Sources of and genetic action of resistance in barley to different virulence types of Pyrenophora teres, the causal organism of net blotch
by Michael Eidis Bjarko

A thesis submitted in partial fulfillment of the requirements for the degree of MASTER OF SCIENCE in Plant Pathology
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Abstract:
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Studies revealed that maximum sporulation of P. teres in culture could be obtained using V8 juice as a growth medium while incubating the cultures under an eight hour photoperiod of 17-18° C.

Twenty-six isolates of P. teres, fifteen from the Middle East and eleven from Montana, were separated into different virulence types based on the differential reactions of different barley lines. Seven distinct Middle East virulence types and five Montana virulence types could be distinguished.

Nine of these virulence types were used to screen 147 barley lines for resistance to P. teres. Nine lines were found to contain resistance to all nine virulence types. These lines were C.I. 1615, C.I. 4207, C.I. 5298, C.I. 5401, C.I. 5845, C.I. 7208, C.I. 9768, C.I. 13262 and Unitan. An additional twenty-two lines were found to contain resistance to eight of the nine virulence types of P. teres.

Crosses involving resistant and susceptible barley lines were made, and the resulting F2 populations were tested with different isolates of P. teres. It was found that at least five major genes exist in barley for resistance to P. teres. Four lines, C.I. 5791, C.I. 9819, Unitan and Steptoe each contain at least three genes for resistance.

Evidence for additive resistance to P. teres was found. Crosses between susceptible parents produced F2 seedlings which were more resistant than either parent to the isolate of P. teres used in the test.

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in

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Approved:

E. L. Sharp
Chairperson, Graduate Committee

E. L. Sharp
Head, Major Department

Henry L. Parsons
Graduate Dean

MONTANA STATE UNIVERSITY
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ABSTRACT

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INTRODUCTION

Net blotch of barley, caused by *Pyrenophora teres*, is a disease common on all commercial varieties. It is found wherever barley is grown, and is especially severe under cool, humid conditions.

Changes in cultural practices, such as the extensive use of irrigation, minimum tillage, continuous cropping and early sowing of barley, and restrictions on chemical treatment, especially the mercury seed treatments, have produced an environment conducive to the growth and spread of this organism. The sowing of susceptible barley varieties then provides a potential for severe disease epidemic.

Net blotch, along with barley scald (*Rhyncosporium secalis*), has produced detrimental effects on the yield and malting quality of barley grown on the Fairfield Bench in Northcentral Montana, where yield losses in 1973 were estimated to be as high as 50% in some fields. During the 1977-78 growing season in California, infection by *P. teres* was so severe that fields were burned rather than harvested.

This study was initiated in order to find and evaluate sources of resistance to *P. teres*. Also, the relationship of different isolates of *P. teres* to different sources of resistance, and the genetics of resistance to *P. teres* was studied.

LITERATURE REVIEW

Pyrenophora teres Drechs. is the causal organism of net blotch of barley. The imperfect stage of this fungus, *Helminthosporium teres* Sacc., syn. *Drechslera teres* Ito., is placed in the class Hyphomycetes, based on the fact that the conidia are not borne in any form of pycnidium or acervulus (Talbot, 1971). The pigmentation of the mucelium and conidia place it in the family Dematiaceae. The genus *Helminthosporium* is recognized chiefly by the presence of brown, septate conidiophores and large, brown, phragmoid conidia (Talbot, 1972). The binomial *Helminthosporium teres* was first applied by Saccardo to a fungus collected on leaves of barley in 1881 (Drechsler, 1923). The fungus was described as having 3-septate condiophores arising in a group of five from a green substratum and bearing at the tip a single conidium. The conidia were described as dark green structures, thick walled, 4-5 septate, ellipsoidal or subcylindrical and tapering perceptibly toward the rounded ends.

The original genus *Helminthosporium* was extremely heterogeneous. Because of this, groups of species have been segregated from it and placed into more discriminating genera. The net blotch organism was placed by Ito in the genus *Drechslera*, based primarily on the origin, development and morphology of the condiophores and conidia (Schoemaker, 1962; Talbot, 1973). *Drechslera* differs from *Helminthosporium* in that the conidia develop at the tips of successive lateral proliferations

of the conidiophores and are pseudopleurogenous. They are phragmoid, cylindrical and subhyaline to pale brown. Conidial germtubes are lateral, usually amphigenous, and may arise from intercalary as well as terminal cells (Luttrell, 1977; Talbot, 1973; Schoemaker, 1962).

The conidiophores of *Pyrenophora teres* occur singly or in groups of two to three. They are straight to flexous, often swollen at the base and are pale to mid-brown or olivaceous in color. They measure 120-200 x 7-11 µm. The conidia are straight, cylindrical, with rounded apical cells and constricted septa. The basal cell is inflated, producing a subglobose appearance. The conidia are 1-11 septate, 30-175 x 15-23 µm and subhyaline to green fuligenous in color. They are sessile and germinate from any or all cells (Drechsler, 1923; Luttrell, 1951; Ellis, 1971). Among the Pyrenophora species, the inflated basal cell and constricted septa are unique to *P. teres*.

