AN ABSTRACT OF THE THESIS OF

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Title: Influence of Site, Soil, and Inoculum Density on Dwarf bunt (Tilletia controversa Kühn) of Winter Wheat.

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Field microplot studies conducted over two seasons (1982-1984) evaluated the influence of four physically and chemically diverse soils collected from Pacific Northwest wheat fields on the incidence of dwarf bunt of winter wheat caused by Tilletia controversa Kühn. These soils were placed at Flora and Pendleton, Oregon to determine the effects of environment (e.g. amount of snow cover) and/or the use of vermiculite to simulate snow cover. In 1982-1983, growing site had a significant ($P \leq 0.001$) affect on the amount of dwarf bunt which developed in these test soils after they were mixed with 5 rates of inoculum (0.0 - 20 g T. controversa teliospores/45 kg of soil). Percent dwarf bunt in these soils ranged from a high of 45% at Flora, with greater than 80 days of snow

cover, to 15% at Pendleton which had 2 days of snow cover.

In 1983-1984, an additional experiment established at Flora, used test soils placed in 61 cm diameter microplots infested with three concentrations of inoculum (0.0 - 1.5 g T. controversa teliospores/0.5 l water) sprayed on the soil surfaces. The modified infestation method in addition to more snow cover days (133) than in the 1982-1983 season resulted in a higher incidence of disease. Percent dwarf bunt reached a high of 89%.

An application of vermiculite was used to simulate snow cover. Vermiculite applied at sowing, delayed plant emergence and reduced disease incidence at Flora when compared to the corresponding no vermiculite treatments. In the absence of vermiculite, a snow cover always increased the incidence of dwarf bunt.

Although a persistent snow cover enhanced infection by dwarf bunt, it was not necessary for ensuring teliospore survival and some infection in the near absence of a snow cover. In 1984, dwarf bunt infected plants were observed at Pendleton in the test soils which had been infested with teliospores in 1982. Also teliospores, buried in the test soils at each site and retrieved at periodic intervals for in vitro germination assessment, remained viable over an 18 month period. The number of soils with teliospores which germinated upon retrieval reflected a seasonal cycle with more teliospores germinating in March

- April than when retrieved in November.

Soil characteristics, either physical or chemical, also influenced the amount of disease which developed. The high clay content (54%) of the Banida, Idaho test soil hindered water infiltration and excess surface water was observed accumulating on the soil surface. In 1984, this soil had less dwarf bunt than in the other three test soils and disease was significantly ($P \leq 0.05$) reduced in this soil as compared to the disease which developed in the silt/clay test soil from Logan, Utah. When the test soils were fumigated with methyl bromide, before infesting them with T. controversa teliospores, no significant ($P \leq 0.05$) differences in percent dwarf bunt were detected among the four soils. The incidence of dwarf bunt increased in all fumigated soils as compared to the non-fumigated treatments.

In vitro germination tests evaluated the affect of chemical and biological soil properties on T. controversa teliospore germination. Teliospore germination on media made from soil extracts of the four test soils was significantly ($P \leq 0.05$) reduced on media with a pH above 7.0 as compared to media with a pH of 5 to 6. An alkaline pH of 8.2 combined with the physical properties of the Banida soil may have contributed to the reduced amount of dwarf bunt observed in this soil.

Additional in vitro studies used teliospores placed on

test soils which had been steamed with moist air at 60° C/30 min. Germination significantly ($P \leq 0.05$) increased on two of the steamed soils as compared to their non-steamed treatments. Fumigation of the soils with methyl bromide significantly ($P \leq 0.05$) increased teliospore germination on three of the four soils. The addition of 1% nonsterile soil to 3 of the 4 fumigated soils reduced but never significantly ($P \leq 0.05$) decreased germination when compared to germination on the respective fumigated soil. This lack of a successful transfer of a suppressive biological factor indicated that the soil sterilization process may have resulted in the release of a compound stimulatory to germination or that increased increments of nonsterile soil need to be added before a significant response is detected.

This study has shown that although snow cover favors infection by dwarf bunt, certain soil characteristics either physical, chemical, and/or biological also influence the final incidence of dwarf bunt.

Influence of Site, Soil, and Inoculum Density on Dwarf Bunt (*Tilletia controversa* Kühn) of Winter Wheat

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

I. Historical Perspective

The genus Tilletia contains some of the historically oldest and most destructive of the cereal fungal pathogens. Three wheat pathogens included in this genus are the common bunt organisms, T. caries (DC.) Tul. and T. foetida (Wall.) Liro. and the causal agent of dwarf bunt, T. controversa Kühn.

Historically the bunts have been significant in developing the germ theory of disease. In 1755, Tillet demonstrated the infective nature of bunt spores. Later, work done by Prevost in 1807, the Tulasne brothers and Berkely in 1847, De Bary in 1853, and Brefeld in 1877 led to the final recognition of bunt teliospores as reproductive bodies capable of infecting young wheat seedlings and the eventual seperation of Tilletia as a distinct genus from that of other smuts (Large, 1940; Fischer and Holton, 1957).

It was not until 1935 that dwarf bunt was recognized

as a disease unique from that of common bunt. Young (1935) then described it as a new variety of Tilletia tritici. Conners (1954) recognized T. contraversa Kühn as the correct epitaph in honor of Kühn's original description in 1874. The currently accepted spelling of T. controversa was adopted by Duran and Fischer (1961).

II. Origin and Distribution

Evidence suggests that dwarf bunt originated in remote areas of eastern Turkey, Iran, and northern Pakistan where bunt diseases are endemic (Niemann, 1956). Early accounts (Merrill and Eliason, 1903; Tingey and Woodward, 1935, Holton and Heald, 1936) described a particularly destructive "short" bunt found occasionally in isolated fields throughout the western United States. These reports are now believed to have been descriptions of dwarf bunt infestations (Purdy, et al., 1963). Herbarium specimens show that dwarf bunt existed in the United States as early as 1860 in New York and was observed in the Pacific Northwest in the early 1900s (Duran and Fischer, 1956).

Presently dwarf bunt occurs not only in the United States but also in Canada and localized areas of South America and several European countries. Within the United States, dwarf bunt is essentially confined to isolated areas throughout the western winter wheat production

region and has not spread appreciably since first reported (Hoffman, 1982).

III. Disease Cycle

Dwarf bunt infections, unlike common bunt, result mainly from soilborne rather than seedborne inoculum (Holton, et al., 1949). Diploid teliospores at or near the soil surface germinate to produce a nonseptate promycelia. Holton (1943) illustrated and described various branched and unbranched promycelia produced by teliospores under laboratory conditions. Gassner-Hoeschst (1953) and Niemann (1956) later confirmed these observations and further described the promycelia as generally developing sporidia terminally on each branch. The number of primary sporidia may vary from fourteen to thirty (Hoffmann, 1982). The haploid sporidia fuse to form H bodies, undergo plasmogamy, and detatch from the promycelium.

Most knowledge concerning the infection process of T. controversa has been gained through histological staining procedures used on artificially inoculated wheat plants. The infection process is not clearly understood, and the current reports may not be applicable to naturally infected plants. Observations have shown that, upon germination of fused sporidia, a binucleate hypha is produced which is pathogenic to susceptible wheat cultivars

(Fischer and Holton, 1957). It is presumed that the young tissue of developing tiller initials provides the primary site of entry for the pathogenic hyphae (Hoffmann and Purdy, 1967). The intercellular fungal hyphae remain in the meristematic apical growing point and upon internodal elongation, the pathogenic hyphae are carried passively upward in the apical tissue (Fernandez, et al., 1978). The fungus remains quiescent until the onset of floral development when it then enters a rapid reproductive phase. The pathogenic hyphae sporulate in the differentiating ovarian tissue and the entire kernel contents are replaced with bunt sori consisting of fungal teliospores. Infected wheat heads and bunt sori which land on the soil surface provide the inocula for subsequent season infection.

An understanding of the pathogen's life cycle was long hindered by the inability to successfully culture all phases of the pathogen. Preliminary investigations (Holton, 1940) failed to obtain teliospore germination on either soil, soil extract agar or water agar. Holton (1943) eventually obtained germination at 10°C after presoaking teliospores in water at 4° C for one to seven months. This germination procedure has never been successfully duplicated and it was not until Lowther (1948) demonstrated an optimum temperature requirement near 5 C that a reliable germination method was obtained for in vitro research.

Even under optimum conditions, teliospore germination is difficult to accurately assess due to the prolonged and variable germination pattern. Dewey and Tyler (1958) unsuccessfully attempted to shorten the time period required for teliospore germination by using various combinations of temperature, base or acid concentrations, and scarification treatments. Germination, however, does not occur until teliospores have been incubated under optimal conditions for three to four weeks. In a typical germination pattern for a population of teliospores, maximum germination may not be obtained until after eight to ten weeks with continued germination lasting up to sixteen weeks (Trione, 1982). Both water and nonwater soluble endogenous germination inhibitors may contribute to spore dormancy (Trione, 1977). Trione (1977) has suggested that an imbalance and low levels of certain free amino acids may impair protein synthesis during the early stages of teliospore germination.

In vitro growth of all phases of the pathogen's life cycle have now been cultured (Trione, 1964; Chung and Trione, 1967; Singh and Trione, 1969; Trione, 1974) enabling closer observation of all nuclear phases of the pathogen. The extreme spore dormancy and the inconsistent and limited amount of infection obtained in greenhouse studies using infested soil have, however, continued to impede pathogenicity studies.

IV. Symptoms

The most notable symptom resulting from T. controversa infection is a drastic dwarfing of the host plant. Infected wheat plants may be only two-thirds to one-third the height of healthy plants (Holton, et al., 1949; Warmbrunn, 1952). Infected wheat heads are generally broader than normal heads due to the more spherical shape of the bunt sori. (Holton and Heald, 1936; Bamberg, et al., 1947; Purdy, et al., 1963).

Some cultivars may show a characteristic leaf flecking reaction in the seedling stage (Kendrick and Purdy, 1959). Infected plants also generally have fifty percent or more tillers than healthy plants (Bamberg, et al., 1947). Leaf flecking and excessive tillering may vary greatly with cultivar, growing conditions, and race of T. controversa and hence are not always reliable diagnostic characteristics of T. controversa infection (Purdy, et al., 1963).

V. Cultural and Environmental Factors Influencing Teliospore Germination and Incidence of Dwarf Bunt.

A. Cultural Practices

Cultural practices which influence emergence and growth of autumn sown wheat also affect the amount of

dwarf bunt infection. Both an extremely early or late planting date, or a deep planting depth have been correlated with a reduced incidence of dwarf bunt (Baylis, 1955; Kendrick, 1956; Meiners, et al., 1956; Tyler and Jensen, 1958; Hoffmann and Purdy, 1967). Kendrick (1956) noted that Orin wheat which was shallow seeded on the soil surface or less than 2.5 cm deep, had a much higher incidence of disease than seed sown at a depth of 6.3 to 10.0 cm.

Hoffmann and Purdy (1967) reported a significant correlation between stage of wheat plant development and amount of disease. Early in the season when plants have only a few tillers, the pathogenic hyphae easily invades the undifferentiated tissue of the growing point thus increasing the chance of infecting most if not all of the tillers (Tyler, 1958). Fewer successful infections occur later in the season with those plants which do become infected having a smaller percentage of infected tillers than younger plants infected earlier in the season (Tyler, 1958; Hoffmann and Purdy, 1967). Tissues in older plants have begun to differentiate and may be beyond the susceptible stage for infection. Stem elongation has also begun in older plants perhaps preventing the fungus from reaching the growing point for a successful infection (Tyler, 1958; Trione, 1982).

Holton and Meiners (1957) related tillage practices to

the cyclic occurrence of dwarf bunt. They theorized that wheat grown in a field where infested surface soil had been plowed under would escape infection until spores were again brought to the surface where optimum germination conditions prevail. Further investigations of other management practices such as minimum or no tillage and spring or fall plowing may provide additional information on T. controversa survival in the field (Hoffmann, 1982).

B. Environmental Factors

1. Groundcover

Holton et al., (1949) noted that not only were occurrences of dwarf bunt localized but the amount of disease varied in successive years within the same field. A greater amount of disease was also generally found on the leeward side of fence rows, hedge rows, and hilltops (Tyler and Jensen, 1953) or wherever there were heavy and persistent deposits of snow (Tyler and Jensen, 1958). A persistent snow cover has been consistently linked with an enhanced level of dwarf bunt (Tyler and Jensen, 1953, 1958; Hoffmann, 1982). An extended snow cover presumably buffers the soil surface, prolonging favorable temperature and moisture periods for infection (Hoffmann, 1982).

