Biology of pyrenophora spot blotch on barley (Hordeum vulgare L.) incited by Pyrenophora teres Drechs. F. Sp. Maculata Smedeg. and genetics of resistance
by Chandra Bahadur Karki

A thesis submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Plant Pathology
Montana State University

Abstract:
Pyrenophora spot blotch (PSB) caused by Pyrenophora teres Drechs. f. sp. macu/ata Smedeg. is a newly reported and an important disease on barley (Hordeum vu/gare L.) in Montana and other parts of the world. Research was initiated to gain a better understanding of the disease, pathogen and host parasite interaction. Tests conducted to determine the pathogenic variation of 14 PSB isolates from Montana, Morocco, Tunisia, and Turkey using 20 barley cuItivars under growth chamber conditions showed smaller lesions and higher amount of chlorosis and necrosis with the Montana isolates than with isolates from North Africa and Turkey. Cultivars Unitan, Cl 9214, Cl 5401, Cl 9440 and Cl 9776 were resistant and Dekap, KIages, Nupana and Cl 13727 were susceptible to all isolates. Cultivars Cl 7584, Cl 9819, Cl 9825 and Tifang showed differential reactions between the PSB isolates from Montana and other countries. Isolates Turk 74-Pt6, Tun 79-30, Mts Plent82 and Mts Eddy 82-5 were the most virulent among 15 isolates. A study on conidium and conidio-phore characteristics of PSB isolates revealed that isolates from Morocco and Tunisia produced significantly longer conidiophores while Montana isolates produced a higher number of geniculations. The basal conidium cell was rounded in Montana isolates and conical or snake-headed in the isolates from North Africa. A comparison of two isolates from Montana and Morocco showed no differential effects on yield components and spike length averaged over four barley cultivars. Infected cuItivar Dekap showed reductions as high as 75% in kernel number and 16% in kernel weight. Symptoms varied between cultivars screened in the growth chamber and in the field. Cultivars Tifang, Minn 21, Himalaya, Oderbrucker, Cl 1615, Cl 5401, Cl 6475, Cl 9214, Cl, 9440 and Cl 9776 were resistant in both conditions. Cultivars showing good sources of resistance were studied in the F1 F2 and BC1 generations to determine gene action and gene number conditioning resistance to a PSB isolate. Crosses involving resistant and susceptible barley cultivars showed often a monogenic or digenic recessive resistance in the growth chamber and often a monogenic or digenic dominant resistance in the field. Among the various crosses evidence was also found for multigenic resistance, maternal inheritance, transgressive segregation, and no allelism.

BIOLOGY OF PYRENOPHORA SPOT BLOTCH ON BARLEY (*HORDEUM VULGARE* L.) INCITED BY *PYRENOPHORA TERES* DRECHS. F. SP. *MACULATA* SMEDEG. AND GENETICS OF RESISTANCE

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APPROVAL

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Chandra Bahadur Karki

This thesis has been read by each member of the thesis committee and has been found to be satisfactory regarding content, English usage, format, citation, bibliographic style, and consistency, and is ready for submission to the College of Graduate Studies.

5-31-85
Date

E. L. Sharp
Chairperson, Graduate Committee

Approved for the Major Department

5-31-85
Date

E. L. Sharp
Head, Major Department

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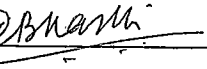
Signature 

Date June 4, 1985

In dedication to:

My wife, son, daughters,
father, and mother.

VITA

Chandra Bahadur Karki, son of Rudra Bahadur and Jagat Kumari Karki, was born on September 5, 1944 in Kothe Gaon, Sindhupalchok District, Nepal. He grew up and received preliminary education in Kothe area where his father farms and ranches.

Chandra graduated from Shanti Vidya Griha High School in Kathmandu, Nepal in 1965. He obtained a scholarship under the USAID-Participant Program to pursue a B.S. degree in Agriculture at University of Udaipur, Udaipur, Rajasthan, India, where he graduated with honors in 1970. He returned to Nepal and was employed as an Assistant Plant Pathologist in the Division of Plant Pathology, Department of Agriculture, Nepal.

In 1975, he went to India to pursue a M.S. degree in Mycology and Plant Pathology at Indian Agricultural Research Institute, New Delhi, and graduated in 1977. While stationed in Nepal, Chandra travelled to England, India, Italy, Mexico, the Netherlands, and the U.S.A. where he participated in short training courses, international meetings or symposia. He came to Montana State University in 1981 as a doctoral candidate in Plant Pathology and anticipates completion of a Ph.D. degree in June 1985.

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ABSTRACT

Pyrenophora spot blotch (PSB) caused by *Pyrenophora teres* Drechs. f. sp. *maculata* Smedeg. is a newly reported and an important disease on barley (*Hordeum vulgare* L.) in Montana and other parts of the world. Research was initiated to gain a better understanding of the disease, pathogen and host parasite interaction. Tests conducted to determine the pathogenic variation of 14 PSB isolates from Montana, Morocco, Tunisia, and Turkey using 20 barley cultivars under growth chamber conditions showed smaller lesions and higher amount of chlorosis and necrosis with the Montana isolates than with isolates from North Africa and Turkey. Cultivars Unitan, CI 9214, CI 5401, CI 9440 and CI 9776 were resistant and Dekap, Klages, Nupana and CI 13727 were susceptible to all isolates. Cultivars CI 7584, CI 9819, CI 9825 and Tifang showed differential reactions between the PSB isolates from Montana and other countries. Isolates Turk 74-Pt6, Tun 79-30, Mts Plent82 and Mts Eddy 82-5 were the most virulent among 15 isolates. A study on conidium and conidiophore characteristics of PSB isolates revealed that isolates from Morocco and Tunisia produced significantly longer conidiophores while Montana isolates produced a higher number of geniculations. The basal conidium cell was rounded in Montana isolates and conical or snake-headed in the isolates from North Africa. A comparison of two isolates from Montana and Morocco showed no differential effects on yield components and spike length averaged over four barley cultivars. Infected cultivar Dekap showed reductions as high as 75% in kernel number and 16% in kernel weight. Symptoms varied between cultivars screened in the growth chamber and in the field. Cultivars Tifang, Minn 21, Himalaya, Oderbrucker, CI 1615, CI 5401, CI 6475, CI 9214, CI 9440 and CI 9776 were resistant in both conditions. Cultivars showing good sources of resistance were studied in the F_1, F_2 and BC_1 generations to determine gene action and gene number conditioning resistance to a PSB isolate. Crosses involving resistant and susceptible barley cultivars showed often a monogenic or digenic recessive resistance in the growth chamber and often a monogenic or digenic dominant resistance in the field. Among the various crosses evidence was also found for multigenic resistance, maternal inheritance, transgressive segregation, and no allelism.

INTRODUCTION

Barley (*Hordeum vulgare* L.) is an important cereal crop and ranks fourth in area among world crops harvested. It is used mainly for animal feed and malt in the brewing industry. In many developing countries, barley is also used for food in different forms such as gruel, soup, bread, roasted grain and flour. It is grown mainly in the temperate regions of the world, requires a moderate climate, and grows well in areas where the ripening season is cool and long. Barley is subject to various fungal, bacterial, viral and noninfectious diseases. Among the many destructive diseases of barley, three *Helminthosporium* disease complexes known for a considerable time are spot blotch caused by *Cochliobolus sativus* (Ito and Kurib.) Drechs. ex Dast., teleomorph of *Bipolaris sorokiniana* (Sacc.) Shoem. (Syn. *Helminthosporium sativum* Pam., King, and Bakke); barley stripe caused by *Pyrenophora graminea* Ito and Kurib., telemorph of *Drechslera graminea* (Rab.) Shoem. (Syn. *Helminthosporium gramineum* Rab.); and net blotch caused by *Pyrenophora teres* Drechs., teleomorph of *Drechslera teres* (Sacc.) Shoem. (Syn. *Helminthosporium teres* Sacc.). Recently, however, one more *Helminthosporium* disease causing spot symptoms, similar to those caused by *C. sativus,* and morphologically similar to *P. teres,* has been occasionally reported in epidemic proportions from diverse areas of the world.

Although the first spot symptoms of the disease were observed by McDonald (1967) in Canada, Smedegard-Petersen (1971) was the first to demonstrate that the spot producing pathogen differed from the net blotch pathogen only in symptom production on barley and designated then by two common names: net-blotch and net-spot blotch. Consequently he described two new forms, *Pyrenophora teres* Drechs f. sp. *teres* Smedeg. for the pathogen

producing net blotch and *Pyrenophora teres* Drechs. f. sp. *maculata* Smedeg. for the pathogen producing net-spot.

In 1981, severe spot symptoms were observed on many barley cultivars at Fairfield, Montana. The spot symptoms were indistinguishable from spot blotch but conidia of the pathogen were similar to those of *P. teres.* Almost all cultures of such spot samples collected from Fairfield, induced spot symptoms on barley seedlings in the growth chamber. Although an accurate determination of losses at Fairfield was not made, it was estimated that the loss of barley grain yield exceeded 5%. A loss of 12-17% in barley grain yield was reported from Canada (Tekauz, 1978), as much as 27% in Australia (Khan and Tekauz, 1982) and up to 11% in Denmark (Smedegard-Petersen, 1976) due to net blotch and the new disease.

The fungus isolated from the spot samples from the field never induced "net" symptoms either in the seedling or adult plant, when tested under growth chamber and field conditions. The common name "net-spot blotch" designated for the new disease by Smedegard-Petersen (1971) may be somewhat misleading to both growers and research scientists. Therefore, a new common name "Pyrenophora spot blotch" for the spot disease caused by *Pyrenophora teres* Drechs. f. sp. *maculata* Smedeg. is proposed and the new common name Pyrenophora spot blotch (PSB) is used throughout this dissertation.

Since PSB is a new disease on barley in Montana, there is a paucity of information pertaining to the morphology of the pathogen, distribution of the disease, pathogenic variation of different isolates, effects on yield components, sources of resistance and inheritance of resistance in barley cultivars. Therefore, the following objectives were established:

1. to determine distribution, origin and pathogenic variation of the different isolates of *Pyrenophora teres* f. sp. *maculata;*
2. to compare the morphological characters of the different isolates;
3. to assess the effects of PSB on yield components of barley;

4. to identify sources of resistance to PSB isolates both in field and growth chamber conditions; and
5. to determine the number of genes and their action involved in conditioning resistance in barley.