The perfect, or sexual, stage of this organism produces asci and ascospores, placing it in the subdivision Ascomycotina. The asci are bitunicate, placing it in the class Loculoascomycetes and the presence of pseudoparaphyses places it in the order Pleosporales (Talbot, 1971). Drechsler (1923) was the first to describe the ascigerous stage of *Pyrenophora teres*. He placed it in the genus *Pyrenophora*, based on the presence of setae on the perithecial surface.

The asci of *P. teres* are club shaped or subcylindrical, rounded at the apex and with a short stalk at the base. They are bitunicate,

and 180-274 x 30-61 µm. The ascospores are light brown, 18-28 x 43-61 µm, ellipsoidal, with three transverse septa and one or two longitudinal septa in the median cells, but not in the terminal cells. The spores are much constricted at the septa, with germtubes forming from any or all cells (Drechsler, 1923; Smedegard-Petersen, 1972).

Pyrenophora teres also produces pycnidia on host tissue and in culture. They are globose to pear-shaped, 64-172 µm in diameter, with a thin fragile wall. They are yellow to brown in color, depending on age. The spores are hyaline, spherical or ellipsoidal, non-septate and 1.0-1.9 x 1.4-3.2 µm (Smedegard-Petersen, 1972). The function of these pycnidiospores is not known, although it is thought they may act as spermatia (McDonald, 1963; Smedegard-Petersen, 1972).

The heterothallic nature of *P. teres* has been shown (McDonald, 1963; Smedegard-Petersen, 1972). Monoconidial or monoascosporic isolates did not produce fertile perithecia when paired with other than their opposite mating types. Mature perithecia of *P. teres* were produced by growing mixed suspensions of two compatible single-spore isolates. A 1:1 ratio of the mating types was obtained when ascospore progeny from perithecia were backcrossed to parental isolates (McDonald, 1963).

P. teres produces three types of spores; ascospores, conidia and pycnidiospores. The ascospores and conidia will readily infect susceptible host tissue. Suspensions of pycnidiospores have never

produced symptoms (Smedegard-Petersen, 1972). All three spore types will germinate and grow on artificial media, although the growth of pycnidiospores is initially very slow. Once mycelium has been established, growth of cultures from pycnidiospores is normal. Suspensions of homogenized mycelium from cultures originating from any of the three spore types will readily infect barley leaf tissue. Conidiophores and conidia are produced from the symptomatic tissue.

The fungus overwinters as mycelium in infested plant debris and infected seed. It also overwinters as perithecia on infested straw.

Although seedling infection resulting from seed is known to occur, the extent to which this happens is unknown and appears to be affected very much by the environment. In Denmark, Smedegard-Petersen (1974) found 18.5% and 13.8% of the seedlings of the susceptible barley cultivars Wing and Lauda, respectively, showing symptoms due to infected seed. Piening (1968), in Canada, found only 0.5% to 1.5% of barley seedlings showing symptoms resulting from seed infection. Seedling infection from infected seed occurs at 10-15° C, but not above 20° C (Drechsler, 1923). Lesions may develop on the coleoptile as a result of infected seed, but more often it is the first, or plumular, leaf which is attacked. Kenneth (in Shipton, et al., 1973) has observed that infection of the hull is inconsequential. For seedling infection to occur the caryopsis itself must be infected.

Even with low percentages (0.5-1.5%) of the plants infected

via seed-borne inoculum, Piening (1968) observed that where leaves with primary infection were not removed, twice as many plants were infected twenty-eight days after emergence as on plots where infected leaves were removed as soon as they were noticed. It therefore appears that the chief danger of seed-borne inoculum is in providing a potential for disease spread throughout the growing season and the ultimate production of contaminated plant debris which could provide abundant inoculum the following year.

Infected straw is of major importance as a source of primary inoculum. Piening (1968) observed 42% of the plants infected with *P. teres* in a field where the previous year the straw and stubble were lightly disced, whereas only 8% of the plants were infected in a field where the straw and stubble were ploughed under during the previous year.

Early in the growing season, when environmental conditions are favorable, conidia are produced on infested plant debris. In addition, perithecia release asci and ascospores. The perithecia are also often densely covered with conidia which form on the tips of setae and conidiophores between the setae. The relative importance of the spore types as primary inoculum is unknown and appears to vary with geographic location and environment (Shipton, 1966; Smedegard-Petersen, 1972; Shipton, et al., 1973; Piening, 1968).

The conidia and ascospores will both germinate readily on host

tissue. The germtubes give rise to appressoria-like structures, and infection may occur after a moist period of as few as five hours, although 10-30 hours of moisture appears necessary for maximum infection (Shipton, et al., 1973). The penetration hyphae pass through the epidermal cells and enlarge slightly upon passing through the inner cell wall. Development of the hyphae is then intercellular, with cell death occurring in advance of the fungus. The respiration rate of susceptible barley leaves increases, significantly exceeding that of non-infected leaves after sixteen hours. The respiration rate continues to increase, reaching a maximum rate coinciding with the appearance of visible necrosis, after which the rate sharply decreases. Externally, symptoms may be evident within two days of infection (Shipton, et al., 1973; Smedegard-Petersen, 1976, 1977b).