The influence of an artificial covering such as vermi-

culite or oat straw on the amount of dwarf bunt depends largely on the time of application (Dewey, 1963). Both straw and vermiculite applied in late fall enhance infection (Tyler and Jensen, 1953, 1958; Dewey, 1963). A straw covering may delay plant maturation thereby exposing susceptible tissues to infective inoculum over a longer period (Tyler and Jensen, 1953, 1958). In addition, an artificial covering may simply help maintain a suitable microenvironment which is favorable to the pathogen over a longer period of time (Tyler, 1958; Dewey, 1963). An artificial covering applied at time of sowing reduces the amount of infection when compared with both uncovered plots or plots covered later in the season (Tyler and Jensen, 1958; Dewey, 1961). An explanation for this reversal in amount of disease has not been conclusively determined.

2. Light and Temperature

Light stimulates germination in at least five other Tilletia spp. that attack grasses and cereals (Meiners and Waldher, 1959). Gassner-Hoeschst (1953) found little or no germination of T. controversa teliospores in the absence of light. Beneath a snow cover, T. controversa teliospores receive continual diffuse light (Hoffmann, 1982). Exposure to 150 - 200 ft-c of fluorescent light,

at least during the latter half of the incubation period, is critical for maximum germination (Baylis, 1958).

Teliospore germination occurs within the temperature range of 2 to 15°C (Lowther, 1948; Baylis, 1958; Meiners and Waldher, 1959; Hoffmann, 1982). An unprotected soil surface exposing teliospores to either below freezing or above optimum temperatures will create unfavorable conditions for germination, fusion, and infection (Baylis, 1958; Hoffmann, 1982; Trione, 1982). Changes in light duration and elevated temperatures outside the optimum range may result in abnormal germination with elongated promycelia and delayed formation of sporidia (Zscheile, 1965).

Preliminary tests suggest that teliospore dormancy is temperature dependent. Teliospores retrieved from the field in late spring germinate in only trace amounts compared to spores retrieved throughout the rest of the year (Hoffmann and Goates, 1981; Hoffmann, 1982). This generally correlates with the occurrence of T. controversa infection which begins after seedling emergence in mid-October and lasts through April with most infections occurring in January and February (Purdy, et al., 1963; Hoffmann, 1982; Trione, 1982). Cool, moist conditions may prolong dormancy until it is broken by warm, dry weather during the summer months (Hoffmann, 1982).

The expression of host resistance in certain cultivars

may also be influenced by temperature. Histological studies of resistance reactions in artificially inoculated plants indicate that for infection to occur the fungus must reach the growing point before internodal elongation (Swinburne, 1963; Fernandez, et al., 1978). In some resistant cultivars, the mycelia becomes established in the coleoptile and leaf primordia but then fungal development ceases. This resistance response coincides with the placement of the wheat plants from colder to warmer temperatures (Fernandez, et al., 1978).

3. Moisture

Teliospores under a snow cover are surrounded by a saturated atmosphere with the occasional presence of free water. Under these moisture conditions, maximum germination and infection occur (Hoffmann, 1982). Tyler and Jensen (1958) observed more infection when wheat plants were growing in low lying areas where free water could accumulate. Baylis (1958), however, noted that sporidia produced on petri dishes containing a clay loam soil disintegrated more rapidly when the moisture level was above forty percent. Trione (1977) further observed that dwarf bunt teliospores germinated poorly on agar when surrounded by a thin film of water. Studies examining the moisture requirements for teliospore germination need to

be conducted to resolve these contradictory observations.

4. Edaphic Factors

Chemical, physical, and biological soil components have not been sufficiently investigated to determine their influence on germination and infection. Baylis (1958) reported that spore germination on a clay loam soil occurred throughout a neutral to acid pH range but was significantly reduced at a pH above 7.8 to 8.2. Meiners and Waldher (1959) obtained maximum germination on soil extract agar as compared to germination on either water agar or soil. They concluded that there was a water soluble soil factor stimulatory to teliospore germination.

Soil type has been inconsistently linked with the incidence of disease. Baylis (1958) observed that most infections in Ontario, Canada occurred on heavy soils whereas Holton, et al (1949) found no indication that soil type was a critical factor when they observed infestations of variable severity on both heavy and light soils.

In a preliminary investigation, Kollmorgen and Jones (1975) found several microorganisms isolated from Australian wheat fields which markedly reduced the in vitro germination of common bunt teliospores. Gassner and Niemann (1955) described two fungi which stimulated dwarf bunt teliospore germination. These early investigations

suggest that soil microflora may play a role in bunt teliospore survival and germination.

VI. Control Methods

Cultural control methods for T. controversa infestations have not proven reliable. An early or late planting date will reduce the amount of dwarf bunt yet any extreme variation from the normal planting time may increase the risk for the development of other diseases (Purdy, et al., 1963). Delayed planting may also lower the yield potential and increase erosion and winter damage. Crop rotation is not a viable control method due to the longevity of spore survival in the soil (Holton, et al., 1949). Inoculum on the soil surface may remain infective for at least two to three years and dwarf bunt has been observed in fields planted to dwarf bunt susceptible cultivars after 7 - 10 years of resistent cultivars (Holton et al., 1949; Tyler, 1953).

The use of seed treatments such as hexachlorobenzene, pentachloronitribenzene, carboxin, and thiabendazole when combined with resistant cultivars has proven effective against both seed and soilborne common bunt inoculum (Purdy, 1965; Hoffmann, 1971; Hoffmann and Waldher, 1981; Hoffmann, 1982). Thiabendazole has been less effective for dwarf bunt control due to the protracted period between

seeding and infection (Tyler, 1958; Purdy et al., 1963; Hoffmann and Purdy, 1967). The effectiveness of thiabendazole increases with lateness of seeding and hence has not been accepted commercially due to growers' preference for early seeding (Hoffmann, 1971, 1982; Hoffmann, et al., 1983). Applying hexachlorobenzene or pentachloronitrobenzene to the soil surface after seeding does control dwarf bunt (Purdy, 1957, 1965) but is not recommended in the United States for economic and environmental reasons (Purdy, 1963; Hoffmann, 1982).

The use of resistant cultivars has proven the most effective means of controlling dwarf bunt. Until the 1960s, the main sources of bunt resistance were obtained from Turkey (Bt4) and Ridit (Bt3). Races with combined virulence against these sources of resistance have appeared in widely seperated areas (Hoffmann and Metzger, 1976; Hoffmann, 1982). Additional new sources of bunt resistance have originated from eastern Turkey yet virulent races have already been detected against some of these sources. It appears that virulent types are rapidly selected in the bunt population making resistance in cultivars non-durable.

VII. Statement of Research Problem

Late in 1974, the Chinese established a quarantine for

T. controversa spores in wheat shipments from the United States. Common bunt is found in China but dwarf bunt has not yet been reported. The Chinese fear that the introduction of dwarf bunt teliospores could establish the pathogen there and thus threaten their wheat crop production. The risk of disease establishment and spread needs to be determined if the Chinese wheat market is to be reopened to the Northwestern United States.

Although seedborne T. controversa spores can infect, they are not an efficient source of inoculum. Infection, therefore, relies mainly on the presence of soilborne teliospores. As previously discussed, teliospore germination and seedling infection are enhanced by certain specific environmental conditions found within areas throughout the winter wheat region. However, only isolated sites within these favorable environmental areas have dwarf bunt infestations with very little spread to adjacent environmentally conducive sites. This suggests that certain sites have unique physical, chemical and/or biological properties for the establishment and survival of the pathogen. A combination of these epidemiological factors will either increase or decrease the probability of infection occurring. A further understanding of factors influencing the spread of dwarf bunt will aid in establishing correct guidelines for the shipment of possibly contaminated wheat.

The initial field research was designed to study the influence of site, soil, and inoculum density on the incidence of dwarf bunt during a two year period. Further in vitro evaluations were conducted to study teliospore germination as influenced by soil leachates and the natural soil microflora found in four soils obtained from sites with differing environmental conditons and histories of dwarf bunt.

Chapter 2

DISEASE INCIDENCE AND SURVIVAL OF DWARF BUNT OF WHEAT AS INFLUENCED BY SITE, SOIL, AND INOCULUM DENSITY

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ABSTRACT

Field microplot studies were conducted over two seasons (1982-1984) with four physically and chemically diverse Pacific Northwest soils. These soils were placed at two locations in eastern Oregon to determine the effects of environment (e.g. amount of snow cover) and the use of vermiculite to simulate snow on the incidence of dwarf bunt of winter wheat caused by Tilletia controversa Kühn.

Experiments using 5 inoculum densities (0.0 - 20 g T. controversa teliospores/45 kg of a test soil) indicated that growing site had a significant ($P \leq 0.001$) affect on the amount of dwarf bunt which developed during the 1982 - 1983 season. Disease incidence in the four test soils ranged from a high of 45% at the Flora, OR site which had

a persistent snow cover (> 80 days) to 16% at the Pendleton, OR site which had only 2 days of snow cover.

At Flora, vermiculite applied as a groundcover at sowing significantly ($P \leq 0.05$) reduced the amount of disease incidence as compared to the corresponding no vermiculite treatments. There was a significant ($P \leq 0.03$) interaction between snow and vermiculite. In the presence of snow and the absence of vermiculite, the percent dwarf bunt always increased compared to the corresponding no snow treatments. In the presence of vermiculite, however, a snow cover did not always increase the percent disease.

Snow cover was not essential for teliospore survival. At both locations in 1984, bunted plants developed in the 1982 microplots which contained the originally infested test soils.

In the 1983-1984 season, additional microplots were established at Flora with test soils infested with one of three teliospore suspensions (0.0 - 1.5 g *T. controversa* teliospores/0.5 l water) sprayed onto each 61 cm diameter microplot soil surface. Use of this modified method coupled with more snow cover days (133) than in the previous season resulted in an increase in the incidence of dwarf bunt (up to 89%) as compared to the previous season. At these higher inoculum densities, the inoculum density X disease incidence curve for each soil was no longer

straight but curved to the right indicating teliospore competition for infection sites.

Test soils obtained from Banida, ID and Flora, OR generally produced fewer dwarf bunt infected spikes than soil obtained from Pendleton, OR or Logan, UT. In 1984, percent dwarf bunt was significantly ($P \leq 0.05$) lower in the Banida soil than in the Logan test soil. During the season there was soil heaving and accumulation of water on the Banida soil surface. The physical characteristics of the Banida soil, such as its high clay content (54%) and its alkaline pH of 8.2, may have influenced teliospore germination and subsequent disease development in this soil.

Fumigation of the test soils with methyl bromide, before infesting them with T. controversa teliospores, resulted in an increase in dwarf bunt as compared to the nonfumigated treatments. Fumigation eliminated any significant ($P \leq 0.05$) differences in percent dwarf bunt among the four soils.

INTRODUCTION

Dwarf bunt (Tilletia controversa Kühn) occurs sporadically and in limited areas throughout the Pacific Northwest winter wheat production region. The Chinese enactment of a quarantine on T. controversa spores in wheat

shipments from the United States has increased the interest in the epidemiology of dwarf bunt.

A persistent snow cover and soil surface temperatures within the range of 0 - 8°C are two major environmental factors consistently linked with an increase in the incidence of dwarf bunt (Tyler and Jensen, 1953, 1958; Baylis, 1955, 1958). Winter wheat is grown over a wide area having these environmentally conducive conditions for infection yet dwarf bunt is limited to localized sites within this region (Fig. 2.1). T. controversa teliospores have no doubt been widely dispersed through contaminated seed and airborne spores. The failure of dwarf bunt to spread to new sites suggests that the establishment and survival of the disease may depend on factors other than the environment such as specific soil characteristics found at a particular site.

Soil moisture (> 40 %) and an alkaline pH (> 7.8) have both been cited as determinants in reducing teliospore germination (Baylis, 1958). Other soil components either chemical, physical, or biological may additionally influence dwarf bunt occurrence and spread. Soils suppressive to the establishment and/or survival of a plant pathogen have been found for numerous diseases. The suppressive nature of some of these soils results from either the failure of the pathogen to become established or the establishment of the pathogen but failure to produce di-

sease (Baker and Cook, 1974).

