THE UNIVERSITY OF MANITOBA

SNOW MOLD DISEASE OF TURFGRASSES IN MANITOBA

THE CAUSAL ORGANISMS AND THEIR CONTROL

BY APPLICATION OF BORAX AND OTHER ORGANIC

NON - MERCURIAL COMPOUNDS

BY

LESLIE R. ALLEN

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With the completion of this study, came an end to soaken-wet feet, aching backs and thoroughly chilled bones.

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GENERAL ABSTRACT

An in vitro test was conducted to determine the effects of different rates of borax on the growth and/or hydrogen cyanide production of various pathogenic low-temperature basidiomycete (LTB) isolates. All rates of borax reduced the mycelial growth of each isolate considerably, but appeared to have no effect on the hydrogen cyanide gas production by the gas producing LTB-Type B (W5) and LTB-Saskatchewan isolates. Borax was found to be more detrimental to the growth of LTB-Manitoba and LTB-Type A (W1), which appeared to be similiar in many ways, than to LTB-Saskatchewan and LTB-Type B (W5).

A second in vitro test investigated the effects of borax concentration and pH on the growth of three pathogenic isolates of the LTB, each being incubated on different culture media. Borax was inhibitory to the growth of all the isolates, particularly at a concentration exceeding 100 ppm. Borax, at all concentrations, was extremely inhibitory to fungal growth with increasing pH on all culture media used. Borax was not a highly lethal compound in vitro, but did have good fungistatic ability against the LTB isolates. On the basis of growth inhibition, the LTB-Manitoba isolate was the most sensitive and the LTB-Saskatchewan least sensitive to the effects of this compound.

Finally a field study, conducted at various locations in southern Manitoba, during the fall and winter of 1973-74 was performed to determine the causal organisms and to find a non-mercurial, organic compound or mixture of compounds which would effectively control snow mold of turfgrasses in our area. Snow

mold damage was caused predominately by Typhula FW[1]. Sclerotinia borealis and the psychrophilic, sterile basidiomycete was also abundant. Damage caused by F. nivale was not excessive. Variation in pathogen abundance did occur and was probably due to a complex of factors which were not revealed in this study. At several of the areas studied and surveyed a complex of 3 or 4 pathogens was found causing damage to the turfgrass. Chemical application towards the end of October provided the most effective control of snow mold in Manitoba. Mercuric chloride (standard control compound) gave excellent control of the snow mold pathogens at all test locations. A combination of borax and Terraclor 75W also provided excellent broad spectrum control of snow mold in 1973-74. Phytotoxicity was a major problem but if this could be eliminated via a more uniform application and reduction in rates used, the borax: Terraclor 75W combination shows promise for snow mold control in Manitoba. All compounds were effective in controlling specific group(s) of pathogens except mercuric chloride and the borax combination treatments which gave a broad spectrum control.

1) Nomenclature of species given by Dr. J. D. Smith, C. D. A. Research Station, Saskatoon, Saskatchewan.

GENERAL INTRODUCTION

Throughout the ages man has always strived to change the environment about him. He has altered the landscape for political, economic and aesthetic achievements. Today there is an increasing demand for such aesthetic areas as parks, golf courses, country clubs, etc., to remove oneself from the tensions and aggravations of modern day life. The major component of these relaxation centres is turf which, in association with ornamental flowers, shrubs and trees, gives beauty and serenity to the area. Nothing is more displeasing than to see the vegetation of these areas obliterated by disease.

The most devastating disease of turf and turfgrasses in Western Canada is the overwintering disease commonly referred to as snow mold. It is to be expected that, with an increased interest in sports and amenity turf, there will develop a better appreciation of the part played by overwintering and other diseases in turf quality and appearance (81). This will lead to the development of simple, inexpensive control techniques that will pose no threat to our environment.

Several fungal pathogens are known to be causal agents of snow mold disease and depending on the conditions prevalent, one or more of the pathogens may predominate at a particular location in a given year. Furthermore, the host range of each of the snow mold pathogens is extremely diverse. Because of these complexities, effective snow mold control is best achieved by chemical treatment of the turf.

In the past and even today mercurial compounds are being used as a general broad spectrum control measure of the snow mold or anisms on golf and bowling greens (53,66). The many disadvantages (3,8,31,53) of mercurials have made researchers realize the importance of finding non-mercurial organic compounds which are fungicidal or fungistatic to all the low-temperature turfgrass pathogens. This concern lead to the present study which investigated the causal organisms of snow mold in Manitoba and the controlling capabilities of several organic compounds and combinations of organic compounds against the pathogens present.

General Review of Literature

Introduction:

The major infectious diseases of turfgrasses are caused by fungi (5). Bacteria and viruses are not important in the total spectrum of turfgrass pathogens (5). A great many fungal diseases are known to occur in turfgrass. Helminthosporium spp., for example, are able to attack the Kentucky bluegrasses, fescues, ryegrasses, bentgrasses, and Bermudagrass and cause diseases such as melting out, zonate eyespot, helminthosporium leaf spot, brown blight and leaf blotch (17). Other turfgrass diseases include brown patch (Rhizoctonia solani), dollar spot (Sclerotinia homeocarpa), red thread, powdery mildew, septoria leaf spot, fairy ring, and last but not least snow mold, the most important disease in our area and in western Canada (17).

Snow mold has been under investigation for many years in North America, Scandinavia and Japan (53). The common psychrophilic organisms which are known to be the causal agents of the disease are: (1) Fusarium nivale (Fr.) Snyder and Hansen (21), (2) Typhula itoana Lasch ex. Fr. (73,74,88), (3) T. idahoensis Remsburg (73,74,88), (4) Sclerotinia borealis Bubak and Vleugal (51,88), (5) An unidentified low-temperature basidiomycete (LTB) (6,7), (6) T. trifolii (23), (7) T. graminum (61,74). In addition, Smith (84) has found a microsclerotium-producing LTB in association with snow mold patches on domestic lawns, golf course fairways and bowling greens in Saskatchewan. A fungus with an orange rindless sclerotium (ORS), believed to be antagonistic to the major snow mold pathogens, and a Typhula sp. which does not fit the description

of T. idahoensis Remsburg or T. incarnata Lach ex. Fr. have also been isolated in Saskatchewan (81).

The majority of the literature deals primarily with the first five mentioned organisms which are known snow mold pathogens of turfgrasses (7,96) and it will be these to which attention will be directed.

Distribution of the Snow Mold Pathogens:

The distribution of these low-temperature pathogens appears to follow a geographical pattern (54). S. borealis Bub and Vleug. is found in the colder regions, the distribution of Typhula spp. is more intermediate in the temperate zone and F. nivale (Fr.) Snyder and Hansen occurs farther south in the slightly warmer regions (51,54). This distribution exists both in Scandinavia and in America (51,54). The unidentified LTB is prevalent in northern and central western Canada (7,15) but has also been isolated in Alaska (54).

However this is only a general distribution pattern and local variations in microclimate may be of more significance in determining pathogen prevalence than the climate generally. For example, Smith (84) has isolated all the causal organisms throughout southern Saskatchewan and it appears that many of these organisms are present also in Manitoba (70). In addition, Vaartnou and Elliott (96) reported severe snow mold damage to lawn caused by F. nivale in the Beaverlodge area of Alberta in 1967. Bruehl (9) reported that Typhula spp. and F. nivale often occur together in the Pacific Northwest to form a disease complex. Also Richardson & Zillinsky (76) reported F. nivale as far south as Mexico (76). Therefore, one can

soon realize that the geographic separation of these psychrophils is not so distinct.

Fusarium nivale:

(A) Taxonomy and Cultural Characteristics:

Fusarium nivale (Fr.) Snyder and Hansen is the causal agent of fusarium patch or pink snow mold of winter cereals, turf and forage grasses (21,88). F. nivale is taxonomically in the class Fungi Imperfecti, order Moniliales (88). The perithecial stage of F. nivale is referred to as Calonectria graminicola (Berk and Br.) Wr. (88).

Cultural studies by Dahl (21) have disclosed that scant aerial, colourless, and sterile mycelial growth is produced on corn-meal agar. However, very abundant mycelial growth developed on oat-meal agar and spores were produced in slimy salmon-coloured masses which often covered a large part of the surface of the agar. On potato-dextrose agar there was an abundant, white fluffy, spore-bearing mycelium. On sterile grass clippings, the fungus covered each leaf with a fluffy white mycelium that in a few cases produced spores.

On potato-dextrose agar F. nivale grows at temperatures from 0°-32° C. with an optimum of 20° C. (21). Dahl (21) has also reported that no sporulation occurs when cultures are kept in total darkness, but when grown at 20° C. in diffuse light, abundant mycelial growth and sporulation occurs.

(B) Hosts:

Turfgrasses which are known hosts of F. nivale are annual bluegrass, Colonial bentgrass, creeping bentgrass, Italian ryegrass,

Kentucky bluegrass, perennial ryegrass, redtop, red fescue, rough bluegrass, sheep fescue, tall fescue, velvet bentgrass and many other grasses (5). Field tests by Dahl (21) and Wernham (106) have indicated that the Seaside strain of creeping bent was highly susceptible to attack by F. nivale, Washington only moderately susceptible, whereas Astoria and Metropolitan bentgrasses were resistant to attack. Lebeau (57) has isolated F. nivale from damaged areas of Penncross bentgrass. Dahl (21) found fescue to be highly susceptible to fusarium snow mold. Ricke and Vargas (77) reported F. nivale on the Merion strain of Poa pratensis. This was also shown by Lebeau in 1968 (57). The host range of F. nivale is indeed very wide (88) and in some cases is not even restricted to Gramineae (9). Bruehl (9) believed this to be presumptive evidence of a low degree of pathogenic specialization in this pathogen.

(C) Symptomatology:

When F. nivale causes disease in the absence of snow, it is referred to as fusarium patch; the damage produced under snow or at the margins of melting snowbanks is called pink snow mold. In the absence of snow, the symptoms appear on turf as irregular pale-yellow to white circular areas ranging from 2 inches to 1 foot or more in diameter (5,17,21). Mycelium is usually not apparent on the leaves under these conditions (5). Under a snow cover, or during prolonged, cool, wet weather, the irregularly circular diseased areas may be covered with a mat of aerial mycelium - at first white and then turning to a faint pink colour with longer exposure to light

(5,17,21). The areas so damaged feel slimy when wet and the surface dries to form a crust (5,17,21). In both situations, the infected areas may coalesce, thus damaging large areas of turfgrass (17).

(D) Life Cycle and Infectivity:

The pathogen survives adverse periods as dormant mycelium in the host or in debris of diseased leaves (17). Couch (17) stated that abundant conidial production occurs soon after the development of optimal environmental conditions and that the conidia are carried to the leaves by wind and/or splashing water where primary infection is accomplished by penetration through stomata. Its progress through the tissue is intercellular until the cells begin to collapse after which the pathogen moves intracellularly (21). After the mycelium becomes abundant in the tissue, sporodochia develop through the stomata in rows (21,76). Usually, only the leaves are attacked, but under severe disease conditions, the pathogen may infect the crowns (17).

(E) Factors Influencing Development:

Several factors favour the development of fusarium patch or pink snow mold. Climatic conditions that favour attacks of the disease are: cloudy weather; abundant moisture in the fall; temperatures of 0° - 5°C; snow falling on unfrozen ground, deep snow; and a prolonged, cold, wet spring (21,66). High nitrogen levels in turfgrass soils favour the development of F. nivale (21,31,62,77). Beard (3) stated that this is because the cell walls of the turfgrass hosts are thinner and more easily penetrated by fungal hyphae. Bruehl (10) and Dahl(21) reported that a high

nitrogen level allows the plants to go under the snow with a higher rate of basal metabolism than normal. This reduces carbohydrate reserves which in turn results in incomplete hardening and increased susceptibility to attack.

Smith (83) reported increased attacks of fusarium patch disease following application of ground limestone and suggested that a high pH in the upper soil regions increased disease incidence. Other reports (82) also have indicated that alkaline soil conditions favour disease development while acidic soil conditions reduce disease severity. Other management practices involving the use of straw mulches, the build-up of deep thatch layers and matting of the turf will prolong wet, cold conditions and thus favour the growth and development of F. nivale (21).

Typhula spp.:

(A) Taxonomy and the confusion which exists:

The genus Typhula is taxonomically positioned in the class Basidiomycetes (88) because of the presence of hyphal clamp connections (18). Until Remsburg's work in 1940 (75) much confusion about the genus Typhula existed in the phytopathological literature. This confusion resulted from the causal organisms of the now known typhula snow molds being reported as Sclerotium rhizodes Averswald (74), Sclerotium fulvum Fries (74), Typhula graminum (74), and T. itoana (74,75,88,106). The confusion had come about partly because of the macroscopic resemblance of the sclerotia of the above-named fungi as described by early mycologists, and partly because of the

inability to collect or otherwise obtain fertile sporophores, which are essential for taxonomic classification (74). These reasons in conjunction with the geographic distribution (North America, Europe, Japan and Scandinavia) of typhula snow molds (73,74), have led to the array of names given to the causal organisms. Typhula species which have been reported in the literature to be the causal agents of snow molds of cereals and grasses are: T. incarnata Lasch ex. Fr. (61,90), T. itoana Imai (16,79), T. graminum Karst (61), T. ishikariensis Imai (61), T. idahoensis Remsberg (75), T. borealis Ekstrand (61), T. hyperborea Ekstrand (61), T. trifolii (23), and Typhula (FW) spp. (81).

According to Corner, as reported by MacDonald (61), T. incarnata and T. itoana are synonyms, but T. incarnata has priority over T. itoana in usage. T. incarnata and T. graminum have been reported to be distinctly different species (61,75). According to Remsberg (75), T. graminum has not been demonstrated as a causal organism of snow mold. MacDonald (61) has reported that Corner believes T. graminum not to be uncommon but that it is a small, inconspicuous pathogen. MacDonald (61) reported that Ekstrand had divided his isolates into two species, T. borealis and T. hyperborea, on the basis of the length-width ratio of their basidiospores only. Some doubt as to the reliability of such a taxonomic character is suggested by Ekstrand's statement that; "the length of the spores is very variable in different fruit bodies and in different collections. The relationship between the length and width of the spores is very variable too, due to the variation in the length which is great even between the spores of fruit bodies from the same collection".

Ekstrand (61) has identified T. borealis on material collected in Sweden, Finland, Canada and Norway. Ekstrand (61) has maintained that his isolates do not fit Remsberg's description of T. idahoensis. Despite this, Jamalainen (41) has since acknowledged that isolates of Typhula occurring in Finland agree with the description of T. idahoensis. In addition, MacDonald (61) stated that the description given by Imai for T. ishikariensis agrees fairly well with those given by Remsberg (75) for T. idahoensis and Ekstrand (61) for T. borealis, considering the variability of the fungus.

Typhula FW is a common snow mold pathogen in Saskatchewan (81). It produces sclerotia which are approximately 0.25 to 0.5 mm in diameter (about half that of T. idahoensis), globular, tawny in colour when young and dark brown to black when mature (81).

In summary, the reported causal agents of typhula blights or snow molds are; T. incarnata (= T. itoana), T. ishikariensis (= T. idahoensis = T. borealis = T. hyperborea), T. graminum and T. trifolii and Typhula FW (61,81). Since T. incarnata and T. ishikariensis are the most commonly reported organisms causing typhula snow molds, the remaining discussion will be confined to them.

(B) Cultural Characteristics:

According to Remsberg (75) T. idahoensis grows over a range of 0° - 18° C., with an optimum temperature of 9 - 12° C. when cultured on potato-dextrose agar. Mycelial growth is abundant, fluffy and concentrically banded (75). In contrast, Sprague (88) has stated that growth is slow and its culture tedious. Dejardin (23) has shown that 5° C. was slightly better than 10° C. for growth

of T. idahoensis and T. trifolii on malt yeast-glucose (MYG) agar and 20° C. was maximum.

Remsburg stated that sclerotia of T. idahoensis are produced in 5 - 10 days, are clustered or in concentric rings and are always produced singly never coalesced into masses (75). Recently, Cunfer (19) has shown that the cultural characteristics of monokaryotic and dikaryotic cultures of T. idahoensis, grown on PDA at - 1 to 20° C. for 20 days, differed primarily in sclerotia production. Generally the dikaryons produced sclerotia 1 - 2 mm in diameter that often coalesced into mounds. Most monokaryons produced fewer sclerotia than did the dikaryons and the sclerotia from the monokaryons were about one-third to two-thirds the size of dikaryotic sclerotia (19). Dejardin (23) has shown that sclerotia of T. idahoensis and T. trifolii first appeared after 4 days when incubated at 10° and 15° C. on MYG agar, but eventually were produced at all temperatures which permitted growth and most abundantly at temperatures above 10° C.

According to Remsburg (75) sterile brown sporophores develop abundantly from the chestnut-brown sclerotia of T. idahoensis in culture. Remsburg (75) however, and many others since, have shown that short rays of light plays an important role in the fructification of these fungi. Through a series of tests Remsburg (75) has shown that fertile sporophores can be produced in culture when exposed to light waves in the region of 2700 to 3250 Angstroms.

In contrast to the growth of T. idahoensis, oxygen uptake was optimal and the respiratory quotients higher at 20° C. than at

5° C. (23). This suggests that growth becomes completely uncoupled from respiration at 20° C. and above (23). Therefore it is possible that the mechanisms responsible for coupling growth to respiration may be abnormally heat-sensitive and thus determine the maximum temperature for growth (23). However the exact coupling mechanisms are not known.

Dejardin (23) has also shown that the optimum pH for growth of T. idahoensis, T. trifolii and T. incarnata lies between 6 and 7.

Dejardin (23) has observed the growth range of T. incarnata, T. idahoensis and T. trifolii to be from temperatures of approximately -5° C. to a maximum of 20° C. Unlike T. idahoensis and T. trifolii, growth of T. incarnata was optimal at 10° C. When grown on potato dextrose agar, T. incarnata has been shown to exhibit growth over a range of 0° - 18° C. with an optimum of 9° - 12° C. (75). Furthermore mycelial growth is abundant, white, webby, radiating, concentrically banded and fan shaped (75). On MYG agar, at 20° C. T. incarnata grew slowly, the resulting colonies being brown instead of white and irregular in appearance (23).

Remsburg (75) has stated that sclerotia of T. incarnata appear in 5 - 10 days, are pinkish orange when young and tawny to hazel brown when mature, single or coalesced with a tendency to develop in concentric rings (75). Sterile sporophores frequently develop from the sclerotia, but unlike T. idahoensis they are white in colour (75). Miss Remsberg (75) has observed that a reddish-brown stromatic crust often develops in culture with small sclerotia arising on it.

(C) Hosts Attacked:

Throughout North America, Northern Europe and Japan, certain fungal species of the genus Typhula are important psychrophilic pathogens of cereals and grasses (8,9,10,40,75). Others have been reported to cause severe damage to legumes such as alfalfa (16) and to strawberries (60). Cormack and Lebeau (16) have reported T. idahoensis to be highly virulent on creeping red fescue, red top and winter wheat, while moderately virulent to Kentucky bluegrass. T. incarnata is known to attack such hosts as quackgrass, annual bluegrass, Kentucky bluegrass, rough bluegrass, and Holcus mollis, colonial bentgrass, creeping bentgrass, Italian ryegrass, perennial ryegrass, red fescue, tall fescue and velvet bentgrass (5,33). Roberts (63) has found differences in Typhula susceptibility among the many bentgrass varieties. From the less to the more susceptible he listed Astoria, Congressional, Penncross, Old Orchard, Washington, Arlington and Seaside bentgrasses. In 1941 Wernham (106) reported the reactions of several varieties of Agrostis palustris and A. tenuis to T. itoana attack. He found the varieties Metropolitan, Astoria and Washington were very susceptible, susceptible and moderately susceptible, respectively, to attack by the pathogen. Beard, as reported by Britton (5), stated that varieties of creeping bentgrass vary widely in susceptibility to T. itoana, Seaside and Cohansey being very susceptible; Penncross, Washington and Toronto moderately susceptible and Congressional and Astoria being quite resistant. Vargas et al. (100) found that the Kentucky bluegrass cultivars of Delta, Merion, Park, Newport, and Prato were quite susceptible to T. itoana attack.