Conidiophores arise directly from between epidermal cells or stomata and produce conidia on the surface of primary lesions. These conidia then cause secondary infection of the developing foliage. This process is repeated throughout the growing season, as long as conditions remain favorable. Complete necrosis of the leaves may occur by flowering time (Schaller, 1955; Shipton, et al., 1973).

Symptoms induced by *P. teres* occur on the blades and sheaths of the leaves and may extend to the flowers and grains. The initial lesions appear as minute spots or streaks which increase in size to form narrow, dark brown, longitudinal and transverse streaks,

producing a net-like pattern. This netting appearance is characteristic of *P. teres* and it is from this appearance that the disease gets its common name of net blotch (Drechsler, 1923; Dickson, 1956).

Isolates of *P. teres* have been collected which do not produce the characteristic netting symptoms on host tissue, but instead produce spot lesions very similar to those produced by *Pyrenophora japonicum*. These isolates were considered to be *P. teres* rather than *P. japonicum* because they could be crossed with isolates of *P. teres* that produce the netting symptoms (McDonald, 1967). Smedegard-Petersen (1971) suggested the designation *Pyrenophora teres* Drechs. forma *maculata* forma nova for the spot type of *P. teres*, and *Pyrenophora teres* Drechs. forma *teres* for the net type.

The symptoms induced by the spot form of *P. teres* also occur on the blades and sheaths of the leaves and consist of dark brown, elliptical or fusiform lesions measuring up to 3 x 6 mm in size and surrounded by a chlorotic zone of varying width (Smedegard-Petersen, 1971).

The spot form of *P. teres* has been found to be more prevalent in Denmark than the net form (Smedegard-Petersen, 1972). Tekauz and Buchannon (1977) have observed the spot form in the Winnipeg, Manitoba region and believe that its distribution indicates this biotype may have been introduced into the region on contaminated barley seed. In crosses between spot and net types of *P. teres*, it was found that

symptom expression is controlled by two independent allelic pairs of genes (Smedegard-Petersen, 1976).

Pyrenophora teres has been found to produce two toxins, both low molecular weight peptides (Smedegard-Petersen, 1976, 1977a). Except for the netting or spotting lesions, both toxins incite most of the symptoms incited by the pathogen itself, i.e.; chlorosis, necrosis and increased respiration. The two toxins do not seem to determine pathogenicity, however, but rather they contribute to the virulence of the organism (Smedegard-Petersen, 1976, 1977a, 1977b).

Under field conditions, the netted lesions of *P. teres* will coalesce with time, forming longitudinal stripes. These stripes can extend the length of the blade and into the leaf sheath. The stripes resemble those normally produced by *Pyrenophora graminea*, the causal organism of barley stripe disease. Often the symptoms of the two diseases are virtually indistinguishable. The morphology of both the sexual and asexual stages of these two species is also very similar, although there are some differences. The ascospores of *P. graminea* are larger than those of *P. teres*, and only four are produced in each ascus (Schoemaker, 1962). The conidia of *P. teres* are constricted at the septa and have an enlarged basal cell. Those of *P. graminea* do not have an enlarged basal cell and are not constricted at the septa. The conidia of *P. graminea* measure 31-111 x 11-24 µm and are 1-7 septate, and are smaller than those of *P. teres* (Drechsler, 1923;

Luttrell, 1951). *P. teres* also sporulates more readily in culture than *P. graminea*.

The variability in these morphological characteristics makes it difficult to distinguish between the two species using the morphological differences as a key (Smedegard-Petersen, 1976; Metz, 1978). The problem is further complicated by the fact that isolates of *P. teres* and *P. graminea* can be crossed in culture and perithecia containing viable ascospores can be obtained (Smedegard-Petersen, 1976). The most reliable method of distinguishing between the two species is in their mode of infection. *P. teres* will cause infection and symptom development when sprayed on the foliage of susceptible barley, while *P. graminea* cannot infect barley in this manner. *P. graminea* is a floral infecting pathogen and becomes systemic, while *P. teres* does not systemically infect barley.

Net blotch is found wherever barley is grown. It is of economic importance in North and South America, Europe, Australia, the Middle East and Africa (Shipton, et al., 1973). The effect on grain yield and quality has frequently been studied. Stakman (1922) estimated losses of 5% and 3% statewide for Iowa and South Dakota, respectively. Shipton (1966) observed a 17.4% decrease in yield in control plots as compared to plots protected with the fungicide Maneb. He also observed a significant reduction in malting quality. Piening and Kaufmann (1969) measured yield losses of 53.3% and a marked reduction in

kernel number per spike, indicating that the effect began in the early stages of plant growth. Caddel and Wilcoxson (1975) reported yield losses of 26% due to *P. teres*. Smedegard-Petersen (1974) reported yield losses of 9.0% and 11.1% in the barley cultivars Wing and Lauda, respectively, due mainly to reduction in grain size and weight.