Previous field studies with T. controversa have been conducted at sites with a known history of dwarf bunt. There has been a lack of research concerning the establishment and spread of T. controversa to sites not known to have had dwarf bunt infestations. The purpose of this study was to evaluate the influence of site and soil on dwarf bunt occurrence.

MATERIALS AND METHODS

Soil locations and preparation

Soil was collected from four winter wheat producing fields in the Pacific Northwest: (1) a commercial field in northeastern Oregon (Flora); (2) the Pendleton Experiment Station (PES) in eastern Oregon; (3) a dwarf bunt research site located at Utah State University (Logan); (4) a commercial field in southern Idaho (Banida). These locations have different histories of dwarf bunt occurrences and also vary environmentally (Table 2.1) and have different soil characteristics (Table 2.2). The soil samples were transported to Oregon State University where each sample was air dried for 48 hrs and then sieved through a 4 mm screen.

A portion of each soil was transferred to a 76 l metal trash can for fumigation with a 0.45 kg can of methyl

bromide (Brom-O-Gas, 98% methyl bromide, 2% chloropicrin, Great Lakes Chemical Co.). To enhance penetration of the fumigant, two 61 cm cardboard mailing tubes with randomly cut 3 mm holes were inserted upright in each can prior to adding the soil. Trash can lids were sealed in place with tape and a 4 mm hole drilled into each lid for placement of the fumigant applicator. After 24 hrs the lids were removed. The soils were then air dried for a minimum of 48 hrs prior to infestation with T. controversa teliospores.

Inoculum preparation, design, and establishment of field experiments (1982-1983)

Inoculum was prepared from dwarf bunt infected wheat heads collected in July, 1982 at Pendleton from 1980 - 1981 test plots which used inoculum provided by Dr. James Hoffmann (Crops Research Lab., Logan, Utah). Sori were broken and sieved through 2 mm and 0.5 mm screens. Inoculum was hand mixed into each test soil at the rate of 0.0, 0.02, 0.2, 2.0, or 20 g of teliospores per 45 kg of nonfumigated soil and 0.0 or 20 g of teliospores per 45 kg of fumigated soil. Each gram of inoculum contained approximately 500 million teliospores (E. J. Trione, personal communication).

In 1982, two microplot experiments were established at both Flora and Pendleton on 27 September and 13 October respectively. Individual microplots were contained in 15

cm wide tin cans with both ends removed (Figure 2.2). The cans were inserted three-quarters into the native field soil and six seeds of the winter wheat cultivar Daws (Triticum aestivum L.) were placed on the soil surface and covered with a 2.5 cm layer of the appropriately infested test soil. Treatments were replicated six times in randomized blocks. Microplots at the Flora site were protected from elk and deer damage by the placement of a 25 cm diameter wire cone constructed from 2.5 cm chicken wire, pop riveted together, and held in place by 12 ga galvinized wire loops (Figure 2.3A). The cones were removed in early spring to allow for plant growth and maturation. All experiments received the appropriate fertilizers and herbicides according to the location.

One experiment at each site consisted of microplots with either nonfumigated or fumigated test soils at the previously specified inoculum densities. The second experiment at each site was designed to further evaluate the influence of snow cover on dwarf bunt incidence and the use of an artificial cover such as vermiculite to simulate a snow cover. Microplots contained either Flora or Pendleton nonfumigated soil infested with either 0.02 or 20 g of teliospores per 45 kg of soil. In addition some of the treatments received a 2.5 cm covering of vermiculite on top of the layer of test soil. Snow was prevented from accumulating on some of the microplots by

the placement of a fiberglass mesh screen over a wire cone (Figure 2.3B). The addition of vermiculite and/or a snow screen resulted in four ground cover treatments consisting of either a natural snow cover or no snow cover in the presence or absence of a layer of vermiculite.

Preparation and planting of the 1982-1983 experiments for a second year disease assessment

All bunt infected spikes were removed from both 1982-1983 experiments at each site. To determine the incidence of dwarf bunt from inoculum remaining in the soil for a second season, microplots were not reinfested with T. controversa teliospores. The experiments were planted on 24 September at Flora and 24 October 1983 at Pendleton. A 2.5 cm furrow was cut into the soil surface of each microplot and planted with six wheat seeds (cv. Daws) which were then recovered with the 1982 infested test soil .

Flora field experiment and inoculum preparation (1983-84)

A field experiment, consisting of larger microplots for each treatment, was established on 25 September 1983 at Flora. Microplots were delineated by strips of 10 cm high aluminum lawn edging pop riveted together to form circles 61 cm in diameter. Approximately 100 seeds (cv. Daws) were broadcast into each microplot and covered with a 2.5 cm layer of either a fumigated or nonfumigated test soil (Figure 2.4).

Inoculum was collected from the 1982-1983 Flora field experiments and sieved as previously described. Teliospores were suspended in water to make three spore suspensions of 0.15, 0.5, or 1.5 g of teliospores per 0.5 l of water plus one drop Tween 20 (Sigma). Inoculum suspensions were sprinkled over the surface of each microplot using a 500 ml Erlenmeyer flask fitted with an aluminum clothes sprinkler head. One-half liter of either the 0.15 or 0.5 g inoculum suspensions was sprinkled over each microplot. The 1.5 g spore suspension was sprinkled only over additional fumigated and nonfumigated PES soil microplots. Control treatments were wetted with 0.5 l of water. With this method, inoculum remained on the soil surface where dwarf bunt teliospores are more likely to germinate. Treatments were replicated six times in randomized blocks and received appropriate fertilizers and herbicides as needed.

Disease assessment and analysis

Disease was assessed in all field experiments as number of infected spikes per total spikes. Disease incidence was transformed using Gregory's multiple infection transformation (Gregory, 1948). Statistical analyses were performed on the transformed data using analysis of variance and Fisher's least significant difference test (FLSD) (Steele and Torrie, 1980).

RESULTS

Influence of site and groundcover on percent dwarf bunt (1982-1983)

Growing site had a significant ($P \leq 0.001$) affect on the overall level of disease which developed. Percent dwarf bunt reached a maximum of 46% at Flora as compared to only 16% at Pendleton. Disease incidence was negligable in both experiments at the Pendleton site and no treatment was significantly ($P \leq 0.05$) different from the 0.0 g inoculum density.

The effect of groundcover (natural snow cover and/or vermiculite) also varied according to site. The natural snow cover at Pendleton in 1982-1983 was negligable (Table 2.1). Only treatments consisting of either both natural snow cover and vermiculite or neither snow or vermiculite resulted in any dwarf bunt infected plants and the disease incidence remained below 5%.

At Flora, in the presence of vermiculite, disease incidence was reduced in both the Pendleton and Flora soils at both inoculum densities as compared to the corresponding no vermiculite treatments (Table 2.3). Disease incidence was significantly reduced ($P \leq 0.05$) in these treatments in the 20 g inoculum density Flora soil microplots and in the 20 g Pendleton soil without a snow screen. There was also a significant ($P \leq 0.03$) inter-

acton between snow and vermiculite, with a higher disease incidence in plots covered with snow without vermiculite than in those plots without both snow and vermiculite. In the presence of vermiculite, however, disease incidence did not necessarily increase with the addition of a snow cover.

Influence of soil on the incidence of dwarf bunt (1982-84)

Differences in the disease incidence trends of the four nonfumigated test soils at Flora can best be visualized by plotting inoculum density X disease incidence (Figures 2.5 and 2.6A). In 1983, disease incidence was not significantly ($P \leq 0.05$) different among the four nonfumigated test soils at the 0.0 - 2.0 g inoculum densities (Figure 2.5). At the 20 g inoculum density, percent disease was significantly ($P \leq 0.05$) reduced in the Pendleton nonfumigated soil as compared to the other three soils (Table 2.4). In the larger 1984 Flora microplots, the disease incidence in the Banida soil was lower than that found in the other three soils and was significantly ($P \leq 0.05$) lower than the disease incidence in the Logan soil (Figure 2.6A and Table 2.5). Similar inoculum density X disease incidence trends occurred among the four nonfumigated soils in both years with disease incidence in the Banida and Flora soils being generally lower than the disease incidence in the Logan or Pendleton soils.

When soils were fumigated with methyl bromide, percent dwarf bunt at Flora generally increased in both the 1982 - 83 and 1983-84 microplots as compared to their corresponding nonfumigated treatments (Tables 2.4 and 2.5). In 1984, fumigation resulted in a significant ($P = 0.001$) overall increase in the amount of disease incidence. There were no significant ($P \leq 0.05$) differences in disease incidence among the four fumigated soils at any inoculum density (Figure 2.6B).

Second year disease assessment of experiments established in 1982-1983

When the 1982-1983 microplots were replanted in the fall of 1983, the soil within the individual cans was compacted and difficult to manage. Stubble in the cans also complicated planting procedures within the confined area of each microplot. A combination of these planting difficulties resulted in a poor stand at each site. Stand count was especially reduced at Flora where only 1/10 of the microplots from both experiments contained mature plants. All groundcover treatment combinations at Flora, except the 20 g infested Pendleton soil with vermiculite and a snow screen, had at least one can containing mature plants. One-third of these cans containing wheat had bunt infected plants. At Pendleton, stands in each can were thin but most cans contained mature plants. Nine cans at Pendleton contained bunt

infected wheat plants.

DISCUSSION

Conducive environmental conditions (e.g. persistent snow cover which provides optimum temperature, moisture, and light for germination) were more prevalent at the Flora site and enhanced disease incidence as compared to the Pendleton site which lacked snow cover in the 1982 - 1983 season (Table 2.1). This increase in percent dwarf bunt at Flora, with its persistent snow cover, agrees with Holton, et al., (1949) who also noted that deposits of drifted snow increased dwarf bunt infection and with Lowther (1948) who demonstrated the low temperature range (0 - 8°C) required for T. controversa teliospore germination. The low conductivity of snow enables it to insulate the soil surface (Oke, 1978). A snow cover, therefore, would buffer the soil and possible T. controversa infection courts from extremes in temperature and moisture fluctuations. The lack of a persistent snow cover at the Pendleton site exposed teliospores on the soil surface to a broad range of temperature and moisture extremes. The Flora site, with its soil surface buffered by a persistent snow cover, was more favorable for dwarf bunt infection.

The increased amount of infection in the 1983-1984 Flora study, as compared to the previous season's experi-

ment, may have been due to both the increased number of days with snow cover and/or the modified method of infesting the soil with T. controversa teliospores. The 1982-1983 winter was milder at both sites with less snow cover than in 1983-1984 (Table 2.1). The longer period of snow cover in the 1983-1984 season would increase the time in which favorable soil temperature and moisture conditions would be conducive for T. controversa germination and subsequent host infection. In addition, dwarf bunt teliospores tend to germinate at or near the soil surface (Baylis, 1955; Meiners, et al., 1956). Teliospore suspensions sprinkled on the soil surface, rather than mixing the inoculum into the soil, would also increase the amount of germination and chance for infection.

A prolonged snow cover, however, was not essential for teliospore survival. It is important to note that dwarf bunt did occur at Pendleton in 1984 in the microplots containing T. controversa teliospores placed in the soil in 1982. Teliospores, therefore, survived in the soil for a second season at a location which is not environmentally conducive for infection.

At Flora, in the absence of vermiculite, a snow cover always increased disease incidence as compared to no snow cover (Table 2.3). An application of vermiculite at sowing, however, reduced the amount of disease incidence

as compared to the corresponding no vermiculite treatments. These results with vermiculite applied at sowing confirm previous observations by Tyler and Jensen (1958) and Dewey (1963) who reported a reduced amount of disease in plots which were covered with straw at sowing and remained covered throughout the winter. Dewey (1963) suggested that straw or vermiculite applied at planting would reduce the amount of light reaching the infection zone and would thus reduce teliospore germination. Vermiculite may also delay plant emergence resulting in conditions similar to a delayed planting date which also reduces the incidence of dwarf bunt (Hoffmann and Purdy, 1967). Conversly, vermiculite or straw applied later in the fall will tend to increase dwarf bunt incidence (Tyler and Jensen, 1953, 1958; Dewey, 1963). They theorized that straw delayed plant emergence and therefore exposed susceptible tissues to possible infection for a longer period of time.