REVIEW OF LITERATURE

Origin and Distribution

Pyrenophora spot blotch (PSB) caused by *Pyrenophora teres* f. sp. *maculata* (*P. teres* f. sp. *maculata*) has been reported from many barley growing areas of the world. Working with isolates of *Pyrenophora teres* f. sp. *teres* (*P. teres* f. sp. *teres*), causal organism of net blotch from North America, Mexico, Israel and Holland, McDonald (1967) first found two isolates, one from Canada and one from Israel, that induced spot symptoms similar to spot symptoms caused by *Pyrenophora japonica* Ito & Kurib. (Ito and Kuribayashi, 1931). He considered those spot causing isolates to be mutant strains of *P. teres* f. sp. *teres.* Later, he confirmed that spot symptoms were a result of mutation at one or two loci of the pathogen. Parmeter et al. (1963) reported that heteroplasmosis, heterokaryosis, parasexuality and mutation may all cause variation in plant pathogenic fungi but believed that spontaneous mutation was the most common phenomenon of variation. Smedegard-Petersen (1976) indicated that inter- or intra-specific crosses are common and could be major sources of variation in *Pyrenophora* spp. However, PSB in Canada and Australia was believed to have originated from infected seed imported from the Scandinavian countries (Khan and Tekauz, 1982; Takauz and Buchanon, 1977).

Pyrenophora spot blotch has been reported in Australia (Khan and Tekauz, 1982); Canada (Tekauz and Mills, 1974); Denmark (Smedegard-Petersen, 1971); Finland (Makela, 1972), Germany and Jordan (Mathre, 1982); Norway (Hansen and Magnus, 1969), Morocco; Tunisia; Turkey; and the U.S.A. (Bockelman et al., 1983). It was more common than net blotch in Denmark, Finland and Norway (Hansen & Magnus, 1969; Makela, 1972; Smedegard-Petersen, 1976). In Canada, the frequency of PSB ranged from 3-4% of

the number of fields surveyed (Tekauz, 1978). In the USA, the disease has been reported only from Montana (Bockelman et al., 1983). Since PSB symptoms are difficult to distinguish from spot blotch and net blotch in the field, its presence in other states cannot be excluded. In 1981, PSB was severe on all barley cultivars at Fairfield, Montana (Bockelman et al., 1983). Since the first report of the disease in Canada (McDonald, 1967) the disease has appeared in many countries.

Disease Symptoms

The symptoms produced by *P. teres* f. sp. *maculata* on barley leaves are spots of various shapes and sizes in contrast to typical dark brown longitudinal and transverse net-like necrotic streaks caused by the net blotch pathogen. Smedegard-Petersen (1971) observed distinct dark brown lesions measuring 3-6 mm in length and surrounded by a chlorotic zone of varying width on the leaf blade. According to him, the chlorosis becomes extended and the leaves eventually become necrotic from the tip to the base of the leaf blade. He emphasized that extensive chlorosis and necrosis are much more important symptoms than necrotic lesions in causing yield reduction in barley. The amount of chlorosis and necrosis reportedly varies with isolates of the pathogen and with the barley cultivars (Smedegard-Petersen, 1971). Hansen and Magnus (1969) pointed out that the spot symptoms caused by the PSB pathogen often resembled the spot blotch caused by *C. sativus* and it was necessary to examine the conidial morphology of both pathogens to determine the true causal agent. Makela (1972) observed two types of dark brown spots on barley in Finland: big spots measuring 0.5-19 X 0.5-3.0 mm and small spots measuring 0.5-3.0 X 0.5-2.00 mm. Elliptical, fusiform or irregularly shaped necrotic lesions were the common symptoms observed with this pathogen in Canada (Tekauz and Mills, 1974). No mention has been made about the infection in other parts of the plant with this pathogen. Smedegard-Petersen

(1976) has described four different kinds of symptoms commonly observed on the leaf blade of barley:

1. dark brown necrotic lesions,
2. chlorosis,
3. water soaking of affected tissues, and
4. general necrosis and blighting.

Disease Rating

Investigators have used different rating scales to denote the incidence and the severity of PSB. Smedegard-Petersen (1971) used five classes to record PSB after modifying the scale devised earlier by Buchanon and McDonald (1965). His rating scale was based on lesion type and amount of chlorosis and is described as follows:

0 = no lesions or very few lesions not exceeding 1 mm in size, no chlorosis;

1 = well defined dark brown lesions, 1-3 mm in size, no chlorosis;

2 = well defined dark brown lesions of varying size, slight necrosis;

3 = dark brown lesions of varying size surrounded by chlorotic tissue; and

4 = necrosis of varying size, large areas of leaves chlorotic or dead.

Tekauz and Buchanon (1977) recorded PSB reactions in the seedling stage using a 0-16 rating scale, in which 0 denoted no observable symptoms and 16 denoted large areas of host tissue affected. They considered relative size of the necrotic and chlorotic portion of the lesions. They also measured the disease reactions based on sporulation on excised diseased leaves after 24-96 hours of incubation.

Pathogen and Systematics

Pyrenophora teres is the teleomorph of *Drechslera teres* (Syn. *Helminthosporium teres*). (Drechsler, 1923; Ellis, 1971; Luttrell, 1977; Shoemaker, 1959). The generic name, "*Helminthosporium*," was said to have evolved from an old genus "*Helmisporium*," which was later changed to *Helminthosporium* by changing the spelling (Shoemaker, 1959). Drechsler (1923) studied and described almost all graminocolous species of *Helminthosporium* and noted clear distinctions of taxonomic separation. Nishikado (1928) as cited by Shoemaker (1959) proposed two subgenera, *Cylindro-Helminthosporium*, characterized by having cylindric conidia that germinated from any cell and *Eu-Helminthosporium* characterized by having fusoid conidia that germinated from an end cell. Ito (1930) proposed a generic name, *Drechslera*, for the graminicolous species with cylindric conidia and Shoemaker (1959) gave a generic name, *Bipolaris*, to the species with fusoid conidia. Luttrell (1963) further differentiated *Drechslera, Bipolaris* and *Helminthosporium* based on conidia formation and pointed out that *Helminthosporium* developed conidia apically and laterally while *Drechslera* and *Bipolaris* developed conidia only at the conidiophore apex. Recently, Alcorn (1983) provided a detailed differentiating descriptions of *Drechslera, Bipolaris* and *Exserohilum* on the basis of septum ontogeny, conidial germination and hilum morphology.

The teleomorph and the anamorph of the pathogen causing net blotch on barley have been studied by various researchers (Drechsler, 1923; Ellis, 1971; Luttrell, 1973, 1977; Shoemaker, 1959, 1962; Smedegard-Petersen, 1971, 1972). The binomial, *Pyrenophora teres,* the causal organism of net-blotch was further divided into sub-species, *Pyrenophora teres* f. sp. *teres* and *P. teres* f. sp. *maculata* for the teleomorph and consequently, *Drechslera teres* was divided into *Drechslera teres* f. sp. *teres* and *D. teres* f. sp. *maculata* for the anamorph based on symptom production on barley (Smedegard-Petersen, 1971).

The genus, *Pyrenophora,* is an Ascomycete, based on ascospore production; Subclass, Loculoascomycetidae because of bitunicate asci; order, Pleosporales, because of presence of pseudoparaphyses; and Family, Pleosporaceae, based on production of dictyosporous ascospores having an anamorph in *Drechslera* (Alexopoulos and Mims, 1979; Luttrell, 1973). The genus, *Drechslera,* is a Deuteromycete, based on unknown sexual reproduction; Form-order, Moniliales, based on absence of fruiting bodies; and Form-family, Dematiaceae, on the basis of pigmented hyphae, conidiophores or conidia (Alexopoulos and Mims, 1979).

Smedegard-Petersen (1972) first reported the teleomorph of *P. teres* f. sp. *maculata* in Denmark. Pseudothecia of the pathogen are dark brown, globose or sclerotia like bodies with rigid setae and measure about 300-700 × 200-400 μ. Asci are bitunicate, hyaline and club shaped. They measure 172-260 × 31-41 μ. Ascopores are yellowish brown, ellipsoidal and rounded at the ends. They are dictyosporous with three transverse septa and 1 or 2 longitudinal septa. They measure 48-61 × 18-25 μ and germinate from any or all cells. Conidiophores of the pathogen arise singly or in small groups of 2-3 from the necrotic leaf tissues. They are light brown, straight or flexuous and 2-8 septate with swollen basal cells. They measure 68-291 × 8-10 μ (Smedegard-Petersen, 1971). Conidia are subhyaline, cylindrical, phragmoid, 2-6 septate and measure 62-138 × 13-18 μ. They are sessile with rounded apical cells and germinate from any or all cells. Catenulation of conidia with short chains of 2 or 3 in culture and on excised leaf tissues has been reported. Smedegard-Petersen (1972) also reported the pycnidial stage of the fungus on host tissue and in culture in Denmark. Pycnidia are globose to pear-shaped, osteolate, yellow to brown, and measure 64-176 μ in diameter. Pycnidiospores are hyaline, spherical or ellipsoidal, nonseptate and measure 1.4-3.1 × 1.0-1.9 μ.

Culture Media and Culture of the Fungus

All three spore types, conidia, ascospores and pycnidiospores have been reported to germinate and grow on artificial media (Smedegard-Petersen, 1972). There has been little work on selective growth media for culturing *P. teres* f. sp. *maculata*. Smedegard-Petersen (1971) used potato glucose agar, $V\text{-}_8$ juice agar and lima bean agar to grow the pathogen in culture. The $V\text{-}_8$ juice agar has been utilized extensively to grow this pathogen and other *Pyrenophora* species (Bockelman et al., 1983; Khan and Tekauz, 1982; Platt et al., 1977; Tekauz and Mills, 1974). Kafi and Tarr (1966) used three natural and three synthetic media to study the effects on conidial characteristics of five species of *Helminthosporium* and found that the growth, sporulation and morphology, color, and dimensions (especially length) of conidia were significantly affected by culture medium, glucose concentration and carbon source. Harding (1975) also noted effects of pH and sucrose concentration on conidium size and septation in four *Bipolaris* species. Sporulation of *H. teres,* the pathogen of net blotch, was reported abundant at 21°C, moderate at 15°C and less or absent in other temperature regimes (Onisirosan and Banttari, 1969). It was also found that ultra-violet radiation in the region between 310-355 nm is required for conidiophore formation as well as a dark period for conidium formation.

Cultural Variation, Cross Fertility and Heterothallism

Variation in morphological characters in culture media, such as color; formation and structure of aerial mycelia, sporulation, etc., have been observed by many investigators (Drechsler, 1923; McDonald, 1967; Shoemaker, 1962; Smedegard-Petersen, 1971). McDonald (1967) found that the original cultural phenotypes of most isolates of *P. teres* f. sp. *teres* could be retained through many generations of monoconidial subculture but the

phenotypes tended to vary in appearance after repeated mycelial transfers. Smedegard-Petersen (1971) reported that many isolates of PSB tended to lose the ability to form mycelial tufts and conidia in culture after two or three mycelial transfers. Ability to form reproductive structures in culture was found to be controlled by one gene and morphological variation by more than one gene (McDonald, 1967).

