
186	4B	240	4.0	.
187	4B	241	4.0	.
188	4B	242	1.0	1
189	4B	243	2.0	3
190	4B	244	3.0	4
191	4B	245	4.0	.
192	4B	246	3.0	.
193	4B	247	4.0	.
194	4B	248	3.0	.
195	4B	249	4.0	.
196	4B	250	2.0	3
197	4B	251	3.0	.
198	4B	252	4.0	.
199	4B	253	.	.
200	4B	254	4.0	.
201	4B	255	4.0	.
202	4B	256	2.0	3
203	4B	257	4.0	.
204	4B	258	5.0	5
205	4B	259	3.0	.
206	4B	261	.	.
207	4B	262	3.0	.
208	4B	263	.	.
209	4B	264	4.0	.
210	4B	265	3.0	.
211	4B	266	3.0	.
212	4B	269	5.0	.
213	4B	277	.	5
214	4B	278	1.0	5
215	4B	279	.	5
216	4B	280	4.0	.
217	4B	281	4.0	5
218	4B	283	4.0	.
219	4B	287	3.5	.
220	4B	288	2.0	.

221	4B	290	1.0	1
222	8B	291	2.0	1
223	4B	293	4.0	.
224	4B	295	2.5	4
225	4B	297	2.5	3
226	6B	298	3.0	.
227	4B	299	5.0	.
228	6B	300	2.0	.
229	6B	301	5.0	.
230	6B	302	4.0	.
231	6B	303	5.0	.
232	6B	304	4.0	.
233	6B	306	5.0	.
234	6B	307	5.0	.
235	6B	309	5.0	.
236	6B	310	4.0	.
237	6B	311	5.0	.
238	6B	312	3.0	.
239	6B	313	4.0	.
240	6B	314	3.0	.
241	6B	315	.	5
242	6B	317	.	4
243	6B	318	.	2
244	6B	319	3.0	.
245	6B	321	3.0	.
246	6B	322	.	.
247	6B	323	.	2
248	6B	324	.	2
249	6B	335	.	1
250	6B	336	.	5
251	6B	337	5.0	.
252	6B	338	5.0	.
253	6B	339	4.0	.
254	6B	340	5.0	.
255	6B	341	5.0	.
256	6B	342	4.0	.
257	6B	343	5.0	.
258	6B	344	5.0	.
259	6B	345	4.0	.
260	6B	347	5.0	.
261	6B	348	4.0	.
262	6B	349	5.0	.
263	6B	350	5.0	.
264	6B	354	4.0	.
265	8B	355	3.0	.
266	6B	356	2.0	1
267	6B	357	1.0	.
268	6B	358	3.0	.
269	6B	359	2.0	.
270	6B	360	4.0	.
271	6B	361	4.0	.
272	6B	362	5.0	.
273	6B	363	2.5	2
274	6B	364	4.0	.
275	6B	365	5.0	5

276	6B	366	3.0	1.50000
277	6B	367	3.0	5.00000
278	6B	368	3.0	3.00000
279	6B	370	3.0	4.00000
280	6B	371	5.0	.
281	6B	373	4.0	.
282	6B	374	5.0	.
283	6B	375	4.0	.
284	6B	376	.	.
285	6B	377	4.0	.
286	6B	378	3.0	.
287	6B	379	4.0	.
288	6B	380	3.0	3.50000
289	6B	382	2.0	3.66667
290	6B	383	3.0	.
291	6B	384	3.0	.
292	6B	385	4.0	.
293	6B	387	3.5	.
294	6B	388	4.0	.
295	6B	389	4.0	.
296	6B	391	5.0	4.00000
297	6B	394	4.0	.
298	6B	395	2.5	4.00000
299	6B	396	2.0	.
300	6B	397	3.0	.
301	6B	399	3.0	.
302	6B	401	3.0	.
303	6B	405	.	.
304	6B	410	4.0	.
305	6B	412	1.0	4.00000
306	6B	413	3.0	.
307	6B	414	5.0	.
308	6B	415	5.0	5.00000
309	6B	416	2.0	5.00000
310	6B	417	5.0	.
311	6B	422	2.0	.
312	6B	424	5.0	.
313	6B	425	4.0	.
314	4B	426	2.0	.
315	6B	427	4.0	.
316	6B	430	5.0	.
317	6B	431	2.0	.
318	6B	434	5.0	.
319	6B	435	2.0	5.00000
320	6B	436	2.0	2.00000
321	6B	437	4.0	.
322	6B	438	3.0	.
323	4B	439	4.0	.
324	6B	440	3.0	4.00000
325	6B	441	3.0	.
326	6B	442	4.0	.
327	6B	443	.	.
328	6B	444	4.0	3.00000
329	6B	445	5.0	5.00000
330	4B	446	4.0	.