These contradictory arguments have not adequately explained the reason for the decrease or increase in disease incidence depending on the time of vermiculite application. A vermiculite cover would alter the microclimate surrounding the infection court. These reactions, therefore, may be the result of a complex interaction among temperature and moisture at the soil surface, an influence of vermiculite on plant growth, and the

duration and quality of light reaching the teliospores.

Although environmental conditons greatly enhance the incidence of dwarf bunt, soil characteristics also influence the amount of disease at a particular site (Figures 2.5, and 2.6A). During both seasons, the highest levels of disease incidence at Flora occurred in microplots with Logan or Pendleton soil. A greater amount of disease expression in the Logan soil is not surprising since this soil is from a site with a consistent history of dwarf bunt occurrence. The textures of both the Logan and Pendleton soils would allow for better moisture drainage than that found in the Banida soil. When the Pendleton soil was placed under more conducive environmental conditions (e.g. the Flora site) a high percent of disease developed in this soil during both growing seasons (Figures 2.5 and 2.6A, Table 2.5). At the Pendleton site, the soil is conducive for dwarf bunt infection but not the site. The better drainage characteristics of these soils could contribute to creating more favorable conditions for teliospore germination and disease development. The Banida and Flora soils both produced the lowest amounts of disease in each of the growing seasons. The Banida and Flora soils are from sites having similar environmental conditions (Table 2.1) yet differ in their histories of naturally occurring dwarf bunt. The Flora site has had a consistent history

of dwarf bunt whereas the Banida soil is from a site with favorable environmental conditions for dwarf bunt development yet only trace amounts of the disease occur.

The reduced amount of disease development in the Banida soil may be explained by some of the physical and chemical properties of this soil (Table 2.2). The Banida soil is a clay soil with 54% of its particles measuring less than 0.002 mm. Soils with a high percentage of clay have reduced water infiltration rates. These reduced rates are due to swelling of the clays which shrinks pore size and thus impedes water movement. A dry clay soil may take up large amounts of water at first but, as it swells and its pores shrink, the infiltration rate quickly drops. In addition, the increased surface area found in clay soils adsorbs water tightly. This combination of reduced pore size and high water tension slows water infiltration so that excess water builds up on the soil surface (Mengel and Kirkby, 1982; Donhue,et al., 1983).

These inherent problems with clay soils were apparent in the Banida soil throughout these experiments. In the spring, when the snow cover was melting and rain was frequent, the Banida soil generally had pools of surface water collecting in each microplot. Although no observations were made during the fall and winter months, excess water probably accumulated on the Banida soil surface throughout these months. T. controversa teliospores tend

to germinate at or near the soil surface. In the Banida soil, teliospores would be surrounded by a saturated atmosphere and would often be lying in a pool of water. The moisture requirements necessary for the germination of T. controversa teliospores are rather unclear. Trione (1977) has observed that dwarf bunt teliospores germinated poorly on agar when lying in a film of water. Baylis (1958) observed that sporidia produced on petri dishes containing a clay loam soil disintegrated more rapidly when the moisture level was above 40%. Water logged, anaerobic conditions would predispose teliospores to lysis. Ethylene, which might be produced by spore-forming bacteria in anaerobic microsites, has been shown to induce soil fungistasis and mycelial lysis (Smith, 1976). With poorer drainage and more accumulated surface water, teliospores on the surface of the Banida soil might germinate but their post-germination products would lyse before successful infection occurred.

Another common problem associated with clay soils develops when these soils dry. The clay particles shrink and cracks tend to develop in the soil structure (Donahue, et al., 1983). Soil heaving resulting in numerous cracks was observed in the Banida soil (Figure 2.7) at both sites. This disruption of the soil structure could redistribute the teliospores further down in the soil layer away from favorable sites for infection and a sufficient

amount of light for germination.

The Banida soil has an alkaline pH of 8.2. Previous in vitro studies (Baylis, 1958) have demonstrated that spore germination is reduced at a pH above 7.8. This high pH could reduce the overall amount of teliospore germination. When combined with excessive moisture, those spores which did germinate would produce sporidia which would quickly disintegrate. The high pH and the moisture characteristics of this soil could contribute to a reduction in the amount of disease which would develop in the Banida soil.

It was rather surprising that the Flora soil developed only a low level of disease in both growing seasons. The Flora soil is classified as a silt/loam with 73% of its particles being between 0.002 - 2.0 mm in diameter. The texture of the Flora soil allows for better drainage than that found in the Banida soil. The pH of the Flora soil (5.4) is much more acidic than the pH of the Banida soil (8.2) and according to Baylis (1958) would be more stimulatory to teliospore germination. An explanation for the low amount of disease which developed in this soil cannot be adequately explained on the basis of its physical and moisture characteristics alone. There are probably other soil factors which influence the survival and infective potential of dwarf bunt teliospores which have yet to be investigated. Factors influencing the

dormancy and long term survival of these teliospores could contribute to a greater resevoir of inoculum for consistent infection year after year.

Unfortunately due to the lack of data from the second field season, these hypotheses could not be further substantiated. A long term survival study needs to be conducted to determine the role that these physical and chemical properties play in the survival of T. controversa teliospores and if soils with a higher clay content would produce fewer and fewer infected plants over successive years.

Plants grown in fumigated soil infested with T. controversa teliospores generally had a higher disease incidence than plants grown in nonfumigated soil (Tables 2.4 and 2.5). Additionally, fumigation eliminated any significant ($P \leq 0.05$) differences in percent dwarf bunt which had been detected among the four nonfumigated soils (Figures 2.6A and 2.6B). Fumigation may eliminate microflora which would be suppressive to teliospore germination and subsequent post-germination growth (Cook and Baker, 1983). Alternatively, the fumigation process itself could release a factor stimulatory to the germination and growth of the teliospores. Both field and in vitro studies to determine the effect of fumigation and the reintroduction of nonsterile soil would aid in ascertaining factors correlated with increased disease incidence in fumigated

soil.

In both seasons, a background level of disease was present in the 0.0 g inoculum density treatments at Flora (Figure 2.5 and Table 2.5). Soil was collected at least 5-6 cm beneath the surface to reduce the probability of obtaining naturally infested field soil. Soil which had been fumigated and not infested with T. controversa teliospores still produced infected plants. These factors indicated that the inoculum source for the noninfested soil treatments was probably not previously present in the soil but resulted from airborne teliospores blown into the air from adjacent infested microplots or from nearby fields which had dwarf bunt infected plants during the previous season.

Baker (1978) points out that at high inoculum densities the competition for single infection sites may override the multiple infection correction factor so that the inoculum density X disease incidence curves are no longer straight but curve to the right. This curve to the right is evident in all the inoculum density X disease incidence soil treatment curves and is most notable in the inoculum density X disease incidence curve for the fumigated Banida soil (Figure 2.6B).

By sprinkling a suspension of teliospores on the soil surface in the 1983-1984 season, inoculum was more concentrated than when premixed into the soil. Com-

petition between teliospores for suitable infection sites and/or the presence of self-inhibitors could have resulted in the decreased ratio of diseased tillers to teliospore number. Endogenous germination inhibitors have been found in Tilletia spp. teliospores (Trione, 1977). Preliminary studies involving both T. controversa and T. caries suggest that teliospores contain water-soluble substances which can inhibit germination . At higher inoculum densities, these substances could contribute to a reduced amount of teliospore germination and thus reduce the chances for host infection.

Baker (1971) described disease with a simple equation:

Disease = inoculum potential X disease potential.

In this equation, inoculum potential consists of a combination of inoculum density and the influence of the environment on inoculum. Dwarf bunt incidence is influenced not only by inoculum density but also by the environmental conditions. The environment consists of the prevailing weather conditions plus the surrounding soil environment.

Disease potential consists of the proneness of the host to contract disease (Baker, 1971, 1978). The susceptibility of a wheat plant to dwarf bunt infection would depend on cultivar susceptibility and cultural practices such as date of seeding (Baylis, 1955) and depth (Kendrick, 1956; Meiners, et al., 1956; Tyler and Jensen, 1958).

No doubt a complex interaction of these factors influence the inoculum potential and disease potential at any particular site and thus affects the final amount of dwarf bunt which will develop. Further studies involving varying soil and site characteristics would provide a better understanding of the epidemiology of dwarf bunt.

Figure 2.1. Locations of dwarf bunt infestations (shown in black) throughout the winter wheat region of the western United States (shaded in grey) and its occurrence in relation to the average annual snowfall (represented in cm).

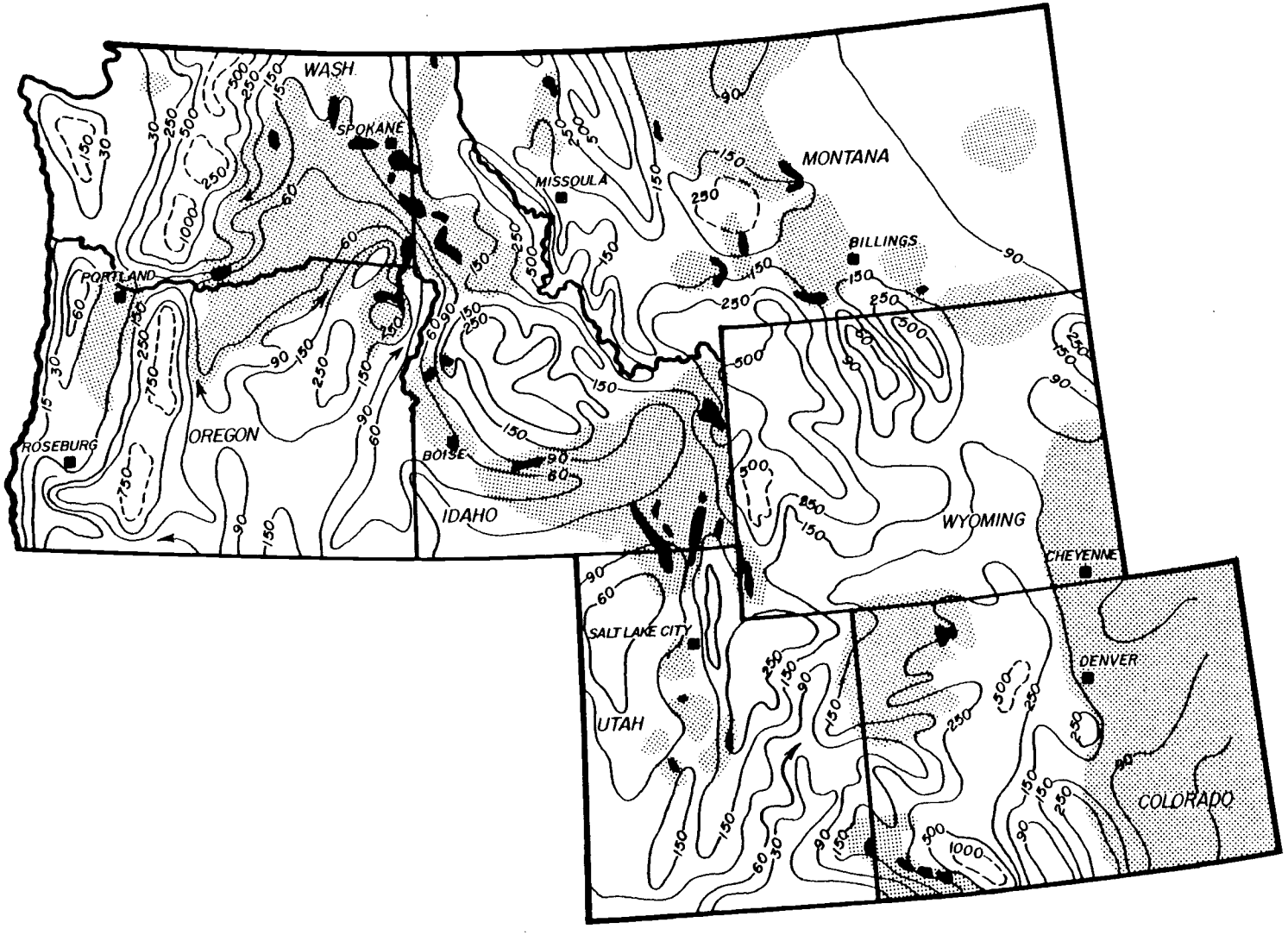
WASH
SPOKANE
MISSOULA
MONTANA
BILLINGS
PORTLAND
OREGON
ROSEBURG
BOISE
IDAHO
WYOMING
CHEYENNE
SALT LAKE CITY
UTAH
DENVER
COLORADO

Figure 2.1

Table 2.1. Number of snow cover days and mean air temperature (°C) for soil sample and/or field experiment locations (1982 - 1984).