(D) Differences between T. itoana and T. idahoensis:

Remsburg (74) has stated that T. idahoensis causes a disease of the same type as that caused by T. itoana. However, in addition to the differences previously referred to T. itoana and T. idahoensis do differ in other respects. T. itoana has been reported to be a facultative parasite and under certain conditions can become well established saprophytically before injuring living tissue (40). Cunfer and Bruehl (18) observed that T. idahoensis grew as a saprophyte only when competition from other micro-organisms was minimal and rarely found it as a saprophyte on straw in the field. The development and virulence of T. idahoensis has been reported to be much greater than that of T. itoana on winter wheats (8,10). Bruehl and Cunfer (10) have shown that T. idahoensis was pathogenic on wheat at temperatures of -1.5° C. whereas T. itoana was not. T. idahoensis is believed to attack leaves primarily with most of its sclerotia being formed on the expanded leaves (22). On the other hand, T. itoana is less restricted to aerial tissues and its sclerotia are formed most frequently below the soil surface between basal leaf sheaths and on roots (22). Holton (38) found T. idahoensis prevalent in areas of heavy snow cover of long duration while T. itoana predominated in areas with light snow cover of relatively short duration.

(E) Disease Symptomatology:

A variety of descriptive terms have been coined for the snow mold disease caused by T. itoana and T. idahoensis, but typhula blight, snow scald, or grey snow mold have had the widest acceptance (40). Jackson and Fenstermacker (40) have noticed that under cool wet conditions in the late fall and spring, mild disease symptoms

appear on turf as small, roughly circular 3 to 6 inch diameter water-soaked patches that are light yellow to fawn in colour. However, the snow mold Typhulas are particularly active under a snow cover, after which the symptoms are intensified and invaded plants are covered with a dense, aerial mycelial growth (17,40). The mycelium is white, but often appears as shades of grey due to atmospheric pollutants in the snow that are deposited on the hyphae as the snow melts (hence the name "grey snow mold"). Affected areas range up to 2 ft. in diameter and frequently coalesce to involve larger areas of turfgrass (17,40). During the spring thaw the presence of numerous ovoid to spherical sclerotia embedded in the leaves and crowns of infected plants is the chief diagnostic feature of typhula blight (17,40).

(F) Life Cycle of the Pathogen:

The pathogen survives the warm summer months in the form of sclerotia (58,75). In late October, November, and early December under the stimulus of cool temperatures (10-17° C.), humidity above 70% and exposure to light rays of short wavelength (2700-3200 Å), the sclerotia germinate to form sporohpores or mycelia (40,75,89). Even though basidiospores are not considered to be important sources of inoculum (18,22,89), they could allow sexual recombination to occur providing added potential for variation in this pathogen (19). In the absence of light, under a snow cover and over an unfrozen ground, mycelial growth progresses and initiates the primary infection centers (17). With rising air temperatures in the spring, the pathogenic capabilities of the fungus are decreased and sclerotia are produced thus serving to

carry the fungus in a dormant condition over the summer months (40). A period of desiccation is necessary before the sclerotia will produce new growth (40). Davidson and Bruehl (22) reported that Lehmann believes this to be the reason new sclerotia do not germinate in the spring.

(G) Factors Influencing Development of the Pathogen:

The environmental conditions which favour typhula blights can be divided into two classes: 1) the cool weather of late October, November, and December, high humidity, and exposure to light rays of short wavelength which stimulate sclerotial germination and 2) early snow falling on wet, unfrozen ground which greatly enhances infection (17,40,89). Davidson and Bruehl (22) found that tillage or burying the sclerotia of T. idahoensis to depths of greater than 2 cm. rendered them ineffective. Lehmann, as reported by Davidson and Bruehl (22), found that sandy soil favoured the development of typhula blights which he attributed to reduced antagonism. Studies by Davidson and Bruehl (22) support Lehmann's hypothesis. This disease is also reported to be favoured by excessive late nitrogen applications and grass left long in the fall (82).

The Unidentified Low Temperature Basidiomycete (LTB):

(A) Interesting Characteristics of the Pathogen:

A basidiomycetous fungus causing snow mold of turfgrass in Alberta was first described by Broadfoot (6). The same fungus was later found to cause a severe winter crown rot of legumes (7) and its pathogenicity on a variety of species of grasses and legumes has been demonstrated under natural (14,15) and artificial (16) conditions. The apparent lack of fruiting bodies or spores, the ability to grow at low temperatures and the presence of hyphal clamp connections has

led to the use of the term "low-temperature basidiomycete" in describing the fungus (7).

The most intriguing of the snow mold organisms is the unidentified LTB. The reasons for this are: 1) at the present this organism has been isolated only in western Canada (14) and Alaska (51); 2) it has a very wide host range which includes a variety of species of grasses (winter wheat and turfgrasses) and legumes (Alfalfa, clovers, etc.) (14,15,16); 3) it produces no fruiting bodies or spores which would allow exact identification of the organism (101), only characteristic basidiomycete hyphal clamp connections (6,7) and 4) as reported by Lebeau and Dickson (48,49), it produces hydrogen cyanide (HCN) gas in culture and on the host in concentrations that are highly toxic to host tissue. These findings have been confirmed by subsequent experiments (50, 52,101) and HCN production is believed to be the major factor in the etiology of diseases caused by this fungus. HCN production would also account for its wide host range (16).

Ward and Lebeau (101) have shown that at least three different isolates of the LTB do exist. They are 1) Type A, which is much more pathogenic on alfalfa than on turfgrasses, produces HCN only in association with the host and has low HCN tolerance in culture, 2) Type B, which is highly pathogenic on turfgrasses, has high HCN tolerance and produces HCN in culture as well as in association with the host, and 3) Type C, which exhibits a high growth rate, has no HCN tolerance, produces no HCN and is believed to be completely saprophytic.

(B) Cultural Characteristics:

The cultural characteristics of these isolates also differ, inspite of the fact that all produce white sterile mycelium with typical clamp connections (7,101). The Type A isolates are slow growing compact colonies, with mycelium mostly appressed to the substratum. This group is also characterized by stroma-like bodies which develop at the surface of the agar medium and are often arranged in concentric rings. Type B isolates are distinguished from that of Type A by production of abundant, fluffy aerial mycelium. The colonies generally grow more rapidly than those of Type A and stromata are not produced under normal cultural conditions. The Type C isolates are a heterogeneous group; in culture the colonies generally grow much more rapidly than those of Type B and produce less aerial mycelium. Ward et al. (101) reported that Garrett has suggested this high growth rate may be a characteristic typical of soil saprophytes. Stromata are produced by certain isolates of the group.

Broadfoot and Cormack (7) have stated that the cardinal temperatures for growth of the LTB are -4, 15 and 26°C. Generally, the best growth of all types and isolates occurs at 12.5° to 17.5°C. on Difco potato dextrose agar, however appreciable growth also occurs at 0° and 5° C. (101). This is consistent with their natural activity under the snow. On malt-yeast-glucose agar the optimal pH for growth of all types was generally similar in that it laid between 6.0 and 7.0 (101).

Ward and Lebeau (101) found that all the types were sensitive to antibiotics which might indicate poor competitive saprophytic ability. This would undoubtedly be offset by their

comparatively high growth rates at temperatures where competition is probably greatly reduced.

(C) Hosts Attacked:

The host range of the LTB is indeed very wide. Cormack (15) found that several varieties of alfalfa were highly susceptible to the pathogen; only the species Medicago falcata was highly resistant. Sweet, red and alsike clovers were all found to be damaged by the LTB. Other legumes, such as bird's foot trefoil and strawberry clover, also were readily attacked by the LTB. Brome grass and Agropyron species were highly resistant, while the rye-grasses and Kentucky bluegrass were moderately to highly resistant. The fescues were moderately resistant. Timothy was moderately susceptible and was frequently damaged under natural conditions. Red top (Agrostis alba L.) and the winter cereals proved to be very susceptible. Horticultural plants such as strawberry, parsnip, iris and tulip were highly susceptible. Cormack (15) has shown that a wide range of native plants and weeds also suffer severe damage from the LTB.

(D) Disease Symptomatology:

The symptomatology of the disease caused by the LTB allows it to be readily distinguised from fusarium or typhula snow molds (7,17). The affected areas of turf, covered with a mat of light-grey hyphal growth, can range up to one foot in diameter (7,17) and these may coalesce and involve large sections of turfgrass (17). The plants beneath the mycelial mat are usually killed (14). With the resumption of plant growth at the first spring thaw, the irregularly shaped areas of grass appear pale yellow at first and then change to straw colour (17).

(E) Factors Influencing Development of the Pathogen:

Since this organism causes severe damage to forage crops and turfgrasses (6) in western Canada, discussion of the factors influencing development is warranted. Saturation of the soil (14) and the atmosphere (16) is detrimental to the development of the low-temperature basidiomycete and subsequent infection of the host. Cormack (14) obtained 77% infection when the soil moisture content was approximately 35% mhc as compared to no infection when the soil moisture was about 90% mhc. Cormack and Lebeau (16) found that the LTB grew best and caused severe infection when the relative humidity was about 80-90%.

There has been much controversey over the importance of a snow cover to snow mold disease development. Observations made by Cormack (14) indicated that the amount of snow cover has much less influence on the development of winter crown rot or snow mold than the time and manner in which it melts. A slow melt in late March or early April can result in very severe snow mold damage to forage crops and turfgrasses (14). Lebeau et al (52) have shown that maximum HCN absorption by host tissue occurs about mid March. Snow removal or melt prior to this time would allow rapid dissipation of HCN and, therefore, reduction in infection and disease damage. Observations by the author indicated that a snow cover would protect the LTB and other snow mold organisms from the drying effects of strong winter winds. Since damage to the host only occurs at temperatures near or at 0° C. (16), snow cover would allow maintenance of this temperature (plus or minus a few degrees) at the

soil surface for the majority of the winter and early spring (26). Bruehl & Cunfer (10) stated that a deep snow cover maintains darkness at the soil surface, prevents photosynthesis, stabilizes humidity and favours vegetative development of snow mold fungi.

Low temperature appears to be the most important single factor favouring snow mold development (16). Although most snow mold fungi grow best at temperatures ranging from 10 - 17° in culture (except F. nivale), they also develop well at 0° C. and cause damage only at low temperatures (16). With the LTB this is apparently the result of increased absorption of HCN by the host at low temperatures (16,49). The relationship of temperature to HCN absorption has been explained on the basis of the gas law which states that the lower the temperature the greater the solubility of a gas in a solvent (49). Furthermore, Lebeau (55) stated that low temperature effects cell permeability in such a way as to make the glycoside substrate from the host available to the enzyme from the fungus. Also low temperatures would reduce competition against the snow mold fungi.

From early studies, Cormack (14) believed that the LTB was strictly a parasite of dormant plants and susceptibility to attack by the pathogen was associated with the physiological changes accompanying dormancy and hardening in the host plant. Results obtained by Lebeau and Dickson (49) indicated that there was no significant difference in susceptibility between dormant and vegetatively active host tissue. Furthermore, Cormack and Lebeau (16) found that a host conditioning period of at least two weeks

at 2 - 5° C., with eight hours light was necessary for maximum disease development in pot inoculation experiments.

An association period of 45 -60 days between the host and pathogen, in the field, is required for absorption of lethal concentrations of HCN and subsequent infection of the host by the pathogen (49,52). Inoculation experiments by Lebeau et al (52) indicated that the period between September and November was critical for development of the disease. Failure to obtain infection when plants were inoculated on or after November 15th showed that the fungus must become established in the fall in order to incite the disease (52). From studies on alfalfa, Cormack (15) has shown that early seeded stands suffered less from the LTB than late seeded stands; also late fall cutting of alfalfa tops retarded the spread of the pathogen, but unfortunately predisposed the plants to winter injury. He also has shown that infection spread was favoured more when there was debris on the ground than when it was bare. He stated that crop rotation with resistant grasses was the most valuable cultural method of controlling winter crown rot, since the pathogen did not survive for longer than two to three years in the absence of a susceptible host (15). In 1959, Cormack & Lebeau (16) found that alfalfa and grass seedlings under three weeks of age proved less susceptible to snow mold than older plants. Observations by Smith (82) indicated that older lawns (2 years or more) can be severely damaged by the LTB whereas new lawns usually escape infection.

Little is known about the manner in which the LTB persists in nature or in the absence of a susceptible host.

(F) The LTB - An Unspecialized Parasite:

Several investigations have implicated disease development to be dependent upon the production of HCN by the fungus and the confinement of the lethal agent in close vicinity of the plant parts until specific tissues are killed (48,49,52,101). Histological studies conducted by Lebeau and Dickson (49) and Lebeau, Cormack and Moffatt (52) showed that mass invasion of the host had not occurred until the tissue had absorbed lethal quantities of HCN - this being about mid-March. Lebeau et al (52) observed that the mycelium was occasionally massed into stromata which functioned as infection pads and initiated penetration through host tissues. On the other hand, Lebeau and Dickson (49) in 1955 stated, "the development of the mycelium through the host cell walls occurred without the formation of special structures of penetration". These contradicting observations may have been due to the existence of different LTB isolates. Regardless, according to Garrett (30) both mechanisms of invasion suggest that the LTB is an unspecialized parasite displaying an extremely low form of parasitism with greater development of the organism on the dead tissues of the host.

(G) Hydrogen Cyanide Production and Its Importance to LTB Pathogenicity:

Many investigations have been done concerning how and when hydrogen cyanide is synthesized and released from the LTB. Much of this work has been focused upon HCN production by the Type B isolate.

Ward and Lebeau in 1962 (102) and Ward in 1964 (103) demonstrated that HCN was a product of autolysis in the Type B isolate. Their results indicated that the release of HCN by the basidiomycete occurred from a cyanogenic compound that accumulated in the mycelium during growth and which broke down chiefly during autolysis.

More recent studies strongly suggest that the amino acid, glycine is involved in the formation of a cyanogenic compound which, upon hydrolysis, leads to HCN formation (104). Furthermore, Ward and Thorn (104) observed that HCN production was not associated with autolysis but occurred throughout the incubation period. Unfortunately no information was given on the exact nature of the pathway linking glycine to HCN production.

In 1968, Stevens and Strobel (91) demonstrated that the amino acids, valine and isoleucine, were utilized by the Type B isolate to produce typical cyanogenic β-glycosides in the mycelium. They identified these as linamarin and lotaustralin. They also found that two β-glycosidases in conjunction with oxynitrilase were capable of hydrolyzing the cyanogenic precursors to form HCN. These researchers proposed a hypothetical pathogenic scheme which indicates that the production of HCN is involved in the reduction of host resistance to a level where mass invasion by the psychrophilic Type B isolate is possible. The importance of HCN gas in reducing host resistance and thereby allowing establishment of an infection court has also been reported for the basidiomycete Marasmius oreades (28).

Mass invasion of the host would be followed by even greater cyanide production. Cyanide could originate from hydrolysis of the fungal cyanogenic glucosides by fungal β-glucosidases, but could also originate from hydrolysis of host cyanogenic glycosides by the fungal enzymes. Work by Lebeau et al (52) substantiated this latter point by the fact that there was a rapid increase in cyanide concentration in the alfalfa crown tissue during the period of mass invasion of the host by the fungal mycelium.

Unlike the Type B isolate, it appears that the release of HCN by the Type A isolate is due only to some specific interaction between the host and the fungus, with the host itself possibly being the source of HCN (102). Work by Colotelo and Ward (13) supports this possibility. They have suggested that the production of HCN in infected alfalfa plants in the field was due to β-glucosidase activity, which was secreted extracellularly, on the part of the fungus and the provision of the cyanogenic substrate by the host.

Sclerotinia borealis

(A) Taxonomy:

There are a number of important diseases of Gramineae caused by members of the Ascomycetes. Several investigations have observed the production of apothecia, asci and ascospores by Sclerotinia borealis Bub & Vleug, thereby taxonomically classifying it as an Ascomycete (29,34,87,107).

(B) Distribution of the Pathogen:

S. borealis was first described by Vleugel in 1917 (34). Since that time it has been isolated as the causal organism of snow mold of grasses and winter cereals in Canada, Alaska and the Yukon, Norway, Sweden, Finland, U.S.S.R., northern Japan and northern United States (41,42,51,79,90,95,105).

(C) Cultural Characteristics:

Cultural studies of S. borealis, or S. graminearum as it is sometimes called (90) have indicated that it is a highly psychrophilic fungus because of its ability to grow on frozen substrates and at temperatures of less than -5^{o} C. (105). This ability is attributed to this organism's capacity to tolerate high osmotic forces (reduced H_2O potentials) which result upon freezing of a substrate (10).

(D) Disease Symptomatology:

Field diagnosis of S. borealis is simple and reliable, based on locating irregularly shaped, black sclerotia 0.5 - 7 mm. in length in the leaf axils and shoot bases of damaged turfgrass (81).

(E) Factors Influencing Development of S. borealis:

It has been suggested that sclerotial germination, apothecial development and ascospore dissemination are favoured by long, moist (RH 70-100%), and warm autumn weather (29,41). Development of S. borealis is further enhanced by freezing of the soil and the presence of a deep, prolonged snow cover (10,29,41,44,90). Bruehl and Cunfer (10) have suggested that the ascospores, not the sclerotia, serve as the major source of inoculum.

(F) Hosts Attacked:

Species of Gramineae appear to be the primary hosts of S. borealis. This pathogen has been reported to attack winter

cereals (41,51,90,95), varieties of Kentucky bluegrass (44,51), bromegrass (51), Agrostis (34), Agropyron (34), Dactylis glomerata (33,76), and many, many others. Smith (81) has stated that Seaside and Penncross creeping bengrass are more susceptible to S. borealis than are the Colonial bentgrasses.

Snow Mold Control:

Since snow mold is a chronic problem of forage crops, winter cereals, and turfgrass throughout many parts of the world, control of this disease is of the utmost importance.

Control of turf disease can be achieved by the use of resistant varieties, cultural practices such as mowing, fertilizer programs, thatch removal, topdressing and syringing, and the use of chemicals (5). Snow mold is probably one of the most difficult diseases to control because 1) several fungal pathogens are known to be causal agents, 2) one or more of the pathogens could predominate at a particular location in a given year depending on the conditions prevalent (24,90), 3) the extent of the host range of each pathogen is very diverse which severely hinders the development of resistant varieties, 4) certain cultural practices may be detrimental to some of the pathogens while favouring the development of others, and 5) the majority of the chemical compounds used today are too specific in fungicidal or fungistatic action.

(A) Chemical Control:

Development of a compound or combination of compounds is probably the most realistic way of achieving effective control of snow mold on a year to year basis. However, many factors should

be realized and taken into consideration before one begins applying chemical compounds to the entire countryside. As stated, the compounds (except the mercurials) being tested and used today are too specific to give effective control of snow mold year after year. Since fungi, like other micro-organisms, possess the capabilities of rapid variation and adaptation, development of resistance to fungicides used on a regular basis can soon occur (12,64). The application of fungicides or other pesticides to turf and ultimately to the soil can benefically or detrimentally affect the dynamic equilibrium of the soil microflora. Smith et al (80) have reported that application of benomyl predisposed turf to a pathogenic unidentified basidiomycete by stimulating its growth and/or reducing the population of its competitors. In 1973, Harder and Troll (35) found that Trichoderma spp., a common soil saprophyte, parasitized the sclerotia and reduced the levels of T. itoana inoculum in the field. Chemical treatment of the soil could physiologically or ecologically stimulate or retard the growth of Trichoderma. Successive applications of mercurial compounds to golf greens have been reported to reduce the numbers of cellulose-decomposing fungi in the soil, thereby allowing an accumulation of litter or thatch (72). Successive applications of chloroneb have been observed to enhance the development of Sclerotinia homeocarpa, the causal organism of the turfgrass disease commonly called dollar spot (11).

The Mercurials

In the past and even today mercurial compounds such as $HgCl_2$, PMAS and mixtures of mercurous and mercuric chloride are being used as a general control measure of the snow mold organisms on golf and bowling greens (53,66). In Washington State, Bruehl (8) has reported that mercurial fungicides will control snow mold caused by F. nivale, T. itoana, T. idahoensis and S. borealis. Smith (84) and Lebeau (53) have reported that effective control of the LTB can be obtained with mercury fungicides applied prior to snow fall.

The mode of action of mercury compounds has been reported to be via the inhibition of respiration and protein synthesis (94). Ward and Thorn (104) reported that mercuric chloride strongly inhibited HCN production by the LTB but this could have been a secondary effect due to inhibition of other metabolic processes.

The high cost of mercurial compounds makes their use uneconomical for treatment of winter cereals, forage crops and golf-course fairways (8,53). Furthermore their high mammalian toxicity makes their use very hazardous (53). Mercury compounds, particularly the inorganics, are not biodegradable and hence have a long residual life in the soil (3,31). Pugh and Williams (72) reported that successive applications of an organic mercury compound to golf greens reduced the population of saprophytic cellulose-decomposing fungi to such a level that thatch build-up became a problem. Another drawback with mercury based compounds is phytotoxicity to putting turf (31,99).