331	6B	447	4.00000	.
332	6B	448	4.00000	.
333	6B	449	2.00000	1.0
334	6B	450	.	.
335	6B	451	2.00000	5.0
336	6B	452	.	.
337	6B	456	3.00000	.
338	6B	457	4.00000	.
339	6B	458	3.50000	.
340	6B	460	3.00000	.
341	6B	461	1.00000	1.0
342	6B	462	2.00000	1.0
343	8B	466	1.00000	2.0
344	8B	467	3.50000	.
345	8B	468	1.66667	2.0
346	4B	469	2.00000	3.0
347	4B	471	2.00000	2.0
348	4B	472	3.00000	1.0
349	6B	474	3.50000	.
350	6B	475	1.00000	2.5
351	6B	476	5.00000	.
352	6B	477	5.00000	.
353	6B	478	4.00000	.
354	4B	479	5.00000	.
355	6B	480	4.00000	.
356	6B	484	2.50000	2.0
357	4B	486	4.00000	.
358	4B	487	3.00000	.
359	4B	488	3.00000	.
360	4B	489	1.00000	1.0
361	2B	490	1.50000	1.0
362	4B	491	4.00000	.
363	4B	492	3.00000	3.0
364	4B	497	4.00000	.
365	4B	498	3.00000	.
366	6B	499	4.00000	.
367	6B	500	4.00000	.
368	6B	501	1.00000	1.0
369	8B	502	1.50000	1.0
370	4B	504	.	.
371	6B	505	1.50000	1.0
372	4B	506	.	.
373	4B	507	4.00000	.
374	6B	512	2.00000	3.0
375	6B	513	3.00000	1.0
376	6B	514	3.00000	.
377	4B	515	2.00000	3.0
378	4B	516	.	.
379	4B	517	1.50000	5.0
380	6B	520	5.00000	.
381	6B	524	3.00000	.
382	4B	525	1.00000	.
383	4B	526	4.00000	.
384	4B	528	4.00000	.
385	4B	529	2.00000	1.0

386	6B	537	3.0	.
387	6B	538	3.0	.
388	6B	539	5.0	.
389	6B	540	3.0	.
390	6B	541	4.0	.
391	4B	543	3.0	.
392	8B	544	.	.
393	6B	546	3.0	.
394	6B	547	3.0	4.0
395	6B	548	1.5	3.5
396	6B	549	1.5	3.5
397	4B	569	4.5	.
398	4B	572	4.0	.
399	4B	573	1.0	1.0
400	4B	588	1.0	.
401	6B	589	3.5	.
402	6B	593	3.5	.
403	6B	596	.	.
404	6B	599	4.0	.
405	6B	607	3.0	.
406	6B	608	5.0	.
407	6B	609	5.0	.
408	6B	613	5.0	.
409	6B	614	3.0	.
410	6B	615	2.0	5.0
411	6B	625	5.0	.
412	6B	626	4.0	.
413	6B	627	5.0	.
414	6B	631	5.0	.
415	6B	632	5.0	.
416	6B	635	4.0	.
417	6B	636	1.0	3.0
418	6B	637	3.0	.
419	6B	638	2.0	2.0
420	6B	639	5.0	.
421	6B	640	4.0	.
422	6B	641	3.0	.
423	6B	642	4.0	.
424	6B	643	4.0	.
425	6B	647	5.0	.
426	6B	648	4.0	.
427	6B	649	4.0	.
428	6B	653	4.0	.
429	4B	654	5.0	.
430	6B	655	3.0	.
431	6B	656	3.0	.
432	6B	657	3.0	3.0
433	6B	658	5.0	.
434	6B	665	2.0	.
435	6B	666	4.0	.
436	6B	667	1.0	2.0
437	4B	689	5.0	.
438	4B	696	4.0	5.0
439	6B	698	3.0	.
440	6B	699	4.0	2.0