Snow cover days	1982 - 83		1983 - 84	
	>2.5 cm	<2.5 cm	>2.5 cm	<2.5 cm
Flora, OR*[y]	43	38	106	27
Logan, UT	60	4	122	4
Pendleton, OR*	2	0	29	17
Banida, ID	96	10	127	6

Temperature	1982 - 83 Avg(°C)[z]	1983 - 83 Avg(°C)
Flora, OR*	0	-2
Logan, UT	-1	-5
Pendleton, OR*	4	0
Banida, ID	-3	-6

[y] Asterik indicates that location was both a field experiment site and a soil collection site.

[z] Average air temperature (°C) from November - February of each growing season.

Table 2.2. Physical characteristics and pH of test soils.[z]

Soil	pH	Particle size distribution (mm) % Sand (2-.05)	Silt (.05-.002)	Clay (<.002)	Textural class
Flora	5.4	23.2	50.0	26.8	Silt/Loam
Logan	7.7	19.6	47.9	32.5	Silt clay/Clay
Pes	6.2	28.1	58.4	13.5	Silt
Banida	8.2	12.5	33.9	53.6	Clay

[z] Data supplied by Soils Testing Laboratory, Oregon State University, Corvallis, Oregon.

Figure 2.2. Individual microplot contained in a 15 cm wide tin can with both ends removed.

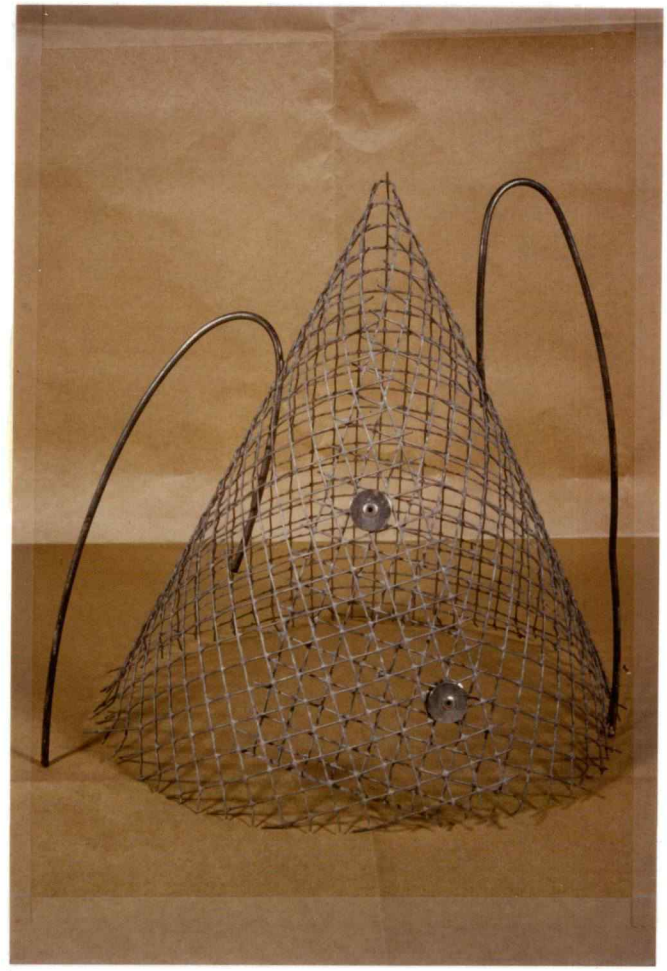

Figure 2.3 A. Chicken wire cone constructed to prevent individual microplots from deer or elk damage at Flora, Oregon.

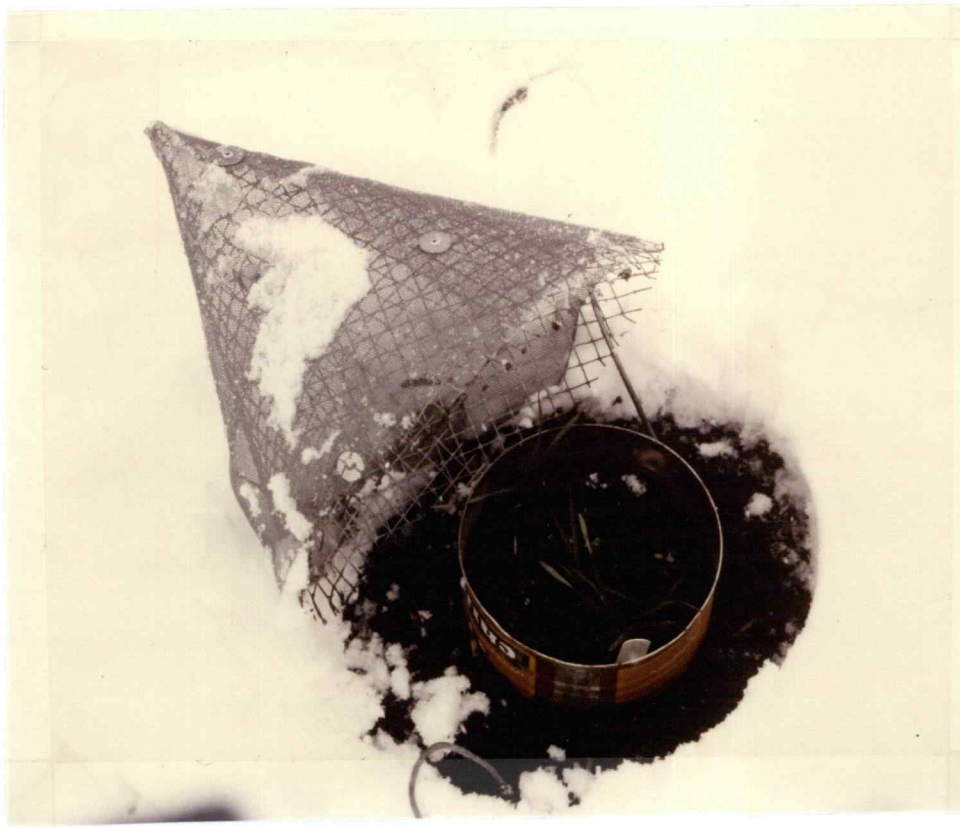

Figure 2.3 B. Chicken wire cone covered with a fiberglass mesh screen to prevent snow from accumulating on designated microplots.

Figure 2.4. Microplots located at Flora, Oregon (1983 - 1984) before (left) and after (right) placement of a test soil over wheat seeds on the native field soil.

Table 2.3. Incidence of dwarf bunt of winter wheat in two soils in relation to the presence or absence of snow cover and/or vermiculite (Flora, Oregon 1983).

Soil	Groundcover	% Dwarf bunt	
		Inoculum density[x] .02	20
Flora	No snow		[z]
	- vermiculite	0.7	14.5 *
	+ vermiculite	0.0	5.3
	Snow		
	- vermiculite	4.9	18.3 *
	+ vermiculite	2.9	2.6
Pendleton	No snow		
	- vermiculite	0.0	8.3
	+ vermiculite	0.0	4.4
	Snow		
	- vermiculite	0.4	45.2 *
	+ vermiculite	0.0	0.5

[x] Inoculum density = grams T. controversa teliospores/45 kg of soil.

[y] Abbreviations: - vermiculite = without vermiculite, + vermiculite = with vermiculite.

[z] Asteriks indicate means of same soil within one inoculum density are significantly different from their corresponding groundcover treatment according to the FLSD test (P = 0.05).

ID	P-VALUE	STANDARD DEVIATION FLORA	LOGAN	PENDLETON	BANIDA
0.0	.1080	.081	.0	.0	.057
.02	.3386	.044	.0	.0	.029
.2	.1358	.023	.113	.068	.033
2	.3083	.152	.219	.368	.161

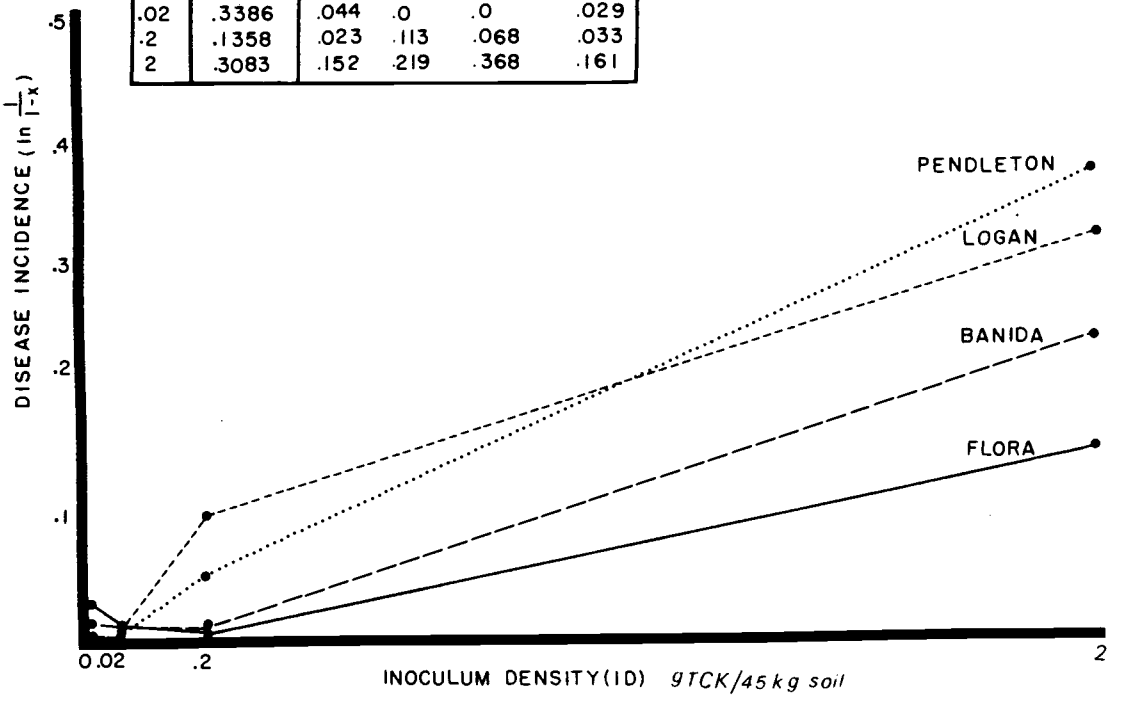

Figure 2.5. Dwarf bunt incidence of winter wheat in four nonfumigated soils (Flora, Oregon 1983). P-values indicate the level of significant difference among the treatment means at each inoculum density.

Figure 2.6A

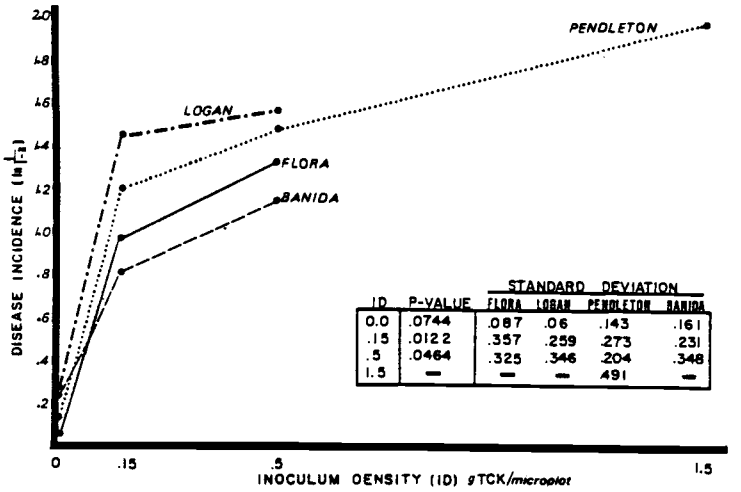

Figure 2.6B

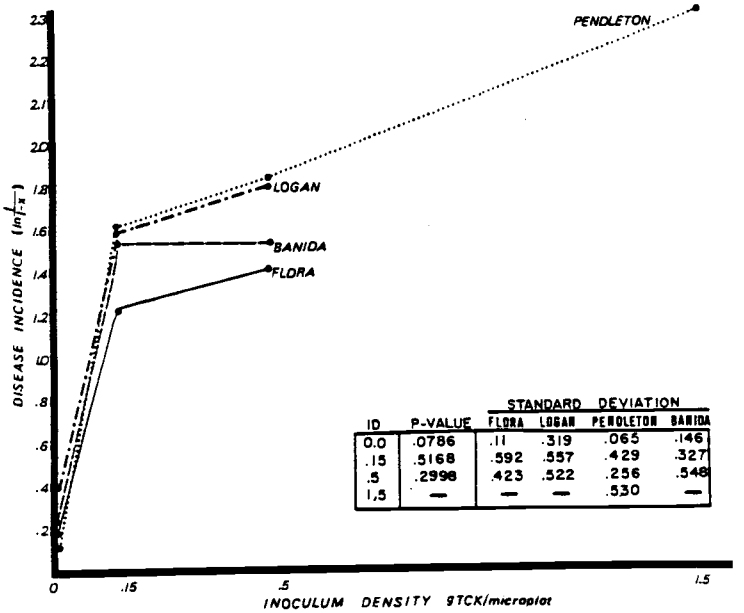

Figure 2.6. Dwarf bunt incidence of winter wheat in four soils (Flora, Oregon 1984). A.) Nonfumigated soils; B.) Fumigated soils. P-values in both figures indicate the level of significant difference among the treatment means at each inoculum density.