The many disadvantages of mercurial compounds has put pressure on researchers to find non-mercurial organic compounds which are fungicidal or fungistatic to the snow mold pathogens.

The Non-Mercurials

(a) Pentachloronitrobenzene:

Pentachloronitrobenzene (PCNB, Terraclor, Quintozene) is a chlorinated hydrocarbon used as a soil fungicide and seed disinfectant (93). This organic compound is usually applied to turfgrasses as a foliar spray and is effective in controlling S. borealis (42,44). Torgeson (94) and Thomson (93) stated that PCNB was ineffective against Fusarium spp. However, Smith (84) reported that the efficacy of quintozene was similiar to phenyl-mercuric acetate (standard of comparison) in controlling F. nivale. Jackson (39) also found that PCNB was effective in controlling F. nivale. In a fungicide study in northern Wisconsin, PCNB formulations and Caloclor (mercuric + mercurous chloride) provided the best overall results in the control of typhula snow mold (109). From Saskatoon, it has been reported that PCNB was not effective in controlling the microsclerotial LTB (84) but did give effective control of the LTB (81). PCNB is the recommended non-mercurial fungicide for the general control of snow mold in Saskatchewan (81).

Pentachloronitrobenzene is considered non-phytotoxic (93). This was substantiated by Kallio (44) who found no injury to turfgrass treated with this compound.

Torgeson (94) reported that the chlorinated hydrocarbons were fungistatic at levels that effectively controlled plant diseases. Torgeson (94) suggested that these compounds somehow altered the host and thus increased disease resistance. This was supported by the work of Pohjakallio (71).

Pentachloronitrobenzene is a very stable compound and therefore, is a long lasting soil fungicide (2).

Some of the recent developed systemic fungicides are now being used effectively in the control of turfgrass diseases.

(b) Chloroneb:

Chloroneb (Demosan, Tersan SP) is a chlorinated hydrocarbon used as a soil fungicide and seed treatment (64,93). The active ingredient is 1, 4-dichloro - 2, 5-dimethoxybenzene (64,93). It is compatible with other pesticides (93), has low mammalian toxicity (64) and does not leach readily from the soil because of its low solubility and volatility (93). It is believed the mode of action is via inhibition of fungal DNA synthesis (64).

Choroneb is readily absorbed and translocated within plant tissues (45). Some studies have revealed that chloroneb is uniformly distributed throughout plant tissue via upward, lateral and downward translocation (45); others have found chloroneb to be localized as well in the hypocotyl and root regions (92). Acoording to Thapliyal and Sinclair (92) this localization would be most effective in controlling root and lower stem infections. This may account for the high effectiveness chloroneb has in controlling

typhula snow mold (59,97,99,108,109) which can damage leaves and/or crown and root tissue (5). Vargas and Beard (97) found that granular chloroneb was more effective in controlling typhula blight on Agrostis spp. than the wettable powder formulation. He stated that this response may have been due to a slower rate of breakdown, and a longer residual effect. Marsh (64) reported that chloroneb gave only poor control of Fusarium spp. whereas Zehr et al (108) reported that Cole et al. found that it provided excellent control of typhula and fusarium snow molds on various bentgrass varieties. Smith (81) found that this compound also significantly reduced infections caused by the LTB, S. borealis and F. nivale.

Chloroneb is non-phytotoxic when used as recommended (93,97,99), however as reported by Zehr et al. (109), Goldberg & Cole observed that a chloroneb/benomyl combination was phytotoxic to the Highland cultivar of bentgrass.

(c) The Oxathiins:

The oxathiin compounds are another group of systemics which are readily absorbed through the foliar cuticle and translocated within the plant (86). A unique feature of the oxathiins, carboxin (Vitavax) (5, 6 - dihydro - 2-methyl - 1, 4 - oxathiin - 3-carboxanilide) and oxycarboxin (Plantvax) (5, 6 - dihydro - 2-methyl - 1, 4-oxathiin - 3-carboxanilide - 4, 4-dioxide) is their specificity for members of the fungal class Basidiomycetes (65). Unfortunately, Smith (84) found that Vitavax gave relatively poor control of the microsclerotial LTB that attacks bluegrasses

and Jackson and Fenstermacher (40) demonstrated that Plantvax was ineffective against T. incarnata infested bentgrass. However, fungicide tests in Saskatoon (84) indicated Vitavax to be effective against F. nivale.

Generally Vitavax is not phytotoxic when used as recommended (93) but it has been observed to induce chlorosis and reduce tillering in Ustilago striiformis infected bluegrass (37).

Translocation studies involving carboxin have shown it to move primarily in an upward and lateral direction when absorbed through seedling roots with localization in the epicotyl tissue (45,92). This would appear to indicate that carboxin would be most effective against pathogens invading these tissues (92).

Mathre (65) believes that carboxin controls sensitive basidiomycetes by inhibiting respiration and nucleic acid synthesis.

(d) The Benzimidazoles:

Benomyl (Benlate or Tersan 1991) or Methyl 1 - (butylcarbamoyl) -2- benzimidazolecarbamate and thiabendazole (Mertect, Tecto, or Thibenzole) or 2 - (4 - thiazolyl) benzimidazole are two systemic compounds which are derivatives of the benzimidazole group of fungicides (64,94). Both have been reported to be broad spectrum systemics (36,64), very safe chemicals to use (64), non-phytotoxic when used as directed (36,93) and growth stimulants (especially benomyl) because of their cytokinin-like activity (64).

Benomyl has been reported to be more effective than thiabendazole (TBZ) in controlling some plant pathogens (86,98).

Solel and Edgington (86) attributed this greater effectiveness to the fact the benomyl is more readily absorbed and translocated throughout plant tissue than is TBZ. Furthermore benomyl has been reported to have excellent residual activity (93) whereas TBZ was found to be short-lived when applied as a soil fungicide (25).

In aqueous solution and within plant tissue benomyl was found to be completely transformed to MBC (methyl benzimidazol-2-yl-carbamate), and it is possible that this stable breakdown product is partly or even wholly responsible for the fungitoxicity (64). There are no indications of any metabolic breakdown of TBZ in vivo (64). The mode of action of MBC appeared to involve the inhibition of DNA synthesis or some closely related process such as nuclear or cell division (64). Allen and Gottlieb (1) concluded from their experiments with Penicillum atrovenetum that inhibition of respiration probably is the primary effect of TBZ.

Benomyl and its breakdown product MBC are apparently translocated upwardly and laterally and therefore are effective against pathogens attacking epicotyl tissue (67,69,92). Thiabenzadole appears to be translocated in an upward and a downward direction (64).

In regard to the major snow mold genera referred to in this review, benomyl and TBZ have been reported effective in controlling Fusarium spp. and Sclerotinia spp. which are the causal agents of turf diseases such as fusarium patch, fusarium blight and dollar spot (Sclerotinia homeocarpa) (20,32,64,68,93,98).

In 1972 Smith (81) found benomyl and thiabendazole to be ineffective against F. nivale on Poa annua turf. In Saskatchewan, TBZ has been shown to be effective in controlling LTB on artifically inoculated turf whereas benomyl provided no significant control (81). Smith (81) stated that benomyl and TBZ significantly reduced infection on Agrostis/ P. annua turf caused by S. borealis. Unfortunately benomyl has also been reported to stimulate or favour the growth of other turfgrass pathogens (78,80).

(e) Borax:

Conflicting reports do exist regarding the effectiveness of borax, the active ingredient being sodium tetraborate, in controlling snow mold of turfgrasses and legumes such as alfalfa (27,53). Specificity is one of the problems, for borax appears to be effective in controlling only the LTB (53,56). Phytotoxicity is another problem associated with the use of borax compounds. Lebeau et al (53) reported this in 1961 when all plots that received sufficient spray to control snow mold showed signs of chlorosis. From this test they also concluded that foliar sprays of borax were more effective than soil treatments in controlling this disease. In addition, it was observed that borax gave effective control of the LTB on alfalfa but gave practically no control of the disease on turfgrass. Results obtained by Ferguson (27) indicated that borax was very effective in controlling snow mold on putting greens and dry application of borax was at least as effective as foliar sprays. Smith (85) found that a dry application of borax to stumps of white fir controlled F. annosus most effectively whereas a water-borax-sticker treatment was the least effective.

Borax does not appear to be a highly lethal compound (46). In 1967 Lebeau and Atkinson (56) stated that it was unlikely that borax acted as a fungicide since the Type B isolate was able to grow at relatively high concentrations of this compound. From studies involving both the Type A and B isolates they presumed that borax somehow interferes with the synthesis of a cyanogenic substrate in the host or fungus rather than inhibiting its enzymic hydrolysis (56).

RESULTS OF RESEARCH

1. The Effects of Borax on Pathogenic Isolates of the Low-Temperature Basidiomycete.

ABSTRACT

The effects of different rates of borax on the growth and/or HCN gas production of pathogenic LTB isolates from Alberta, Saskatchewan and Manitoba were investigated. The LTB-Man and Type A (W_1) isolates appeared to be similar in many ways. All rates of borax reduced the linear mycelial growth of each isolate considerably, but appeared to have no effect on HCN gas production by the gas producing LTB - Type B (W_5) and LTB-Sask isolates. Borax was found to be more detrimental to the linear growth of LTB-Man and Type A (W_1) than to LTB-Sask and Type B (W_5).

INTRODUCTION

The low temperature basidiomycete (LTB) is an important snow mold pathogen of turf and forage grasses in western Canada (1,2) and Alaska (5).

Borax (sodium tetraborate), an inexpensive, non-hazardous compound is known to control this organism (4,6). Control is believed to be achieved by inhibition of HCN gas evolution via interference with the synthesis of a cyanogenic substrate in the fungus or host rather than by blockage of the enzymic hydrolysis of the substrate (6). Lebeau et al (4) reported that foliar spray applications of borax are more effective than soil treatment, but did not recommend the former because of phytotoxicity which occasionally results. Ferguson (3) stated that borax has been very effective in controlling snow mold on experimental putting greens at the University of Manitoba and that dry applications of borax are at least as effective as foliar sprays.

Isolates of the LTB have been collected in Manitoba (7), Saskatchewan (8), and Alberta (9). Preliminary studies by the author indicated that the cultural characteristics of the Manitoba isolate were very similiar to those of the Alberta Type A (W_1) isolate when grown on a soil plus whole ground soybean medium. The Saskatchewan isolate produced white, fluffy mycelium in greater abundance and began producing hydrogen cyanide gas earlier than the Alberta LTB-Type B (W_5) isolate and appeared to have a growth rate at least equal to that of Alberta's Type C (W_{14}) isolate.

This study was conducted to qualitively test the effects of different concentrations of borax on the linear growth and/or HCN production of the various pathogenic LTB isolates.

MATERIALS AND METHODS

The low temperature basidiomycete (LTB) isolates used in this study were: 1) LTB - Type A (W_1) 2) LTB - Type B (W_5) 3) LTB - Manitoba 4) LTB - Saskatchewan. The Type A & B isolates were obtained from Dr. J. B. Lebeau of the CDA Research Station, Lethbridge, Alberta, the Saskatchewan isolate from Dr. J. D. Smith of the Canada Department of Agriculture Research Station, Saskatoon, Saskatchewan and the Manitoba isolate was isolated by Mr. G. Platford from infected bentgrass greens at the University of Manitoba Turf Research plots.

The study was conducted using sterile 100 X 15 mm petri plates with a center well containing sodium picrate solution which is an indicator of hydrogen cyanide (HCN) gas production (9). Sodium picrate solution changes from its normal colour of bright yellow to yellow orange, orange, orange-red, red, dark red or very dark red depending on the concentration of HCN gas produced.

Preliminary studies indicated the individual isolates grew and/or produced HCN optimally when grown on the following media: 1) LTB-Man.- 20 gm of a 10:1 mixture of air dried, sterile soil (1:1:1 sand, soil and peat moss) and whole ground soybean. 2) LTB-Sask.- 20 gms of a 10:1 mixture of air dried, sterile soil (1:1:1 of sand, soil and peat moss) and soybean meal. 3) LTB-Type A- 19 gms of a 10:1 mixture of air dried, sterile soil (1:1:1 sand, soil, peat moss) and whole ground soybean plus 1.0 gm of incorporated fresh bentgrass clippings. 4) LTB-Type B- 19 gms of a 10:1 mixture of air dried, sterile soil (1:1:1 sand, soil, and peat moss) and soybean

meal plus 1.0 gm of incorporated fresh bentgrass clippings. These media/organism combinations were used in this study and all of the above weights were calculated on a per plate basis. To all media four milliliters of sterile, distilled water were added per plate in order to aid isolate growth.

Prior to inoculation, borax (Borax-Granular Technical, United States Borax and Chemical Corporation, New York and Los Angeles) was applied (on a per plate basis) as uniformly as possible to the surfaces of the culture media at concentrations equivalent to field rates of 1, 2 and 3 lbs./1000 ft^2. At each of the previously mentioned field rates, borax concentrations (on a per plate basis) were 0.15%, 0.30% and 0.45%, respectively of the total culture medium. In all cases, each plate was inoculated with two mycelial plugs (7 mm. in diameter) cut from the periphery of 12 day old cultures of the isolates used. The stock cultures of the LTB - A, Man. and B were grown on Difco potato-dextrose agar medium and LTB-Sask. was grown on a medium composed of 17 gm. Difco corn meal (without dextrose), 1 gm Difco malt extract and 1 gm. Difco yeast extract in 1 litre of distilled H_2O. All stock cultures were grown at 12° C in the dark.

After inoculation all plates were sealed with masking tape and incubated in total darkness at 15° C for a period of 24 days.

All isolates were tested against the three concentrations of borax. Three plates per isolate/concentration were used and observations were compared with those of the untreated check plates at 6 day intervals up to 24 days inclusive.

The extent of linear mycelial growth for all isolates was determined by measuring, in millimeters, the distance between the

farthest points of visible hyphal development extending from each of the inoculum plugs. Then, the total amount of linear mycelial growth per petri plate was simply computed by summing together the amounts of growth emanating from each of the two inoculum plugs.

RESULTS

The amount of mycelial growth emanating from each of the two inoculum plugs in the untreated LTB-Sask.,Man., and Type A plates was equal or nearly so throughout the entire incubation period. Therefore, it may be assumed that the untreated LTB-B inoculum plugs also would have produced equal amounts of growth if it had not been for the contamination of one plug by Trichoderma sp. (Fig. 1B). Hence, the amount of mycelial growth produced by the LTB-B should be approximately twice the amount shown in Table 1. Despite the contamination of this plug, mycelial growth did not appear to be severely hindered. The only drawback was that the presence of Trichoderma sp. made measurment of LTB-B growth extremely difficult.

On optimal medium, at optimal temperature, and in the absence of light, the linear growth of all non-treated isolates was approximately equal for the first 12 days of incubation (Table 1). At the termination of the incubation period, the LTB-Man. and LTB-Type A (W_1) isolates had totally over-grown their respective substrates with approximately 254 mm. of mycelial development, whereas the LTB-Sask. and LTB - Type B (W_5) isolates had not (Table 1). However, throughout the entire incubation period the growth of the untreated Saskatchewan isolate exceeded that of the Alberta Type B isolate (Table 1). The linear growth rates of the LTB-Man. and LTB - Type A isolates were equal (Table 1). The colonies of LTB-Sask. consisted of an abundant, white, fluffy, aerial growth of mycelium; LTB-B and LTB-Man. produced a grey-white mycelium which was appressed to the substrate whereas LTB-A

produced a dense white mycelium which was slightly appressed to the substrate (Fig. 1 A, B, C, D). Both the LTB-Man and LTB-A isolates produced dense, white stroma-like bodies (Fig. 1 C, D).

All concentrations of borax reduced the total amount of growth of each isolate to a level much below that of their corresponding untreated colonies (Table 1). The inhibitory effects of borax (at all concentrations) on the growth of the Manitoba and Type A isolates were more pronounced than on the growth of the LTB-Sask and LTB-B isolates. At the concentration of 1 lbs. of borax/1000 ft^2, the average growth at the end of the incubation period (24 days) of the LTB-Sask was 162.7 mm. whereas that of LTB-B, LTB-Man and Type A isolates were 117, 17.3 and 82.3, respectively. This is probably to be expected since the LTB-Sask isolate normally appears to be more vigorous than any of these other isolates. This greater vigor has been repeatedly observed in many preliminary studies conducted by the author. At the termination of the incubation period the average growth of the LTB-B (102 mm.) strikingly exceeded the growths of LTB-Sask, Man and Type A (the latter essentially totally inhibited) at the concentration of 2 lbs. of borax/1000 ft^2. At the concentration of 3 lbs./1000 ft^2 the average growths of LTB-B and LTB-Sask were equal (104.3 mm.) whereas the growths of LTB-Man and LTB-A isolates were completely inhibited or nearly so with 0.0 mm. and 8.7 mm., respectively, at the end of the incubation period.

Comparing the average growths of LTB-Sask and LTB-B at the concentration of 2 lbs borax/1000 ft^2, it appears that the Saskatchewan isolate was slightly more sensitive to borax than

the LTB-B isolate (Table 1). However, the low average growth of LTB-Sask (54.3 mm.) isolate at the end of the incubation period was the result of several zero growth inoculum plugs which probably resulted from placement of those plugs in direct contact with borax granules which had been unevenly distributed over the surface of the soil media.

Regardless of the LTB isolate or the rate of borax, mycelial growth in the majority of instances was irregular and appeared to be avoiding specific areas of the substrate (Fig. 1 A, B, C, D). After 12 days of incubation and particularly at concentrations equivalent to 3 lb. of borax/1000 ft^2 the LTB-Sask mycelium completely avoided the substrate by growing along the glass wall of the petri plates (Fig. 1A).

Under the cultural conditions of this study LTB-Sask and LTB-B were very vigorous HCN gas producers while the Manitoba and Type A isolates failed to liberate gas at any time (Table 1). The cultural production and non-production of HCN by LTB-B and LTB-A respectively, agrees with the findings of Ward et al (9).

Despite the inhibitory effects on the growth of all isolates, borax did not appear to be hindering HCN gas production by the LTB-Sask and LTB-B isolates. Within 12 days of incubation colour changes in the sodium picrate solution were observed and after 18 and 24 days incubation many of the LTB-Sask and Type B cultures had produced enough HCN gas to change the sodium picrate solution to a very dark red (VDR) colour, regardless of the concentration of borax (Table 1). The VDR colour of the borax-treated plates after 18 and 24 days incubation was comparable to that of the check plates, yet, in the case of LTB-Sask, the amount

of mycelial growth in plates treated at concentrations of 2 and 3 lbs. of borax/1000 ft^2 was always much less than the growth in the untreated plates indicating an earlier or stimulated HCN production in the borax treated plates. Because one inoculum plug in the untreated LTB-B isolate became contaminated, it was impossible to determine the exact amount of linear mycelial growth. As a result, a realistic comparison of HCN production relative to mycelial growth between the treated and untreated colonies was not possible.

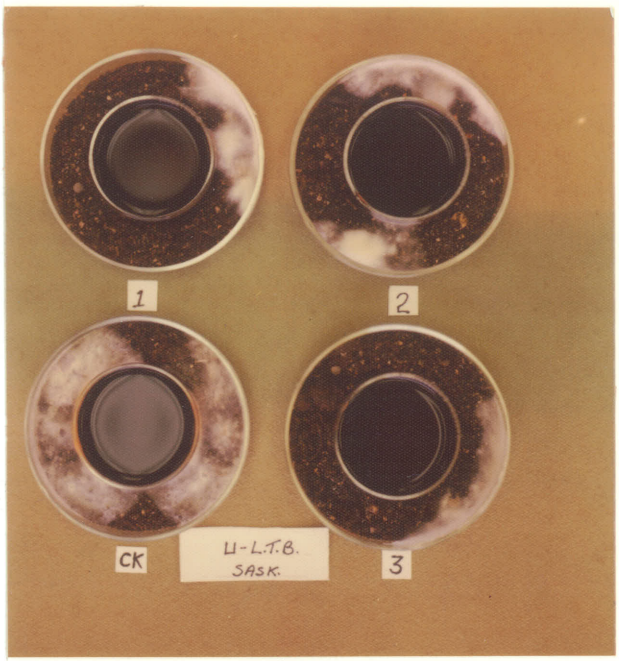
Table 1. Effect of borax on growth and/or HCN gas production of four LTB isolates.