Smedegard-Petersen (1977) observed fertile progenies resulting from crosses between *P. teres* f. sp. *maculata* and *P. teres* f. sp. *teres* and between *P. teres* f. sp. *maculata* and *P. graminea* and suggested that the capacity of *P. teres* to produce spot and net lesions and of *P. graminea* to produce stripe lesions on barley was controlled by three gene pairs, Ss to produce spot lesions; Nn to produce net lesions; and Gg to produce stripe lesions. He also indicated that the loci for Ss and Nn segregated independently while those for Ss and Gg were closely linked.

McDonald (1963) first detected heterothallism in *P. teres* f. sp. *teres.* Fertile pseudothecia developed when different mating types were grown together in plates. Smedegard-Petersen (1978) also observed heterothallism in PSB isolates and indicated that the mating type in *P. teres* f. sp. *maculata* is controlled by a single gene pair and additional steps in the reproductive mechanisms to produce pseudothecia and then ascospores are controlled by yet other gene pairs.

Toxin Theory

Smedegard-Petersen (1977) isolated and purified two toxins from culture filtrate of different *P. teres* f. sp. *teres* and *P. teres* f. sp. *maculata* isolates. He designated A and B toxins and demonstrated that each could produce disease symptoms on barley without the presence of the pathogens. Toxin A was more effective than toxin B in producing symptoms. Recently, Bach et al. (1979) characterized three toxins in both PSB and net blotch

isolates and determined their chemical and structural properties. There was a high relationship between the host range of the toxins and those of the pathogen and between the virulence of isolates and concentration of toxins. However, toxins never produced the characteristics necrotic lesions similar to those produced by the pathogen (Smedegard-Petersen, 1976). Therefore, he suggested that toxins do not determine pathogenecity but contribute to the virulence of individual isolates.

Pathogenicity and Virulence

The pathogenic nature of PSB isolates was first confirmed by Smedegard-Petersen (1971). He observed no difference in morphological characters of the pathogens causing PSB and net blotch, but found two distinct symptom types on barley in Denmark. He tested 12 barley cultivars, resistant to North American net blotch isolates (Buchanon and McDonald, 1965; Schaller and Wiebe, 1952) and observed differences in reactions of those cultivars with net blotch and PSB isolates in Denmark. Subsequently, the pathogenic nature of PSB was reported from Australia, Canada, Finland, Morocco, Tunisia, Turkey and the USA (Bockelman et al., 1983; Khan and Tekauz, 1982; Makela, 1972; Tekauz and Mills, 1974). Disease reactions between barley cultivars to the PSB isolates were quite uniform and the range of reactions was narrower than that caused by net blotch isolates (Smedegard-Petersen, 1971; Tekauz and Buchanon, 1977). Cultivars with totally resistant and totally susceptible reactions were not observed with PSB isolates and generally, the PSB pathogen was considered less pathogenic than the net blotch pathogen based on lesion characteristics and extent of chlorosis and necrosis. Tekauz and Buchanon (1977) noted that the average symptom ratings of some CI lines were lower than those of other commercial cultivars and the incidence of the disease was higher in two rowed than in six rowed barleys. On the other hand Makela (1972) found net blotch higher in six rowed and PSB higher in two rowed barley. Barley cultivars, CI 5791 and CI 7584, were resistant to

net blotch isolates but intermediate or susceptible to PSB isolates (Khan and Tekauz, 1982). Differences in disease reactions were also observed among isolates of PSB from different countries. Khan and Tekauz (1982) noted that the disease reactions with Australian isolates were different from those from Canada on some barley cultivars. Bockelman et al. (1983) observed that isolates from Morocco, Tunisia and Turkey generally induced bigger lesions and different reactions than those produced by an isolate from Montana. Differences in pathogenicity and disease reactions on barley cultivars have also been reported with net blotch isolates originating from different countries (Buchanon and McDonald, 1965). Bjarko (1979) studied 26 net blotch isolates from Montana and the Middle East countries and separated seven virulence groups among the Middle Eastern isolates and five virulence groups among Montana isolates. Recently, Khan (1982) studied and identified changes in pathogenicity of some net blotch isolates with time.

Little research has been done on the pathogenicity of *P. teres* f. sp. *maculata* on various cereals and grasses. However, Smedegard-Petersen (1976) reported that rye, and to a lesser extent wheat, showed disease symptoms with PSB isolates but he did not observe characteristic lesions as in barley. Earlier, Makela (1972) observed infection on winter wheat, winter rye, spring wheat and to a lesser degree on oats with PSB isolates in Finland. Host specialization mechanisms are known to occur in isolates of *P. teres* f. sp. *teres* since isolates virulent on barley grass (*Hordeum leporinum*) did not attack barley and vice versa (Khan, 1973).

Virulence of the pathogen has been found to depend on inoculum type (conidia, ascospores, pycnidiospores or mycelia), culture age, concentration of inoculum and other environmental factors (Shipton et al., 1973). Smedegard-Petersen (1976) reported that conidia, ascospores and mycelia of *P. teres* f. sp. *maculata* could infect barley and produce disease symptoms. He observed no difference in symptoms and severity of the disease whether the inoculum was conidia or mycelia. However, McDonald (1967) reported less

virulence in net blotch isolates which produced fewer conidia and found fleck type symptoms when aconidial inoculum was used. Pathogenicity and apathogenicity of *P. teres* f. sp. *maculata* was controlled by a single factor pair (Smedegard-Petersen, 1976). Khan and Boyd (1969b) obtained susceptible type reactions on some barley lines when 15-25 day old inoculum of the net blotch pathogen was used and pin-point lesions on the same cultivar when 10 day old cultures were used as inoculum. However, Singh (1963) observed greatest infectivity with 10 day old inoculum of the same pathogen. Information on inoculum concentration required for infection by *P. teres* f. sp. *maculata* is limited. However, a spore concentration of 10^3-10^4 conidia/ml has been found satisfactory to differentiate resistance and susceptibility of barley cultivars to net blotch and PSB isolates (Bockelman et al., 1983; Khan and Boyd, 1969b; Tekauz and Mills, 1974).

Disease Cycle and Epidemiology

Studies on the disease cycle of PSB are lacking. As the disease is very similar to net blotch, some stages of the disease cycle and the environmental requirements of net blotch are reviewed. Conidia and ascospores germinate and produce germ tubes and appressoria on the host surface. When there is a moist period of at least five hours, conidia and ascospores infect the host epidermis directly after forming infection pegs and vesicles form within 24 hours after inoculation (Keon and Hargreaves, 1983). Infection hyphae are initiated within the epidermal cells following the development of the secondary infection vesicles and subsequent hypal growth develops intercellularly in the mesophyll tissue. Host mesophyll cells and internal organelles show various degrees of disruption two days after infection and a portion of the entire leaf becomes necrotic or chlorotic. Conidiophores and conidia develop from the necrotic lesions and serve as secondary inoculum of the disease.

Although seedling infection from infected seed is known to occur (Piening, 1968; Smedegard-Petersen, 1979), the extent of such infection is variable and appears to be

affected by environmental factors (Shipton et al., 1973). The infection of barley seedlings from seed borne inoculum of the net blotch pathogen occurs at 10-15°C but not at 20°C. Infested straw and stubble are considered major sources of primary infection as compared to infected seeds (Evans, 1969; Piening, 1968; Smedegard-Petersen, 1972). Dispersal distance of conidia and ascospores of *P. teres* f. sp. *teres* is largely unknown, however, Piening (1968) reported the spores can be dispersed up to seven meters from the source. Environmental requirements for infection and disease development are variable. In general, the disease needs cool and damp weather and requires 15-25°C for infection and 25-29°C for colonization. Khan and Boyd (1969b) observed a breakdown of resistance at 36°C and increased lesion number with increased light intensity. Age of the host, pathogen and nutritional status of the host have also been reported to affect the host-parasite interaction (Shipton et al., 1973).

Control Measures

Exchange of seeds between regions or countries, minimum tillage and other changes in cultivation practices have led to an increased incidence and severity of net blotch and PSB. There is no report of any control measure applied for PSB. However, various measures such as cultural, chemical, and varietal resistance used to control net blotch disease (Shipton et al., 1973) might be applied to PSB.

Sources and Inheritance of Resistance

Although the use of some fungicides as seed treatment or foliar sprays might be effective to control net blotch and PSB, the high cost of fungicide application and chemical hazard have limited their use. Control by resistant cultivars is desirable because it is the cheapest, the easiest and the most reliable. Although an extensive screening of barley cultivars against the net blotch pathogen has been done by several researchers (Bjarko,

1979; Buchanon and McDonald, 1965; Caddel and Wilcoxson, 1975; Khan, 1971; Schaller and Weibe, 1952), the report on sources of resistance in barley to PSB are limited. However, Smedegard-Petersen (1971) found four barley cultivars, CI 4795, CI 4941, CI 6094 and CI 9820, which had some resistance to PSB in Denmark. Although complete resistance was not found to the spot form of *P. teres,* Tekauz and Buchanon (1977) observed that cultivars CI 9214, CI 5401 and CI 9440 showed some resistance in Canada. Later, Khan and Tekauz (1982) found CI 9214 and CI 16225 more resistant than 11 other cultivars they tested with Canadian and Australian isolates. Barley cultivars, CI 9776, CI 9819 and Unitan were resistant to a Montana PSB isolate but intermediate or susceptible to the isolates from North Africa and Turkey (Bockelman et al., 1983).

Several people have worked on inheritance of resistance to net blotch. The first study to determine the genetics of resistance to *P. teres* f. sp. *teres* was made by Schaller (1955) who found that Tifang contained one incompletely dominant gene, designated as "Pt" for resistance to a net blotch isolate from California. Subsequently, several researchers have reported inheritance of resistance to net blotch and found different gene action and gene numbers with several barley cultivars (Bjarko, 1979; Bockelman et al., 1977; Khan and Boyd, 1969a; Mode and Schaller, 1958). However, there is no published information on inheritance of resistance to PSB in barley.

MATERIALS AND METHODS

Isolate Origin and Designation

Many leaf spot samples of barley were collected from Montana, Morocco, Nepal and Syria and pathogenicity tests were conducted to identify and study the pathogen causing the spot symptoms. Confirmed pathogens of Pyrenophora spot blotch (PSB) from different spot samples were designated by names based on State or Country of origin, place and year of collection and referred to as "isolates" of the pathogen (Table 1). New names also were designated for the isolates of *P. teres* f. sp. *teres* and *P. teres* f. sp. *maculata* previously described (Bockelman et al., 1983).

Table 1. Isolates of *Pyrenophora teres* f. sp. *maculata* from Different Countries.