441	6B	702	5	.
442	4B	706	1	2
443	6B	718	5	.
444	6B	719	5	.
445	6B	720	5	.
446	6B	721	5	.
447	6B	722	5	.
448	4B	723	5	.
449	4B	724	5	.
450	6B	725	5	.
451	4B	726	4	.
452	4B	727	5	.
453	4B	728	4	.
454	4B	729	5	.
455	4B	730	4	.
456	4B	731	4	.
457	4B	732	5	.
458	6B	733	4	.
459	4B	734	4	.
460	6B	735	4	.
461	6B	736	5	.
462	6B	737	5	.
463	6B	738	3	.
464	4B	739	5	.
465	4B	740	5	.
466	4B	741	5	.
467	4B	742	5	.
468	4B	743	5	.
469	4B	744	4	.
470	4B	745	4	.
471	4B	746	4	.
472	4B	747	5	.
473	4B	748	5	.
474	4B	749	4	.
475	4B	750	5	.
476	4B	751	3	.
477	6B	753	4	.
478	4B	754	2	.
479	4B	755	4	.
480	4B	756	3	.
481	4B	757	5	.
482	6B	758	5	.
483	6B	759	5	.
484	6B	760	5	.
485	6B	762	5	.
486	6B	763	5	.
487	4B	764	4	.
488	4B	765	.	.
489	4B	766	.	.
490	4B	767	2	.
491	4B	768	1	.
492	4B	769	4	.
493	4B	770	.	4
494	4B	771	.	5
495	4B	772	.	3

496	4B	773	.	5
497	4B	774	5	.
498	4B	775	.	5
499	4B	776	5	3
500	6B	777	5	.
501	6B	778	5	.
502	6B	779	.	.
503	6B	780	5	.
504	6B	781	1	.
505	6B	782	4	.
506	6B	783	3	.
507	6B	785	4	.
508	6B	786	4	.
509	6B	787	3	.
510	6B	790	5	.
511	6B	791	3	.
512	6B	792	4	.
513	8B	802	.	2
514	6B	901	4	.
515	6B	902	3	.
516	4B	903	5	.
517	4B	904	3	.
518	4B	905	4	.
519	4B	906	5	.
520	4B	907	4	.
521	4B	908	5	.
522	6B	909	5	.
523	4B	910	.	.
524	4B	911	3	.
525	4B	912	5	.
526	4B	913	5	.
527	4B	914	3	.
528	6B	915	4	.
529	6B	916	5	.
530	6B	917	5	.
531	6B	918	4	.
532	6B	919	3	.
533	6B	920	5	.
534	4B	921	5	.
535	6B	922	4	.
536	6B	923	2	.
537	4B	924	5	.
538	4B	925	5	.
539	6B	930	4	.
540	6B	931	5	.
541	6B	932	3	.
542	6B	947	2	.
543	6B	961	4	.
544	6B	962	5	.
545	6B	963	5	.
546	6B	964	5	.
547	6B	965	5	.
548	6B	966	3	.
549	6B	967	5	.
550	6B	968	5	.

551	6B	969	3	.
552	6B	970	1	.
553	6B	971	5	.
554	6B	972	4	.
555	6B	973	4	.
556	6B	974	4	.
557	6B	975	5	.
558	6B	976	4	.
559	6B	977	5	.
560	6B	978	2	.
561	6B	979	4	.
562	6B	980	4	.
563	6B	981	3	.
564	6B	982	5	.
565	6B	983	2	.
566	6B	984	3	.
567	6B	985	4	.
568	6B	986	5	.
569	6B	987	5	.
570	6B	988	5	.
571	6B	989	5	.
572	6B	990	5	.
573	6B	991	3	.
574	6B	992	3	.
575	6B	993	4	.
576	6B	994	.	.
577	6B	995	4	.
578	6B	996	5	.
579	4B	997	4	.
580	4B	998	5	.
581	4B	999	5	.
582	4B	1000	4	.
583	4B	1001	5	.
584	2B	1012	.	.
585	2B	1013	5	.
586	4B	1014	2	.
587	2B	1015	4	.
588	2B	1016	4	.
589	4B	1017	5	.
590	6B	1018	5	.
591	6B	1019	5	.
592	6B	1020	5	.
593	6B	1021	3	.
594	6B	1022	5	.
595	6B	1023	5	.
596	6B	1024	4	.
597	6B	1025	5	.
598	6B	1026	5	.
599	6B	1027	4	.
600	6B	1028	5	.
601	4B	1029	4	.
602	4B	1030	4	.
603	4B	1031	3	.
604	4B	1032	4	.
605	4B	1033	5	.