CONCENTRATION[1] (lbs./1000 ft.[2])

		0		1		2		3	
	IP[2] (Days)	Mycelial Growth[3]	HCN Production[4]	Average Range Mycelial Growth	HCN Production	Average Range Mycelial Growth	HCN Production	Average Range Mycelial Growth	HCN Production
LTB - Sask	6	65	Or[5]	45.7	Y-YO	0	Y	17.3	Y
	12	117	DR	80.3	R-DR	17.3	Y-OR	50.3	OR-R
	18	208	VDR	125.7	VDR	32.7	Y-VDR	76.0	VDR
	24	234	VDR	162.7	VDR	54.3	Y-VDR	104.3	VDR
-B	6	52	Y	30.3	Y	30.3	Y	19.7	Y
	12	52 (104)[6]	R	67.3	OR-DR	43.3	Or-R	41.3	Y-R
	18	78 (156)[6]	VDR	93.3	VDR	76.0	DR-VDR	67.7	YO-VDR
	24	104 (208)[6]	VDR	117.	VDR	102.0	VDR	104.3	OR-VDR
- Man	6	52	Y	11.0	Y	14.3	Y	0	Y
	12	117	Y	13.0	Y	24.0	Y	0	Y
	18	234	Y	15.3	Y	32.7	Y	0	Y
	24	254	Y	17.3	Y	37.0	Y	0	Y
-A	6	59	Y	19.7	Y	6.7	Y	6.7	Y
	12	130	Y	39.3	Y	6.7	Y	6.7	Y
	18	234	Y	69.3	Y	6.7	Y	8.7	Y
	24	254	Y	82.3	Y	6.7	Y	8.7	Y

1) Borax applied as uniformly as possible to the surface of the soil culture media at concentrations equivalent to field rates of 1, 2 and 3 lbs./1000 ft.[2]. 2) IP = the days of the incubation period at which measurements were taken. 3) The check (0) values represent the total linear mycelial growth (in mm.) of one petri plate only. The values of the borax treated plates represent the average total linear mycelial growth for 3 replicate plates. All petri plates were inoculated with two inoculum discs. 4) The colours indicating amount HCN gas production in the check cultures are for one petri plate. The colour reactions given for the borax treated plates are a range over 3 petri plates. 5) HCN gas production measured by colour change of Na picrate solution (Y = yellow = no gas production; YO = yellow/orange; Or = orange; OR = orange/red; R = red; DR = dark red; VDR = very dark red). 6) Because of contamination, the non bracketed values represent growth of one inoculum plug only. The bracketed values are more realistic of LTB-B growth.

Figure 1. The effects of borax on the growth, HCN production and mycelial characteristics of four pathogenic LTB isolates after 24 days incubation. CK = untreated, Plates 1, 2 and 3 = each are identical as to isolate and concentration of borax treatment: A) LTB-Sask. treated with borax at concentration of 3 lbs./1000 $ft.^2$. All plates indicating development of abundant, white, fluffy mycelium and very dark red colour of Na picrate sol'n due to high conc'n of HCN. Plates 1 and 3 - each show zero growth at one inoculum plug. Plate 1, 2 and 3 - indicate irregular mycelial growth patterns. B) LTB - B treated at concentration of 2 lbs. borax/1000 $ft.^2$. All plates show the development of appressed grey-white mycelium and the very dark red colour of Na picrate sol'n due to high HCN conc'n. CK plates show presence of green Trichoderma sp. contaminating and emanating from upper inoculum plug; C) LTB - Man. treated at concentration of 2 lbs. borax/ 1000 $ft.^2$. All plates show yellow colour of Na picrate solution due to lack of HCN production. Plate 1, 2 and 3 - show poor, zero and irregular mycelial growth, respectively. CK plate - indicates appressed, grey-white mycelium and the presence of stroma - like bodies; D) LTB - A treated at concentration of 1 lb. borax/1000 $ft.^2$. All plates indicate lack of HCN production. Plates 1, 2 and 3 show inhibitory effects of borax on growth. Plates 2 and 3 show irregular mycelial growth patterns. CK plate shows development of dense, white mycelium and presence of stroma - like bodies. Peculiar appearance of Na picrate sol'n in CK and #1 plates of A and B is due to a shadow effect. All plates in A and B were identical to the very dark red colour shown in plate 2 of A and B.



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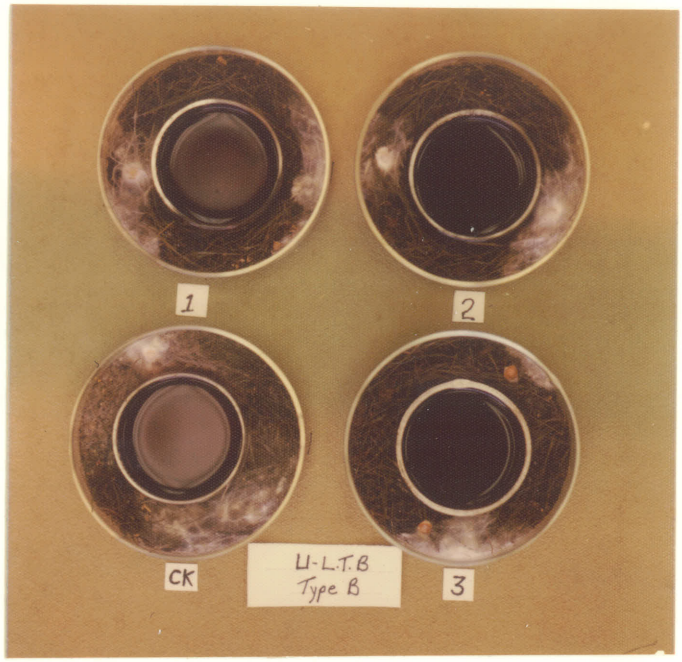

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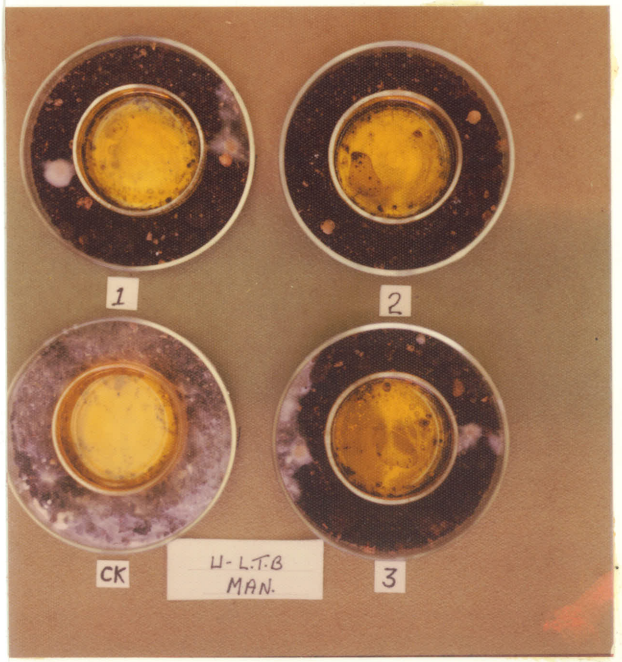

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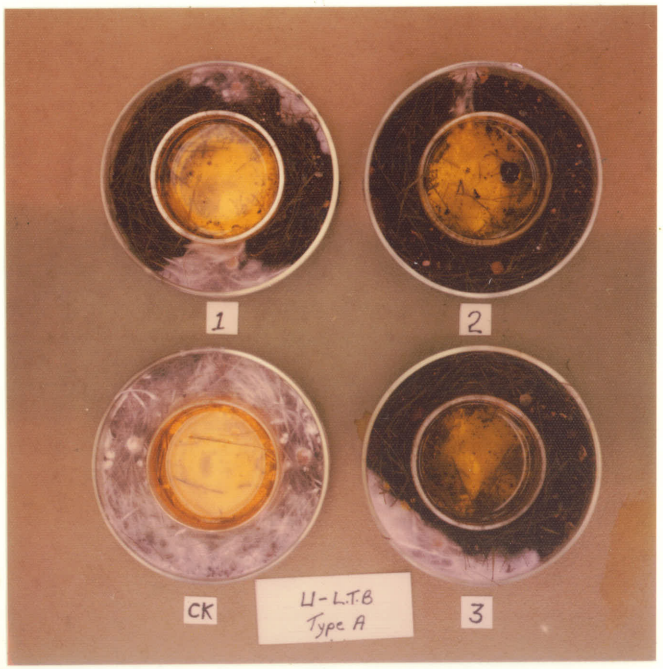

D

Figure 1

DISCUSSION

Ward et al (9) observed that the LTB-B isolates produced abundant, fluffy aerial mycelium and generally grew more rapidly than the LTB-A isolates which produced an appressed mycelial growth. Furthermore, the Type B isolates produced HCN gas readily in culture and in association with the host, produced no stroma-like bodies and were highly pathogenic to grass species, whereas the LTB-A produced gas only in association with the host, produced stroma-like bodies in culture and were extremely virulent on alfalfa species. Results of this experiment support Ward et al (9) in regard to cultural HCN gas production and the presence or absence of stroma-like bodies of the respective LTB isolates. However, the linear growth rates of the untreated LTB-A greatly exceeded that of the LTB-B but both isolates produced an appressed-type of mycelial growth. Preliminary tests by the author found the LTB-B isolate to produce fluffy, aerial mycelium as opposed to the more appressed growth of the LTB-A isolate when both were grown on a medium of soil plus whole ground soybean. Therefore, any contradictions as to cultural characteristics of the various isolates in this study probably can be explained on the basis of the differences between the "optimal" media used for each isolate. The values of Table 1 are average measurments of the linear growth of the isolates and did not take into account the development of aerial mycelium. Since the LTB-Sask. produced very dense, white, fluffy, aerial mycelium and nearly had overgrown the substrate (untreated colony)

at the end of the incubation period, it would appear that this isolate displays more vigor than any of the other pathogenic LTB isolates. On the basis of linear growth rate, absence of HCN gas in culture, the development of dense, white, stroma-like bodies, and sensitivity to borax, the LTB-A and LTB-Man. isolates appeared to be quite similiar.

The growth of the LTB-A and LTB-Man. isolates, which did not produce HCN gas, was inhibited to a much greater extent by borax than that of the HCN producing isolates. An explanation for this range in sensitivity is not offered, however, the resistance mechanisms of the LTB-B and LTB-Sask. are apparently very active and complex since these isolates are able to exist and grow well when subjected to high concentrations of extremely lethal HCN gas. Under cultural conditions, Ward et al (9) have shown that the tolerance of the LTB-B to HCN exceeds that of LTB-A isolates which will produce HCN only in association with host tissue. Possibly the differences in resistance capabilities among the isolates may be used to explain the range in sensitivity to borax. Unfortunately it is not known if the Manitoba isolate produces HCN in association with the host.

Studies by Lebeau and Atkinson (6) have shown that borax did reduce the growth of LTB-B (W_2) and that it inhibited the autolytic production of HCN by this isolate. Results of this experiment indicate that borax reduced and, in some cases, inhibited the growth of the LTB isolates tested. However a comparison of colour range to amount of growth between the treated and untreated

colonies of the LTB-Sask. and LTB-B (W5) indicate that HCN production was at least equal in all cases. Therefore, borax did not appear to have any inhibitory effects on HCN production by the gas producing isolates. As mentioned, there were instances in which the LTB-Sask, treated with 2 and 3 lbs. of borax/1000 $ft.^2$, appeared to have produced HCN gas earlier, relative to the amount of mycelial growth, than did the untreated colonies (Table 1). Whether this was due directly to a borax effect, to increased autolysis (10,11) which resulted from a borax reduction of growth, or if it was an adaptive and/or preservation characteristic is not known. Regardless, the amount of HCN produced appears to be dependent on the amount of mycelium produced; whether this relationship is directly proportionate is not known. Discrepancies between the present study and that of Lebeau and Atkinson (6) may be explained on the basis that the latter utilized 1) a malt-yeast-glucose broth medium, 2) the W2 form of the LTB-B and, 3) incubated at 10° C. for a period of 6 weeks.

Differences have been reported with regard to the capabilities of borax in controlling snow mold of turfgrasses. Lebeau et al (4) stated that borax, when used as foliar spray, gives effective control on LTB infected alfalfa but gives practically no control of LTB mold on turfgrass. Ferguson (3) found that borax (foliar spray or dry application) is effective in controlling snow mold of turfgrasses in Manitoba. As stated, this study indicated the growth of the LTB-A to be more sensitive to borax than that of the LTB-B. These results, combined with the observations of Ward et al (9) which

indicated the LTB-A to be extremely virulent on alfalfa species whereas the LTB-B was highly pathogenic on grass species may explain, in part, why Lebeau et al (4) found borax to be effective in controlling LTB attacking alfalfa but ineffective when applied to LTB infested turfgrass. Furthermore, it appears that borax is very inhibitory to the growth of LTB-Man. This may explain why Ferguson (3) has found borax to be effective in controlling snow mold of turfgrasses in Manitoba.

CONCLUSION

In the last 15 years several different isolates of the low-temperature basidiomycete have been found. The differences between them appear to range from a physiological and biochemical basis to the type(s) of host they will invade. This study has illustrated that differences among the pathogenic isolates exist also in their sensitivity to borax. Therefore, these differences may explain why erratic borax control of snow mold has been reported from different areas of western Canada.

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2. The Interacting Effects of Borax Concentration, pH and Culture Substrate on the Growth of Pathogenic Isolates of the Unidentified Low-Temperature Basidiomycete.

Abstract

The influence of borax concentration, pH and culture substrate on the growth of low-temperature basidiomycete isolates obtained from Manitoba, Saskatchewan and Alberta was investigated. Isolate substrate preference, optimal pH for growth, tolerance to the inhibitory effects of borax, and pH and nutrient status of the substrate appeared to be some of the interacting factors which determined the extent of isolate growth. Borax at all concentrations became more inhibitory to LTB growth with increasing substrate pH, particularly on soil extract medium, indicating the importance of a borax-pH interaction. Borax was not highly lethal but had good fungistatic ability against the LTB isolates, particularly on the unadjusted media. LTB-Man was the most sensitive and LTB-Sask appeared to be the least sensitive to the effects of borax and the borax-pH interaction.

INTRODUCTION

Snow mold or crown rot of alfalfa, caused by a psychrophilic sterile basidiomycete (LTB) (3,5) can be controlled by foliar sprays of borax (13). In addition, effective control of snow mold has been achieved by dry applications of borax to bent grass putting greens at the University of Manitoba (8). Other investigations have demonstrated that borax will inhibit the growth of Fomes annosus, a basidiomyceteous pathogen causing root and butt rots of fir trees (7,6,2,9,15). Some of these studies have shown that borax increases the pH of the medium or substrate, but that its effectiveness on the growth of F. annosus is due primarily to toxic concentration levels (10,12). Recent in vitro studies (1) have indicated that borax is very inhibitory to the growth of various pathogenic isolates of the LTB, but does not hinder the hydrogen cyanide producing isolates from synthesizing and releasing this highly toxic gas.

This study was conducted to investigate the interacting effects of different concentrations of borax, pH and media on the growth of three pathogenic LTB isolates.

MATERIALS AND METHODS

Three isolates of the low temperature basidiomycete (LTB) were used in this investigation. They were (1) LTB-Manitoba (Man) obtained from Mr. G. Platford, Manitoba Department of Agriculture, Winnipeg, Manitoba, (2) LTB-Saskatchewan (Sask), from Dr. J. D. Smith, Canada Department of Agriculture Research Station, Saskatoon, Saskatchewan, and (3) LTB-Type B (W5), from Dr. J. B. Lebeau of the Canada Department of Agriculture Research Station, Lethbridge Alberta.

The effects of borax (Borax-Granular Technical, United States Borax and Chemical Corporation, New York and Los Angeles) and borax-pH interaction on the growth of the LTB isolates were studied first by culturing the pathogens on synthetic media and at temperatures optimal for their growth. LTB-Man and LTB-B were grown in 15 X 60 mm. petri plates on Difco potato dextrose agar (PDA) and LTB-Sask on a medium (CMMY) composed of 17 gm. Difco corn meal (without dextrose), 1 gm. of malt extract, 1 gm. of yeast extract in one litre of distilled water; autoclaved for 15 min. at 15 lbs/in.^{2} (16). Since preliminary screening of some media indicated that the above media were optimal for growth of the respective isolates, it was assumed that any reduction in growth would have been due to borax toxicity alone or a borax-pH interaction. Also, borax and the borax-pH interaction effects were studied by culturing the isolates on soil extract agar (SE). This was prepared by autoclaving 1 kg. of soil in 1 litre of distilled water for 30 min. at 15 lbs/in.^{2}; the supernatant

was filtered off and distilled water was added to make a litre; then 15 gm. of agar were added after which the entire medium was re-autoclaved for 15 min. at 15 lbs/in.^{2} (16). The soil (a sandy loam) used was of a type which is normally used in the construction of putting greens in Manitoba. By utilizing a sterile soil extract medium prepared from this type of soil, it was assumed that stresses such as nutritional deficiencies (in comparison to the synthetic media), pH and possible interaction of borax with soil constituents would have produced results which would have been more indicative of borax and pH effects on the LTB isolates in the field than would have been the results obtained from the synthetic media.

Pre-determined aliquots of borax stock solutions were added to medicine bottles containing specified amounts of media, to give final concentrations of 100, 500, 1000 and 5000 ppm. The stock solutions were added to cooled media (50^{o}C) after autoclaving to assure that no change or hydrolysis of the borax occurred. For each concentration two series of plates were poured; one series contained media with an unadjusted (unbuffered) pH and one with media in which the pH was adjusted (buffered) to that of the normal medium with 25% lactic acid solution. All pH readings and adjustments were done after autoclaving when the media had cooled to 40^{o} C. Twenty millilitre aliquots of the final media were dispensed per petri plate in the order of lowest to highest concentration. The automatic syringe was repeatedly rinsed with sterile distilled water to insure that the concentration and pH of all treatments remained accurate.

Each plate was inoculated with a disc (7 millimeters in diameter) cut from the periphery of a colony grown on the medium and

at the temperature optimal for its growth (1). Four replicate plates were used per isolate per borax concentration -pH level. All treatments were incubated for an eight day period at 12° C. in the dark. At the end of the incubation period the average amount of growth (in millimeters) was determined by measurement of colony diameter on two different axes.

If no growth occurred, the inoculum plugs were removed to normal (borax-free) media and incubated for a further eight days at temperatures optimal for growth. This was performed only with LTB inoculum cultured on synthetic media and was to determine if borax and/or the borax-pH interaction was fungistatic or fungicidal.

RESULTS AND DISCUSSION

Borax is known to be inhibitory to the growth of the low-temperature basidiomycete (14), but its exact mode of action is still uncertain (1,14). Interactions between the borax and substrate or pH may, in part, determine the inhibitory effects on LTB growth.

The pH Differences Between the Soil Extract (SE) and Synthetic Media and Their Effects on LTB Growth.

Even though this study was not designed to determine the optimal pH for LTB growth, such information was partially revealed and could be useful in explaining borax control of the LTB isolates. According to Ward et al (17) the optimal pH for LTB growth generally lies between 6.0 and 7.0. Specifically these workers found that the LTB - B (W5) and LTB - A (W1) grew best at a pH of 6.0 with growth declining beyond and below this point. Evidence in support of these observations is revealed in Tables 2 and 3. For example, on PDA (pH = 5.8) the growth of the LTB - B was 29.5 mm, whereas on the unadjusted PDA at 100 ppm borax (pH = 6.4) growth of mycelium was 32.9 mm. Since borax at 100 ppm on adjusted PDA appeared to be somewhat toxic to LTB - B growth (pH = 5.8), it is probable that the greater growth on the unadjusted medium was the result of a shift to a more favourable pH which counteracted the toxic effects of borax at this concentration. Furthermore, pH of the SE medium was 7.1 and although there were nutritional deficiencies (in comparison with the synthetic media), this "high" pH may have been largely responsible for the lower amount of LTB growth (Table 3). Despite the preference

of the LTB-B for slightly acidic conditions, Ward et al (17) observed that its tolerance to alkaline conditions was greater than that of the Type A isolate since considerable LTB-B growth occurred even at a pH of 9.0.