Isolate Designation	State or Country of Origin	Place of Collection	Year of Collection
Mts FF81E$_1$ *	Montana	Fairfield	1981
Mts FF82-12	Montana	Fairfield	1982
Mts Sun82-2	Montana	Sun River	1982
Mts Conrd82	Montana	Conrad	1982
Mts Lewis82-7	Montana	Lewistown	1982
Mts Eddy82-5	Montana	Eddy's Corner	1982
Mts Plent82	Montana	Plentywood	1982
Mts Lav82	Montana	Lavina	1982
Mts Fromb82	Montana	Fromberg	1982
Mor 82-1	Morocco	Rabat	1982
Mor 82-2†	Morocco	Rabat	1982
Mor ELS77-5†*	Morocco	—	1977
Mor 79-27*†	Morocco	Merchouch	1979
Tun 75*	Tunisia	Tunis	1975
Tun 79-30*	Tunisia	Tunis	1979
Tun 79-32*†	Tunisia	Fahs	1979
Tun 79-38*	Tunisia	—	1979
Turk 74-Pt6*	Turkey	—	1974
Mtn 77-51*n	Montana	Sidney	1977

* = Isolates previously described; † = Isolates not used in pathogenic variation study; n = Net blotch isolate.

Pathogenic Variation

Isolate Selection and Maintenance

Based on pathogenicity tests, nine new PSB isolates, one from Morocco and eight from Montana, were chosen for the study. Additionally, one isolate each from Montana and Turkey, three from Tunisia and one net blotch isolate from Montana, which were previously described in Table 1, were included. Leaf tissue containing Pyrenophora spot blotch symptoms was maintained at about 5°C.

Pathogen Isolation

Leaf tissue containing PSB symptoms was cut into 10-15 mm long pieces and surface sterilized in 1% sodium hypochlorite solution for 1-2 minutes. Three to four leaf pieces of each sample were then transferred into Petri plates containing 2% water agar and incubated at 21°C with alternating 8 hours light (fluorescent) and 16 hours dark conditions for a total of 24-72 hours. Conidia emerging from the leaf tissue were singly transferred onto the center of Petri plates containing V-$_8$-agar (V-$_8$ juice—170 ml; Bacto agar—20 g; calcium carbonate—3 g; and distilled water—830 ml). The V-$_8$ agar (VA) plates, each containing a single conidium, were incubated for 15-20 days under the same conditions as mentioned above. Monoconidial cultures, up to two months old, maintained on VA plates were used for further subculturing. Subculturing was done by transferring masses of mycelia containing conidiophores and conidia onto several spots of fresh VA plates. The subculturing was done only once from the original monoconidial culture plates, since further subculturing resulted in reduced conidial production. The subcultured plates were incubated for 10-11 days. Isolates from Morocco, Tunisia, and Turkey did not produce sufficient conidia even after the first subculturing. Therefore, 17-18 day old monoconidial cultures of these isolates were used for inoculum.

Inoculum Preparation

Inoculum was prepared by adding 50 ml of double distilled water to each culture plate and scraping mycelia, conidiophores, and conidia with a microscopic glass slide. The conidial suspension, including mycelial fragments, was stirred with a magnetic stirrer for 4-5 minutes to help dislodge and separate conidia from conidiophores and mycelia. The conidial suspension was then strained through a gauze filter and about 0.1 ml of Tween 20 (polyoxyethylene sorbitan monolaurate) was added per 100 ml of the inoculum suspension as a spreading agent. The conidial concentration was standardized at 3.2×10^4 conidia/ml using a mold counting chamber (Hausser Scientific).

Cultivar Selection and Planting

Twenty barley cultivars (Table 2) were chosen based on preliminary testing with PSB isolates (C. B. Karki, unpublished data) and earlier reports with PSB and net blotch isolates (Bockelman et al., 1983; Bjarko, 1979). As a planting medium, fresh Bozeman clay loam soil was mixed with sand at a ratio of four parts clay loam soil to one part sand. About 10-12 seeds each of 10 cultivars were planted in a plastic flat (18 cm × 18 cm × 5 cm) and replicated four times. Since space in the dew chamber and the growth chamber was limited, studies were performed in six batches using 10 cultivars and five isolates in each batch. Pre and post inoculation incubations were in a growth chamber maintained at 21°C with 12 hours light (3.9×10^4 erg/cm^2-sec) and 13°C (dark) using a completely randomized design.

Inoculation

Four flats with nine to 10 day old barley seedlings were placed on a turntable and inoculated by spraying 60 ml of the inoculum suspension using a DeVilbis atomizer driven by compressed air. The atomizer was hand-held at 30-40 cm from the plants and at an angle of 30-45°. Inoculated plants were immediately transferred to a dark dew chamber

Table 2. List of Barley Cultivars or Lines used in Determining Pathogenic Variation in Isolates of *Pyrenophora teres* f. sp. *maculata*.

Cultivar	CI Number	Row Type
Dekap	3351	2
Klages	15478	2
Nupana	16559	2
Steptoe	15229	6
Clark	15857	2
Tifang	4407-1	6
Galt	11770	6
Unitan	10421	6
Arimont	15509	6
	5401	6
	5791	2
	5845	6
	7584	6
	9214	6
	9440	6
	9699	6
	9776	6
	9819	2
	9825	2
	13727	2

maintained at 21°C±2°C and incubated for 24 hours before returning to the growth chamber.

Disease Rating Scale

Disease reactions were recorded for each plant 10 days after inoculation using the following rating scale:

1 — Minute pin-point or fleck type lesions, without any visible chlorosis and necrosis on the leaf blade.

2 — Minute pin-point or small (1-2 mm long) necrotic lesions, with slight chlorosis on the leaf blade and less than 20% leaf tip necrosis.

3 — Small to large (1-2 mm or larger) necrotic lesions with slight chlorosis on the leaf blade and 20-30% leaf tip necrosis.

4 — Small to large necrotic lesions with slight to moderate chlorosis on the leaf blade and about 31-40% leaf tip necrosis.

5 — Small to large necrotic lesions with slight to moderate chlorosis on the leaf blade and about 41-50% leaf tip necrosis.

6 — Coalescing lesions with moderate chlorosis on the leaf blade and about 51-60% leaf necrosis.

7 — Coalescing lesions with moderate to severe chlorosis and about 61-70% leaf necrosis.

8 — Coalescing lesions with severe chlorosis and about 71-80% of leaf necrosis.

9 — Coalescing lesions with more necrosis than chlorosis and about 81-90% of leaf necrosis.

10 — Coalescing lesions, more necrosis than chlorosis and less than 10% green area visible on the leaf blade.

Statistical Analysis

Means of the disease ratings of each cultivar were calculated by averaging the ratings of all plants infected. Cluster analysis of mean disease ratings of the four replications was performed using BMDP software packages (Engelman and Hartigan, 1981). Also, an analysis of variance was performed on mean disease ratings (Little and Hills, 1978).

Conidium and Conidiophore Characteristics

Barley leaf specimens infected with three *P. teres* f. sp. *maculata* isolates, Mts FF81E_1, Mor 82-1, and Tun 79-38 and one *P. teres* f. sp. *teres* isolate, Mtn 77-51, were allowed to sporulate on excised leaf sections in Petri plates containing water agar. Monoconidial cultures of each isolate were produced in VA as described earlier. A mass of conidiophores and conidia of each isolate, from excised leaf sections after 72 hours and from monoconidial

culture after 10 days, was separately placed in a drop of distilled water on a glass slide. Four slides were prepared representing samples from different areas of the culture plate for each isolate. Measurements were taken on 30 conidia and conidiophores produced on excised leaf tissue and on 100 conidia and conidiophores produced in V_8 agar culture for length, width, and number of septa of conidia and conidiophores; shape of the basal conidium cell, and the number of geniculations (number of bends) in the conidiophores.

Means and standard error of the means for length and width of conidia and conidiophores were calculated. The range of measurements of each character was noted. Modal classes were noted for characters such as shape of the basal conidium cell, the number of conidiophore geniculations, and the number of conidia and conidiophore septa.

Effects on Yield Components and Spike Length

Based on seedling reactions, four barley cultivars, Dekap (susceptible), Galt (intermediate), Unitan and CI 9776 (resistant) were chosen for greenhouse study along with two isolates, Mts FF81E_1 (Montana) and Mor 82-1 (Morocco). Fifteen seeds of each cultivar were planted in 20 cm diameter plastic pots, containing sterilized Bozeman sandy loam soil. Each treatment combination was replicated three times. An additional three pots were planted for each cultivar as controls. All 36 pots were maintained on greenhouse benches at 24°C and 16°C for day and night, respectively, in a completely randomized design. Supplemental light was provided for 15 hours a day by fluorescent tubes fixed about one meter above the benches. After germination, the pots were thinned to five plants and a mixed NPK (10-10-10) liquid fertilizer was provided in the water every 20-25 days.

Inoculum of the monoconidial cultures of Mts FF81E_1 and Mor 82-1 was prepared as described earlier. The first inoculation was performed at the tillering stage (growth stages (GS) 25-26; Zadoks et al., 1974). The inoculation was done as described earlier using an inoculum suspension of 20 ml/pot. The same quantity of double distilled water was

sprayed on the control pots. The inoculated plants were incubated in a dew chamber maintained at 20°C for 24 hours before returning them to the greenhouse benches. A second inoculation was done at the boot stage (GS 45-46). Inoculum preparation and inoculation were done as before but the inoculum quantity was increased to 30 ml/pot.

The severity of the disease was rated at GS 82-85, when the flag leaves were still green. The disease ratings were based mainly on percent of necrotic lesions, chlorosis and necrosis on 10 flag leaves of each replication. Ten spikes were harvested from plants previously rated for the disease. The length of spikes, the number of kernels/spike, and 1000-kernel weight were determined from the 10 spike samples. Total seed bearing spikes (except for Dekap which contained both seed bearing and nonseed bearing spikes) in each pot were recorded. Average disease severity, yield components, and spike length of the above samples were used for analysis. An analysis of variance was performed for disease ratings, yield components and spike length (Little and Hills, 1978). Correlation was made between disease severity and yield components and between disease severity and spike length (Snedecor and Cochran, 1980). Percent change in yield components and spike length of barley cultivars was calculated by comparison with control pots.

Sources of Resistance

One hundred ninety-five commercial cultivars, CI and PI lines were obtained from different sources, including about 100 lines which originated from Nepal. They were screened under growth chamber and some were screened under field condition.

Growth Chamber Studies

Ten to twelve seeds of each cultivar and line were planted in unreplicated plastic flats. One isolate, Mts FF81E_1, from Montana was used for inoculation. Inoculum preparation,

inoculation and disease rating have been described in the previous section. A minimum of two but usually 10 seedlings were evaluated and modal PSB ratings were recorded.

Field Studies

About 20-25 seeds of the most promising cultivars based on growth chamber results were planted in 3 m rows in the field. Inoculum was a mixture of four monoconidial isolates from Fairfield, Montana. Inoculum preparation was done by blending both culture colony and VA in a Waring blender. Eight culture plates used per liter of distilled water, were blended for 2-3 minutes and sieved through a gauze filter. The spore concentration was 5.0×10^4 conidia/ml.