Table 2.4. Incidence of dwarf bunt of winter wheat in four soils either nonfumigated or fumigated with methyl bromide (Flora, Oregon 1983).

Soil[x]	% Dwarf bunt	
	Nonfumigated	Fumigated
Flora	35.3 a[y]	43.2 a
Logan	37.7 a	32.9 ab
Pendleton	6.0 c	43.9 a*[z]
Banida	35.2 ab	17.9 b

[x] Soils were infested with 20 g T. controversa teliospores/45 kg of soil.

[y] Means followed by the same letter within a column are not significantly different according to the FLSD test (P = 0.05). Gregory's multiple infection transformation was used in the analysis.

[z] Asterik indicates fumigated teatment mean is significantly different from its corresponding nonfumigated treatment.

Table 2.5 Incidence of dwarf bunt of winter wheat in four soils either nonfumigated or fumigated with methyl bromide (Flora, Oregon 1984).

Fumigation treatment/Soil	% Dwarf bunt			
	Inoculum Density[w]			
	0.0	0.15	0.5	1.5
Nonfumigated				
Flora	8.0 a[x]	60.2 ab	71.7 ab	-[y]
Logan	23.1 a	75.5 a	78.5 a	-
Pendleton	14.7 a	69.7 ab	76.9 ab	84.9 a
Banida	18.7 a	54.7 bc	66.9 b	-
Fumigated				
Flora	15.6	67.6	74.8	-
Logan	30.4	77.8	82.3	-
Pendleton	13.2	78.9	84.3	88.9
Banida	17.8	78.2 *[z]	76.5	-

[w] Inoculum density = grams T. controversa teliospores/45 kg of soil.

[x] Nonfumigated soil means followed by the same letter within columns are not significantly different according to the FLSD test (P = 0.05). Fumigated soil means were not significantly different at any inoculum density. Gregory's multiple infection transformation was used in the analysis.

[y] Symbol: - = soil not infested at this inoculum density.

[z] Asterik indicates fumigated treatment mean is significantly different from its corresponding nonfumigated treatment.

Figure 2.7. Soil heaving in a Banida soil microplot.

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Chapter 3

SURVIVAL AND GERMINATION STUDIES OF *TILLETIA CONTROVERSA* TELIOSPORES IN FOUR PACIFIC NORTHWEST SOILS.

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ABSTRACT

Studies were designed to evaluate the field survival and in vitro germination of *Tilletia controversa* Kühn teliospores in four physically and chemically diverse soils obtained from Pacific Northwest winter wheat fields. Field studies involved placing teliospores on polycarbonate membranes which were buried in these soils at two eastern Oregon locations which differed environmentally (e.g. in amount of snowfall).

A prolonged snow cover is not required for ensuring teliospore survival. Teliospores survived for 18 months at both locations and survived similarly in all four test soils. Teliospore germination, upon retrieval from the soils, followed a seasonal trend with fewer soils containing germinable teliospores in early spring (March -

April) than later in the season (November).

The pH of a soil extract had a significant ($P \leq 0.05$) affect on the amount of in vitro teliospore germination and the area under the spore germination curve (AUSGC). Less germination occurred on soil extracts with a pH of 7.0 or above compared to the germination on soil extract media with pHs of 5 to 6. When soil extract media were all adjusted to a pH of 6.5, there was no significant ($P \leq 0.05$) difference in percent germination among the four media.

Teliospore germination and the AUSGC on soil which had been steam heated at 60°C/30 min significantly ($P \leq 0.05$) increased for two of the four test soils as compared to their respective nonsteamed treatments. When these soils had been fumigated with methyl bromide or had 1% nonsterile soil added back to the fumigated soil, teliospore germination significantly ($P \leq 0.05$) increased on three of the four soils. Germination did not significantly ($P \leq 0.05$) decrease with the addition of 1% nonsterile soil when compared to germination on the respective fumigated treatments.

INTRODUCTION

Interest in factors affecting the survival and germination of dwarf bunt (Tilletia controversa Kühn) has increased in recent years since the Chinese established a quarantine on T. controversa spores in wheat shipments from the United States. In general, germination and the post-germination growth stages prior to infection may be the weakest stages in the pathogen's life cycle (Trione, 1982). Optimum in vitro teliospore germination requires a combination of several unique environmental conditions which include: low temperatures within the optimum range of 2 - 8°C (Lowther, 1948; Baylis, 1958; Meiners and Waldher, 1959; Zscheile, 1965); and the presence of light during at leaśt part of the incubation period. Zscheile (1965) reported abnormal germination resulting in elongated promycelia and delayed sporidia formation due to elevated temperatures or prolonged in vitro incubation in the absence of light. Even under optimum conditions, dwarf bunt teliospores begin to germinate only after 3 - 4 weeks (Dewey and Tyler, 1958) and a population of teliospores may continue to germinate over a sixteen week period (Trione, 1982). Further studies on factors influencing these stages are crucial to a clearer understanding of the biology of T. controversa and the development of control measures.

T. controversa has failed to spread to new sites which have conducive environmental conditions for infection and this suggests that there are additional factors involved in the ability of the pathogen to survive and germinate. Watson and Ford (1972) in their review on soil fungistasis described an organism's ability to germinate as being at least partially dependent on exogenous stimulators and inhibitors present within all soils. Meiners and Waldher (1959) obtained an increase in dwarf bunt teliospore germination on soil extract agar and concluded that there was a water soluble soil factor stimulatory to teliospore germination. Earlier studies by Gassner and Niemann (1955) succeeded in enhancing teliospore germination in the presence of two fungi. These preliminary investigations suggest that there are additional soil factors, either chemical and/or biological, which may contribute to teliospore survival and germination.

The following research describes a series of studies designed to evaluate field survival and in vitro germination of T. controversa teliospores in soils obtained from four Pacific Northwest winter wheat producing fields. Teliospore survival in the field was assessed over an eighteen month period. In vitro tests evaluated the role of the natural soil biomass or water soluble soil leachates found in the four test soils on teliospore germination.

MATERIALS AND METHODS

Preparation of teliospores

Teliospores used in the experiments were collected in July, 1982 at the Pendleton Experiment Station from 1980 - 1981 test plots which used inoculum provided by Dr. James Hoffmann (Crops Research Lab, Logan, Utah). Inoculum was stored as intact bunted wheat heads until needed. Bunted heads were then broken and sori were sieved through a 2 mm followed by a 0.5 mm screen.

Teliospores were surface sterilized with 95% ethanol for 15 min and then rinsed twice with sterile distilled water (SDW) before suspending 0.8 g of the teliospores in 500 ml of SDW with one drop of Tween 20 (Sigma). The spores were kept in continuous suspension with a stir bar. A 0.5 ml of spore suspension was combined with four ml SDW and then vacuum filtered onto a 25 mm, 10.0 um, polycarbonate (PC) membrane (Nuclepore Corp., Pleasanton, CA). This dilution resulted in a thin, single layer of teliospores adhering to the membrane surface.

Evaluation of teliospore germination

The teliospore bearing membranes were incubated on their respective treatments in petri plates placed at 5°C under a continuous low fluorescent light. After 4 weeks, the plates were examined under a compound microscope at 40X to determine percent germination out of a random

sample of 400 spores per membrane. A teliospore was counted as germinated if the promycelium was at least the diameter of the teliospore. Germinated spores were counted at 10 day intervals until it was too difficult to distinguish the individual germinated spores.

Soil collection and preparation

Soil was collected from four wheat producing fields in the Pacific Northwest: (1) a commercial field located at Flora, OR, with a consistent history of dwarf bunt and having a silt/loam soil of pH 5.4; (2) the Pendleton Experiment Station (Pes), Pendleton, OR, with no natural history of dwarf bunt and with a silt soil of pH 6.2; (3) Utah State University, Logan, UT, in a test nursery with a history of dwarf bunt and having a silt clay/clay soil of pH 7.7; (4) a commercial field in southern Idaho (Banida) with only trace amounts of dwarf bunt reported on a clay soil with a pH of 8.2. All four soil samples were transported to Oregon State University where they were air dried for 48 hrs and then sieved through a 4 mm followed by a 2 mm screen.

For experiments requiring fumigated soil, a portion of the appropriate soil was transferred to a 76 l metal trash can for fumigation with a 0.45 lb can of methyl bromide (Bromo-O-Gas, 98% methyl bromide, 2% chloropicrin, Great Lakes Chemical Co).

Field survival study

Teliospores were buried in the field using a modified version of a membrane filter method (Adams, 1967) and a microholder (Gochenaur and Sheehan, 1980) designed for studying soil fungi in situ. The spore holder was constructed from two 75 X 25 X .05 mm plastic microscope slides. A pilot hole was drilled in the center of each slide before enlarging it to a 16 mm circle. A section of dacron mesh was affixed over each hole with a cyanoacrylate adhesive (Krazy Glue, Inc., Chicago, IL). A sterile PC membrane was placed over the spore bearing side of a second PC membrane. These two PC membranes were inserted over the openings of two slides placed with their mesh sides back to back. All slide edges were sealed with time tape (Professional Tape Co., Ill.) The bottom end of a 12.5 cm plastic pot label was glued to one end of the microholder. The pot label extended above ground allowing for easy retrieval of the microholder from the soil.

The test soils and spore microholders were taken to experimental plots located at Flora and Pendleton, Oregon. At each site, 15 cm wide tin cans with both ends removed were inserted three-quarters into the field soil. A 2.5 cm layer of one of the test soils was spread over the surface. Five microholders were placed on top of the test soil in each can and covered with a 3.8 cm layer of the same soil. Spores were buried on 27 September and 13

October 1982 at Flora and Pendleton respectively. Five treatments were located at each site which consisted of the four nonfumigated soils and the fumigated native soil. Each treatment was replicated twice. One microholder was retrieved from each can after 2, 7, 10, 14, and 18 months.

In 1983, additional spore microholders were buried at the Flora site on 24 September. Treatments included either nonfumigated or fumigated soil from each of the four test soils. Each treatment contained six microholders and two microholders from each treatment were retrieved after 2, 7, and 10 months.

After retrieval, the microholders were transported to Oregon State University and dusted with a paintbrush to remove outside soil. The edges were cut and the two PC membranes removed. The membranes were cut in half and placed in culture tubes containing two glass beads and 5 ml of 95% ethanol. After 10 min, the tube was vortexed for 30 sec and the spores placed on a sterile PC membrane mounted on a filter holder. The ethanol was removed by vacuum filtration and the spores rinsed with two washes of SDW. The spore containing membranes were incubated on streptomyocin (Sigma) 2% water agar (Bacto-agar; Difco) adjusted to a pH of 6.5.

Germination on soil extract agar

One thousand grams of each test soil were mixed with 1

l of distilled water. This soil and water mixture was incubated for 24 hrs at room temperature and then filtered through a fluted, crepe filter paper (VWR). Five hundred ml of the soil extract solution were added to 500 ml of distilled water. The pH for each solution was recorded before adding 20 g of Bacto-agar and autoclaving. Teliospores placed on PC membranes were incubated on these soil extract agars. Spores were incubated on 2% water agar as a control. Each treatment was replicated 3 times. The entire experiment was repeated once.