Cultural changes have led to a recent increase in the prevalence of net blotch. Changes resulting in an increase in overwintering inoculum, such as minimum tillage and continuous cropping, and changes which have helped produce an environment more conducive to disease spread, i.e., irrigation, early sowing, and the planting of susceptible varieties have all helped to produce a situation which lends itself to potential disease epidemics (Smedegard-Petersen, 1971: Shipton, et al., 1973).

These cultural practices are more beneficial than harmful. Control of this disease must therefore utilize other methods. Foliar sprays are effective, but the cost is prohibitive. Seed treatment is helpful, but will not completely control the disease. The use of resistant varieties is the most efficient and desirable means of controlling net blotch. Resistant varieties, in addition to suffering less yield loss, also slow the spread of disease during the growing season and provide less infected plant debris, thus reducing the source of primary inoculum for the following year.

Resistant varieties apparently have no effect on spore germination,

the number of germtubes produced, the length and branching of germtubes or the number of penetrations (Keeling and Banttari, 1975). Inhibition of hyphal growth is pronounced after penetration and many infections do not progress beyond the penetrated cell. The organism therefore causes fewer and smaller lesions on resistant plants than on susceptible plants. Sporulation of the fungus on excised leaves of resistant plants was also found to be less than on leaves of susceptible plants (Keeling and Banttari, 1975). Tekauz and Buchannon (1977) found that the time required for sporulation of conidia on excised leaves varied from less than twenty-four hours with susceptible varieties to ninety-six hours for resistant varieties.

The first extensive screening of barley lines for sources of resistance was done by Schaller and Wiebe (1952). A total of 4,526 barley lines was tested to California isolates of *P. teres*. Twenty-five highly resistant lines were found, with the greatest percentage of these originating in Manchuria. Buchannon and McDonald (1965) tested 6,174 barley lines for resistance to net blotch. Of these they found forty lines, seventeen originating in Ethiopia, which were resistant in the seedling stage to isolates of *P. teres* from Canada, Mexico and the United States. Khan and Boyd (1969a) used seventeen Australian isolates to screen 142 barley lines which has previously been reported to be resistant to net blotch. Twelve highly resistant and twenty-two resistant lines, from both Manchuria and Ethiopia,

were found, with the Ethiopian lines generally exhibiting a higher degree of resistance than the Manchurian lines. Eighty barley lines, out of 2,608 lines tested, were found by Caddel and Wilcoxson (1975) to be resistant to Moroccan isolates of *P. teres*. Metcalfe, et al. (1978), evaluated 226 Ethiopian barley lines and found this region to be a good source of resistance to isolates of *P. teres* from Canada.

Isolates from various geographic areas differ significantly in pathogenicity. The physiologic specialization of *P. teres* became apparent with the screening of different barley lines. The lines found by Schaller and Wiebe (1952) to be resistant to California isolates of *P. teres* were susceptible to Canadian isolates (Buchannon and McDonald, 1965). Most barley lines resistant to *P. teres* elsewhere in the world proved to be susceptible or moderately susceptible to Moroccan isolates (Caddel and Wilcoxson, 1975). In general, the spot form of *P. teres* found in Canada is less virulent than the net types when lesion characteristics and extent of chlorosis are compared (Tekauz and Mills, 1974). This is not the case in Denmark, where the spot form is more prevalent than the net form (Smedegard-Petersen, 1971, 1976). Khan and Boyd (1969a) separated isolates of *P. teres* from Western Australia into three different virulence types using two differential varieties.

The first study to determine the genetics of resistance to *P. teres* was done by Schaller (1955). He found that Tifang contains a single

incompletely dominant gene for resistance effective against a California isolate. The symbol Pt was suggested for this gene. Mode and Schaller (1958) designated the resistance gene in Tifang Pt_1. They found C.I. 4797, C.I. 739 and C.I. 4929 contain a single gene for resistance, designated Pt_2, and C.I. 2750 and C.I. 4922 contain two genes for resistance, designated Pt_2 and Pt_3. It was found that Pt_1 and Pt_2 are closely linked, with a 2.57% recombination, Pt_3 is independent of Pt_1 and Pt_2. Khan and Boyd (1969c) found C.I. 2330, C.I. 4797 and Tifang to contain a single gene for resistance to a Western Australian isolate of *P. teres*. C.I. 9819 and C.I. 5791 contained two dominant genes for resistance to this isolate. All five varieties have a common gene for resistance to this isolate of *P. teres*.

Tifang and C.I. 4797 carry linked genes, Pt_1 and Pt_2, respectively, for resistance to a California isolate of *P. teres*. These two lines also carry an allelic gene for resistance to isolates of *P. teres* from Western Australia. Since neither Tifang nor C.I. 4797 carry the gene Pt_3, the common gene effective against the Australian isolate would need to be a fourth gene. This gene was designated Pt_a by Khan and Boyd (1969c).

Bockleman, et al. (1977), utilizing trisomic analysis, found Tifang to contain a single dominant gene for resistance effective against a Tunisian isolate of *P. teres*. The gene was located on chromosome 3. C.I. 7584 also contains a single dominant gene for

resistance, located on chromosome 2, effective against this isolate. C.I. 9819 contains two dominant genes, on chromosomes 3 and 5, effective against this isolate of *P. teres*.