Prior to inoculation, the field was irrigated and inoculation was performed at the stem elongation stage (GS 30-35), in the evening, at a rate of 1 liter/2 sq. m area with a Solo Mist Sprayer (Solokleinmotoren, GMBH, W. Germany). Plants were then covered with plastic tarps immediately after inoculation for 12-13 hours to create a humid environment. A second inoculation was made one week later using the same mixtures of isolates and similar inoculation methods. Disease ratings were made at GS 75-80 using a 1-10 scale. Disease ratings were based mainly on percentage of chlorosis and necrosis on the second leaf below the flag leaf. Ten leaves were rated on ten plants and the modal classes were recorded.

Inheritance of Resistance

The best sources of resistance identified in the field tests were utilized in crosses between (a) susceptible and resistant, (b) resistant and resistant, and (c) susceptible and susceptible cultivars. Reciprocal crosses were made in some cases. Dekap and Klages were used extensively as susceptible parents in the study and all crosses were made at the Horticultural Farm, Bozeman during the summer of 1982. Ten to 20 F_1 seeds of each cross were planted at Mesa, Arizona during the winter of 1982-83. F_1 plants of many crosses

were backcrossed to Dekap *msg* 7cg or Klages *msg* 25dz in Arizona and F_2 seeds were harvested from five F_1 plants.

Parents, F_1, F_2 and BC_1 (F_1 backcrossed to female parent) were tested together in the growth chamber and only F_2 generation was tested in the field. Seeds from all parents used in the test were from five bagged spikes from the row in which crossings were made. However, bulk harvested and threshed seeds were used in the case of Dekap, Unitan and CI 9214, when the bagged seeds were depleted.

Growth Chamber Studies

About eight to ten seeds of each parent, one to six seeds of each F_1, eight to 14 seeds of each BC_1 and 80-90 seeds of each F_2 were planted in plastic flats. Three flats were used for each cross. The environment in the growth chamber was as described earlier. Due to space limitations only parents and progenies of six crosses were tested at one time. One monoconidial isolate, Mts FF81E_1, was used throughout the experiment. Inoculum preparation, inoculation and disease ratings were done as described earlier. About 50 ml of inoculum was used for 3 flats and inoculum concentration was standardized at 3.0×10^4 conidia/ml.

Field Studies

In the summer of 1983, 200 F_2 seeds of each cross were space-planted in 4 rows each 6 m long. A mixture of 4 isolates from Fairfield, Montana were used for the tests. Inoculation was first performed at GS 30-35 and a second inoculation followed one week after the first inoculation using similar inoculum preparation and inoculation procedures described in the previous section on field studies. The disease ratings of the F_2 plants were made at GS 75-80 using the 1-10 disease rating scale. In the summer of 1984, the parents, and F_2 seeds of 20 crosses were space planted and inoculated once with isolate Mts FF81E_1.

Statistical Analysis

Genetic analysis was made using the disease reactions of the parents, F_1, F_2, and BC_1. In most crosses tested in the growth chamber, disease reactions in F_2 and BC_1 were grouped into two classes, resistant (disease ratings, 1-5) and susceptible (disease ratings, 6-10). However, due to some cases of variable disease reactions of susceptible parents and F_1 the disease ratings 1-4 or 1-3 were sometimes considered as a resistant class and the remainder (disease ratings, 5-10 or 4-10) were considered as a susceptible class. Additionally when the disease ratings of F_1 plants were intermediate between the two parents, disease reactions of F_2 and BC_1 populations were classified into three groups, resistant (disease ratings, 1-3), intermediate (disease ratings, 4-6) and susceptible (disease ratings, 7-10) (see Tables 22-24, Appendix, for detail). For each cross of the resistant X susceptible category, a degree of dominance (level of dominance) was calculated using the following formula: Degree of dominance = $\frac{F_1 - MP}{P_1 \text{ or } P_2 - MP}$, where F_1 = disease reactions in F_1, MP = mid-parent disease reactions, P_1 or P_2 = disease reactions in P_1 (Susceptible) or P_2 (Resistant) (Halleur and Miranda, 1981; Falconer, 1981). Any degree of dominance value of 0.3 or less was considered no dominance and disease reactions of progenies were classified into three classes. If the value was 0.4 or more, disease reactions of the progenies were classified into two classes. Gene action and the number of genes involved in each cross were estimated by a hypothesized ratio and chi square probability values.

Disease severity ratings in the field were generally higher than in the growth chamber and thus susceptible and resistant classes were adjusted accordingly. Except in crosses involving Unitan and Oderbrucker, the disease ratings 1-3 were considered a resistant class, 4-6 as an intermediate, and 7-10 as susceptible. In crosses involving Unitan, disease ratings 1-7 were considered a resistant class and in a cross with Oderbrucker only 1-3 disease ratings were considered as a resistant class because of environmental variation. In many

cases, the resistant and intermediate classes were grouped into one resistant class (see Table 25, Appendix, for detail) and the genetic analyses were performed accordingly.

RESULTS

Pathogenic Variation

Pathogenicity tests of isolates obtained from barley exhibiting leaf spot symptoms collected from different areas of Montana and other countries showed that not all spot symptoms were caused by *P. teres* f. sp. *maculata*. Of many samples from Montana, only eight were confirmed to be caused by this pathogen. Most of the spot samples from Nepal were found to be caused by *Cochliobolus sativus*, while those from Syria were caused by either *C. sativus* or *P. teres* f. sp. *teres*. Two of five samples from Morocco were confirmed as *P. teres* f. sp. *maculata*.

Although small water soaked areas were observed on the leaf blade 24 hours after inoculation, visible necrotic lesions appeared three to four days later. The lesions appeared initially as grayish or brownish, small, circular or elliptical areas of less than 1 mm in diameter. Later, the lesions enlarged to elliptical, fusiform or circular areas with a size of 1-6 mm in length and 1-3 mm in width or 1-2 mm in diameter depending on the isolate and the cultivar. In general, the lesions induced by PSB isolates from Montana were small grayish or brownish and circular and varied in size from pinpoint to 1-2 mm in diameter. In some cases, the lesions forming on some cultivars were dark brown and fusiform in shape and were 2-3 mm X 1-2 mm in size. The lesions produced by the isolates from Morocco, Tunisia, and Turkey were dark brown, elliptical or fusiform and generally larger than those produced by Montana isolates. These lesions were in the size range of 2-8 mm X 1-4 mm.

In most cases, the lesions were surrounded by chlorosis four to five days after inoculation with the extent of chlorosis depending on the cultivar and isolate. Isolates from Montana had a tendency to produce more chlorosis than the isolates from North Africa

and Turkey, and some cultivars produced more chlorosis than others. Seven to eight days after inoculation the chlorotic areas extended and necrosis developed in some cultivars. In most cases, the necrotic areas developed at the tip of the leaf blade and extended down to the leaf sheath. The isolates from Montana produced the largest lesions on CI 5791, Clark, Dekap, and Klages. Occasionally, dark brownish discoloration was observed on the leaf sheaths and basal culms of the seedlings of some cultivars.

Table 3 shows the mean disease ratings 10 days after inoculation of 20 barley cultivars inoculated with 14 isolates of *P. teres* f. sp. *maculata* and one isolate of *P. teres* f. sp. *teres.* Entry CI 9214 showed the lowest disease rating to all isolates. Other cultivars showing low disease ratings were Unitan, CI 5401, CI 9440, and CI 9776. However, the disease ratings on Unitan were much more uniform than on the other four cultivars. Cultivars CI 9825, Tifang, CI 7584 and CI 9819 showed low disease ratings to all Montana isolates and high disease ratings to isolates of North Africa and Turkey. Nupana, Klages, CI 13727 and Dekap showed high disease ratings to all isolates. Disease ratings on Clark, CI 9699, Arimont, and CI 5845 varied with the different isolates, and Galt, Steptoe and CI 5791 showed intermediate or high disease ratings with most of the isolates. Cultivar Tifang showed a high disease rating to Mt n77-5l and CI 5845 showed a lower disease rating to the same isolate.

An analysis of variance was used to determine differences and possible interactions among the various cultivars and isolates. Table 4 shows highly significant differences among the main effects and interaction effects. The higher mean square for the main effects indicates that the differences among cultivars and isolates were much larger than the interaction effects. Nupana and Klages showed significantly higher disease ratings than the other cultivars (Table 5). Although the mean disease rating on CI 9214 was the lowest, the rating did not differ with that of Unitan at the 5% level of significance using least significant difference.

Table 3. Mean Disease Ratings[1] of Pyrenophora Spot Blotch caused by *Pyrenophora teres* f. sp. *maculata* and Net Blotch caused by *Pyrenophora teres* f. sp. *teres* from Montana, Morocco, Tunisia and Turkey on Barley Cultivars or Lines (*Hordeum vulgare*).

	Isolates														
Cultivar	Mts FF $81E_1$	Mts FF 82-12	Mts Sun 82-2	Mts Conrd 82	Mts Lewis 82-7	Mts Eddy 82-5	Mts Plent 82	Mts Lav 82	Mts Fromb 82	Mor 82-1	Turk 74-Pt6	Tun 75	Tun 79-30	Tun 79-38	Mtn[2] 77-51
Nupana	8.6	9.4	7.0	9.3	9.8	7.8	8.7	7.7	8.3	5.3	8.5	5.6	9.0	7.0	8.7
Klages	9.1	9.9	7.9	9.8	9.3	9.6	9.1	8.2	9.0	4.5	7.5	4.5	6.4	5.9	10.0
CI 13727	8.6	7.2	7.1	8.9	8.2	9.3	9.6	9.0	8.9	5.4	6.3	5.4	7.6	4.1	10.0
Dekap	8.5	7.4	6.5	7.9	8.9	8.6	8.7	8.9	6.5	5.5	6.5	7.1	6.2	5.7	9.8
Clark	7.2	8.5	6.0	9.0	7.9	9.0	9.1	6.4	8.1	4.0	6.7	5.0	5.5	6.7	9.8
CI 9699	5.2	8.1	6.6	7.2	7.4	8.5	9.4	7.7	6.2	3.3	7.4	8.5	6.5	5.9	6.1
Arimont	6.4	6.3	2.6	7.5	6.5	8.9	7.8	7.8	6.3	3.4	7.7	3.6	6.7	5.8	9.0
CI 5845	4.3	6.4	6.5	7.3	6.9	8.8	9.4	5.9	6.6	3.8	6.4	7.1	6.3	5.5	2.8
Galt	3.6	5.6	2.9	5.5	4.4	5.1	6.6	5.8	5.6	5.1	5.4	4.3	6.1	5.3	6.3
Steptoe	2.7	3.9	2.0	4.1	3.3	4.8	4.0	7.3	3.2	3.2	5.6	3.3	5.3	3.4	1.5
CI 5791	4.6	4.3	4.0	3.9	4.3	5.6	5.3	3.0	3.4	2.6	2.7	3.5	2.7	2.9	1.5
CI 9819	2.1	2.6	1.3	2.1	2.1	1.7	1.8	2.2	1.3	5.6	7.9	5.0	7.3	7.1	1.2
CI 7584	1.2	2.2	1.3	1.9	1.8	1.7	1.8	2.5	1.4	4.4	8.1	4.4	6.9	6.3	1.1
Tifang	1.9	1.9	1.0	2.1	1.5	1.8	2.2	2.0	1.3	3.7	6.2	4.1	4.6	5.4	7.1
CI 9825	1.1	1.7	1.6	1.9	2.2	2.1	2.5	2.1	1.8	3.6	4.4	6.8	2.4	5.8	1.8
CI 9776	1.9	3.7	1.7	1.9	2.4	4.0	5.1	3.7	2.4	2.1	2.5	3.1	1.2	3.0	2.3
CI 9440	1.1	2.1	1.8	2.1	2.1	4.0	4.7	1.7	2.4	2.0	4.1	4.0	1.8	3.5	2.6
CI 5401	2.2	2.1	1.4	2.3	1.9	2.0	2.7	1.9	1.6	2.2	4.9	2.1	3.3	3.3	1.1
Unitan	1.4	1.9	2.0	2.1	2.6	2.9	2.7	3.0	2.0	1.2	2.1	2.2	1.3	1.6	1.7
CI 9214	1.1	1.8	1.6	1.6	1.8	3.1	3.1	2.3	1.8	1.4	1.6	1.5	1.1	1.4	3.03