606	4B	1034	5.0	.
607	4B	1035	4.0	.
608	4B	1036	4.0	.
609	4B	1037	5.0	.
610	4B	1038	3.0	.
611	6B	1040	4.0	.
612	6B	1041	5.0	.
613	6B	1042	1.0	.
614	6B	1043	1.0	.
615	6B	1045	2.0	.
616	6B	1046	1.0	.
617	4B	1047	2.0	.
618	6B	1048	2.0	.
619	6B	1049	4.0	.
620	6B	1050	4.5	.
621	6B	1051	5.0	.
622	6B	1052	3.0	.
623	6B	1053	5.0	.
624	6B	1054	5.0	.
625	6B	1055	4.0	.
626	6B	1056	5.0	.
627	6B	1057	5.0	.
628	6B	1058	5.0	.
629	6B	1059	5.0	.
630	6B	1060	4.0	.
631	6B	1061	4.0	.
632	6B	1062	5.0	.
633	6B	1063	4.0	.
634	6B	1064	4.0	.
635	6B	1065	4.0	.
636	6B	1066	2.0	.
637	6B	1067	4.0	.
638	6B	1068	3.0	.
639	6B	1069	4.0	.
640	6B	1070	3.0	.
641	6B	1071	3.0	.
642	6B	1072	5.0	.
643	6B	1073	4.0	.
644	6B	1074	4.0	.
645	6B	1075	2.0	.
646	6B	1076	5.0	.
647	6B	1077	5.0	.
648	6B	1078	4.0	.
649	4B	1079	.	.
650	6B	1080	2.0	.
651	4B	1081	1.0	.
652	4B	1082	5.0	.
653	6B	1083	4.0	.
654	6B	1084	2.0	.
655	6B	1085	3.0	.
656	6B	1086	4.0	.
657	6B	1087	5.0	.
658	6B	1088	1.0	.
659	6B	1089	2.0	.
660	6B	1090	5.0	.

661	6B	1091	2.0	.
662	4B	1092	1.0	.
663	4B	1107	1.0	.
664	4B	1110	2.0	5
665	4B	1111	2.5	.
666	4B	1112	3.0	.
667	4B	1113	4.0	.
668	6B	1114	3.5	.
669	6B	1115	2.0	.
670	6B	1116	2.0	.
671	6B	1117	5.0	.
672	6B	1118	1.0	.
673	6B	1119	4.0	.
674	6B	1120	1.0	.
675	6B	1121	3.0	.
676	6B	1122	1.0	.
677	6B	1123	4.0	.
678	6B	1124	4.0	.
679	6B	1125	3.0	.
680	6B	1126	5.0	.
681	6B	1127	3.0	.
682	6B	1128	5.0	.
683	6B	1129	4.5	.
684	6B	1130	3.5	.
685	6B	1131	3.0	.
686	6B	1132	3.5	.
687	6B	1133	1.0	.
688	2B	1134	1.0	.
689	6B	1135	4.0	.
690	6B	1136	5.0	.
691	6B	1137	4.0	.
692	4B	1138	3.0	.
693	4B	1139	3.0	.
694	4B	1140	3.0	.
695	4B	1141	4.0	.
696	4B	1142	3.0	.
697	4B	1143	4.0	.
698	6B	1144	2.0	.
699	6B	1145	3.0	.
700	6B	1146	2.0	.
701	4B	1147	3.0	.
702	4B	1148	4.0	.
703	4B	1149	1.0	.
704	4B	1150	3.0	.
705	6B	1152	4.0	.
706	6B	1153	3.0	.
707	6B	1154	4.0	.
708	6B	1155	3.0	.
709	6B	1156	4.0	.
710	6B	1157	3.0	.
711	6B	1158	4.0	.
712	4B	1159	3.0	.
713	4B	1160	3.0	.

Appendix 2

Chi square tests.

	observed ratio	expected ratio	Chi^2	Prob.*
Diploid vs Tetraploid	133.4:57	95.7:95.7	21.37	< 0.005
Diploid vs Hexaploid	136.4:52	94.2:94.2	36.91	< 0.005
Tetraploid vs Hexaploid	65.1:52	58.5:58.5	1.25	0.950-0.50

Data represent pooled and adjusted numbers of resistant (score 1) and moderately resistant (score 2) accessions within each ploidy group.

* level of significance

Appendix 3

Discrepancies in scores between tests at the 2 and 4-6 leaf stages.

Accessions rated resistant (scores 1-2) at the 4-6 leaf stage and susceptible (scores 4-5) at the 2 leaf stage.

ACCESSION	SCORE1	SCORE2
4B74	2	4
4B90	2	4
4B110	2	4
4B156	2	5
4B278	1	5
6B412	1	4
6B416	2	5
6B435	2	5
6B451	2	5
6B517	1.5	5
6B615	2	5
4B1110	2	5

Accessions rated susceptible (scores 4-5) at the 4-6 leaf stage and resistant (scores 1-2) at the 2 leaf stage.