In vitro studies (1) have indicated that the LTB-Man and LTB-A (W1) were very similar on the basis of cultural characteristics, lack of HCN production in culture and borax sensitivity. The data in Table 2 indicate that the Manitoba isolate, like that of LTB-A and LTB-B was favoured by a slightly acidic environment with a pH in the vicinity of 6.0. When cultured on adjusted PDA with a borax concentration of 100 ppm. growth of the LTB-Man was slightly greater than when cultured on unadjusted PDA media, indicating that a pH of 6.4 was not optimal for LTB-Man growth. Therefore, it is possible that the Manitoba isolate, like that of the LTB-A isolate may be intolerant of pH levels greater than 6.0, particularly in the vicinity of 7.0 and higher (17). This preference for a slightly acidic environment and intolerance of higher pH levels would explain why growth of LTB-Man on the SE agar (SEA) (pH = 7.1) was only 23.3 mm. but while on PDA it was 36.6 mm. As with the LTB-B, growth discrepancies between the synthetic and SE media were undoubtedly influenced by nutritional differences, but the pH difference also did appear to play an important role.

The LTB-Sask isolate appeared to be unique in regard to optimal pH levels for growth. At 0 ppm. borax, the growth of the LTB-Sask on CMMY agar exceeded that of the SE agar by only 4.0 mm (Table 2 and 3). Since the range of growth of this isolate on these media was relatively small, it appeared that the LTB-Sask was less

sensitive to nutritional and/or pH differences than were the other two isolates. It is also possible that the optimal pH for LTB-Sask growth is near 7.0 and that the higher nutritional status of CMMY agar counteracted its lower pH (6.3) effect thereby allowing growth to be slightly greater on this than on the SE agar.

Although this study did not indicate clearly the pH optimum of each isolate, it did reveal; (1) the LTB-B and Manitoba isolates appeared to prefer slightly acidic conditions, possibly in the vicinity of pH = 6.0. (2) the LTB-Sask was possibly more tolerant of the alkalinity and/or nutritional status of the SE media than were the Type B and Manitoba isolates. (3) the SE agar was more detrimental to isolate growth than were the more favourable synthetic media. This was probably the result of nutritional and pH differences between the substrates. In the natural environment many more stresses are present thus creating even greater limitations on LTB growth than were represented in the SE medium. These natural restrictions may possibly aid in the control on the LTB pathogen.

Borax: Its Influence on Substrate pH and on Isolate Growth.

Johnson and Cowling (10) found that borax increased the pH of the substrate, but only investigated the actual toxicity of borax to Fomes annosus without its corresponding pH effect. Koenigs (12) found that borax raised the pH of wood (substrate) from 4.8 to 7.6 - 8.1, but did not consider this to be important in preventing F. annosus from colonizing stump surfaces since a supratoxic concentration of borax was required to achieve these alkaline conditions. Tables 2 and 3 show that pH of all media increased progressively

with each increment in borax concentration (except in unadjusted SE medium in which no pH change occurred beyond 1000 ppm) thereby supporting the results of the previous studies (10,12). Generally, as borax concentration on the adjusted media increased, the growth of the LTB isolates was correspondingly reduced. This supports previous reports regarding the inhibitory effects of borax on fungal growth (10,12,14).

Regarding the importance of a borax-pH interaction in inhibiting pathogen growth, the results of this study did not agree with those of Koenigs (12). The data (Tables 2 and 3) reveal the importance of a borax-pH interaction in inhibiting LTB growth. At each concentration of borax, the growth of each LTB isolate on the unadjusted synthetic and SE media was significantly less than the growth on the respective adjusted media with the exception of the LTB-B on PDA at 100 ppm borax and the LTB-Man on PDA and SE media at 5000 ppm borax (Tables 2 and 3). Total growth inhibition occurred at lower borax concentrations on the unadjusted media than on the adjusted media (Tables 2 and 3). Furthermore at the higher borax concentrations (i.e. LTB-Sask $\geq$ 500 ppm; LTB-Man $\geq$ 1000 ppm; and LTB-B $\geq$ 1000 ppm), LTB growth on the adjusted SE medium was greater than that on the unadjusted synthetic media at corresponding concentrations. A comparison of the percent growth inhibition (% G.I.) of every isolate on each of these media indicates also the importance of a borax-pH interaction in providing effective control of the LTB isolates.

As mentioned, the above results indicate a borax-pH interaction was operative in inhibiting the growth of the LTB isolates. Unfortunately extrapolation of these results to the natural soil environment is not possible. In the field such factors as cation exchange capacities (C.E.C.) influence the pH buffering capacities of the soil (4) which, depending upon soil type, could severely reduce or entirely eliminate the borax-pH interaction effect. If so, LTB control in the field may be due to borax concentration only, and, as found by Koenigs (12), any shift in pH (detrimental to isolate growth) may be of no significance in control since a supratoxic concentration of borax would be required to cause such a shift. However, the soils used in putting green construction in Manitoba are usually sandy loams which have low C.E.C. and therefore low buffering capacities (4). Only field studies in which the soil pH and borax concentrations are continually monitored will verify or disprove the importance of a borax-pH interaction in control of LTB induced snow mold.

Variation in the Sensitivity of Isolates to the Effects of Borax and the Borax - pH Interaction

The effectiveness of borax and the borax-pH interaction in inhibiting growth varied among the LTB isolates.

(a) Sensitivity to Borax

An examination of growth on the adjusted media indicates that the LTB-Man was the most sensitive to the inhibitory effects of borax since it had the highest average percent G.I. and was the only

isolate to be totally inhibited at 5000 ppm borax on both the SE and synthetic adjusted media (Tables 2 and 3). The average percent G.I. of the LTB-Sask, when cultured on adjusted SE and synthetic media, was essentially equal to that of the LTB-B indicating that its borax tolerance was at least equal to that of LTB-B (Tables 2 and 3). This supports the findings of a previous in vitro study (1). However, since the LTB-Sask appeared to prefer a substrate pH of 7.0 (pH of adjusted SE agar) and was possibly more tolerant of the nutritional status of the adjusted SE agar than LTB-B, it would have been natural to expect the average percent G.I. of the Saskatchewan isolate to be less than that of the Type B isolate. This was not the case, but this expectation might have been fulfilled if it had not been for the unexplainable stimulation of LTB-B growth, on the adjusted SE medium at 1000 ppm borax (Table 3). The borax tolerance of the LTB-Sask might thus be greater than that of the LTB-B.

When the isolates were cultured on their "optimal" adjusted synthetic media, the average percent G.I. of the LTB-Sask was 31.5 mm. whereas that of the LTB-B was 30.6 mm., indicating the borax tolerance of these isolates was about equal (Table 2). However, a consideration of the pH of the adjusted CMMY (6.3) and adjusted PDA (5.8) media indicates that CMMY may have not been totally optimal for LTB-Sask growth whereas the PDA was optimal for LTB-B growth. Since LTB-Sask appeared to have been subjected to two stresses (borax and pH) and LTB-B to only one stress (borax), it would appear that the Saskatchewan isolate has a greater borax tolerance than does the Type B isolate.

(b) Sensitivity to the Borax - pH Interaction

The high average percent G.I. of the LTB-Man on unadjusted synthetic and SE media indicate that it was the most sensitive isolate to the inhibitory effects of the borax-pH interaction (Tables 2 and 3). Table 3 also shows that the average percent G.I. of the LTB-Sask was only 58.9 mm. whereas that of the LTB-B was 64.3 mm. when cultured on unadjusted SE agar indicating that the Saskatchewan isolate was slightly more tolerant of the borax-pH interaction than was the LTB-B.

Undoubtedly a complex of physiological, biochemical and physical factors interact to determine the sensitivities of the LTB isolates to the effects of borax. From this study it appears that pH and/or nutritional preferences of the isolates in conjunction with the high alkalinity of the borax-treated media are some of the factors interacting to determine the borax tolerance of the various LTB isolates. It is obvious that the LTB-Man is extremely sensitive to borax and the high substrate pH levels it creates. Furthermore the sensitivity of the Saskatchewan isolate is at least equal to that of the Type B isolate, but since the former seems to be more tolerant of higher pH and/or nutritional deficiencies, as found in the SE media, it is likely that the LTB-Sask would have the greatest tolerance in an unbuffered borax-treated environment. As stated, the results of this study cannot be extrapolated to field conditions, but since the natural soil environment would undoubtedly subject the LTB isolates to many more stresses than were present in the SE media it is likely that the order of isolate sensitivity to borax established in this study, would exist in the field.

Borax - Fungicidal or Fungistatic?

Koenigs (11) has stated that borax is not a highly lethal compound in vitro. Table 2 shows that on optimal synthetic media a borax concentration of 5000 ppm was required to completely inhibit LTB growth (except with LTB-Man on unadjusted PDA at 1000 ppm). Furthermore, with the exception of LTB-Man on adjusted PDA at 5000 ppm, total inhibition of LTB growth on synthetic media occurred only on the unadjusted series indicating a lethal pH effect rather than a lethal borax effect (Table 2). Therefore, these results substantiate Koenigs (11) findings that the lethality of borax is low.

In every instance (except No. 1 and 4 plugs of LTB-Man at 5000 ppm unadjusted and No. 1 plug of LTB-Sask at 5000 ppm unadjusted) growth resumed from the totally inhibited inoculum plugs when they were transferred to their respective normal (borax-free) media (Table 4). This indicates that the inhibitory effects of borax and the borax-pH interaction were fungistatic rather than fungicidal.

Table 2. The effects of borax concentration and borax - pH interaction on the growth of LTB isolates cultured on synthetic media[1].

Concentration (ppm)	LINEAR MYCELIAL GROWTH IN MM[2] LTB - B (W5)	LTB - Man	LTB - Sask	MEDIA pH PDA	CMMY
0	29.5[g][4]	36.6[f]	33.9[h]	5.8	6.3
100 AD[3]	26.3[e] (11.0)[5]	37.1[g] (-1.4)	33.4[g] (1.5)	5.8	6.3
100 UA	32.9[h] (-11.4)	36.0[e] (1.7)	32.9[f] (3.0)	6.4	7.5
500 AD	28.3[f] (4.2)	21.6[d] (41.0)	28.3[e] (16.6)	5.8	6.3
500 UA	26.3[e] (11.0)	14.1[c] (61.4)	23.9[d] (29.5)	7.5	8.6
1000 AD	22.4[d] (24.2)	9.9[b] (73)	24.3[d] (28.4)	5.8	6.3
1000 UA	15.0[c] (49.2)	0.0[a] (100)	13.5[c] (60.1)	7.9	8.9
5000 AD	5.0[b] (83.1)	0.0[a] (100)	7.0[b] (79.3)	5.8	6.3
5000 UA	0.0[a] (100)	0.0[a] (100)	0.0[a] (100)	8.2	9.1
$\bar{x}$ % GI	AD = 30.6	AD = 53.2	AD = 31.5		
	UA = 37.2	UA = 65.8	UA = 48.2		

(1) LTB - B (W5) and LTB - Man cultured on potato-dextrose agar and LTB - Sask on cornmeal - malt extract - yeast extract agar.

(2) Statistical analysis done independently for each isolate. Values shown represent average colony diameter (mm) measured on two different axes for each of four plates.

(3) AD = pH of medium has been buffered back to normal (PDA = 5.8; CMMY = 6.3) with 25% lactic acid; UA = medium is unbuffered.

(4) Means within columns followed by the same letters are not significantly different at the 1.0% level using Duncan's Multiple Range Test.

(5) Values in ( ) = growth inhibition for each treatment as a percentage (% GI) of the check (0 ppm) growth.

Table 3. The effects of borax concentration and borax - pH interaction on the growth of LTB isolates cultured on soil extract medium.

Concentration (ppm)	LINEAR MYCELIAL GROWTH IN MM[1] LTB - B (W5)	LTB - Man	LTB - Sask	pH
0	16.6[e][3]	23.3[e]	29.9[h]	7.1
100 - AD[2]	18.8[f] (-12.8)[4]	30.4[f] (-30.6)	30.5[i] (-2.1)	7.1
100 - UA	15.5[e] (6.8)	22.0[d] (5.4)	27.5[g] (7.9)	8.3
500 - AD	11.9[d] (28.6)	1.3[b] (94.6)	25.0[f] (16.3)	7.1
500 - UA	8.3[c] (50.4)	0.0[a] (100)	18.1[d] (39.3)	8.9
1000 - AD	16.1[e] (3.0)	1.6[c] (93.3)	22.5[e] (24.7)	7.1
1000 - UA	0.0[a] (100.0)	0.0[a] (100)	3.4[b] (88.5)	9.1
5000 - AD	3.4[b] (79.7)	0.0[a] (100)	8.4[c] (72)	7.1
5000 - UA	0.0[a] (100)	0.0[a] (100)	0.0[a] (100)	9.1
$\bar{x}$ % GI	AD = 24.6	AD = 64.3	AD = 27.7	
	UA = 64.3	UA = 76.4	UA = 58.9	

(1) Statistical analysis done independently for each isolate. Values shown represent average colony diameter (mm) measured on two different axes for each of four plates.

(2) AD = pH of medium has been buffered back to normal medium pH (7.1) with 25% lactic acid; UA = medium is unbuffered.

(3) Means within columns followed by same letters are not significantly different at the 1.0% level using Duncan's Multiple Range Test.

(4) Values in ( ) = growth inhibition for each treatment as a percentage (% GI) of the check (0 ppm) growth.

Table 4. The viability of the zero growth LTB inoculum plugs transferred from borax supplemented to unsupplemented synthetic media.

Isolate	Culture Media	Concentration[1] (ppm)	Activity Inoculum Plug # 1	2	3	4
LTB - Man	PDA	1000 - UA[2]	G[3]	G	G	G
		5000 - AD	G	G	G	G
		5000 - UA	NG	G	G	NG
LTB-B (W5)	PDA	5000 - UA	G	G	G	G
LTB-Sask	CMMY	5000 - UA	NG	G	G	G

(1) Borax concentration at which no mycelial growth occurred.

(2) AD = pH of borax treated media buffered back to normal media pH (PDA = 5.8; CMMY = 6.3) with 25% lactic acid; UA = pH is unbuffered.

(3) G = growth occurred on normal (borax-free) media; NG = no growth occurred on normal media.

CONCLUSION

This study has shown that borax will reduce and totally inhibit growth of known pathogenic LTB isolates in a fungistatic manner and that isolates of LTB vary in their sensitivity to borax. The inhibitory effect of borax on fungal growth appears to be the result of direct toxicity as well as an increase in substrate pH.

Extrapolation of the results of this study to field conditions is not possible. However, since the pH preference for LTB growth appears to be in the vicinity of 6.0 - 6.4 (except LTB-Sask), a putting-green soil with a base or normal pH of 7.0 or higher, in conjunction with other natural stresses, would undoubtedly aid in borax control of the LTB and depending upon the buffering capacity of the soil, could enhance the toxic effects of the borax - pH interaction.

Since borax appears to enhance its own toxicity by increasing substrate pH, an investigation of increasing only the pH levels of the soil and thatch layers without resulting phytotoxicity to the turf is another avenue of research in the control of snow mold caused by the unidentified low-temperature basidiomycete.

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3. A Field Study of Snow Mold of Turfgrasses: The Causal Organisms and Their Chemical Control in Southern Manitoba.

ABSTRACT

The causal organisms of snow mold and their chemical control in southern Manitoba were investigated during the fall and winter of 1973 - 74. Snow mold damage to turfgrasses was caused primarily by Typhula FW. S. borealis and the psychrophilic sterile basidiomycete (LTB) were also highly abundant. Damage caused by F. nivale was not excessive. Variation in pathogen occurrence was probably due to a complex of factors which were not revealed in this study. At several of the areas surveyed a complex of 3 or 4 pathogens were associated with damage to the turfgrass. Mercuric chloride which is used as the standard control compound in Manitoba and a combination of borax and Terraclor 75W provided excellent control of all the snow mold pathogens when applied in late October. Unfortunately phytotoxicity was a problem but if this could be eliminated by more even distribution of the chemical and/or by a reduction in the rates used, the borax: Terraclor 75W combination would be a very adequate substitute for mercury in the control of snow mold in Manitoba. All compounds were effective in controlling one or more specific group(s) of pathogens but only $HgCl_2$ and the borax combination treatments gave broad spectrum control.

INTRODUCTION

Snow mold is probably the most devastating disease of golf and bowling greens in western Canada (7,26,33,46). The extensive use and cost of establishment of this type of turf justifies the use of fungicides in controlling this disease.

Sclerotinia borealis (23,41), Typhula spp. (35,41), an unidentified low-temperature basidiomycete (LTB) (6,7) and Fusarium nivale (13) are known causal agents of the disease. Because several of these pathogens can predominate yearly in one locality (33,38) control measures such as breeding for resistance or use of non-mercurial fungicides are difficult to employ.

Inorganic mercurial compounds satisfactorily control the snow mold pathogens attacking turfgrass. However, high cost (25), high mammalian toxicity (24), possible deleterious effects on the soil microflora (34) and in some instances phytotoxity have stimulated a search for compounds which will be at least as effective but more economical and safer to use than the mercurials.

Many non-mercurial organic compounds have been reported to be effective in controlling specific groups of snow mold pathogens (21,38,42,47) but none appears to be as broad in spectrum of activity as the mercurials. Borax (sodium tetraborate) has effectively controlled the LTB (24), a major snow mold pathogen of alfalfa in western Canada (7,11). Furthermore, Ferguson (17) has found borax to be very effective in controlling snow mold on bentgrass turf but has never recommended it for general use because of potential phytotoxicity. Other organic compounds such as chloroneb (47),

PCNB (19,21,38), oxathiins (30,38) and benzimidazoles (29,44) are apparently effective in controlling specific groups of these pathogens. Moreover in Manitoba the effectiveness of all compounds recommended, including the mercurials, is believed to be enhanced greatly when they are applied as late in the fall as possible. This greatly increases the risk of snowfall prior to treatment of golf and bowling greens thus complicating application.

The following study was conducted (1) to test and compare the effectiveness of borax alone and in combination with several other organic compounds against mercuric chloride in controlling this disease; (2) to determine the time and number of applications which effectively control snow mold of turfgrasses; and (3) to survey the causal organisms of snow mold in different localities of southern Manitoba.

MATERIALS AND METHODS

Experiments were conducted at five locations in southern Manitoba during the fall and winter of 1973-74. The locations were the University of Manitoba turf research plots, Pine Ridge Golf and Country Club, Wildwood Golf Club, Breezy Bend Golf Club - all within a ten mile radius of the city of Winnipeg - and Sandy Hook Golf Course, fifty miles to the north.

Availability of the test sites determined the number of treatments and times of application. At the University of Manitoba, Breezy Bend and Pine Ridge the sites were available for use in early fall and thirty-three treatments including "early" (the third week of September) and "late" (the last week of October) applications were made (Table 5). Wildwood and Sandy Hook sites were not available until play had ended, hence only the "late" treatments were applied (Table 5).

The turfgrasses varied somewhat between locations: Seaside and Penncross creeping bentgrasses were predominant at the University of Manitoba; Washington bentgrass and Poa annua at Breezy Bend; Cohansey at Wildwood; a mixture of Kentucky bluegrass and native bentgrass at Pine Ridge and Poa annua at Sandy Hook.

The compounds tested were: Borax-Granular Technical, United States Borax and Chemical Corporation, New York and Los Angeles; Mercuric chloride (granular), Mallinckrodt Chemical Works, St. Louis; Terraclor 75W (Pentachloronitrobenezene) (PCNB 75%),

Olin Agricultural Division, Little Rock, Arkansas; Tersan SP (1,4 - dichloro - 2,5 dimethoxy-benezene) (chloroneb 65%), E. I. DuPont de Nemours & Co. Inc., Wilmington, Delaware; Vitavax 75W (5,6 - dihydro - 2 - methyl - 1, 4 - oxathiin - 3 - carboxanilide) (Carboxin 75%), Uni 2010 and Uni 2002 from UniRoyal Chemical Division, Elmira, Ontario; Tecto 40 Flowable (Thiabendazole 42.28%), Merck Chemical Division, Merck & Co., Inc., Rahway, New Jersey.

All compounds were tested alone and all, except mercuric chloride, in combination with borax in a completely randomized block experiment with three replications.

All compounds, except borax, were applied to the turf as a liquid spray with a Green Cross No. 10 hand pump sprayer. Borax was applied dry, without a carrier, using a hand shaker made from a tobacco tin. In the combined treatments, the sprayed compounds were applied first and allowed to dry before application of the borax. The untreated check plots were sprayed with an equivalent amount of water.