Environment appears to affect the expression of resistance to *P. teres*. High inoculation and post-inoculation temperatures bring about a breakdown in resistance (Khan and Boyd, 1969; Tekauz and Buchannon, 1977). Light intensity, high pre-inoculation temperatures and spore concentration will also affect the expression of resistance in some varieties (Khan and Boyd, 1969b).

Consistency of expression of resistance is also a varietal characteristic and is dependent on the genetic background of the host. This is especially important when lines are crossed. The penetrance and expressivity of the resistance genes are highly dependent upon the genetic background of the parents used in the cross, even the background of a susceptible parent (Khan, 1969; Khan and Boyd, 1969b, 1969c). Khan (1969), using C.I. 5791 as a resistant parent and Atlas and Dampier as susceptible parents showed that F_1 host reaction and F_2 segregation patterns are considerably influenced by the choice of susceptible parent, with less resistance being expressed in the Atlas x C.I. 5791 cross than the Dampier x C.I. 5791 cross.

Chapter 1

THE EFFECT OF DIFFERENT GROWTH MEDIA ON THE SPORULATION OF *Pyrenophora teres*

For all inoculations done in this study, condial suspensions were used as inoculum. Since an adequate concentration of conidia in the suspension is needed to obtain a satisfactory infection, and hence good symptom development, it is important that the cultures of *P. teres* produce adequate amounts of conidia. In this experiment, different types of growth media were tested to determine which type would be most favorable for sporulation of *P. teres*.

Materials and Methods

One isolate of *P. teres*, Pt S (Sidney, Mt.), was used in the experiment. Eight different types of growth media were tested, V8 juice agar, Potato dextrose agar (Difco), Modified Eckert's Medium, Czapeck's agar (Difco), Barley leaf extract agar, Mycophil agar (BBL), Lima bean agar (Difco), and Rice cereal agar.[1]

Six replications of the experiment were carried out. For the initial transfer of *P. teres* to the different agar types, single spore transfers from leaf tissue were made. Transfers for subsequent replications were done as mass spore transfers using a dissecting needle, and

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[1]Recipes of these agar types are included in the appendix.

transfering spores to four spots on each petri plate. Transfers to fresh plates were made using spores only from plates of the same agar type. Spores were transferred to four plates of each agar type, three of these to be used for counting and the fourth as a source for the next transfer.

Spores were counted seven days after transferring. Counts were made by flooding the plates with distilled water, scraping the spores loose with a microscope slide and straining the suspension through three layers of cheesecloth. A total of 65 ml of distilled water was used per three plates. The spore concentration of the resulting suspension was then measured using a Howard Mold Counting Chamber (Hausser Scientific). Three counts were made, with the average of these used as the spore concentration for that agar type.

The cultures were grown in an incubation chamber with a 12 hour photoperiod and a temperature of 17-18° C.

Results and Discussion

Table 1 shows the spore concentrations for each agar type at each transfer and also the average number of spores per ml for the different agar types. An analysis of variance was performed on the data in this table, as shown below.

SOURCE	SS	DF	MS	F	$F_{.05}$
Total	244.293×10^8	47	----	----	----
Agar Type	132.745×10^8	7	18.964×10^8	6.8	2.25
Error	111.548×10^8	40	2.789×10^8	----	----

The results indicate that there is a significant difference in the sporulation of *P. teres* with respect to agar type.

To determine which averages were significantly different, a Duncan's Multiple Range test was utilized. The results of this test are indicated in Table 1.

Lima bean agar (LBA), V8 juice agar (V8), Mycophil agar (MyA) and Barley leaf extract agar (BLEA) all allowed abundant sporulation of *P. teres*. Potato dextrose agar (PDA), Modified Eckert's Medium (MEM) and Rice cereal agar (RCA) were less favorable for sporulation, while Czapeck's agar (CzA) was least suitable as a growth medium.

The results indicate that the type of growth medium used has a significant effect on the sporulation of *P. teres*. *P. teres* sporulates well enough on LBA, V8, BLEA and MyA that any of these should be adequate as a growth medium for this organism.

Table 1-1. Differential sporulation (spores/ml) of *P. teres* on eight types of growth media.

MEDIUM	1st transfer	2nd transfer	3rd transfer	4th transfer	5th transfer	6th transfer	average (spores/ml)
LBA[1]	59,081.9	49,928.4	21,828.6	52,207.7	62,388.1	49,819.9	49,200.8 A[2]
V8	66,100.9	47,649.1	33,285.6	48,951.5	54,161.5	42,330.6	48,746.5 A
MyA	57,634.7	32,923.8	28,461.6	34,117.7	78,510.6	10,492.2	40,356.8 A
BLEA	41,245.2	62,844.7	8,321.4	27,750.1	73,807.2	26,664.7	40,105.6 A
PDA	47,395.8	23,046.7	12,060.0	19,899.0	75,145.9	27,967.1	34,253.9 AB
MEM	20,875.9	8,321.4	7,839.0	25,579.3	6,982.7	22,069.8	15,278.0 BC
RCA	18,922.1	13,922.1	7,839.0	10,962.5	33,647.4	3,256.2	14,687.1 BC
CzA	3,002.9	1,085.4	1,688.4	1,447.2	1,085.4	361.8	1,445.2 C

1) LBA: Lima bean agar, V8: V8 juice agar, MyA: Mycophil agar, BLEA: Barley leaf extract agar, PDA: Potato dextrose agar, MEM: Modified Eckert's Medium, RCA: Rice cereal agar, CzA: Czapeck's agar

2) Averages followed by different letters differ significantly at P = .05 probability level.