Baylis (1958) reported that spore germination is adequate throughout the pH range of 5.0 to 7.0 but is significantly reduced at a pH above 7.8. To determine if soil pH influenced germination, an additional soil extract experiment was conducted with all soil extract media adjusted to a pH of 6.5 before autoclaving. The treatments were replicated four times and percent germination determined as previously described.

Teliospore germination on steamed or fumigated soil

Moisture content of the four test soils was determined by drying three 5 - 10 g weighed samples of each soil at 110°C for 24 hrs. The oven dried samples were weighed and the percent moisture based on the weight of dry soil was calculated (Johnson and Curl, 1972).

Based upon their calculated percent moisture content, the test soils were adjusted to -0.2 bars based on mois-

ture tension curves calculated by the Soil Physics Lab, Oregon State University (Table 3.1). Moisture levels of the soils were adjusted by adding the appropriate amount of crushed ice and incubating the ice and soil in mason jars for 24 hrs. Half of each soil, approximately 200 g, was placed in additional mason jars and sealed with parafilm. These jars were placed in a water bath and the soil heated at 60°C for 30 min.

Fifty g of each soil, either steam heated or non-heated, were placed in a 10 cm pyrex petri dish. The surface of each plate was smoothed with a spatula and 5 PC membranes carrying a thin layer of teliospores were placed on the surface of each soil plate. Plates were air dried under a sterile hood for 15 min to remove excess surface moisture. Each treatment was replicated twice and the entire experiment was repeated once.

After 28 days, one filter was removed from each treatment, mounted on 2% water agar and percent germination determined. One of each of the remaining membranes was examined at each 10 day interval.

A second experiment used nonfumigated and fumigated soils. Fifty g of each test soil were placed in a 10 cm pyrex petri dish. Some of each fumigated test soil had 1% of the nonfumigated soil premixed back into it (49.5 g fumigated soil + 0.5 g nonfumigated soil) before placement in a petri dish. All soils were adjusted to -0.2 bars by

adding the desired amount of water per plate and thoroughly mixing it with the soil. The surface of each plate was smoothed and 4 PC membranes with teliospores were placed on the surface, air dried, incubated, and percent germination determined. Each treatment was replicated three times.

Statistical analysis

Statistical analyses on all in vitro germination tests were performed by using an analysis of variance followed by Fisher's least significant difference test (FLSD) (Steele and Torrie, 1980). The area under the spore germination curve (AUSGC) was determined by the formula suggested by Dreiseitl and Hlavac (1984).

RESULTS

Germination of teliospores recovered from four Pacific Northwest soils.

Quantitative in vitro germination counts of recovered teliospores were hindered due to fungal and bacterial contamination which developed throughout the extended in vitro incubation period. In both the 1982 and 1983 experiments a considerable number of replicates were entirely lost or were uncountable after one or two observations due to these contaminants. Germination was therefore recorded as a plus (germination) or minus (no ger-

mination).

Teliospores buried in all soils at both sites germinated upon retrieval after 2 months (Table 3.2). Germination was observed more frequently for teliospores recovered from nonfumigated Flora, Logan, and Banida soils at the Pendleton rather than the Flora site. Teliospores buried in the nonfumigated Flora soil at Flora failed to germinate at the 7 and 10 month sampling dates but did germinate at these times when retrieved from this soil at Pendleton. After 18 months, teliospores recovered from nonfumigated Pendleton, Logan, and Banida soils at the Pendleton site still germinated whereas teliospores retrieved from Flora germinated only from the nonfumigated Pendleton and fumigated Flora soils. Teliospore germination occurred in the nonfumigated Pendleton soil at all sampling dates for both sites.

Teliospores were buried in fumigated and nonfumigated soils at Flora in 1983 to obtain more information on survival at this site (Table 3.3). Teliospores again germinated upon recovery from all test soils at the 2 month sampling date. There was a noticeable drop in observed germination upon teliospore recovery at 7 months and germination was then observed in only the nonfumigated Logan and Pendleton soils and the fumigated Banida soil. Teliospores recovered from fumigated Flora, Logan, and Pendleton soils at 7 months failed to germinate but telio-

spores did germinate when recovered from these soils after 10 months.

Teliospore germination on soil extract media

Significant ($P \leq 0.05$) differences were observed in the percent germination on media containing soil extracts from the four test soils (Table 3.4). Teliospore germination on the water agar control was significantly ($P \leq 0.05$) greater than on the Logan or Banida soil extract media and the AUSGC for water agar was significantly ($P \leq 0.05$) greater than the AUSGC on any of the soil extract media. Germination was highest on the Flora and Pendleton soil extract media. Germination on the Logan soil extract medium was significantly ($P \leq 0.05$) lower than on the Flora soil extract medium and germination on the Banida soil extract medium had significantly ($P \leq 0.05$) less germination than on either the Flora or Pendleton soil extract media. The AUSGC for the Flora soil extract medium was significantly ($P \leq 0.05$) greater than the AUSGC for the other three soil extract media. There was no significant difference between the two lowest AUSGC for the Banida and Logan soil extract media.

Previous studies involving the germination of Tilletia spp. teliospores have generally used media adjusted to a pH of 6.5 (Trione, 1972, 1977). When all the soil extract media were adjusted to this pH prior to autoclaving, no significant ($P \leq 0.05$) difference was observed in the

percent teliospore germination or the AUSGC among these media (Table 3.5).

Teliospore germination on steam heated or fumigated soils.

Germination and the AUSGC on the steam heated Logan and Banida soils was significantly ($P \leq 0.05$) greater than on their nonsteamed controls (Table 3.6). Percent germination and the AUSGC on either the Flora or Pendleton steamed soils decreased slightly as compared to their nonsteamed treatments. Germination was not significantly ($P \leq 0.05$) different among the four steam heated treatments.

Percent teliospore germination and the AUSGC on the Logan, Pendleton, and Banida test soils was significantly ($P \leq 0.05$) increased when these soils were fumigated with methyl bromide regardless if 1% nonsterile soil was reintroduced (Table 3.7) Percent germination on the Flora soil did not significantly ($P \leq 0.05$) increase when the soil was fumigated and the AUSGC was significantly ($P \leq 0.05$) reduced on the fumigated and fumigated plus 1% nonsterile Flora soil.

DISCUSSION

Edaphic factors influencing T. controversa teliospore survival and germination have not been adequately investigated. In 1930, Kienholz and Heald commented that clay soil extracts were not quite as satisfactory for growth of Tilletia tritici as agars made from loam soils. Specific characteristics of these test soils were not compared for a more detailed analysis. Meiners and Walder (1959) suggested a water soluble factor in a Palouse silt-loam might be stimulatory to germination. No definitive study has been conducted to investigate the effect of the resident microflora, as a whole, on the amount of teliospore survival and germination. Chemical and biological soil factors need to be considered, in conjunction with the prevailing environmental conditions, for a clearer understanding of the epidemiology of dwarf bunt.

Previous dwarf bunt survival studies have been conducted at sites with known histories of the disease and thus have had environmental conditions considered conducive for the fungus. The T. controversa survival study discussed in this paper was designed to further evaluate the role of soil and environmental factors on teliospore survival in the field and on in vitro germination. Experimental sites included the environmentally differing locations of Flora, with a long history of dwarf bunt, and Pendleton, which

has no natural history of the disease (Table 3.8). Environmental conditions which enhance dwarf bunt incidence (e.g. persistent snow cover and low temperatures) are not necessarily required for ensuring spore survival. Teliospores buried in the nonfumigated Pendleton soil survived equally well at both the Flora and the Pendleton sites.

There was more seasonal variation as to when teliospores did or did not germinate when retrieved from the Flora site. Teliospores buried in the nonfumigated Flora soil at Flora failed to germinate when recovered in April or July (seven and ten months) but again germinated after recovery at 14 months in November (Table 3.2). At Flora, only two soils had teliospores which germinated when retrieved in the spring (March) after eighteen months whereas the Pendleton site had three soils with germinable teliospores. The 1983 set of buried teliospores had a noticeable drop in the number of soils containing teliospores which germinated when recovered in the spring (April) but there was an increase in soils with germinable teliospores in July (Table 3.3).

Hoffmann and Goates (1981) and Hoffmann (1982) have suggested that certain environmental conditions play a role in teliospore dormancy. They retrieved spores monthly from the soil and showed that maximum germination in vitro occurred throughout the summer to late winter months

with the least amount of germination occurring from March to May. They have hypothesized that teliospore dormancy is initiated by protracted, cool periods and broken by warm, dry conditions during the summer months. The seasonal variation for germination of recovered teliospores at Flora in both the 1982 and 1983 set of buried teliospores may be correlated with these proposed environmental conditions affecting teliospore dormancy. A lack of snow cover at the Pendleton site may cause temperature fluctuations which hinder the initiation of teliospore dormancy.

The perennial survival of dwarf bunt inoculum has previously been observed and tested by using either bunted wheat heads or sori. Tyler (1953), Baylis (1958), and Tyler and Jensen (1958) buried either whole wheat heads or intact sori in field soil and obtained at least trace amounts of germination after 3 - 5 years of exposure. Holton, et al. (1949) observed dwarf bunt infected plants in a naturally infested field which was planted to a susceptible cultivar after seven years of resistant cultivars. He further observed infection in plots that were artificially infested with a combination of bunted wheat heads, sori, and powdered teliospores and then protected from outside inoculum sources. Bunted plants were observed in these plots for up to three years after infestation.

These earlier studies, however, did not definitively determine if free teliospores can remain viable and cause infection after field exposure over several successive years. The present survival study conclusively demonstrates that free, individual T. controversa teliospores can remain viable after exposure to field conditions for at least eighteen months. Furthermore, teliospores are capable of surviving over this time period in a broad range of soil types, in fumigated or nonfumigated soil, and at a site with no previous history of dwarf bunt (e.g. the Pendleton site).

Although varying soil characteristics may not play a crucial role in teliospore survival, the pH of a soil extract does have a pronounced affect on the amount of in vitro teliospore germination and the total AUSGC (Table 3.4). Significant ($P \leq 0.05$) differences in percent teliospore germination and the AUSGC among the various soil extract media were observed only when the media were not adjusted to a common pH of 6.5. The differences observed further substantiate observations made by Baylis (1958) who reported a reduction in germination on a clay loam soil when adjusted to a pH above 7.8. Germination on the Banida soil extract medium, with a pH of 7.8, was less than on the other soil extract media. The Flora and Pendleton soil extract media, which were the most acidic soils, had the highest levels of germination and the

greatest AUSGC. pH appears to be at least one soil factor which affects the amount of teliospore germination.

Previous studies (Meiners and Waldher, 1959) reported increased germination on soil extract agar as compared to 2% water agar. Our data indicated that there was no advantage to using soil extract media regardless of pH adjustment.

In an attempt to find a particular portion of the microbial biomass which might influence teliospore germination, the four test soils were steamed in a hot water bath at 60°C/30 min. The use of moist heat can selectively eliminate various groups of soil organisms (Baker and Cook, 1974) and has been successfully used to identify specific groups of bacteria and fungi antagonistic to Gaeumannomyces graminis (Gerlagh, 1968; Shipton, et al., 1973) and Phytopthora cinamomi (Broadbent, et al., 1971).

No significant ($P \leq 0.05$) differences in germination or AUSGC were detected between nonsteamed or steam heated Flora or Pendleton soils. However, germination and the AUSGC on the steamed Logan and Banida soil plates (Table 3.6) did significantly increase compared to their respective nonsteamed soils. This increased germination could indicate the influence of non-spore forming bacteria in suppressing teliospore germination. Certain fluorescent Pseudomonas spp. produce siderophores on iron deficient substrates and successfully chelate iron making it

unavailabe for other organisms (Baker and Chet, 1982). The alkaline pHs of the Banida and Logan soils could create this sort of iron deficient environment and thus steam heating at 60°C/30 min would eliminate the competitive Pseudomonas spp. and teliospore germination would increase.