ACCESSION	SCORE1	SCORE2
2B27	4	1

score1 = 4-6 leaf stage
score2 = 2 leaf stage

Appendix 4. List of accessions tested in the field in 1986.

OBS	LINE	* DS1	DS2	DS3	** LT1	LT2	LT3	*** P1	P2	P3
1	ARCOLA	5.50000	5.75000	6.50000	4.00000	5.00000	5	4.00000	4.00000	5.00000
2	A178	4.00000	4.00000	7.00000	1.00000	1.00000	2	4.00000	5.00000	8.00000
3	BENITO	6.75000	7.75000	8.50000	5.00000	5.00000	5	.	.	.
4	BH1146	4.87500	5.25000	7.00000	3.20000	4.40000	5	3.80000	4.10000	4.50000
5	BT201	1.00000	1.00000	2.00000	1.00000	1.00000	1	.	.	.
6	CARIFEN12	3.50000	7.50000	.	4.00000	5.00000	.	.	.	.
7	CELTIC	6.00000	9.00000	9.00000	3.00000	5.00000	5	6.00000	6.50000	8.00000
8	CHINESE/EMMER	5.00000	6.00000	6.00000	3.00000	4.00000	4	5.00000	5.00000	8.00000
9	COLOMBUS	7.08333	7.81818	8.61111	4.58333	5.00000	5	4.53846	5.88462	6.61538
10	COULTER	6.92857	7.57692	8.27273	4.28571	4.84615	5	4.42857	4.67857	6.32143
11	DURI	7.00000	8.00000	8.00000	4.00000	5.00000	5	6.00000	7.00000	8.00000
12	E. INFLATUS	6.50000	6.00000	9.00000	3.00000	5.00000	5	4.00000	4.00000	6.00000
13	ERIK	3.25000	.	.	1.00000	1.00000	.	4.00000	4.00000	5.00000
14	GLENLEA	6.25000	7.25000	7.00000	4.00000	5.00000	5	4.00000	5.00000	6.00000
15	HY320	4.90000	5.10000	7.16667	4.00000	4.60000	5	.	.	.
16	IAS	3.00000	6.50000	7.50000	1.00000	4.00000	5	4.00000	4.00000	6.00000
17	KAHLA	5.00000	6.00000	7.00000	4.00000	4.00000	5	3.00000	3.50000	4.50000
18	KAHLI	3.00000	6.00000	6.00000	2.00000	2.00000	4	3.00000	4.00000	4.50000
19	KATEPAWA	7.66667	8.33333	9.00000	5.00000	5.00000	5	5.00000	6.50000	8.00000
20	KENYA FARMER	9.00000	9.00000	9.00000	5.00000	5.00000	5	6.00000	6.50000	8.50000
21	KENYA 117.A	7.00000	7.50000	8.00000	5.00000	5.00000	5	6.00000	6.00000	6.50000
22	KENYA 294B2A3	7.00000	7.50000	8.00000	4.00000	4.00000	5	5.00000	5.00000	6.00000
23	MEDORA	6.50000	6.00000	7.00000	4.00000	5.00000	5	.	.	.
24	MERCURY	7.00000	7.00000	8.50000	4.00000	5.00000	5	5.00000	5.75000	8.50000
25	MOURISCO	8.00000	8.00000	9.00000	4.00000	4.00000	5	7.50000	9.00000	8.00000
26	NAHS 82-175	7.50000	8.00000	8.00000	4.00000	5.00000	5	.	.	.