Chapter 2

EFFECT OF PHOTOPERIOD AND TYPE OF GROWTH MEDIUM ON THE SPORULATION OF *P. teres*

In the previous experiment different types of growth media were tested for their effectiveness in inducing the sporulation of *P. teres*. Based on the amount of sporulation, four agar types were found to be particularly effective.

In this experiment, these four agar types, Mycophil agar (BBL), Lima bean agar (Difco), V8 juice agar and Barley leaf extract agar, were again used. These were tested over a range of photoperiods in an effort to determine an optimum photoperiod and agar type.

Materials and Methods

The same isolate used previously, Pt S (Sidney, MT), was used in this experiment. The four types of growth media were prepared following the procedures in Appendix A. Seven different photoperiods were used; no light, 8 hr., 12 hr., 16 hr., 20 hr., 22 hr., and 24 hr. of light. Temperature in the growth chamber was maintained at 17-18° C, with a photoperiod of 24 hr. of light. Cultures grown under shorter photoperiods were placed in cardboard boxes and wrapped in aluminum foil during the dark period. All four agar types were tested at each photoperiod. Spores were initially transferred from leaf tissue to plates of the four agar types. These cultures were then grown at the different photoperiods for seven days. The amount of sporulation was then

determined in the manner described previously. Four successive replications were run.

Results and Discussion

Table 2-1 (a-g) shows the sporulation of *P. teres* on the different agar types at each photoperiod. The values were used to determine the average sporulation at each photoperiod over all agar types (Table 2-2) and for each agar type over all photoperiods (Table 2-3). An analysis of variance test was then computed to determine which averages were significantly different (Table 2-4). Because of the extremely low sporulation of *P. teres* under conditions of no light, the date in Table 2-1 (a) were not included.

The results of the analysis of variance test indicate that the differences within replications, photoperiods, replications x photoperiod interaction and replication x agar type interaction were statistically significant, while the differences within agar types and agar type x photoperiod interaction were not statistically significant. These results indicate that the different agar types did not significantly affect the sporulation of *P. teres*, while the photoperiod under which the cultures of *P. teres* were grown did have a significant effect upon sporulation.

A Duncan's Multiple Range Test was utilized to determine which photoperiods differed significantly (Table 2-2). The results of this test show that the sporulation of *P. teres* under a photoperiod of eight hours

was significantly higher than the sporulation under any other photoperiod. The results also indicate that sporulation increases significantly as the photoperiod decreases. Because of the very low sporulation with no light, it is evident that some light is necessary. The results obtained in this test indicate that of the seven different photoperiods tested, an eight hour photoperiod is best for sporulation of *P. teres*. These results also indicate that it would be worthwhile to test the sporulation of *P. teres* grown under photoperiods shorter than eight hours.

There is a paucity of studies involving the effect of environment on sporulation of *P. teres*. Onesirosan and Banttari (1969) studied the effect of light and temperature on sporulation. Using photoperiods of no light, 12 hr. light and 24 hr. light, they found a 12 hr. photoperiod most conducive to sporulation. They also studied the effect of light quality, and found that ultraviolet light with wavelengths of 310-355 μm was necessary for conidiophore formation. Conidia production was most favored in the absence of light or in the absence of light of wavelengths 355-495 μm. It appears that stimulation of conidiophore production is critical, and once this is accomplished, production of conidia is not light dependent. One might be able to obtain maximum sporulation by growing the cultures in only ultraviolet light.

Although not significantly so, the sporulation of *P. teres* on mycophil agar was higher than on the other agar types. For this

reason the entire collection was transferred to plates of mycophil agar. This resulted in a differential range of reactions of the isolates to this agar type. It appeared that certain isolates grew and sporulated well on it while others sporulated very poorly. Since all isolates sporulated reasonably well on V8 juice agar and since the sporulation of *P. teres* on the two agars in this test was not significantly different, it was decided that the cultures would be maintained on V8 juice agar.

Using V8 juice agar as a growth medium, and using a photoperiod of 8 hr. produces highly sporulating cultures of *P. teres*. Adequately high concentrations of conidia are obtained to produce readable, differentiating symptoms on barley plants.

Table 2-1. Sporulation (spores/ml) of *P. teres* on different agar types at different photoperiods.