1 = Mean disease ratings of 20-40 plants of each cultivar based on 1-10 disease rating scale, from 1 = minute pin-point lesions to 10 = coalescing lesions. See text for details of rating scale.
2 = *P. teres* f. sp. *teres* isolate.

Table 4. Analysis of Variance of Mean Disease Ratings of 14 Isolates of *Pyrenophora teres* f. sp. *maculata* and One Isolate of *P. teres* f. sp. *teres* on 20 Barley Cultivars or Lines.

Source	Degrees of Freedom	Mean Square[1]
Cultivars	19	305.3**
Isolates	14	30.6**
Cultivars X Isolates	266	8.5**
Error	900	0.8

[1] Mean squares with suffix ** indicates significant at P = 0.01.

Table 5. Mean Disease Ratings Due to 14 Isolates of *Pyrenophora teres* f. sp. *maculata* and One Isolate of *P. teres* f. sp. *teres* on 20 Barley Cultivars or Lines.

Cultivar	Disease Ratings[1]
Nupana	8.3 A
Klages	8.3 A
CI 13727	7.6 B
Dekap	7.5 BC
Clark	7.3 C
CI 9699	6.9 D
Arimont	6.4 E
CI 5845	6.3 E
Galt	5.2 F
Steptoe	3.8 G
CI 5791	3.6 GH
CI 9819	3.4 HI
CI 7584	3.1 I
Tifang	3.1 I
CI 9825	2.8 J
CI 9776	2.7 J
CI 9440	2.6 JK
CI 5401	2.3 KL
Unitan	2.1 LM
CI 9214	1.9 M

[1] Mean disease ratings with the same letter are not significantly different at P = 0.05 using least significant difference.

The highest degree of virulence was shown by Mts Plent82 (5.7), Turk 74-Pt6 (5.6), and Mts Eddy82-5 (5.44) (Table 6) and the degree of virulence of these three isolates was not different at the 5% level. The lowest virulence was shown by the isolate, Mor 82-1. Isolate Mts FF81E_1, used in many experiments, had relatively low virulence. Comparisons

Table 6. Degree of Virulence of 14 Isolates of *Pyrenophora teres* f. sp. *maculata* and One Isolate of *P. teres* f. sp. *teres* on 20 Barley Cultivars or Lines.

Isolate	Mean Disease Rating[1]
Mts Plent82	5.7 A
Turk 74-Pt6	5.6 A
Mts Eddy82-5	5.4 A
Mts Lav82	4.9 B
Mts Conrd82	4.9 B
Tun 79-30	4.9 B
Mtn 77-5I	4.8 B
Mts FF82-12	4.8 B
Tun 79-38	4.8 B
Mts Lewis82-7	4.8 B
Tun 75	4.5 CD
Mts Fromb82	4.3 DE
Mts FF81-E_1	4.1 E
Mts Sun82-2	3.6 F
Mor 82-1	3.6 F

[1] Mean disease ratings followed by same letters are not significantly different at P = 0.05.

made between the isolates from Montana and the isolates from Morocco, Tunisia, and Turkey showed significant differences in all cases.

A cluster analysis was performed using means of the disease ratings to distinguish and separate resistant and susceptible reactions of barley into a 1-10 rating scale. In this regard, twenty cultivars were clustered into 6 groups or classes (Table 7). Each response class was then designated as highly resistant (HR), resistant (R), moderately resistant (MR), moderately susceptible (MS), susceptible (S), and very susceptible (VS) reaction. A separation point for resistant and susceptible classes was calculated by taking the average of disease ratings of two middle clusters and adding the standard error of the means (Eyal et al., 1985). The average disease rating of the two middle clusters were calculated as 4.0. A standard error of the mean was calculated by taking the square root of the error mean square and dividing it by the square root of replications [$SE = \sqrt{\text{Error MS}/r} = \sqrt{0.80/4} = 0.5$]. The final separation point was then 4.0 ± 0.5. Since very few cultivars with an intermediate disease reaction were included in the experiment, the final separation point was

Table 7. Cluster Analysis of Mean Disease Ratings of 14 Isolates of *Pyrenophora teres* f. sp. *maculata* and One Isolate of *P. teres* f. sp. *teres* on 20 Barley Cultivars or Lines.

Cluster No.	Cultivars in the Cluster	Frequency of Cultivars in the Cluster in %	Mean Disease Rating[2]	Response Classes[1]
1	CI 9214, CI 9776, CI 9440 CI 5401, CI 5791 & Unitan	30.0	2.4	HR
2	CI 9825	5.0	2.7	R
3	CI 9819, CI 7584 & Steptoe	15.0	3.6	MR
4	Tifang & Galt	10.0	4.5	MS
5	CI 5845 & CI 9699	10.0	6.6	S
6	CI 13727, Dekap, Arimont, Clark, Klages & Nupana	30.0	7.6	VS

[1] Response classes abbreviated as HR = Highly resistant, R = Resistant, MR = Moderately resistant, MS = Moderately susceptible, S = Susceptible, and VS = Very susceptible.
[2] Mean disease ratings based on average disease ratings of 20-40 plants in each cultivar in 1-10 rating scale. See text for details.

calculated by adding the standard error to the average value instead of subtracting (Eyal et al., 1985). Therefore, a value of 4.50 was determined to be a separation point for resistant and susceptible classes. Cultivars with ratings below 4.5 were considered resistant and cultivars rated above 4.5 were considered susceptible.

The frequency of virulence of each isolate was calculated by dividing number of cultivars with a compatible reaction (4.5 or above) by total number of cultivars (20). The frequency of virulence of Turk 74-Pt6 was the highest (70%). With the exception of Mor 82-1 and Tun 75, the virulence frequency of isolates from Tunisia and Turkey was higher than for the isolates from Montana (Table 8). Among the isolates from Montana, Mts Plent82 (55%) had the highest frequency of virulence. In general, only two virulence groups were detected. Cultivars, Tifang, CI 7584, CI 9819, and CI 9825 showed a resistant rating to isolates from Montana, whereas the same cultivars were susceptible to isolates from Morocco, Tunisia and Turkey (Table 3).

Table 8. Frequency of Virulence of *P. teres* f. sp. *maculata* and *P. teres* f. sp. *teres* Isolates on 20 Barley Cultivars and Lines.

Isolate	Frequency of Virulence (in %)
Turk 74-Pt6	70
Tun 79-30	65
Tun 79-38	60
Mts Plant82	55
Mts Eddy82-5	50
Mts Lav82	50
Mts FF82-12	45
Mts Conrd82	45
Mts Fromb82	45
Tun 75	45
Mts Lewis82-7	40
Mts $FF81E_1$	35
Mts Sun82-2	35
Mor 82-1	25

Conidium and Conidiophore Characteristics

Numerous light brown conidiophores arising singly or in groups of two or three from the necrotic leaf tissue were observed 24-72 hours after incubation. The number of conidiophores that emerged from a unit area of leaf tissue varied with the isolates and depended on whether the leaf tissue was necrotic or chlorotic. More conidiophores formed in necrotic areas than in the chlorotic areas. Leaf tissues infected with Mtn 77-5I (net blotch) produced more conidiophores than the leaf tissues infected with PSB isolates and Mts $FF81E_1$ produced more conidiophores than Mor 82-1 and Tun 79-38 in similar leaf areas. Conidiophores of Mtn 77-5I and Mts $FF81E_1$ appeared 24 hours after incubation while those of Mor 82-1 and Tun 79-38 appeared after 48 hours.

Ranges and means or modal classes (in parenthesis) of length and width, the number of septa and the number of geniculations of conidiophores of each isolate are shown in Table 9.

Generally, mean conidiophore length was longer in culture than on the excised leaves for all isolates and also the range was greater in culture than on excised leaves. Mean conidiophore length of PSB isolates was longer than that produced by net blotch isolate (Mtn 77-51). However, Mts FF81E$_1$ produced shorter conidiophores on excised leaves than that produced by Mtn 77-51 in culture. The conidiophores of Mts FF81E$_1$ were significantly shorter than those of Mor 82-1 and Tun 79-38 both on excised leaves and in cultures. Differences in conidiophore length were also observed between Mor 82-1 and Tun 79-38. The greatest range in conidiophore length was observed in Tun 79-38. Conidiophore width was similar for all isolates but slightly wider conidiophores were observed in cultures than on excised leaves and slightly wider conidiophores were produced by Tun 79-38. Conidiophore width of both net blotch and PSB isolates from Montana was slightly narrower than that produced by PSB isolates from Tunisia and Morocco.

The number of septa in the conidiophores varied greatly in all isolates and differed between culture and excised leaf tissue (Table 9). As many as 21 septa were observed in some conidiophores produced in culture by Tun 79-38. However, on excised leaf tissue the most frequent number of septa was 11 in Tun 79-38, 7 in Mor 82-1, 6 in Mts FF81E$_1$ and 4 in Mtn 77-5I. Septa number appeared to be highly correlated with conidiophore length.