27	NAHS87-175	.	.	.	.	.	.	4.00000	4.50000	6.00000
28	ND4/LEE/ND905	9.00000	9.00000	9.00000	5.00000	5.00000	5	6.00000	7.00000	8.50000
29	ND611	6.00000	6.00000	5.50000	3.00000	3.00000	5	3.00000	4.00000	5.50000
30	NEEPAWA	4.50000	5.00000	8.00000	4.00000	4.00000	5	.	.	.
31	ORGAZ	.	.	3.00000	2.00000	.	.	4.00000	3.50000	4.00000
32	OSLO	4.50000	5.00000	8.00000	2.00000	3.00000	5	4.00000	4.00000	6.50000
33	PEL 1373268	6.00000	5.50000	6.00000	3.00000	4.00000	5	4.00000	4.00000	4.00000
34	PI 166308	1.00000	1.50000	4.00000	1.00000	2.00000	5	.	.	.
35	PILOT2/THATCHER	6.00000	6.00000	6.00000	2.00000	2.00000	3	5.00000	5.00000	7.00000
36	PI136575	6.00000	.	.	5.00000	.	.	5.00000	5.00000	5.00000
37	PI166308	.	.	.	.	.	.	4.00000	5.00000	6.50000
38	PI298586	4.50000	4.00000	5.00000	1.00000	2.00000	3	4.00000	4.00000	7.00000
39	PI306557	1.00000	1.00000	2.00000	1.00000	1.00000	1	2.00000	2.00000	3.00000
40	PI306577	1.00000	1.00000	2.00000	1.00000	1.00000	1	1.50000	1.50000	2.50000
41	PR2348	7.00000	7.00000	8.00000	3.00000	4.00000	5	6.00000	7.00000	8.00000
42	PR2360	4.50000	5.00000	5.50000	2.00000	2.00000	3	4.00000	4.75000	6.75000
43	PR2369	6.00000	7.00000	8.00000	3.00000	4.00000	5	.	.	.
44	RL4452	7.00000	8.00000	9.00000	5.00000	5.00000	5	4.00000	5.00000	6.00000
45	SALAMOUNI	4.00000	4.00000	3.50000	2.00000	2.00000	2	3.50000	3.50000	4.00000
46	SAUNDERS	5.00000	6.00000	9.00000	2.00000	3.00000	5	5.00000	7.00000	8.50000
47	SCEPTRE	6.00000	7.00000	8.00000	5.00000	5.00000	5	.	.	.
48	SELKIRK	5.00000	5.50000	8.00000	4.00000	4.00000	5	.	.	.
49	SINTON	7.00000	8.00000	8.00000	5.00000	5.00000	5	.	.	.
50	T. PERSICUM	1.00000	1.00000	2.00000	1.00000	1.00000	1	4.00000	4.00000	4.50000
51	T. PERSICUM STRAM.	1.00000	1.00000	1.50000	1.00000	1.00000	1	.	.	.
52	T. SPELTA	1.00000	4.00000	4.00000	1.00000	1.00000	1	3.00000	3.00000	4.00000
53	T. TIMO/AE. SQUAR	4.50000	8.00000	9.00000	3.00000	5.00000	5	5.00000	6.00000	8.00000
54	T. TIMOPHEEVI	6.00000	6.00000	9.00000	2.00000	3.00000	.	4.00000	5.00000	8.00000
55	T. TURGIDUM	4.00000	.	2.00000	3.00000	3.00000	5	.	.	.