2-1(a); No light

Agar type[1]	1st transfer	2nd transfer	3rd transfer	4th transfer	average (spores/ml)
V8	0.0	108.5	108.5	0.0	54.3
LBA	0.0	470.3	0.0	108.5	144.7
MyA	0.0	253.3	470.3	242.4	241.5
BLEA	0.0	108.5	1,193.9	1,085.4	597.0

[1]V8 = V8 juice agar, LBA = Lima bean agar, MyA = Mycophil agar, BLEA = Barley leaf extract agar

2-1(b); 8 Hours of light

Agar type	1st transfer	2nd transfer	3rd transfer	4th transfer	average (spores/ml)
V8	361.8	62,591.4	45,104.4	79,133.6	46,792.8
LBA	5,427.0	55,234.8	58,129.2	79,234.2	49,506.3
MyA	2,062.3	99,615.8	78,402.1	70,792.2	62,718.1
BLEA	6,150.6	47,395.8	41,607.0	91,656.0	46,702.4

2-1(c); 12 Hours of light

Agar type	1st transfer	2nd transfer	3rd transfer	4th transfer	average (spores/ml)
V8	46,057.1	17,366.4	5,897.3	64,641.6	33,490.6
LBA	40,159.8	17,619.7	9,298.3	61,867.8	32,236.4
MyA	16,642.8	11,577.6	361.8	84,781.8	28,333.5
BLEA	68,870.7	8,936.5	723.6	42,330.6	30,215.4

2-1(d); 16 Hours of light

Agar type	1st transfer	2nd transfer	3rd transfer	4th transfer	average (spores/ml)
V8	34,732.8	5,427.0	14,363.7	36,059.4	22,645.7
LBA	39,327.7	10,492.2	7,344.5	34,853.4	23,054.5
MyA	19,537.2	13,133.3	3,509.5	86,349.6	30,632.4
BLEA	51,013.8	3,979.8	5,427.0	31,476.6	22,974.3

2-1(e); 20 Hours of light

Agar type	1st transfer	2nd transfer	3rd transfer	4th transfer	average (spores/ml)
V8	21,961.3	7,489.3	4,956.7	20,140.2	13,636.9
LBA	15,448.9	12,771.5	9,298.3	41,848.2	19,841.7
MyA	832.1	30,282.7	3,364.7	60,420.6	23,725.0
BLEA	54,378.5	3,364.7	1,700.5	37,386.0	24,207.4

2-1(f); 22 Hours of light

Agar type	1st transfer	2nd transfer	3rd transfer	4th transfer	average (spores/ml)
V8	25,217.5	20,069.8	20,622.6	11,577.6	19,371.9
LBA	14,725.3	6,620.9	10,854.0	10,612.8	10,703.3
MyA	2,170.8	12,301.2	20,875.9	32,320.8	16,917.2
BLEA	61,614.5	6,512.4	16,642.8	11,577.6	24,086.8

2-1(g); 24 Hours of light

Agar type	1st transfer	2nd transfer	3rd transfer	4th transfer	average (spores/ml)
V8	15,810.7	17,113.1	13,278.1	3,738.6	12,485.1
LBA	6,982.7	5,173.7	8,212.9	12,180.6	8,137.5
MyA	1,193.9	10,492.2	17,728.2	18,451.8	11,966.5
BLEA	5,318.5	12,301.2	4,088.3	13,024.8	8,683.2

Table 2.2 Average sporulation (spores/ml) of *P. teres* at each photoperiod.

Photo period	1st transfer	2nd transfer	3rd transfer	4th transfer	average	Duncan's Multiple Range Test P=.05*	P=.01**
8 hr	3,500.4	66,209.4	55,810.4	80,199.0	51,429.8	A	A
12 hr	42,932.6	13,875.1	4,070.3	63,405.5	31,070.9	B	B
16 hr	36,152.9	8,258.1	7,661.2	47,184.8	24,814.3	BC	BC
20 hr	23,155.2	13,477.1	4,830.1	39,948.8	20,352.8	CD	BCD
22 hr	25,932.0	11,376.1	17,248.8	16,522.2	17,769.8	CDE	CD
24 hr	7,326.5	11,270.1	10,826.0	11,849.0	10,318.1	E	D

Table 2-3. Average sporulation (spores/ml) of *P. teres* on each agar type.

Agar type	1st transfer	2nd transfer	3rd transfer	4th transfer	average	Duncan's Multiple Range Test P=.05*
MyA	7,073.2	29,567.1	20,717.0	58,852.8	29,052.5	A
LBA	20,345.2	17,985.5	17,189.5	40,099.5	23,904.9	A
V8	24,023.5	21,676.2	17,370.5	35,878.5	24,737.2	A
BLEA	41,224.4	13,748.4	11,698.2	37,908.6	26,144.9	A

*, ** Those values not followed by similar letters differ significantly at probability P=.05, or P=.01, respectively.

Table 4. Analysis of Variance Test.