The best character differentiating the various isolates was the number of geniculations (bends) in conidiophores. Although occasionally one or two geniculations were observed in the conidiophores produced in cultures of Tun 79-38 and Mor 82-1 isolates, as many as six geniculations occurred in the conidiophores of Mts FF81E$_1$. Conidiophores of Tun 79-38 and Mor 82-1 on excised leaf tissue did not show any geniculation and Mtn 77-5I and Mts FF81E$_1$ had one or two geniculations in most cases. It was interesting that the isolates that produced few or no geniculations, also produced fewer conidia per unit volume of spore suspension. In addition to producing conidia at the tip of conidiophores isolates, Mts FF81E$_1$ and Mtn 77-5I produced secondary conidia on the side of the end cell of each

Table 9. Characteristics of Conidia and Conidiophores of *Pyrenophora teres* f. sp. *teres* (Mtn 77-51) and *Pyrenophora teres* f. sp. *maculata* Isolates Observed on Excised Leaves and V_8 Agar.

	Conidium				Conidiophore			
Isolate	Length[1] (μ)	Width[1] (μ)	Septa[1] #	Basal Cell[2] (kind)	Length[1] (μ)	Width[1] (μ)	Septa[1] #	Geniculation[1] #
MTn 77-51								
Leaves	76.9-128.2 (101.2±2.2)[1]	14.0-18.6 (16.3±0.3)	3-5 (4)	R	112.2-224.4 (140.4±3.8)	7.0-9.3 (8.3±0.1)	3-5 (4)	0-2 (1)
V_8	39.6-123.5 (76.3±3.6)	12.8-18.6 (16.0±0.3)	0-6 (4)	R	142.8-255.0 (190.7±5.4)	8.2-10.5 (8.9±0.1)	5-9 (7)	2-3 (2)
Mts $FF81E_1$								
Leaves	79.2-128.2 (105.0±2.0)	15.1-18.6 (16.8±0.2)	3-6 (4)	R	132.6-214.2 (180.2±5.0)	7.0-9.3 (8.4±0.1)	4-8 (6)	0-3 (1,2)
V_8	32.6-111.8 (71.1±.6)	12.8-21.0 (16.5±0.2)	1-7 (3)	R	113.2-357.0 (222.7±4.4)	7.0-11.7 (8.7±0.2)	3-12 (8)	1-6 (3)
Mor 82-1								
Leaves	86.2-130.5 (115.0±2.6)	14.0-18.6 (16.3±0.3)	3-5 (4)	R-S (S)	204.0-418.2 (283.1±12.7)	7.0-9.3 (8.9±0.2)	5-14 (7)	0-0 (0)
V_8	72.2-139.8 (104.5±1.5)	11.7-18.6 (14.9±0.1)	2-6 (4)	R-S (S)	163.2-479.4 (311.6±11.0)	8.2-11.7 (9.7±0.2)	5-19 (11)	0-2 (0)
Tun 79-38								
Leaves	116.5-144.5 (131.8±1.5)	14.0-18.6 (16.0±0.2)	4-7 (6)	S-S (S)	173.4-428.4 (319.6±10.2)	8.2-11.7 (9.4±0.2)	6-14 (11)	0-0 (0)
V_8	41.9-139.8 (88.5±4.3)	12.8-18.6 (15.7±0.3)	1-6 (3)	R-S (S)	224.4-612.0 (379.6±16.6)	7.0-11.7 (9.4±0.2)	6-21 (12,14)	0-1 (0)

[1] Number in parenthesis is mean or mode of 30-100 observations and is followed by standard error of the mean.
[2] R = Round basal cell, S = Snake headed or conical basal cell.

conidiophore. This resulted in zig zag geniculations and more conidia than in isolates of Mor 82-1 and Tun 79-38, which produced straight conidiophores and single conidia at the tip of each conidiophore.

Hyaline to light brown conidia were initially singly produced at the tip of each conidiophore and were elliptical to cylindrical in shape. One to seven septa developed as the conidia matured and they became dark brown. In general, mean conidial length was larger

on excised leaves but the ranges were greater in culture for all isolates. Conidia of the net blotch pathogen (Mtn 77-51) were shorter than those of PSB pathogens. However, the difference in length was not significant at 5% level between Mtn 77-51 and Mts $FF81E_1$. Conidia produced by the Tun 79-38 were significantly longer than those produced by Mts $FF81E_1$ but the conidia produced by Mor 82-1 were not different statistically even though slightly longer conidia were produced by Tun 79-38 on excised leaves. Conversely, the mean conidial length of Mor 82-1 was greater than that of Tun 79-38 in cultures.

Conidium width was similar in all isolates. The number of septa in the conidia ranged from 0 to 7 and showed more variability in culture than on excised leaves. The number of septa depended on length of conidia. The most frequent number of septa observed in conidia on excised leaves was 4 and thus was not a distinguishing character. However, the basal conidium cell was a distinguishing character of some isolates of *P. teres* f. sp. *maculata*. Isolates Mor 82-1 and Tun 79-38 frequently produced snake headed or conical shaped and slightly inflated basal cells but the conidia of Mts $FF81E_1$ and Mtn 77-5l were rounded and sometimes inflated. These results showed that the morphology of the pathogens from Morocco and Tunisia was different from the PSB and net blotch pathogens of Montana.

Effects on Yield Components and Spike Length

Greenhouse trials conducted to determine the relative effect on yield components and spike length between an isolate (Mts $FF81E_1$) causing smaller lesions and a higher amount of chlorosis and necrosis and an isolate (Mor 82-1) causing bigger lesions and less chlorosis and necrosis is illustrated in Table 10. The mean disease severity caused by both isolates was the highest on Dekap and the lowest on CI 9776. The mean disease severity between the two types was statistically different at the 5% level. Means for yield components and spike length averaged over three replications and four cultivars were not different

Table 10. Mean Disease Severity of Isolates Mts FF81E$_1$ and Mor 82-1 of *Pyrenophora teres* f. sp. *maculata* and Their Effects on Yield Components and Spike Length of Four Barley Cultivars.

Isolate	Disease Severity and Yield Components	Cultivars				Mean[1]
		Dekap	Unitan	Galt	CI9776	
Mts FF81E$_1$	disease severity (%)	92.3	69.9	49.1	35.0	61.6 A
Mor 82-1	disease severity (%)	82.7	61.4	53.1	25.9	55.8 B
Control	disease severity (%)	0.0	0.0	0.0	0.0	0.0 C
Mts FF81E$_1$	spike length (cm)	6.8	6.5	8.4	5.7	6.8 A
Mor 82-1	spike length (cm)	6.9	6.7	8.3	5.3	6.9 A
Control	spike length (cm)	8.0	7.4	7.8	6.4	7.4 A
Mts FF81E$_1$	kernel number/spike	6.9	18.3	12.6	12.2	12.5 A
Mor 82-1	kernel number/spike	4.6	18.8	18.2	17.9	14.9 A
Control	kernel number/spike	18.1	38.1	39.2	23.3	29.4 B
Mts FF81E$_1$	1000 grain wt. (g)	28.1	40.8	38.5	43.4	37.7 A
Mor 82-1	1000 grain wt. (g)	34.9	42.8	33.9	44.7	39.1 A B
Control	1000 grain wt. (g)	33.4	46.3	37.1	45.6	40.6 B
Mts FF81E$_1$	total spike no./pot	45.3	25.3	16.3	21.0	27.0
Mor 82-1	total spike no./pot	53.7	19.3	17.0	16.0	26.5
Control	total spike no./pot	27.3	22.0	18.0	22.7	22.6

[1] Means followed by same letters are not significantly different at P = 0.05 using least significant difference.

with both isolates but individual cultivars responded differently. Spike length was reduced by both isolates on Dekap, Unitan, and CI 9776. Mean kernel number per spike of all cultivars was reduced by both isolates but no differential effect of the isolates was observed (Table 10). Thousand kernel weight was also reduced significantly in Dekap, Unitan, and CI 9776 but no differential effect of the isolates was observed. Total spike number was significantly increased in Dekap with both isolates. Increased kernel weight and spike length were observed in Galt infected with Mts FF81E$_1$ and both isolates respectively.

A highly significant negative correlation was observed between disease severity and kernel number, and 1000 kernel weight (Table 11). A significant positive correlation

Table 11. Correlation Among Percent Infection of Pyrenophora Spot Blotch and Yield Components and Spike Length of Four Barley Cultivars.

Variable Compared	r-value[1]
Disease vs kernel number	-0.69**
Disease vs kernel weight	-0.47**
Disease vs spike length	-0.12ns
Kernel number vs kernel weight	0.40*
Kernel number vs spike length	0.26ns
Kernel weight vs spike length	-0.37*

[1] r-value with suffix, ** = significant at P = 0.01, * = significant at P = 0.05, and ns = non-significant.

occurred between kernel number and 1000-kernel weight. A nonsignificant correlation was observed between the kernel number and spike length.

Analysis of variance indicated significant main effects (cultivars and isolates) on disease severity and other yield components and spike length (Table 12). Significant interaction effects were observed only in disease severity, 1000-kernel weight and total spike number.

Table 12. Analysis of Variance for Barley Yield Components and Spike Length and Percent Pyrenophora Spot Blotch Infection.

		Mean Square[1]				
Source	df	% Infection	Kernel No.	Kernel Weight	Spike Length	Total Spike No.
Cultivars	3	2310.0**	416.6**	308.5**	8.5**	1160.0**
Isolates	2	13860.0**	999.1**	25.3*	1.3**	70.2*
Cultivars X Isolates	6	609.9**	56.2ns	18.8*	0.7ns	179.9**
Error	24	36.0	29.6	5.4	0.6	17.6

[1] Mean square with suffix, ** indicates significant at P = 0.01, * indicates significant at P = 0.05 and ns indicates nonsignificant.

Percentage change in yield components and spike length by the two isolates is shown in Table 13. Percentage reduction in kernel number was considerably higher than percent reduction in kernel weight and spike length in all cultivars with both isolates. The highest percentage reduction of kernel number (74.6%) was observed in Dekap with Mor 82-1. A

reduction of 15.9% in 1000-kernel weight was also observed in Dekap with Mts FF81E$_1$. However, 1000-kernel weight increased in the same cultivar with Mor 82-1. Spike length was increased in Galt by both isolates. The number of total spikes was increased in Dekap by both isolates but it was reduced as much as 29.5% in CI 9776 with Mor 82-1. It was found that a 35% and 26% disease severity of CI 9776 with isolates Mts FF81E$_1$ and Mor 82-1, respectively, caused a considerable reduction in yield components and spike length. In general, both isolates caused significant reduction in kernel number and 1000-kernel weight in all cultivars but reduction in kernel number was most evident. Increased total spike number in Dekap may partially compensate for high disease severity.