In early May all test plots were rated on the basis of percent infected area using a twelve point scale[1] and then were converted and reported in terms of disease control as a percentage of the check plots. Each test location was rated twice with an interval of approximately 1-1½ weeks between ratings. Because disease and phytotoxicity damage were more apparent upon the resumption of growth, only the values from the last rating are

1) Elanco Conversion Tables for Barratt-Horsfall Rating Numbers - Eli Lilly & Co., Greenfield Laboratories, Box 708, Greenfield, Ind. 46140.

reported. All diseased areas were thoroughly examined for the presence or absence of fruiting structures and sclerotia. At all locations representative samples of turf were taken from diseased areas and sclerotia extracted.

Isolation of F. nivale was attempted by plating infected tissue onto PCNB medium (45), of LTB by incubating diseased tissue on potato-dextrose agar (PDA) plus 0.5% Tersan 1991 (36) and by plating superficial mycelium onto PDA medium (11). Tissue suspected of infection by Typhula sp. or S. borealis was plated on PDA directly and after momentary surface sterilization in 0.5% sodium hypochlorite solution (28). All inoculated plates were incubated at 2°C. for a period of approximately two weeks. All sclerotia were thoroughly air dried, immersed in a 1:1 solution of 6% sodium hypochlorite and 95% ethyl alcohol, plus two drops of polyoxyethylene sorbitan monolaurate (Tween 20) for 60 sec., rinsed for 5-10 sec. in sterile water, and blotted. Four to five sclerotia were placed on acidified (pH 4.8) cornmeal agar medium. Dishes were enclosed in clear polyethylene bags, left at 25°C. for 30 hours to allow sclerotia to hydrate and then placed at 3°C. until sclerotial germination had occurred (14). Mycelium radiating over the agar was transferred to PDA medium and incubated at 3°C.

Disease control data were subjected to an Analysis of Variance (ANOVA) and to Friedman's Nonparametric Analysis for a Two-Way Classification (42). Analysis of the data was performed for each individual location and, an overall analysis was not performed. Only the significantly equal and best treatments at each

location are lettered in Tables 7-9. The statistical equality of these treatments was verified by both of the above techniques at all locations with the exception of the University of Manitoba. The significantly equal and best treatments at the University of Manitoba, which were determined from ANOVA technique and using a LSD of 5%, are shown in Table 7. However, Friedman's Nonparametric Analysis indicated the treatments Tecto 40 - "late", Uni 2002 (Vitaflo) - "early + late" and $HgCl_2$ - "early" did not provide control equal to that of the other lettered treatments and therefore should have been excluded from the list.

RESULTS & DISCUSSION

A) Snow Mold Pathogens found in Southern Manitoba:

Isolations of Typhula sp. and the unidentified low temperature basidiomycete (LTB) from infected turf tissue were very successful. Typhula sp. was successfully isolated by plating surface sterilized tissue onto PDA medium and incubating at 2°C. This pathogen was successfully isolated from plots at Pine Ridge, Wildwood, Sandy Hook and the University of Manitoba (Table 6). Typhula sp. was the only snow mold pathogen isolated from the turf samples taken from Wildwood and was believed to be the predominant pathogen in the test sites at Pine Ridge, Sandy Hook's No. 5 green, and the University of Manitoba (Table 6). Dr. J. D. Smith, CDA Research Station, Saskatoon, Saskatchewan, identified this organism as Typhula FW, a common typhula snow mold pathogen in Saskatchewan. The sclerotia are approximately 0.25 to 0.5 mm in diameter, globular, tawny in colour when young and dark brown to black when mature (Figure 2 A). They were very loosely attached to the plants, sometimes being suspended in mycelial wefts (Figure 3B). The characteristic halo of grayish-white mycelium (one to several inches in width) at the margin of infected areas on turfgrass aided in the diagnosis of typhula snow mold in the early spring (Figure 3A).

The LTB was isolated from the University of Manitoba test area and from the No. 2 green at Sandy Hook, but for some reason unknown it was not isolated or believed to be present on No. 5 green at Sandy Hook where the test plots were located (Table 6). It was easily isolated from tissue directly plated onto PDA or PDA plus Tersan 1991 (0.5%) media and from surface sterilized tissue incubated

on PDA medium alone. Because the symptomatology of the diseased areas at Breezy Bend resembled that of the LTB (Figure 4A) described by Couch (12) and the lack of sclerotia in these areas, it was believed that this organism was the predominant pathogen at this location (Table 6). At the University of Manitoba the LTB was isolated from the area where the snow was deepest and was believed to be the second most predominant pathogen in this test area. In culture the mycelium was sterile, white, fluffy and contained numerous clamp connections (Figure 5). Identification was confirmed by Dr. J. D. Smith.

Neither Sclerotinia borealis nor Fusarium nivale was recovered from diseased tissue at any of the locations; however, large, black, irregularly-shaped sclerotia of S. borealis (Figure 2B) were found and collected from four of the five test locations (Table 6). It was particularly abundant at Breezy Bend and, in association with the LTB, caused severe damage to the turfgrass on the test site. Also, it was the predominant pathogen on the No. 2 green at Sandy Hook and throughout all the putting greens surveyed at Breezy Bend. Although these greens had been treated with mercuric chloride in late fall, the presence of very deep snow favoured the development of this organism. At Pine Ridge, the University of Manitoba and on No. 5 green at Sandy Hook S. borealis was present, but not as prevalent as Typhula FW. At all locations damaged areas were circular, whitish-bleached in colour, and varied from 1"-12" in diameter (Figure 4B). Within the infected areas large, black sclerotia were either lying loosely upon the dead turf or embedded in leaf axils or shoot bases.

F. nivale was believed to have caused damage to the turf on the No. 2 green at Sandy Hook and at the Pine Ridge, Breezy Bend and Sandy Hook test areas (Table 6), since, at the time of snow melt, the presence of perithecia on infected leaves and the pinkish colour of the damaged areas indicated the presence of this pathogen. The author found F. nivale to be abundant at only the Pine Ridge and Sandy Hook test sites. Platford (32) reported F. nivale damage on some of the putting greens at the Wildwood Golf Course in the spring of 1974. He successfully isolated the pathogen from infected tissue incubated on PDA medium at 40°C.

A fungus producing an orange, rindless sclerotium (ORS) was found only at Pine Ridge Golf Course on the fairway turf surrounding the test plot. The spherical or ovoid regular or flattened sclerotia were 0.25 - 2 mm in size and were found completely embedded and covered by foliar tissue (Figure 2C). Identification of the ORS fungus was confirmed by Dr. J. D. Smith. Attempts to show pathogenicity have failed but the significance of this fungus may be in its antagonism to F. nivale, LTB, Typhula spp. and S. borealis at low temperatures (37).

In summary, Typhula FW was isolated from all locations. The LTB was isolated from two locations and believed to be the predominant pathogen at a third site. S. borealis was also widespread with sclerotia being collected from four of the five test sites. F. nivale was believed to be present at four locations but generally the total damage incurred was not severe.

B) Snow Cover & Disease Incidence at the Various Test Locations

Disease incidence was on the average lower and variation greater between replicate plots at the University of Manitoba than at the other locations. The wide variation in disease was believed to be the result of an uneven distribution of snow cover over the test area. Plots located on the extreme west and southwest, where snow cover was deepest, had the highest incidence of disease whereas even the untreated check plots on the extreme east and northeast areas of the site, where snow cover was light, had little if any disease damage. Presumably the environmental conditions in the exposed areas were less than optimum for disease development.

Snow cover at the other test locations was relatively deeper and more uniformly distributed, because of protection from the wind, than at the University of Manitoba. Also snow melt was considerably slower thus providing ideal conditions for snow mold development and hence disease damage was proportionally greater.

(C) Fungicide Effectiveness

The University of Manitoba test location was unique because it had the lowest disease incidence of all locations and received four treatments which were not tested at the other locations (Table 5). Except for mercuric chloride and borax in combination with Terraclor 75W, all the "early" treatments were inadequate in controlling the disease (Table 7). Tersan SP, Uni 2002 and Tecto 40, applied alone and "early" were completely ineffective. All treatments, except Tersan SP (57%) and Tecto 40 (0%), gave good to excellent control when applied both "early" and

"late" (½ rate each time) (Table 7). Control ranged from 80% for Uni 2002 to 99% for mercuric chloride and the borax:Terraclor 75W combination. All treatments, except Tecto 40 (see page 82) were highly effective in controlling snow mold when applied "late" (Table 7). Table 7 shows that, regardless of the time of application, the Terraclor 75W: borax treatment always gave control which was at least as good as that by mercuric chloride.

Comparing the "early + late" treatments to the other two application categories, it appears that the "late" application of the "early + late" category was largely responsible for the control given by all chemical treatments (except Tecto 40) (Table 7). Therefore, a reduction in the application rates appears to be feasible and should be further tested, particularly with the mercuric chloride and borax combination treatments.

At the remaining four locations all the compounds and combinations of compounds tested were similar with variation in application dates being the only difference (Table 5). At Pine Ridge and Breezy Bend Golf Courses (Table 8), all "early" treatments (except mercuric chloride at Pine Ridge) gave inadequate control and are not worth further consideration. However, at Breezy Bend the "early" applied borax combination treatments gave much better control than all the individual "early" treatments. As at the University of Manitoba, borax: Terraclor 75W - "early" at Breezy Bend controlled snow mold better than any of the other "early" treatments and was exceeded only by mercuric chloride - "early" and Terraclor 75W - "early" at Pine Ridge. In the "early + late" category, only the borax combination treatments gave excellent control of snow mold at Breezy Bend. When applied "late", excellent control was given by mercuric chloride, borax and the borax combination treatments.

At Pine Ridge, the best treatments were 1) "early + late" and "late" - $HgCl_2$, Terraclor 75W, borax: Tersan SP, borax: Terraclor 75W and borax: Vitavax 75W 2) "late" only - borax, Vitavax 75W and borax: Uni 2010. Generally, $HgCl_2$ gave the best control over all application categories.

As at the University of Manitoba, effective control given by many of the "early + late" treatments at Pine Ridge and Breezy Bend appeared to be largely the result of the one-half dosage "late" application and therefore a reduction in the application rates of these compounds appears feasible and should be further tested. This was particularly true of all the "early + late" borax combination treatments at Breezy Bend and of the "early + late" mercuric chloride, Terraclor 75W, borax: Terraclor 75W, borax: Tersan SP and borax: Vitavax 75W treatments at Pine Ridge.

As mentioned, all treatments at Sandy Hook and Wildwood were applied "late" only. As at the other locations, mercuric chloride provided excellent control in these tests, with the borax: Terraclor 75W combination being equally effective at both locations (Table 9). Borax: Tersan SP and borax: Uni 2010 gave 83% and 70% disease control respectively, while borax: Terraclor 75W and mercuric chloride each give 98.5% disease control at Sandy Hook. Individual compounds, other than mercuric chloride, at Sandy Hook gave poor to no control, borax (58% control) being the most effective. The best treatments at Wildwood were mercuric chloride, Vitavax 75W and all the borax combinations. Of these, only mercuric chloride borax: Terraclor 75W and borax: Vitavax 75W gave almost perfect control. Vitavax 75W and borax were the most effective of the individually applied treatments with 78% and 68% disease control

respectively.

All results indicate there was some advantage in combining borax with certain other compounds, especially with Terraclor 75W. Regardless of the time of application, borax: Terraclor 75W always provided control that was as good as or better (except when applied "early" at Pine Ridge) than that given by mercuric chloride (standard control compound) (Figure 6A, B).

D) Fungicide Phytotoxicity

Phytotoxicity of the borax and borax combination treatments when applied "early + late" or "late" at Breezy Bend and the University of Manitoba, or "late" at Wildwood and Sandy Hook, was very noticeable in the spring (Table 10). However, the damage was not permanent and with the exception of Wildwood, the turf recovered in a very short time. As long as control is not adversely affected it may be possible to eliminate or at least sharply reduce the phytotoxicity problems associated with these treatments by using lower rates and uniform application. Possibly a dilution effect caused by spring flooding was responsible for the lack of phytotoxicity at the Pine Ridge site (Table 10).

Shortly after the "early" treatment of the plots at Breezy Bend, rain wetted the whole test area and within four to five days all borax and borax combination plots showed signs of severe bleaching. However, in the spring these phytotoxic effects had almost completely disappeared (Table 10).

E) Time of Fungicide Application in Manitoba

"Early" application (approximately third week of September) of compounds generally provided inadequate snow mold control at all test locations. "Early" application is poor possibly because of the higher amounts of rainfall, usually experienced in September and early October, which could dilute fungicide potency by washing the compound(s) from the zones or areas in the turf where it is needed (15). Since several of the organic compounds are short-lived in the soil (3,16), chemical decomposition may be another cause of the ineffectiveness of "early" applied compounds at the time of pathogen activity.

Ordinarily in Manitoba snow mold control chemicals are not applied to putting greens until just before the onset of winter. In several of the past few years, heavy snowfall, earlier than usual, has interfered with this operation and the tendency now is for some superintendents to treat in mid-October. While a critical date for effective control was not established in this experiment results do indicate that late fall treatment is a sound practice.

F) Factors Favouring Development of the Pathogenic Organisms

The long winter of 1973-74 with an abundance of snow cover over slightly frozen ground and a slow thaw in the spring, provided excellent conditions for the development of snow mold organisms in Manitoba. With the exception of the Wildwood location, all test sites and areas surveyed received damage from a complex of low temperature pathogens including Typhula FW, S. borealis, F. nivale and the LTB (Table 6). Lebeau (26) reported that snow mold in southern Alberta is caused by a disease complex whose

principal components are F. nivale and the LTB. Complexes of F. nivale and Typhula spp. have also been found occurring in the Pacific northwest (9). Furthermore, Smith (37) has found many snow mold pathogens existing in harmony throughout many parts of Saskatchewan. Exploratory studies have indicated that little antagonism exists between F. nivale, LTB, S. borealis, and Typhula spp. in pure culture (37).

Local variations in microclimate are probably of more significance in determining pathogen prevalence than climate generally (37). The occurrence of the LTB at the University of Manitoba, Breezy Bend Golf Course, and Sandy Hook's #2 green, but not at Pine Ridge, Wildwood or Sandy Hook's #5 (test site) green substantiates this theory. However, microclimate variation may be in part due to turfgrass management practices. The use of snow fencing or covering greens with branches allows snow to accumulate in greater quantities. This, in conjunction with a slow thaw in the spring is advantageous to all the snow mold organisms (10,11,13,31). The soil base of putting greens is usually quite sandy in order to prevent such problems as compaction and to increase aeration and drainage of the area. Unfortunately, sandy soils appear to favour the development of typhula blights (14) which were the major cause of damage to putting greens in the areas of Manitoba tested and surveyed. Prior to snowfall, top-dressing of the putting greens with a highly organic soil is a common practice at Sandy Hook Golf Course. Just prior to snowfall the top-dressing was removed from Sandy Hook's No. 2 green to facilitate spraying operations since

this green was also to be used as a test site. Since removal was very laborious, the top-dressing on the other test site (No. 5 green) was worked into the turf with a metal mat prior to chemical application. In the spring all the greens, except No. 2, were severely damaged by the low temperature pathogens (primarily Typhula FW) (Table 6). Infection was so low on No. 2 green that it was abandoned as a test site. This highly organic soil appeared to enhance disease development at Sandy Hook Golf Course, but no concrete explanations as to why were revealed in this study.

In 1972, the LTB was observed to be the most serious pathogen of turfgreas in Manitoba (33). Platford et al (33) stated that typhula snow mold was not a major problem in golf greens and caused scattered damage in Winnipeg. However, in this study Typhula FW was isolated and found to be the predominant pathogen at four test sites and was at least present at the fifth location (Breezy Bend) (Table 6). S. borealis was present at four locations and was particularly abundant at Breezy Bend Golf Course. The ability of S. borealis to withstand high osmotic forces allows this pathogen to develop on frozen substrate (10). Whether its abundance at Breezy Bend Golf Course was due to substrate temperature, variety susceptibility, high inoculum levels or a combination of factors is not known. The answers to why Typhula FW was the only pathogen present on the Wildwood test site, why the LTB was found at the University of Manitoba, Breezy Bend Golf Course and on No. 2 green at Sandy Hook, but not at Pine Ridge, Wildwood and on No. 5 green at Sandy Hook and why F. nivale was believed to be present at only Pine Ridge, Breezy Bend and Sandy Hook are undoubtedly very complex and were not revealed in this study.

G) The Fungicides and the Pathogens Controlled

Mercuric chloride ($HgCl_2$ - corrosive sublimate)

Mercuric chloride is being used effectively by many of the golf courses in Manitoba to attain a broad spectrum control of the snow mold pathogens on their putting greens. As expected, the control achieved with mercuric chloride - "late" at 6 oz/1000 sq. ft. ranged from excellent to perfect at all locations (Tables 7-9). Even when applied "early" and "early + late", mercuric chloride effectively controlled the LTB at Breezy Bend. However, S. borealis appeared to be less inhibited and caused some damage to these plots. The 6% disease incidence level of the $HgCl_2$ "late" treatments at Breezy Bend was due primarily to the presence of S. borealis in these plots. A survey of other greens at Breezy Bend which had all been treated with $HgCl_2$ revealed that while total damage was not severe, that which did occur was caused by S. borealis. It seems reasonable to conclude that S. borealis has some tolerance to mercury.

At Pine Ridge considerable mice damage occurred to turf within the test area. Significantly all plots treated with mercury were untouched by mice possibly because of its high mammalian toxicity (24). This feature of mercuric chloride along with its possible long term residual effects on soil ecology (5,34), its high cost (8,24) and phytotoxicity (18,48) are disadvantages that must be considered in the use of this chemical. In this study, phytoxicity effects of $HgCl_2$ ranged from very slight to moderate but were very short lived (Table 10).

Terraclor 75W

Terraclor 75W (PCNB) is usually applied to turfgrasses as a foliar spray and is extremely effective in controlling S. borealis (20,21). It has also been reported to control F. nivale (19,38), typhula snow mold (50) and the LTB (38). However the extremely poor control by Terraclor 75W - "late" at Breezy Bend (Table 8) can be attributed to the high incidence of LTB at this location (Table 6). At Breezy Bend, Sandy Hook, Pine Ridge and the University of Manitoba no S. borealis was found in any of the "late" treated PCNB plots. PCNB "late" also appeared to be effective against F. nivale at Sandy Hook and Breezy Bend, but not at Pine Ridge. At Wildwood, which was infested with Typhula FW only, PCNB alone gave 59% control and therefore must be considered at least partially effective against this pathogen.

PCNB is considered and has been reported to be non-phytotoxic (21,44) and results of this study support these findings (Table 10).

Tersan SP

Tersan SP (chloroneb) is a systemic fungicide (22) which usually becomes localized in the hypocotyl and root regions of plants (43). This may account for the many reports of its high effectiveness in controlling typhula snow mold (27,47,48,49,50). Chloroneb also has been reported to significantly reduce infections caused by S. borealis, F. nivale and the LTB (37,49).

At Sandy Hook, Breezy Bend, Pine Ridge and the University of Manitoba test sites, no evidence of S. borealis was found in any of the chloroneb - "late" treated plots. At Breezy Bend all the chloroneb-treated plots were severely infected with the LTB which accounts for its poor degree of control at this location (Table 8).

At the University of Manitoba where damage in the check plots was associated primarily with Typhula FW, chloroneb - "late" gave approximately 91% disease control (Table 7). However as stated earlier, disease incidence at this location was generally low. At Pine Ridge, Wildwood and Sandy Hook where Typhula FW predominated, chloroneb - "late" was not very effective, giving only 45%, 28%, 43.5% disease control, respectively (Tables 8 and 9). It also appeared to be ineffective against F. nivale at Sandy Hook and Pine Ridge. The ineffectiveness of chloroneb may be explained on the basis of formulation used. Chloroneb in this study was applied as a liquid spray whereas Vargas & Beard (47) found that granular chloroneb was more effective than the wettable powder formulation in controlling typhula blight. They believed that this might be due to a slower rate of breakdown and longer residual effect of the former. On the other hand, chloroneb simply may not be effective against Typhula FW in Manitoba. Snow mold fungicide tests on a turf nursery at Prince Albert, Saskatchewan, in 1973-74, indicated that chloroneb was only moderately effective against Typhula FW (37).

Chooroneb was not phytotoxic to the turf at any of the test locations in this study (Table 10).