	DF	SS	MS	F value
Reps (R)	3	1,000,120.0	333,374.0	27.135+
Photoperiod (P)	5	1,631,030.0	326,207.0	26.551+
R x P	15	2,029,070.0	135,271.0	11.010+
Agar (A)	3	36,718.4	12,239.5	0.996‡
R x A	9	627,475.0	69,719.5	5.675+
P x A	15	129,328.0	8,261.84	0.702‡
RPA (Error)	45	552,863.0	12,285.9	-- +
	95	6,006,610.0		

+ Significant at P=.01

‡ Non-significant at P=.05 or P=.01

Chapter 3

DETERMINATION OF DIFFERENT VIRULENCE TYPES OF *Pyrenophora teres*

In the spring of 1977, when testing of barley varieties for their reaction to net blotch was first initiated, seven different isolates of *Pyrenophora teres* were available. Four of these isolates were from Montana and three had been collected in the Middle East. By the summer of 1977, twenty-six isolates were available, fifteen originating in the Middle East and eleven from various parts of Montana.

It was possible to test a limited number of varieties to all twenty-six isolates, but when a screening test was set up to screen large numbers of barley lines to *P. teres*, limits on space and time made it necessary to use fewer isolates. To insure that the reduced number of isolates would be representative of the entire collection, the collection was separated into different virulence types.

Materials and Methods

The twenty-six isolates were separated into different virulence groups based on the differential reactions of fifteen barley varieties which had been tested to the isolates during the summer of 1977.

The varieties were grown in metal flats, 14"x10"x3", with twelve varieties planted per flat. Ten seeds of each variety were planted in six rows across the width of the flat, with two varieties per row. The plants were grown in a growth chamber with a 15°/24°C dark/light temperature, and a 12 hour photoperiod.

The isolates of *P. teres* were grown on V8-juice agar, in an incubation chamber with a temperature of 17-18°C and a 12 hour photoperiod. The isolates were subcultured utilizing the mass spore transfer method mentioned previously. Isolates to be used as inocula were transferred on the same day the barley to be inoculated was planted. The barley was then inoculated seven days later with the seven day old cultures. The inoculum consisted of a suspension of conidia and some mycelial fragments. As a surfactant, a solution of 1% Tween 20 at approximately 0.1 ml of surfactant to 100 ml of inoculum (0.1%) was used. The inoculum was applied using an atomizer driven by compressed air. Approximately 15-18 ml of inoculum were used per flat. A minimum of 10^4 spores/ml seemed necessary to obtain adequate infection. Spore concentrations of 20-30 x 10^3 spores/ml were not difficult to obtain. No standardization of the spore concentration was attempted. with concentrations approaching 80-90 x 10^3 spores/ml used in the inoculations.

Inoculated plants were incubated in a dew chamber at 100% relative humidity in darkness for 24 hours. The temperature of the water bath was 35°C and the wall temperature of 7°C. The resulting air temperature was 24-27°C.

Readings on plant infection were made seven days after inoculation. Reaction types were based on a scale of 0-4:

0: no observable infection

1: pin-point to very slightly elongated lesions (1-2 mm) with no observable chlorosis

2: slightly elongated lesions, with no netting symptoms, limited amounts of chlorosis

3: elongated lesions forming netting symptoms, easily observable chlorosis, no necrosis

4: well developed, netted lesions, with extensive chlorosis and necrosis of leaf tissue

The isolates were then grouped, based on the similarities and differences of the reactions of the barley varieties and C.I. numbers. The isolates and their origin are listed in Table 3-1. The barley varieties utilized as differentials are listed in Table 3-2.

Results and Discussion

The Middle Eastern isolates were separated from the Montana isolates, since the two groups as a whole produced different reactions on several varieties. For example, Shabet, Firlbecks III, Betzes, and Ingrid were susceptible to all Montana isolates, yet contained resistance to several Middle Eastern isolates. C.I. 7584, C.I. 9776 and Steptoe were resistant to all Montana isolates, yet were susceptible to some Middle Eastern isolates. Because of the nondiscriminatory reactions of these varieties and lines to the Montana isolates, they were of little use as differentials. Hence, it was necessary to utilize other varieties

in order to determine virulence types of the Montana isolates.

Table 3-3 shows the Middle Eastern isolates grouped into their respective virulence types, based on the reactions of fourteen barley lines. Virulence type A contains five isolates, the other types contain from one to three isolates. The reactions of those varieties and lines which are most useful in separating the isolates into the different virulence types are circled. For example, C.I. 9776 is susceptible to type B, while Shabet, Betzes and Ingrid are all resistant. Type D is the only virulence type to which Mona-Arivat is resistant. Both C.I. 7584 and Tifang are susceptible to type E, while type F is the only virulence type to which C.I. 7584, C.I. 9776 and Steptoe are all susceptible and both Shabet and Ingrid are resistant. Arimont is resistant only to virulence type G.

Table 3-4 shows the virulence types of the Montana isolates of *P. teres*, based on the reactions of eight barley varieties and lines. Here again, those lines and varieties most useful in separating the different virulence types are circled. Tifang is susceptible only to type C. C.I. 9819 exhibits type 4 symptoms only to those isolates in type D. Both Lud and Piroline are intermediate in reaction to type E, while Lud is resistant to intermediate in reaction to type B and Piroline is susceptible.

Using differential varieties a dichotomous key was set up with which it was possible to distinguish the virulence types of the Middle