OBS	LINE	DS1	DS2	DS3	LT1	LT2	LT3	P1	P2	P3
56	T. TURGIDUM 10	.	.	.	.	.	.	10.1	10.53	11.1
57	T.PERSICUM	.	.	.	.	.	.	2.0	2.00	4.0
58	TC/K338331/TRIUMPH	9.00000	9.00	9.0	5	5.00	5.0	6.0	7.00	11.1
59	TP X TM	1.66667	1.00	3.0	1	1.25	1.0	3.0	3.00	3.5
60	V-525	6.50000	.	6.0	4	4.00	4.0	3.0	3.50	4.0
61	V-557	6.00000	7.00	6.0	4	5.00	5.0	4.0	4.00	5.0
62	V-627	.	.	.	.	.	.	4.0	3.50	5.0
63	V-637	6.00000	6.00	6.5	4	5.00	5.0	4.0	3.50	4.5
64	V-648	5.00000	6.00	6.0	2	4.00	4.0	4.0	4.00	5.0
65	V-672	6.00000	6.00	6.0	4	4.00	5.0	.	.	.
66	VIR53360	2.50000	3.00	3.5	1	1.00	2.0	2.0	2.50	4.0
67	WAKOOMA	4.00000	4.00	5.0	5	5.00	5.0	.	.	.
68	WASCANA	6.50000	6.50	7.0	5	5.00	5.0	4.0	5.00	6.0
69	11869	6.00000	7.50	8.0	3	5.00	5.0	6.0	7.00	8.0
70	2B26	0.50000	.	3.0	1	1.00	1.0	.	.	.
71	272522	1.50000	4.00	5.0	1	2.00	2.0	4.0	4.00	7.0
72	283887	5.00000	6.50	7.0	2	3.00	3.0	4.0	5.00	8.0
73	283890	3.50000	5.00	6.0	2	3.00	3.0	4.0	5.00	7.0
74	4B108	5.00000	5.00	.	2	1.00	.	4.0	5.00	6.0
75	4B115	7.00000	7.00	8.0	5	5.00	5.0	.	.	.
76	4B146	7.00000	.	.	5	.	.	.	.	.
77	4B149	8.00000	6.00	9.0	5	5.00	5.0	4.0	6.50	6.5
78	4B151	4.00000	4.00	4.5	2	3.00	4.0	2.0	2.00	4.0
79	4B152	6.00000	3.00	3.0	3	3.00	4.0	3.0	4.00	4.5
80	4B175	5.50000	6.00	8.0	4	5.00	5.0	4.0	4.00	5.5
81	4B177	7.00000	6.00	6.5	3	5.00	5.0	4.0	4.00	5.0
82	4B180	7.00000	7.00	8.0	4	5.00	5.0	5.0	6.50	6.5
83	4B188	6.25000	6.25	8.5	3	4.00	5.0	.	.	.
84	4B190	2.50000	3.00	7.5	1	1.00	2.0	3.0	3.00	3.0
85	4B191	4.00000	4.00	6.0	2	2.00	2.0	3.5	3.50	4.5
86	4B193	5.00000	4.00	4.5	5	5.00	5.0	4.0	5.00	5.0
87	4B212	.	.	.	.	.	.	3.0	3.00	3.0
88	4B233	4.00000	4.50	.	2	3.00	.	4.0	4.00	5.0
89	4B235	8.00000	9.00	9.0	5	5.00	5.0	6.0	6.00	8.0
90	4B242	3.75000	4.00	4.0	2	2.00	2.5	4.0	4.00	6.0
91	4B243	4.00000	.	.	3	.	.	.	.	.
92	4B248	7.00000	7.00	7.5	5	5.00	5.0	.	.	.
93	4B250	8.00000	8.00	9.0	5	5.00	5.0	5.0	6.00	6.0
94	4B258	9.00000	.	.	5	5.00	5.0	7.0	7.50	8.0
95	4B277	5.00000	6.00	.	3	5.00	.	3.0	4.00	8.0
96	4B278	6.00000	7.00	.	5	5.00	.	4.0	5.00	6.5
97	4B285	.	.	.	.	.	.	3.0	3.00	4.0
98	4B287	1.00000	1.00	.	1	1.00	3.0	2.0	2.00	3.0
99	4B288	1.00000	1.00	5.0	1	1.00	1.0	.	.	.
100	4B290	1.00000	1.50	.	1	1.00	1.0	.	.	.
101	4B295	9.00000	6.00	9.0	5	5.00	5.0	5.0	6.00	9.0
102	4B297	5.50000	6.00	6.0	4	4.00	.	3.0	3.00	4.0
103	4B493	9.00000	9.00	9.0	5	5.00	5.0	5.0	5.00	6.0
104	4B495	6.00000	6.00	.	4	4.00	.	.	.	.
105	4B515	5.00000	4.50	4.0	3	3.00	3.0	3.0	3.00	5.0
106	4B517	8.00000	9.00	9.0	4	5.00	5.0	5.0	5.00	7.0
107	4B549	4.00000	4.00	4.0	3	5.00	5.0	.	.	.
108	4B559	6.00000	6.00	6.0	4	5.00	5.0	3.0	3.00	5.0
109	4B56	8.00000	8.50	.	4	5.00	.	.	.	.
110	4B561	6.00000	6.00	6.0	5	5.00	5.0	3.0	3.00	3.5