Vitavax 75W

Vitavax 75W is an oxathiin compound which is systemic and readily translocated within the plant (40). When applied "late" it gave effective control of Typhula FW at Wildwood and Pine Ridge and was extremely effective against the LTB at Breezy Bend. These results are in agreement with the unique feature of the oxathiin;

their specificity for the fungal class Basidiomycetes (30). For reasons unknown Vitavax 75W did not give effective control of Typhula FW at Sandy Hook. It appeared ineffective against S. borealis whenever it was present. Smith (38) found Vitavax to be effective against F. nivale. This was borne out by results at Pine Ridge and Breezy Bend, but not at Sandy Hook.

Vitavax 75W increases the plant's nitrogen absorption and promotes early growth (3). The dark green growth of many of the plots treated with this compound is proof of this most desirable characteristic. From an ecological viewpoint, Vitavax 75W is also desirable because it is short lived in the soil (3). This probably explains why the "early" application of this compound did so poorly at all sites.

Uni 2010:

Uni 2010 is a new, experimental turf and soil fungicide which was formulated to control root rot and damping-off pathogens (4). According to UniRoyal Chemical, it will control snow mold, fairy ring, brown patch, fusarium patch, dollar spot and stripe smut diseases of turf grasses. Uni 2010 was almost totally ineffective against Typhula FW at Wildwood and Pine Ridge test sites. At Sandy Hook, Typhula FW incidence in Uni 2010 treated areas was extremely high indicating that this compound was ineffective against the pathogen. At Breezy Bend, Uni 2010 was reasonably effective against the LTB, which accounts for the 73.5% disease control attained at this location (Table 8). Disease damage which was present within the Uni 2010 plots was due only to S. borealis. On the other hand,

for reasons unknown, Uni 2010 did appear to be effective against S. borealis at the Sandy Hook and Pine Ridge locations. Fortunately this study indicated that the phytotoxicity of Uni 2010 was nil (Table 10).

Tecto 40 (TBZ) and Uni 2002 (Vitaflo)

These compounds were tested only at the University of Manitoba during the fall and winter of 1973-74. TBZ is a systemic and is a derivative of the benzimidazole group of fungicides (29,44). It has been reported effective in controlling Fusarium and Sclerotinia spp. (29,37,44), and has been shown to be effective on turf artificially inoculated with LTB (37). In this study TBZ appeared to be effective against S. borealis but not against Typhula FW or the LTB. Table 7 indicates that Tecto 40 - "late" gave sixty-nine percent disease control at the University of Manitoba test site. However, this level of control may not be accurate because the incidence of disease throughout the entire University of Manitoba test site was generally low. Despite reports of its non-phytotoxic nature (44), phytotoxicity ranged from nil to moderate in this experiment (Table 10). Another problem with TBZ is that it has been found to be short-lived in the soil (16).

Vitaflo is a combination of Vitavax and thiram formulated as a liquid suspension (3). At the University of Manitoba, it appeared to be effective against S. borealis and the LTB but not against Typhula FW. Vitaflo was slightly phytotoxic to the turf but the effects were very short-lived. Vitaflo also breaks down easily in the soil (3), which may explain why no disease control was given by the "early" treatment (Table 7). Where mice cause severe damage

to turf, Vitaflo may be beneficial since it contains thiram, a non-toxic repellent of birds, deer and rodents (3).

Borax

Borax has been reported effective in controlling snow mold caused by the LTB (24,25). This was substantiated by in vitro studies by the author (1,2) and by the results of this study. The high levels of control attained by borax - "late" at the University of Manitoba (98%) and at Breezy Bend (94%) were probably due to its high effectiveness against the LTB. Borax also gave 68% control at Wildwood (a pure Typhula FW infection), 88% at Pine Ridge (predominantly Typhula FW) and 58% at Sandy Hook (predominantly Typhula FW) and therefore must be considered somewhat effective against Typhula FW. However, borax appeared to be ineffective against F. nivale and S. borealis since these pathogens were found causing damage to plots treated with this compound.

The advantages of borax are 1) its availability, 2) low cost, 3) low mammalian toxicity and, 4) high solubility which does not allow it to persist in well-drained soils for extended periods of time. Phytotoxicity appeared to be the major problem of this compound. Liquid sprays of borax are known to be very injurious to vegetative hosts (24). Fortunately dry application of borax has been found to be effective in controlling snow mold (17) and other diseases (39). However, when applied dry at the rate of 1 lb/ 1000 sq. ft., snow mold control is poor and applied dry at 3 lbs/ 1000 sq. ft., phytotoxicity effects appear (17). This low margin of safety dictates that borax must be applied uniformly at approximately 2 lbs/1000 sq. ft. It was observed that the phytotoxic effects of borax on turfgrass, if not extremely severe, were usually short-lived.

Borax Combinations

The borax combination treatments appeared to be very compatible in terms of disease control. When applied "late" all the combination treatments (except borax: Vitavax 75W at Sandy Hook) at all the test sites gave control that equalled mercuric chloride - "late"(Tables 7-9). Regardless of the time of application, the combination treatments generally gave much better control than the individual compounds comprising the combinations. This effect was probably the result of a broader spectrum control and of an additive effect resulting from the combination-treated plots receiving applications of two compounds (i.e. borax and Terraclor 75W or Tersan SP or Uni 2002 or Tecto 40 or Vitavax 75W or Uni 2010), each at the respective rates indicated on Table 5. Whether or not some of the combination treatments reacted synergistically to provide control is not known. Borax: Terraclor 75W and borax: Tersan SP combinations - "early + late" and "late" in all cases gave very good to excellent control. Also the borax: Tecto 40 - "late" combination and particularly borax: Vitaflo - "early + late" and "late" combination gave good control at the University of Manitoba.

At Sandy Hook and Breezy Bend, the borax: Vitavax 75W - "late" combination appeared to be ineffective against S. borealis. This is logical since both of the individual - "late" applied compounds were also ineffective against this pathogen. Even though borax: Uni 2010 gave 70% control at Sandy Hook, incidence of S. borealis and Typhula FW, particularly the former, was common in plots treated with the chemical combination. Generally, the

phytotoxicity problem with the borax combination - "early + late" and "late" treatments was great but fortunately short-lived. The phytotoxic effects were usually seen as blackened areas on the turf (Figure 7A), but occasionally appeared as severe bleaching (Figure 7B), both of which were probably caused by the borax. However, since rate reductions appear feasible it is probable that phytotoxicity problems of the borax combination treatments could be eliminated.

Table 5. Test sites with times and rates of application for each of the compounds tested.

Compound(s)	Location[1]	Rate of Application[2]		
		E[3]	E + L	L
Borax	A	2	1 + 1	2
Mercuric Chloride	A	6	3 + 3	6
Terraclor 75W	A	8	4 + 4	8
Tersan SP	A	9	4.5 + 4.5	9
Uni 2002	1	8	4 + 4	8
Tecto 40	1	2	1 + 1	2
Vitavax 75W	2; 3; 4; 5	4	2 + 2	4
Uni 2010	2; 3; 4; 5	8	4 + 4	8
Terraclor 75W: Borax	A	R A T E S	S A M E	A S
Tersan SP:Borax	A			
Uni 2002: Borax	1			
Tecto 40:Borax	1			
Vitavax 75W:Borax	2; 3; 4; 5	INDIVIDUAL RATES		
Uni 2010:Borax	2; 3; 4; 5			

(1) Locations used A = all locations; 1=University of Manitoba; 2=Pine Ridge Golf Course; 3=Wildwood Golf Course (late application only); 4=Breezy Bend Golf Course; 5=Sandy Hook Golf Course (late application only).

(2) All compounds, except borax, applied with water at 3 gals/1000 sq. ft. Borax applied dry. All rates in ozs./1000 sq. ft., except borax which is pounds/1000 sq. ft.

(3) E = early application third week of September (full rate).
L = late application fourth week of October (full rate).
E + L = ½ rates applied at both early and late applications.

Table 6. Snow mold pathogens occurring at various locations in southern Manitoba.

Location	Predominant Pathogen	Other Pathogens Present
University of Manitoba	Typhula FW[1]	LTB[1] (In high abundance) S. borealis[1]
Breezy Bend Golf Course	LTB	S. borealis[1] (in high abundance); F. nivale and Typhula FW[1] (very low incidence)
Pine Ridge Golf Course	Typhula FW[1]	F. nivale (high incidence); S. borealis[1]
Wildwood Golf Course	Typhula FW[1]	None
Sandy Hook Golf Course (Green No. 5)	Typhula FW[1]	F. nivale and S. borealis[1] (both organisms in high incidence)
Sandy Hook Golf Course (Green No. 2)	S. borealis[1]	LTB[1] (high incidence); Typhula FW[1] (high incidence); F. nivale (low incidence)
Breezy Bend Golf Course (Putting Greens)	S. borealis[1]	None

(1) Pathogen isolated in culture or identified from sclerotia present in damaged turf areas; otherwise the pathogens were believed to be present from symptomatology of diseased areas.

Table 7. Snow mold control provided by the various chemical treatments at the University of Manitoba[1].

Compound	"Early"[2]	"Early + Late"	"Late"
Mercuric Chloride	74[a][3]	99[a]	98[a]
Borax	33[4]	81[a]	98[a]
Terraclor 75W	23	84[a]	93[a]
Tersan SP	0	57	91[a]
Uni 2002 (Vitaflo)	0	80[a]	94[a]
Tecto 40	0	0	69[a]
Borax:Terraclor 75W	86[a]	99[a]	100[a]
Borax:Tersan SP	25	95[a]	99[a]
Borax:Uni 2002	67	95[a]	100[a]
Borax:Tecto 40	61	97[a]	100[a]

(1) Test green primarily Seaside and Penncross creeping bentgrass.

(2) Time of application; "early" and "late" = full rate of application of each compound third week of September and end of October, respectively; "early + late" = each compound applied "early" at ½ rate and then "late" at ½ rate.

(3) Means within columns followed by the same letter indicate chemicals which gave best control and are statistically equal at the 5% level using the Least Significant Difference method.

(4) Disease control values expressed as a percentage of the check plots. Values were obtained by conversion from field rating numbers (12 point scale). Conversion tables used were obtained from Elanco Conversion Tables of Barrett - Horsfall Rating Numbers. Eli Lilly and Company., Greenfield Laboratories, Greenfield, Ind.

Table 8. Snow mold control provided by the various chemical treatments at Pine Ridge (PR)[1] and Breezy Bend (BB)[2] Golf Courses.

Compound	"Early"[3]		"Early + Late"		"Late"	
	PR	BB	PR	BB	PR	BB
Mercuric Chloride	85[4]	38.5	100[b5]	43	100[b]	94[c]
Borax	0	15.5	74.5	75	88[b]	94[c]
Terraclor 75W	39	29	92[b]	22	96[b]	14
Tersan SP	1.5	7	28	13	45	11
Vitavax 75W	7.5	32	74.5	47	92[b]	78
Uni 2010	12	6	1.5	78	16	73.5
Borax:Terraclor 75W	26	74.5	96[b]	99[c]	99[b]	97.5[c]
Borax:Tersan SP	14	59	94[b]	97.5[c]	100[b]	98[c]
Borax: Vitavax 75W	0	65	97.5[b]	95.5[c]	99[b]	98[c]
Borax:Uni 2010	0	58	78	90[c]	99.5[b]	98[c]

(1&2) Test areas primarily Washington bentgrass and Poa annua at BB and Kentucky bluegrass and native bentgrass at PR.

(3) Time of application; "early" and "late" = full rate of application of each compound third week of September and end of October, respectively; "early + late" = each compound applied "early" at ½ rate and then "late" at ½ rate.

(4) Disease control values expressed as a percentage of the check plots. Values were obtained by conversion from field rating numbers (12 point scale). Conversion tables used were obtained from Elanco Conversion Tables of Barratt - Horsfall Rating Numbers. Eli Lilly and Co., Greenfield Laboratories, Greenfield, Ind.

(5) Means within columns followed by the same letter indicate chemicals which gave best control and are statistically equal at the 5% level using the Least Significant Difference method.

Table 9. Snow mold control provided by the various chemical treatments at Sandy Hook[1] and Wildwood[2] Golf Courses.

Compounds Used	Sandy Hook[3]	Wildwood[4]
Mercuric Chloride	98.5[d5]	99.5[e]
Borax	58[6]	68
Terraclor 75W	30.5	59
Tersan SP	43.5	28
Vitavax 75W	30.5	78[e]
Uni 2010	0	8.5
Borax:Terraclor 75W	98.5[d]	98.5[e]
Borax:Tersan SP	83[d]	86.5[e]
Borax:Vitavax 75W	48	98[e]
Borax:Uni 2010	70[d]	90[e]

(1&2) Test areas primarily Poa annua at Sandy Hook and Cohansey bentgrass at Wildwood.

(3&4) Treatments applied at end of October.

(5) Means within columns followed by the same letter indicate treatments which gave best control and are statistically equal at the 5% level using the Least Significant Difference Method.

(6) Disease control values expressed as a percentage of the check plots. Values were obtained by conversion from field rating numbers (12 point scale). Conversion tables used were obtained from Elanco Conversion Tables of Barrett - Horsfall Rating Numbers. Eli Lilly and Co., Greenfield Laboratories, Greenfield, Ind.

Table 10. Chemical phytotoxicity of turfgrass treated with the various fungicides at the different times of application at each of the test locations.

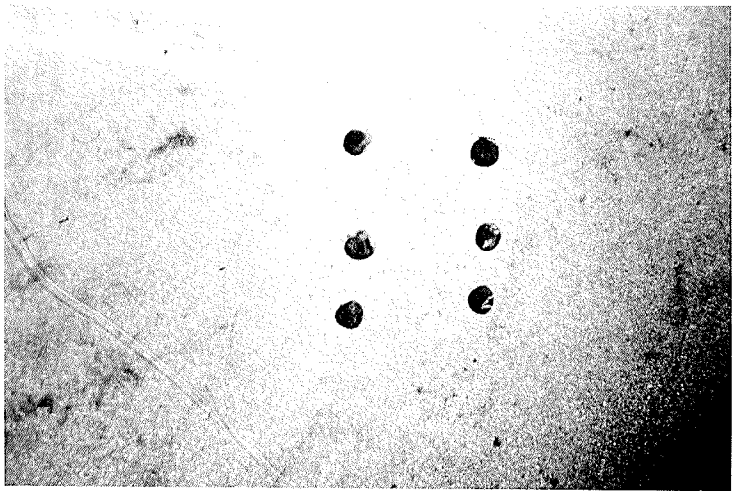
	"Early"[1]			"Early + Late"			"Late"				
Treatment	UM[2]	BB	PR	UM	BB	PR	UM	BB	PR	WW	SH
Mercuric Chloride	N[3]	N	N	N-S	N-M	N	VS-M	S-M	N	N-S	VS-S
Borax	N	N	N	S-M	N-H	N	M-H	M-H	M	H	N
Terraclor 75W	N	N	N	N-S	N	N	N-S	N	N	N	N
Tersan SP	N	N	N	N	N	N	N-S	N	N	N	N
Uni 2002	N			N-S			S				
Tecto 40	N			N			N-M				
Vitavax 75W		N	N		N	N		N	N	N	N
Uni 2010		N	N		N	N		N	N	N	N
Borax:Terraclor 75W	N	N-S	N	S-H	N	M-H	M-H	M-H	N-S	N-H	S-M
Borax:Tersan SP	N	N-S	N	S-M	M-H	N	M-VH	M-H	M	N-H	N-S
Borax:Uni 2002	N			S-M			M-VH				
Borax:Tecto 40	N			M-H			H				
Borax:Vitavax 75W		N	N		S-M	N		S-M	N-M	M-H	N
Borax:Uni 2010		N-S	N		M-H	N		M	N-M	N-H	N-VS

(1) "Early" = Compounds applied third week of September (full rate); "Late" - Compounds applied fourth week of October (full rate); "Early + Late" = Each compound applied "early" at half rate and then "late" at half rate.

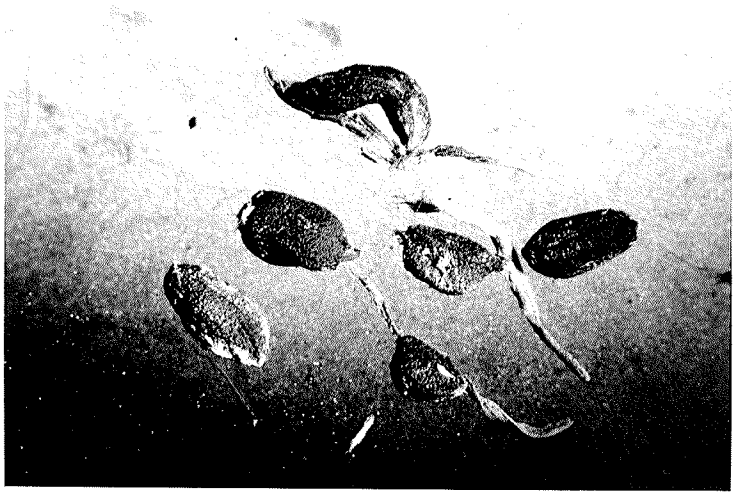
(2) UM - University of Manitoba; BB = Breezy Bend Golf Course; PR = Pine Ridge Golf Course; WW - Wildwood Golf Course; SH - Sandy Hook Golf Course.

(3) Phytotoxicity range over 3 replications/locations. N = None; VS = very slight; S = slight; M - Moderate; H = Heavy; VH = Very Heavy.

Figure 2. Sclerotia of three low-temperature fungi collected from various areas of southern Manitoba in the early spring of 1974. (A) The black, mature, globular sclerotia of the pathogenic fungus, Typhula FW. Magnified (X6). (B) The large, black, irregularly shaped sclerotia of the common turfgrass pathogen, S. borealis. Magnified (X6). (C) The flattened and spherical shaped sclerotia of the unidentified ORS fungus. The sclerotia are orange in colour and were found as shown - completely embedded in and surrounded by foliar tissue. Magnified (X6).



A



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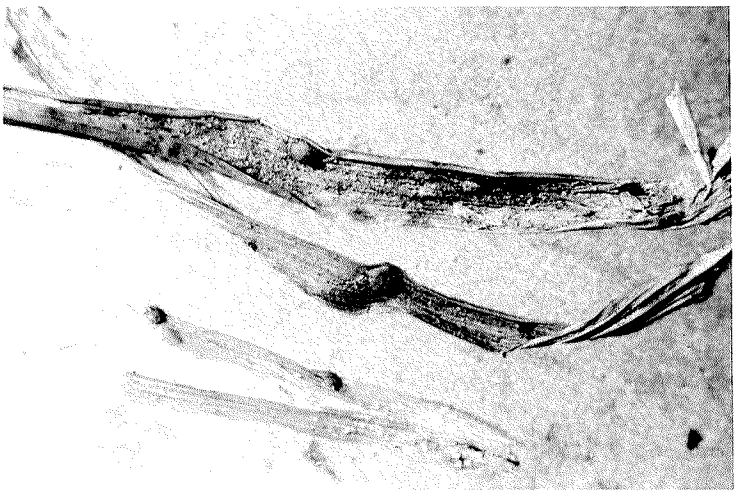


C

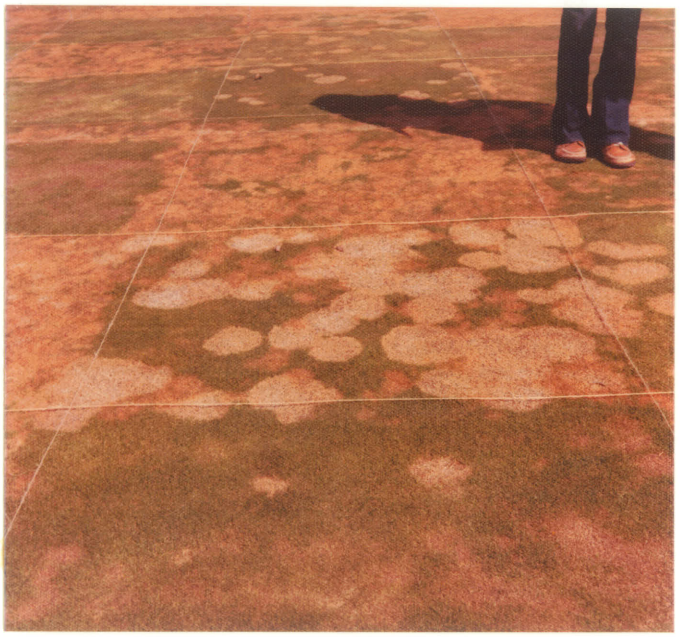
Figure 2

A

B

Figure 3. Showing the early spring diagnostic characteristics of Typhula FW attacking Agrostis turf. (A) The characteristic halo of grayish-white mycelium at the margin of the infected area. (B) The tiny, globular, black sclerotia being suspended in mycelial wefts and on leaf blades of the turf.