Table 13. Percentage Change in Yield Components and Spike Length of Four Barley Cultivars Due to Two Isolates of *Pyrenophora teres* f. sp. *maculata.*

	Isolates							
	Mts FF81E$_1$				Mor 82-1			
Cultivar	Spike Length	Kernel Number	1000 Kernel Weight	Total Spike Number	Spike Length	Kernel Number	1000 Kernel Weight	Total Spike Number
Dekap	-15.0	-61.9	-15.9	+65.9	-13.8	-74.6	+4.5	+96.7
Unitan	-12.2	-52.0	-11.9	+15.0	-9.5	-50.7	-7.6	-12.3
Galt	+7.7	-67.9	+3.8	-10.9	+6.4	-53.6	-8.6	-7.1
CI 9776	-10.9	-47.6	-4.8	-7.5	-17.2	-23.2	-2.0	-29.5

- = percentage reduction, + = percentage increase.

Sources of Resistance

Considering the limited known sources of resistance to PSB, evaluation of disease resistance in barley was undertaken both in the seedling and the adult stage utilizing different barley cultivars or lines originated from various countries (Table 14). In the seedling stage, barley cultivars, Klages, Dekap, Clark, Steptoe, Hector, and CI 5791 showed comparatively bigger lesions than others. Cultivars such as CI 9214, CI 5276, CI 5298, CI 5401, CI 9647, Minn 21 and Himalaya often showed only fleck-like hypersensitive lesions.

In the field, initially dark brown pin-point lesions appeared on the leaf blade 7-8 days after inoculation. Those lesions increased in size, and changed in shape with time. The lesions appeared as dark brown, fusiform or elongated lesions measuring 8-10 mm in length and 2-4 mm in width on susceptible and moderately susceptible cultivars about 25-30 days after inoculation. On resistant cultivars, the lesions were rounded or fusiform in shape with sizes of 1-2 mm diameter or 2-4 mm long and 1-2 mm wide. In most cases, necrotic lesions were surrounded by chlorosis, thus forming a yellow halo with a dark brown center. The extent of chlorosis depended on time and cultivar. In some cases, the necrotic lesions were 15-20 mm long and 4-5 mm wide. About 30 days after inoculation additional necrotic lesions of various shapes and sizes developed on the leaf blade and often coalesced to form long lesions or big blotches. In very susceptible cultivars, long lesions coalesced from end to end forming long brown stripes appearing similar to the stripe disease caused by *P. graminea*. As time advanced, chlorosis and necrosis developed extensively. Generally, necrosis developed downward from the tip of the leaf blade and extended to the leaf sheath. On resistant cultivars, only chlorosis surrounded the necrotic lesions. In some cases, however, dark brown pin-point lesions first developed then disappeared and were replaced by grayish or yellowish flecks.

The spot symptoms progressed from the tip of the leaves to leaf sheaths and from lower leaves to upper leaves. On susceptible cultivars, even flag leaves were completely blotched and symptoms appeared on the leaf sheaths, glumes and awns. However, no detectable symptoms were observed on the seed surface. On resistant cultivars, the symptoms rarely appeared on the flag leaves or above. On some cultivars, chlorosis and necrosis developed very late but on others, leaves were completely blighted and dried at an early stage. Cultivars, such as Dekap, Klages, Summit, Emir, Wapana, Nupana, and CI 13727 showed large lesions and large amounts of chlorosis and necrosis. Cultivars such as CI 5845, CI 9159, CI 9698, Steptoe, Hector, CI 13262, CI 12860 and many others produced larger

lesions but did not show early chlorosis and necrosis. Unitan and CI 5791, which showed a resistant rating in the growth chamber produced large lesions in the field. The later appearing chlorosis and necrosis resulted in a moderately susceptible or susceptible reaction.

Cultivars, such as Minn 21, Oderbrucker, Tifang, CI 1615, CI 5276, CI 5401, CI 6475, CI 9214, CI 9440, CI 9776 and Himalaya were resistant both in the growth chamber and in the field (Table 14). However, cultivars such as Unitan, Steptoe, Bedford, Clipper, CI 5822, CI 6225, CI 7504, CI 8332, CI 9647, CI 9819, CI 9825, and CI 14023 were resistant in the growth chamber but intermediate or susceptible in the field. Conversely, cultivars Cumhunyet 50, Arimont, CI 12860, and Galt were resistant in the field but were intermediate or susceptible in the growth chamber. Nupana, Dekap, Klages, Wapana, and CI 13727 were susceptible in both conditions and Ingrid, Vireo, Bruen's Wisa, Galt, Shabet, CI 9751, Bonus, Bigo and Union were intermediate. Unitan was consistently resistant in the growth chamber, but moderately susceptible in the field. Of the 72 cultivars tested both in the growth chamber and the field, 35 cultivars (with 1-3 ratings) in the growth chamber and 25 cultivars (with 1-4 ratings) in the field were resistant to the disease.

Of the one hundred twenty-three barley cultivars, CI or PI lines tested in the growth chamber using isolate Mts FF81E_1, Zephyr, Watan, Atlas 68, Nuvan, OAC 21, Karl (all from the USA), CI 9505 (from Korea) and CI 10379 (from Ethiopia) plus 21 PI or CI lines originating from Nepal showed a resistant reaction to the disease (Table 15). Most of the cultivars originated from Nepal produced comparatively larger lesions than cultivars originating from other countries.

Table 14. Disease Ratings of Barley Cultivars or Lines to a Pyrenophora Spot Blotch Isolate Mts FF81E$_1$ Seedlings (growth chamber) and a Mixture of Isolates as Adult (field).

Cultivar	CI or PI No.	Origin	Row Type	Disease Rating[1]	
				Seedling	Adult Plant
Ingrid	10083	Sweden	2	5	5
Erbet	13826	USA	2	8	8
Dekap	3351	Turkey	2	9	10
Hector	15514	Canada	2	8	5
Vireo	15555	USA	2	5	7
Summit	2248	UK	2	8	8
Nupana	16559	USA	2	10	10
Cumhuriyet 50	—	Turkey	2	6	3
Gem	7243	USA	6	3	8
Unitan	10421	USA	6	2	7
Arimont	15509	USA	6	6	2
Galt	11770	Canada	6	5	4
Steptoe	15229	USA	6	3	7
Minn 21	15481	USA	6	1	2
Athenais[2]	10501	Cyprus	6	2,6	7
Atsel	6250	USA	6	4	8
Cm 67	13782	USA	6	6	8
Manchuria (*msg*)[2]	—	Egypt	6	7,10	3,6
Oderbrucker	4666	USA	6	2	1
Betzes	6398	Poland	2	7	7
Klages	15478	USA	2	9	9
Mona-arivat[2]	—	—	6	6,10	3,7
Compana	5438	USA	2	5	10
Trebi	936	Turkey	6	3	4
Turk	14400	USA	6	3	5
Clipper	—	Australia	2	3	8
Bonanza	14003	Canada	6	3	4
Bedford	15774	—	6	2	6
Bruen's Wisa	10089	Germany	2	5	7
Wapana	—	USA	2	9	8
Tifang	14373	Egypt	6	2	2
Beacher	6566	—	6	3	10
Shabet	13827	USA	2	5	6
	1615	China	6	2	1
	2221	Nepal	2	1	3
	4207[2]	USSR	6	2	2,6
	5276	USA	6	1	2
	5298	USA	6	1	3
	5401	USA	6	1	2
	5791	Ethiopia	2	4	6
	5822	Ethiopia	6	3	6
	5845	Ethiopia	6	6	6
	6225	Turkey	2	2	4
	6475	Poland	2	2	2
	7208	Ethiopia	6	2	4

Table 14 (continued).

Cultivar	CI or PI No.	Origin	Row Type	Disease Rating[1]	
				Seedling	Adult Plant
	7272	Morocco	6	2	4
	7504	Mexico	6	2	6
	7584	USA	6	2	4
	8332	France	6	2	5
	9159	Turkey	2	6	8
	9214	Korea	6	1	2
	9440	Korea	6	2	2
	9647	Ethiopia	2	1	4
	9699	Ethiopia	6	9	4
	9751	Ethiopia	2	7	6
	9758	Ethiopia	6	10	6
	9768	Ethiopia	6	8	8
	9776	Morocco	6	2	2
	9819	Ethiopia	2	2	6
	9825	Ethiopia	2	3	5
	10448	Colombia	6	3	4
	12860	Ethiopia		6	3
	13262	Germany	6	8	–
	13727	Turkey	2	10	9
	14023	USA	2	2	5
Himalaya	2257	Nepal	6	1	2
Bonus	11308	Sweden	2	6	5
Bigo	328754	Israel	6	5	5
Ketch	14845	Australia	2	6	8
Emir	13541	Yugoslavia	2	8	5
Clark	15857	USA	2	7	–
Union	3124	UK	2	5	6

[1] Disease ratings based on 1-10 rating scale from 1 = minute pin-point or fleck type lesions, without any visible chlorosis and necrosis on the leaf blade to 10 = coalescing lesions, more necrosis than chlorosis and less than 10% green area visible on the leaf blade. See text for details.

[2] Cultivars or lines showing two types of disease ratings.

Table 15. Disease Ratings Reactions of Barley Cultivars or Lines to a Pyrenophora Spot Blotch Isolate Mts FF81E$_1$ as Seedlings (growth chamber).

Cultivar or Line	CI or PI	Source	Disease Rating[1]
Herta	8097	Austria	4
Zephyr	13667	USA	1
Marismink	15556	USA	8
Toonucier	—	USA	4
Watan	—	USA	2
Nordic	15216	USA	3
Nucier	13831	USA	3
Atlas 68	13824	USA	2
Nuvan	13831	USA	2
OAC 21	1470	Canada	2
Coast	2235	USA	3
Harbin	4929	Egypt	5
Can Lake Shore	2750	USA	4
Manker	15549	USA	4
Manchuria	2330	Egypt	5
Kombar	15694	USA	4
Palliser	10860	Canada	4
Vanguard	11868	USA	7
Jau	12108	Nepal	8
Karl	15487	USA	2
Suweon 188	—	Korea	3
Bonus	8093	Sweden	5
Bonus	11189	Sweden	6
Bigo	13611	—	4
Bigo	11795	Netherlands	3
NBA3	403975	Nepal	5
NBA4	403976	Nepal	6
NBA5	403977	Nepal	4
NBA6	403978	Nepal	5
NBY7	403979	Nepal	5
NB 1003-3	403980	Nepal	5
NB 1003-37	403981	Nepal	5
NB 1003-11	403982	Nepal	5
NB 1003-47	403983	Nepal	5
NB 1003-53	403984	Nepal	7
NB 1003-59	403985	Nepal	6
NB 1003-64	403986	Nepal	5
NB 1003-68	403987	Nepal	7
NB 1003-86	403988	Nepal	5
NB 1003-88	403989	Nepal	5
NB 1003-89	403990	Nepal	7
NB 1003-91	403991	Nepal	7
NB 1003-108	403992	Nepal	5
NB 1003-109	403993	Nepal	6
B1A	429495	Nepal	6
B3A	429496	Nepal	5
B4A	429497	Nepal	3