Since Dobbs and Hinson (1953) first reported the occurrence of a widespread fungistasis in soils, much interest has been focused on the role of the microbial population in this phenomenom. Cook and Baker (1983) discuss the general suppression of a soil pathogen as it is directly related to the total amount of microbial activity. The microbial biomass competes with the pathogen for energy sources. Microbial competition could result from the usage of spore exudates. The nutrient sink hypothesis (Ko and Lockwood, 1967) proposes that a nutrient gradient is established whereby a propagule is drained of its endogenous energy reserves. Nutrient independent spores, such as T. controversa teliospores which readily germinate on water agar, would become nutrient dependent. This form of fungistasis is directly dependent on the microbial activity of the soil.

The elimination of the resident soil microflora by methyl bromide fumigation with or without the addition of 1% nonsterile soil increased the amount of teliospore germination and the total AUSGC for the Logan, Pendleton,

and Banida soils compared to the respective nonfumigated treatments (Table 3.7). Fumigation could have freed compounds previously unavailable to the teliospores due to the nutrient gradient created by microbial competition. Both fumigation and steam heat treatment may have enhanced teliospore germination by eliminating microbial competition and thus freeing nutrients stimulatory to teliospore germination.

The addition of 1% nonsterile soil, however, did not significantly ($P \leq 0.05$) decrease germination when compared to the germination on the corresponding fumigated soils. This amount of added soil may not have been a sufficient amount to successfully transfer any biological suppressing component. Menzies (1959) reported that the transfer of as much as 10% suppressive soil did not successfully suppress the disease incidence of potato scab caused by Streptomyces scabies. A transfer of up to 50% nonsterile soil was needed to adequately reduce disease incidence. Further experiments transferring various amounts of nonsterile soil could demonstrate a biological component which contributes to the suppression of T. controversa teliospore germination.

Environmental conditions are a major factor in determining the survival of T. controversa teliospores, the germination of these spores, and their ability to infect the host. The Flora and Logan sites are environmentally

conducive (e.g. have a persistent snow cover) for dwarf bunt infection yet the soils have widely differing pHs and significantly ($P \leq 0.05$) different AUSGCs on soil extract agars. Additionally, differing responses to soil fumigation or steam heat treatment occurred between these soils. Both these sites, however, have consistent histories of dwarf bunt.

Although favorable environmental factors play a strong role in enhancing the level of disease incidence at a specific site, these factors are not crucial for the survival of T. controversa teliospores as noted by the amount of teliospore survival at both the Flora and Pendleton sites. These preliminary tests also indicate that soil factors such as pH and perhaps the microbial biomass do influence the amount of teliospore germination. The comparision of in vitro teliospore germination on soil extracts obtained from nonsterile, fumigated, fumigated plus 1% nonsterile, heat treated, and heat treated plus 1% nonsterile soils could provide a clearer understanding of the soil biomass role in T. controversa teliospore germination. A complex interaction among these varying soil components and the prevailing environmental conditions probably determines the final level of T. controversa teliospore survival, germination, and subsequent host infection.

Table 3.1. Soil moisture tensions[y]

Soil sample	(-) Bars			
	0.1	0.8	2.0	15.0
Flora	45.7[z]	24.0	21.7	14.1
Logan	37.4	21.2	18.7	9.5
Pendleton	47.6	17.3	14.9	6.3
Banida	40.9	24.1	23.2	18.8

[y] Calculated by the Soil Physics Laboratory, Oregon State University, Corvallis, Oregon.

[z] Calculated as % moisture by dry weight.

Table 3.2. In vitro germination of Tilletia controversa teliospores recovered at periodic intervals from four Pacific Northwest soils placed at two eastern Oregon sites (1982 - 1984).

Site/ Soil	Teliospore germination[z]				
	Nov 1982 (2 mos)	Apr 1983 (7 mos)	July 1983 (10 mos)	Nov 1983 (14 mos)	Mar 1984 (18 mos)
Flora					
Flora	+	-	-	+	-
Logan	+	x	x	-	-
Pendleton	+	+	+	+	+
Banida	+	+	-	+	-
Flora fumigated	+	x	x	+	+
Pendleton					
Flora	+	+	+	+	-
Logan	+	+	+	+	+
Pendleton	+	+	+	+	+
Banida	+	+	+	+	+
Pendleton fumigated	+	+	+	+	-

[z] Symbols: + = germination observed, - = no germination observed, x = germination not determined due to contamination. Germination was observed on 2% water agar.

Table 3.3. In vitro germination of Tilletia controversa teliospores recovered at periodic intervals from four Pacific Northwest soils placed at Flora, Oregon (1983 - 1984).

Soil/treatment		Teliospore germination[y] Nov 1983 (2 mos)	Apr 1984 (7 mos)	July 1984 (10 mos)
Flora	NF[z]	+	-	-
Flora	F	+	-	+
Logan	NF	+	+	+
Logan	F	+	-	+
Pendleton	NF	+	+	x
Pendleton	F	+	-	+
Banida	NF	+	x	+
Banida	F	+	+	+

[y] Symbols: + = germination observed, - = no germination observed, x = germination not determined due to contamination. Germination was observed on 2% water agar.

[z] Abbreviations: NF = soil not fumigated with methyl bromide, F = soil fumigated with methyl bromide.

Table 3.4. Influence of four soil extract media on percent germination of teliospores of Tilletia controversa.

Media	pH[x]	% germination after 58 days:[w]	AUSGC[y]
Flora	5.7	37.3 a[z]	795.2 b
Logan	7.0	24.0 bc	489.2 d
Pendleton	6.1	32.5 ab	649.9 c
Banida	7.8	20.2 c	433.4 d
Water agar	-	37.5 a	1147.9 a

[w] Percent germination based on 400 spores per replicate.

[x] pH of soil extract media prior to autoclaving.

[y] AUSGC = area under the spore germination curve (0 - 58 days).

[z] Means followed by the same letter are not significantly different according to the FLSD test (P = 0.05).

Table 3.5. Influence of four soil extract media adjusted to pH 6.5 on percent germination of teliospores of Tilletia controversa.

Media	% germination after 48 days:[x]	AUSGC[y]
Flora	40.1[z]	153.4
Logan	39.4	146.5
Pendleton	35.1	209.9
Banida	35.7	123.7

[x] Percent germination based on 400 spores per replicate.

[y] AUSGC = area under the spore germination curve (0 - 48 days).

[z] Means within columns are not significantly different according to the FLSD test (P = 0.05).

Table 3.6. The influence of soil steaming of four Pacific Northwest soils on the germination of *Tilletia controversa* teliospores.

Soil	% germination after 48 days[v]: Steam treatment[x] -	Steam treatment[x] +	AUSGC[w] -	AUSGC[w] +
Flora	37.8 b[y]	36.0 a	630.5 b	469.5 b
Logan	22.0 c	52.5 a*[z]	484.6 b	878.8 a*
Pendleton	56.6 a	53.1 a	963.4 a	785.5 a
Banida	25.8 bc	45.7 a*	462.5 b	811.3 a*

[v] Percent germination based on 400 spores per replicate.

[w] AUSGC = area under the spore germination curve (0 - 48 days).

[x] Symbols: - = soil not steam heated, + = soil steamed with moist air at 60 C/30 min.

[y] Means followed by the same letter within columns are not significantly different according to the FLSD test (P = 0.05).

[z] Asteriks indicate that steam heated means are significantly different from their corresponding nonheated treatments.

Table 3.7. The influence of methyl bromide fumigation of four Pacific Northwest soils on the germination of *Tilletia controversa* teliospores.

Soil/treatment[y]	% germination after 48 days:[w]	AUSGC[x]
Flora NF	51.0 a[z]	905.9 a
F	50.0 a	763.3 b
1%	47.5 a	695.9 c
Logan NF	22.2 b	499.2 c
F	62.3 a	1086.4 a
1%	55.2 a	909.4 b
Pes NF	50.0 b	722.3 b
F	62.8 a	996.3 a
1%	61.7 a	946.6 a
Banida NF	23.1 b	412.0 c
F	43.2 a	662.1 b
1%	51.5 a	821.6 a

[w] Percent germination based on 400 spores per replicate.

[x] AUSGC = area under the spore germination curve (0 - 48 days).

[y] Abbreviations: Pes = Pendleton Experiment Station; NF = soil not fumigated with methyl bromide, F = soil fumigated with methyl bromide, 1% = soil fumigated with methyl bromide and then 1% nonsterile soil reintroduced.

[z] Means followed by the same letter within columns of the same soil are not significantly different according to the FLSD test (P = 0.05).

Table 3.8. Site characteristics

Location	Month	Mean Temp.[y]	Climatological Data 1982-83 Snow cover days[z]	1983-84 Snow cover days[z]	
Flora/	Oct	7	4	9	0
	Nov	0	19	3	9
	Dec	-2	22	-5	31
	Jan	0	15	-4	31
	Feb	2	7	-1	29
	Mar	4	7	4	24
	Apr	6	7	5	9
Pendleton/	Oct	10	0	10	0
	Nov	3	0	7	1
	Dec	3	2	-5	28
	Jan	5	0	-5	17
	Feb	6	0	4	0
	Mar	8	0	8	0
	Apr	9	0	9	0

[y] Mean air temperature in degrees Celsius.

[z] Numbers represent days per month.

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Chapter 4

CONCLUSIONS

Growing site had a pronounced effect on the amount of dwarf bunt development. The Flora location, with its prolonged snow cover, increased the overall amount of disease which developed in each soil as compared to the disease which occurred at the Pendleton site with its limited amount of snow. Likewise, microplots exposed to the natural snow cover always developed more bunted spikes than in microplots protected from the snow. A snow cover buffered the soil surface and protected T. controversa teliospores from extremes in moisture and temperature fluctuations and thus provided a more suitable micro-environment for germination and infection. The use of vermiculite to simulate a snow cover, however, reduced disease incidence when applied at sowing. Plant emergence was delayed and the microclimate surrounding the teliospores was probably altered thus influencing the final amount of infection.

A snow cover, however, was not necessary for teliospore survival. Teliospores buried in the test soils survived for 18 months at both the Flora and Pendleton sites. Likewise, dwarf bunt occurred at Pendleton from inoculum which had been in the test soils since the

beginning of the previous season.

Both in vitro germination tests and field studies also suggested that certain chemical, physical, and perhaps biological soil characteristics influenced the amount of T. controversa teliospore germination and subsequent host infection. Soil moisture may play a crucial role in determining the final amount of disease incidence. The Banida test soil, with its high clay content (54%), had poor drainage characteristics which allowed for the build up of excess surface water. Anaerobic conditions resulting from the excess water may have predisposed teliospores and/or their post-germination products to lysis thus resulting in the reduced amount of disease observed in this soil during both seasons and perhaps exhausting the inoculum resevoir for the subsequent season's infection. Likewise, the soil heaving which occurred in this soil could have redistributed the teliospores away from favorable infection sites. In vitro germination tests demonstrated that teliospore germination was reduced on soil extracts with a pH of 7.0 or above as compared to more acidic pHs of 5 to 6. Both the physical characteristics of this soil plus its more alkaline pH (8.2) contributed to the reduced amount of disease.

The Pendleton soil, from a site with no natural history of dwarf bunt, was conducive for infection as demonstrated by the high incidence of disease which

developed in this soil at Flora. At the Pendleton site, the soil was conducive for dwarf bunt but not the site. The Flora and Logan soils, from sites with favorable environmental conditions for infection, differed in the amount of dwarf bunt infected plants which they produced. Both soils had higher sand and silt contents (73% and 68% respectively) and thus better water drainage characteristics than the Banida soil. The Flora soil, however, with a favorable pH for teliospore germination (5.4) produced fewer bunted plants than found in the Logan soil with a more alkaline pH of 7.7.

Fumigation of the test soils with methyl bromide eliminated any significant differences in percent disease incidence which had been detected among the four non-fumigated soils. Likewise, in vitro germination tests using teliospores placed on fumigated or steam heated (60° C/30 min) soil resulted in an increase in percent germination on 3 and 2 of the test soils respectively as compared to the nontreated controls. Germination did not significantly decrease, however, on fumigated soils with 1% nonsterile soil reintroduced. Studies using increased percentages of reintroduced nonsterile soil or soil extracts from fumigated or steamed soils could further indicate if the increased germination response is due to the elimination of microbial competition and the release of compounds stimulatory to germination. The final amount of

dwarf bunt which occurs at any given site is no doubt the result of a complex interaction between both the prevailing environmental conditions and chemical, physical, and biological soil components.

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