A



B

Figure 4. Disease symptomatology of two snow mold pathogens attacking Agrostis turf in southern Manitoba. (A) Showing the straw-colour appearance of completely killed Washington bentgrass infected with the unidentified low-temperature basidiomycete (B) Showing the circular, white to bleached in colour, damaged areas of Washington bentgrass attached by S. borealis.

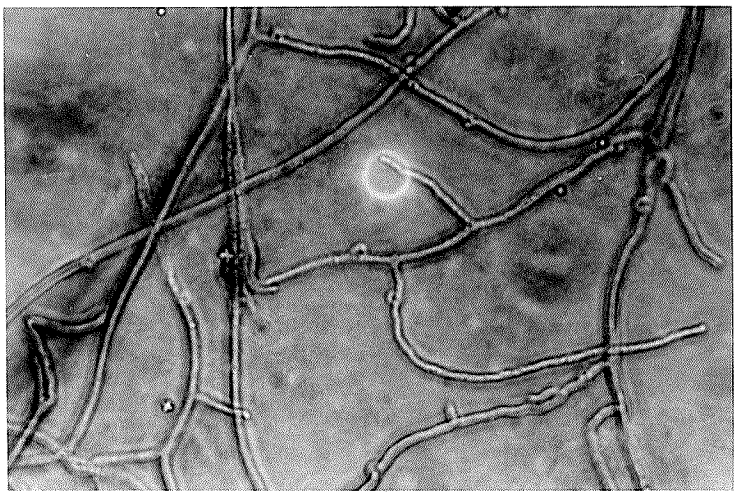


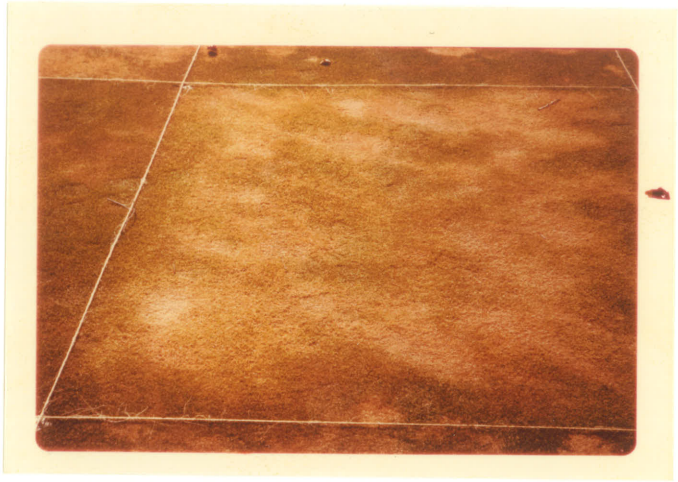
Figure 5. A photomicrograph showing the numerous clamp connections found on the hyphae of the sterile, psychrophilic basidiomycete isolated from infected turfgrass in southern Manitoba. Magnified (X400).

A

B

Figure 6. Showing the high level of snow mold control on Poa annua treated with mercuric chloride and a combination of borax and Terraclor 75W at the end of October. (A) Borax:Terraclor 75W. Note the localized areas of phytotoxicity. (B) Mercuric chloride Note the general discolouration of the turf.

A



B

Figure 7. Showing severe phytotoxicity of Agrostis turf resulting from an uneven application of borax and borax-containing mixtures of compounds. (A) Early spring symptoms of borax phytoxicity showing the localized, blackened areas of damage on Cohansey bentgrass. (B) Severe bleached-out effect of late applied borax: Terraclor 75 on a mixture of Seaside and Penncross bentgrass turf.

CONCLUSION

Snow mold of amenity turf in Manitoba was caused by several different pathogens. The pathogens isolated and/or recognized in this study were 1) Typhula FW (the most predominant pathogen) 2) S. borealis (found in relatively high incidence at four of the five locations tested and surveyed), 3) the LTB (found at Breezy Bend and the University of Manitoba and on the No. 2 green at Sandy Hook), and 4) F. nivale (believed to have been present at Pine Ridge and Sandy Hook in relatively high incidence and in low incidence at Breezy Bend). The generally poor control achieved in the "early" applications and the good control obtained when the same chemicals were used at the same rate later in the fall supports the practice of delaying chemical treatment until as late in fall as possible. Furthermore "early" application of borax and borax - containing compounds or mixtures, if followed by limited precipitation or even very heavy dew, could result in severe phytotoxicity. The lack of broad spectrum control by the individually applied non-mercurial compounds and their specificity towards certain pathogens resulted in erratic control over all the locations tested.

Borax - "late" was highly effective against the LTB and slightly effective against Typhula FW whereas Terraclor 75W - "late" was extremely effective against S. borealis and slightly effective against Typhula FW and F. nivale. The combined antifungal capabilities of these compounds resulted in the borax: Terraclor

75W - "late" combination being as good as or more effective than mercuric chloride - "late". The disadvantage of this combination treatment is that it can be phytotoxic to the turf. As explained earlier, a rate reduction of the borax: Terraclor 75W treatment appears feasible which, in conjunction with uniform application, may reduce or eliminate the phytotoxic effects. If so, this organic combination treatment would probably be more advantageous than mercuric chloride in all respects.

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GENERAL DISCUSSION

The psychrophilic snow mold pathogens, if not controlled, will cause extensive winter damage to the fine turf of golf and bowling greens and also to a lesser degree to domestic lawns, park grass and playing fields. The high cost involved in re-establishing these damaged areas to grass and the inconvenience to its users dictates that this overwintering destruction be minimized in an economical way.

As stated earlier, snow mold is a very complex disease (see Page 29), but can be effectively controlled in most years by applying mercurial compounds to the turf in the late fall (53,66). However, public clamor and prospective restrictive legislation against the use of mercurial compounds placed their future in jeopardy (4). Thus, this study was undertaken - with the ultimate goal of finding a non-mercurial, organic compound or combination of compounds which would effectively control snow mold of turfgrasses in Manitoba thereby eliminating the use of the mercurials.

Borax (sodium tetraborate) is one organic compound in which attention has been directed in regard to snow mold control. Conflicting reports have arisen regarding the effectiveness of borax in controlling this devastating disease. Lebeau et al (53) reported foliar sprays of borax to be effective against the low-temperature basidiomycete (LTB) on alfalfa, but not on turfgrasses; whereas in Manitoba it has been very effective as a dry or foliar-spray application, in controlling snow mold on putting greens (27). This study has focused on the effectiveness of borax and borax combined with other non-mercurial compounds in controlling this major disease of turfgrasses.

Platford et al (70) have observed the presence of the LTB in damaged turf for several years and reported this to be the most serious of the psychrophilic fungal pathogens attacking turfgrasses in Manitoba. From this arose the assumption that the usefulness of borax in this province was due to its inhibitory effect on the LTB. This assumption was reinforced by reports that borax was effective in controlling only the LTB snow mold pathogen (53,56). Ward et al (101) found that at least three different LTB isolates exist in the province of Alberta when compared on the basis of cultural behaviour and pathogenicity. One striking discovery of their work was the LTB-Type B isolates were highly virulent on turf grasses whereas LTB-Type A isolates were most pathogenic on alfalfa. Since Lebeau et al (53) found foliar applications of borax effective against LTB infested alfalfa but not against LTB damaged turfgrass, the obvious query which comes to mind is - does the antifungal activities of borax vary between the different LTB isolates?

The first part of the study was performed to investigate if and how different concentrations of borax affected the growth and/or hydrogen cyanide (HCN) production of four pathogenic isolates of the LTB. All concentrations of borax reduced the total amount of growth of all isolates to a level much below that of the untreated check colonies. However, the growth of the non-hydrogen cyanide-producing LTB isolates (LTB-Type A & LTB-Man.) was inhibited to a much greater extent by borax than that of the HCN-producing isolates (LTB-Sask. & LTB-Type B (W5)). This variation in sensitivity towards borax may be related to the variation in the resistance mechanisms between

the HCN-producing and non-HCN-producing isolates. For example, the resistance mechanisms of the LTB-B and LTB-Sask. were apparently very active and complex since they were able to exist and grow normally when subjected to high concentrations of extremely lethal HCN gas. Furthermore, Ward et al (101) has shown that the tolerance of the LTB-B to HCN exceeds that of LTB-A which will produce HCN only when in association with host tissue.

Table 1 indicates that borax had no effect on the HCN production of the LTB-B (W5) and LTB-Sask. isolates when the relationship of the colour range to the amount of growth are compared between the treated and untreated colonies. It appeared that the amount of HCN produced was dependent upon the amount of mycelium produced by the isolates. This was in complete contradiction to the studies performed by Lebeau and Atkinson (56) , but the discrepancies might be explained on the basis of differences in techniques (see Page 53 Study #1).

On the basis of their growth rates, their failure to produce HCN gas in culture, the development of dense, white, stroma-like bodies, and borax sensitivity, the LTB-Type A (Alberta) and LTB-Man (Manitoba) isolates appeared to be quite similar. These similarities and the finding of Ward et al (101) regarding the host virulence of the LTB-B and LTB-A isolates could be used as a logical explanation of why Ferguson (27) found borax to be effective in Manitoba and why Lebeau found it to be effective only in controlling the LTB attacking alfalfa (53).

Since borax was highly inhibitory to the growth of the LTB isolates, particularly LTB-A and LTB-Man, it seemed worthwhile to

begin an investigation into some of the factors responsible for its fungitoxicity. Other studies have indicated that borax increases the alkalinity of a substrate (43,47), but none have indicated that borax toxicity and increased substrate alkalinity are primarily responsible for control of the pathogens involved. Using synthetic media optimal for the growth of the LTB isolates (LTB-Sask, Type B and LTB-Man) and a soil extract medium (SE) prepared from soil used as putting green base, the inhibitory effects of borax and borax plus increasing substrate pH on the growth of these isolates were studied. By using synthetic media, optimal for the growth of the LTB isolates, it was assumed that any reduction in growth would have been due to borax toxicity only or to borax toxicity plus a pH affect. By utilizing a soil extract medium prepared from actual putting green-soil, it was assumed that stresses such as nutritional deficiencies (in comparison to synthetic media), pH (pH = 7.1 for the normal (borax-free) SE media and higher for borax incorporated SE media) and possible interaction of borax with soil constituents, would have produced results which would have been more indicative of borax-pH effects on the LTB isolates in the field than results obtained from the synthetic media. As expected the growth of all the isolates was poorer on the normal SE medium than on the normal synthetic media. However, regardless of the media used and throughout the concentration regime studied, a general effect of borax on the growth of all LTB isolates was apparent - an increase in borax concentration, without a corresponding increase in pH (i.e. pH equal to pH of normal media), resulted in a reduction of fungal growth; but a corresponding increase in borax concentration and pH resulted in drastic reduction of growth to the extent that total growth inhibition finally resulted at a lower

borax concentration than in the corresponding adjusted pH series (Table 2 & 3). This indicated the importance of increased alkalinity of the culture substrate in conjunction with borax toxicity in inhibiting the growth of the LTB isolates. Other interesting aspects which were revealed were that the LTB-Man isolate was much more sensitive to borax and borax-pH interaction than were LTB-B and LTB-Sask isolates. Borax was not fungicidal, but only fungistatic to the growth of all the LTB isolates (Table 4).

These studies provided additional support to the previous findings (53,56) that borax was inhibitory to the growth of the LTB even though it was not highly fungicidal to this pathogen. The variation in growth sensitivities of pathogenic LTB isolates could be used as an explanation of why borax control of snow mold in Western Canada has been so erratic.

As mentioned many times before, snow mold disease of turfgrass is very complex and is probably one of the most difficult to control. Difficulties in finding non-mercurial compounds which will effectively control this disease arise because several different fungal pathogens are known to be causal agents and each varies in its sensitivity toward many of the non-mercurial fungicides now available. Variation in sensitivity towards these compounds also exists between different isolates of the same pathogen (see Studies 1 & 2; Pages 39 and 57 respectively) making the search for a non-mercurial fungicide or combination of such compounds an extremely difficult task.

The field study conducted during the fall and winter of 1973-74 revealed that many different snow mold pathogens were responsible for turfgrass damage in southern Manitoba. Platford et al (70) reported

that typhula snow mold had not been a major problem to golf greens in Winnipeg, Manitoba. However, typhula snow mold, caused by Typhula FW, was the predominant disease found to be causing damage to turfgrass in Manitoba during this past winter. S. borealis and the psychrophilic, sterile basidiomycete were also highly abundant. F. nivale was believed to have been present in some areas, but damage caused by it was generally very minor. A complex of 3 or 4 pathogens was found causing damage to the golf greens in several different areas. The conflicting reports of this study with those of Platford et al (70), the variation in the abundance of the pathogens at different locations, and the different pathogen complexes which existed at the different locations (Table 6) were probably the result of local variation in microclimate (81). Furthermore, management practices such as the use of snow fencing or branches to accumulate snow on the greens, fall fertilization or lack of it, top-dressing, turfgrass varieties, etc. may have been responsible in part for the local variation in microclimate and ultimately in the prevalence of the pathogens.

In Manitoba, as elsewhere, mercurial compounds (mercuric chloride) are being used effectively to give a broad spectrum control of all the snow mold pathogens. Unfortunately the field study showed that all the non-mercurial, organic compounds tested, generally gave much poorer control than did mercuric chloride. These results are probably a reflection of the fungicidal or fungistatic specificity of the non-mercurial compounds which would severely hinder the effectiveness of such chemicals applied to an area infected with a complex of 3 or 4 pathogens. On the basis of percent disease control, borax, when applied at the end of October, generally was the most effective of all the individually applied non-mercurial compounds. In the field,

borax effectively controlled the LTB and also was slightly detrimental to the development of typhula snow mold. As with borax, all the other individually applied compounds (except $HgCl_2$) were effective in controlling only specific pathogens and therefore gave generally poor control of snow mold during the 1973-74 winter.

The borax combination treatments, particularly borax:Terraclor 75W, provided excellent control of the low temperature pathogens. All borax combination treatments were compatible and since borax alone was effective against the LTB and partially against Typhula FW and the other compounds were effective against the same and/or other pathogens, (Terraclor 75W was effective against S. borealis, F. nivale and partially against Typhula FW) it follows that these treatments would have given an effective broad spectrum control of snow mold.

Regardless of the compound, the treatment of the turf near the end of October (late application) provided much more control than did treatment around the third week of September (early application). Because of chemical breakdown and/or the abundant amount of rainfall which usually occurs during the fall, the effectiveness of the earlier treatments would undoubtedly be diminished. When applied "early + late", the borax combination treatments also gave effective disease control. Since the "early" application was relatively ineffective, it appeared that the "late" treatment of the "early + late" application was primarily responsible for control. Therefore, when applied "late" only, rate reductions of the borax combination treatments do appear to be very feasible. This would make the use of these treatments more economical - a highly desired characteristic of a good fungicide.

Phytotoxicity problems were severe particularly with the borax and borax combination treatments. Fortunately, much of the phytotoxic damage which occurred to "late" treated turf was due to an uneven distribution of the borax (Figure 7). Uniform application of the borax and borax combination treatments via use of a mechanical spreader in conjunction with a possible rate reduction of the compounds comprising the combination treatments would greatly reduce or eliminate this problem.

In conclusion, it appears that effective substitutes of mercurial compounds for the control of snow mold of turfgrasses in Manitoba are available by formulating mixtures of non-mercurial, organic compounds, such as borax and Terraclor 75W, and by applying them as late in the fall as possible.

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APPENDIX 1

Analysis of Variance for the Effects of Borax Concentration and Borax - pH interaction on the Growth of LTB - Manitoba Cultured on Potato - Dextrose Agar Medium (Study No. 2).

Source of Variation	D.F.	S.S.	M.S.	F.
TREATMENTS	8	8391.8056	1048.9757	3001.36**
ERROR	27	9.4375	0.3495	
TOTAL	35	8401.2431		

APPENDIX 2

Analysis of Variance for the Effects of Borax Concentration and Borax - pH Interaction on the Growth of LTB - Type B (W5) Cultured on Potato - Dextrose Agar Medium Study No. 2).

Source of Variation	D.F.	S.S.	M.S.	F.
TREATMENTS	8	4226.8750	528.3593	758.37**
ERROR	27	18.8125	0.6967	
TOTAL	35	4245.6875		

** 1.0 percent level of Significance.

APPENDIX 3

Analysis of Variance for the effect of Borax Concentration and Borax - pH Interaction on the Growth of LTB - Saskatchewan Cultured on Corn Meal - Malt Yeast Extract Agar Medium (Study No. 2).

Source of Variation	D.F.	S.S.	M.S.	F.
TREATMENTS	8	4869.8056	608.7257	1289.13**
ERROR	27	12.7500	0.4722	
TOTAL	35	4882.5556		

APPENDIX 4

Analysis of Variance for the Effects of Borax Concentration and Borax - pH Interaction on the Growth of LTB - Manitoba Cultured on Soil Extract Agar Medium (Study No. 2).

Source of Variation	D.F.	S.S.	M.S.	F.
TREATMENTS	8	5070.4097	633.8012	2590.12**
ERROR	27	6.6094	0.2447	
TOTAL	35	5077.0191		

** 1.0 percent level of Significance

APPENDIX 5

Analysis of Variance for the Effect of Borax Concentration and Borax - pH Interaction on the Growth of LTB - Type B (W5) Cultured on Soil Extract Agar Medium (Study No. 2).

Source of Variation	D.F.	S.S.	M.S.	F.
TREATMENTS	8	1754.6389	219.3298	186.52**
ERROR	27	31.7500	1.1759	
TOTAL	35	1786.3889		

APPENDIX 6

Analysis of Variance for the Effect of Borax Concentration and Borax - pH Interaction on the Growth of LTB - Saskatchewan Cultured on Soil Extract Agar Medium (Study No. 2).

Source of Variation	D.F.	S.S.	M.S.	F.
TREATMENTS	8	4346.2639	543.2829	943.53**
ERROR	27	15.5469	0.5758	
TOTAL	35	4361.8108		

** 1.0 percent level of Significance

APPENDIX 7

Analysis of Variance for Snow Mold Control Provided by the Various Chemical Treatments at the University of Manitoba. (Study No. 3)[1].

Source of Variation	D.F.	S.S.	M.S.	F.
TREATMENTS	32	45921.4492	1435.0452	7.01**
REPS.	2	1666.8867	833.4434	
ERROR	64	13111.0508	204.8602	
TOTAL	98	60699.3867		

APPENDIX 8

Analysis of Variance for Snow Mold Control Provided by the Various Chemical Treatments at Breezy Bend Golf Course (Study No. 3)[1].

Source of Variation	D.F.	S.S.	M.S.	F.
TREATMENTS	32	65821.5625	2056.9238	15.03**
REPS.	2	2330.3984	1165.1992	
ERROR	64	8759.7500	136.8711	
TOTAL	98	76911.7500		

1 The Arc - sin transformation was used prior to statistical analysis.

** 1.0 per cent level of Significance.

APPENDIX 9

Analysis of Variance for Snow Mold Control Provided by the Various Chemical Treatments at Pine Ridge Golf Course (Study No. 3)[1].

Source of Variation	D.F.	S.S.	M.S.	F.
TREATMENTS	32	76706.6875	2397.0840	16.37**
REPS.	2	417.0430	208.5215	
ERROR	64	9372.5625	146.4463	
TOTAL	98	86496.3125		

APPENDIX 10

Analysis of Variance for Snow Mold Control Provided by the Various Chemical Treatments at Sandy Hook Golf Course (Study No. 3)[1].

Source of Variation	D.F.	S.S.	M.S.	F.
TREATMENTS	10	15561.5898	1556.1589	7.38**
REPS.	2	234.2500	117.1250	
ERROR	20	4219.7695	210.9885	
TOTAL	32	20015.6094		

1 The Arc - sin transformation was used prior to statistical analysis.

** 1.0 percent level of Significance.

APPENDIX 11

Analysis of Variance for Snow Mold Control Provided by the Various Chemical Treatments at Wildwood Golf Course (Study No. 3)[1].

Source of Variation	D.F.	S.S.	M.S.	F.
TREATMENTS	10	20934.7266	2093.4727	10.06**
REPS.	2	469.1016	234.5508	
ERROR	20	4163.5234	208.1762	
TOTAL	32	25567.3516		

1 The Arc - sin transformation was used prior to statistical analysis.

** 1.0 percent level of Significance.