OBS	LINE	DS1	DS2	DS3	LT1	LT2	LT3	P1	P2	P3
111	4B566	7.00000	6.50000	7.00000	5.00000	4.00000	5.00000	3.0	3.00	5.5
112	4B573	1.00000	1.00000	2.00000	1.00000	1.00000	1.00000	2.0	2.00	3.5
113	4B71	4.00000	4.00000	4.00000	4.00000	4.00000	5.00000	4.0	4.50	6.5
114	4B74	2.50000	3.00000	3.00000	1.00000	1.00000	1.00000	4.0	4.50	.
115	4B771	7.50000	7.50000	.	5.00000	5.00000	.	.	.	.
116	4B772	6.50000	.	.	4.00000	.	.	3.0	4.50	6.5
117	4B773	7.00000	8.00000	.	5.00000	5.00000	.	.	.	.
118	4B775	7.00000	7.50000	.	5.00000	5.00000	.	.	.	.
119	4B776	7.50000	7.00000	9.00000	5.00000	5.00000	5.00000	3.0	3.00	.
120	4B81	6.00000	6.00000	7.00000	2.00000	2.00000	5.00000	4.0	4.00	6.0
121	4B85	6.00000	6.00000	9.00000	2.00000	2.00000	5.00000	4.0	4.00	6.0
122	4B96	2.00000	2.00000	.	1.00000	1.00000	1.00000	.	.	.
123	6B290	1.66667	1.66667	1.83333	1.00000	1.00000	1.33333	2.5	2.50	2.5
124	6B312	3.00000	.	.	2.00000	.	.	4.0	4.00	5.0
125	6B317	4.50000	8.50000	.	3.00000	2.00000	.	5.0	6.50	.
126	6B319	3.83333	5.16667	6.25000	2.00000	3.33333	4.00000	4.0	4.00	5.5
127	6B359	4.25000	4.25000	6.00000	1.50000	2.00000	3.00000	.	.	.
128	6B363	2.50000	.	.	3.00000	3.00000	.	4.0	4.25	5.0
129	6B364	6.00000	6.00000	.	3.00000	2.00000	.	4.0	4.00	.
130	6B366	3.00000	4.00000	.	3.00000	3.00000	.	.	.	.
131	6B368	4.00000	3.00000	5.00000	2.00000	2.00000	.	5.0	6.00	5.5
132	6B375	6.00000	4.00000	.	3.00000	5.00000	.	4.0	6.50	.
133	6B382	3.00000	3.50000	.	2.00000	3.00000	.	5.0	6.00	8.5
134	6B392	5.00000	.	.	3.00000	.	.	4.0	4.50	6.0
135	6B393	7.00000	7.00000	7.00000	4.00000	5.00000	5.00000	5.0	5.00	5.0
136	6B396	4.00000	6.00000	9.00000	2.00000	4.00000	5.00000	6.0	6.50	.
137	6B415	7.00000	6.00000	7.00000	4.00000	4.00000	.	4.0	4.00	6.0
138	6B436	4.00000	3.00000	3.00000	4.00000	3.00000	4.00000	3.0	2.50	2.5
139	6B440	5.00000	6.00000	7.50000	4.00000	4.00000	5.00000	.	.	.
140	6B461	1.00000	6.00000	7.50000	1.00000	3.00000	.	2.0	2.00	2.0
141	6B501	3.26667	3.26667	4.00000	1.33333	2.00000	3.50000	.	.	.
142	6B511	2.25000	4.00000	7.00000	1.00000	1.00000	4.00000	4.0	5.00	7.0
143	6B519	4.00000	4.00000	4.00000	2.00000	.	.	.	.	.
144	6B521	5.00000	4.50000	6.00000	3.00000	3.00000	4.00000	.	3.00	6.5
145	6B534	3.00000	4.00000	.	1.00000	1.00000	.	4.0	4.00	.
146	6B535	7.00000	.	6.50000	5.00000	.	5.00000	.	.	.
147	6B538	4.50000	4.00000	.	1.00000	3.00000	.	4.0	4.00	4.5
148	6B548	4.00000	4.00000	4.50000	3.00000	4.00000	5.00000	3.0	2.50	3.0
149	6B549	.	.	.	.	.	.	3.0	3.00	3.5
150	6B579	3.00000	3.00000	3.00000	2.00000	2.00000	3.00000	3.0	3.00	3.0
151	6B589	8.00000	7.50000	8.00000	5.00000	5.00000	5.00000	6.0	7.00	6.0
152	6B593	7.00000	.	.	5.00000	.	.	.	.	.
153	6B616	6.00000	6.00000	.	2.00000	2.00000	.	5.0	7.00	.
154	8B468	2.00000	2.50000	3.00000	2.00000	2.00000	2.00000	.	.	.
155	8B502	1.00000	1.00000	.	1.00000	1.00000	.	.	.	.
156	899	.	.	.	.	.	.	4.0	5.00	6.5

* Disease severity; ** LT= lesion type ; *** P: disease severity in stubble sown plots.

1,2,3 with DS, LT and P indicate rating number (12 days after inoculation and every 10 days thereafter.

Appendix 5.

Fries Medium # 66 in Dhingra and Sinclair (1985).

NH4 tartrate	5 g
NH4NO3	1 g
MgSO4.7H2O	0.5 g
KH2PO4	1.3 g
K2HPO4	2.6 g
Sucrose	30.0 g
Yeast extract	1.0 g
Trace element Stock solution	2.0 mL
Water	1 L

Stock solution: /L

LiCl	167 mg
CuCl2.H2O	107 mg
H2MoO4	34 mg
MnCl2.4H2O	72 mg
CoCl2.4H2O	80 mg

Reference:

Dhingra O.D. and Sinclair J.B. 1985. Basic Plant Pathology Methods. CRC Press, Boca Raton, Florida. pp.355.