A STUDY OF THE SEVERITY OF PYTHIUM BLIGHT OF DROUGHT STRESSED AND NON-DROUGHT STRESSED TURFGRASSES IN KANSAS

by

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INTRODUCTION AND OBJECTIVES

During the summer of 1977, an unusually large number of cases of Pythium blight of turfgrass occurred in eastern and central Kansas. The disease occurred during hot humid rainy periods of June through August following a general drought. However, its severity appeared to be greater on turfgrass stands recently drought stressed while those stands which had received ample irrigation during the drought had less disease (47). Such a situation occurred in Johnson county (Kansas City area) where water was available for lawn irrigation on the east side of the state line road but was unavailable during the drought on the west side due to repairs being made in the municipal water system. The outbreak of Pythium blight developed in July following heavy rains during a hot humid period. Approximately the same number of patches occurred on the lawns (which were predominantly bluegrass) in both areas but those on the irrigated east side were one-half to one-tenth the size of those on the west side. In August of that same summer, a similar situation developed on some municipal golf courses in Wichita, Kansas. Pythium blight was severe during a hot rainy period preceded by a general drought and was especially noticeable on non-irrigated bluegrass fairways as opposed to the irrigated bentgrass greens. It should be noted that bluegrasses and bentgrasses are equally susceptible to Pythium blight (30). Preventative fungicides had not been used on any of these areas. These observations suggest that, under hot humid conditions, drought stressed turf may be more susceptible to Pythium blight than non-drought stressed turf. Since periods of drought stress followed by heavy rains are common in Kansas, it appeared reasonable to test the above hypothesis.

Before attempting to investigate this relationship, one should be aware of the conditions necessary for the occurrence of Pythium blight. Equally important are the pathogens involved, their survival, germination, and growth potentials, the etiology of the disease, and finally, the effects of drought stress on the pathogen, plant, and soil. These conditions will be elucidated in the following literature review and in discussion of the results of this study.

The objectives of this thesis were developed as a result of the observations of Pythium blight in Kansas during the summer of 1977. These objectives were:

a) to determine which Pythium species were causing blight of turfgrass in Kansas, and

b) to compare the severity of Pythium blight on drought stressed vs non-drought stressed turfgrass using zoospores as a source of inoculum.

The latter study was divided into three phases: a test of the severity of the disease on drought stressed vs non-drought stressed turfgrass under greenhouse conditions, a similar test under field conditions, and observations of Pythium blight on hydroponically-grown turfgrass subjected to a controlled water stress with the use of polyethlyene glycol.

I. Literature Review

A. The History and Occurrence of Pythium Blight on Turfgrasses in the U.S.

1. Disease Symptoms:

Pythium blight of turfgrass (commonly called cottony blight or grease spot) has received much attention in the southern states (26, 27,28,29,30,32,38,39,46,60,67,69,77,78,115,116) where it often occurs as a foliage blight destroying young stands of annual ryegrass used in overseeding bermudagrass. Foliage blighting often begins as a watersoaking of the leaves in 5-15 cm patches followed by production of aerial "cottony" mycelium observable under high humidity early in the morning. The turf appears greasy and patches may coalesce, often destroying large areas within 12-24 hr. In the north, the pathogens often occur as a complex of Pythium spp. (90) resulting in a foliage blight (80,91,112) or, more commonly, thinning of the turf as a foot and crown rot (90). Root rotting is responsible for seedling damping-off or thinning of older turfgrass stands.

2. History:

Pythium blight was first reported in 1926 on creeping bentgrass at the Arlington turf gardens, Rosslyn, Virginia (75). Within the following six years, the disease was observed in Washington D.C., Philadelphia, St. Louis, and Chicago (75). In 1952, ryegrass was observed to be a host to this pathogen, followed by bermudagrass in 1957 and an extensive list of turfgrass cultivars within the subsequent twenty year period (App. A).

The first official report of Pythium blight on turfgrass in Kansas was made during the summer of 1977 (47). Home lawns and

golf courses in Johnson and Sedgwick counties were attacked by the pathogen which was later isolated and identified as P. aphanidermatum. Prior to 1977, the disease had been known to occur on golf course turf but had not been documented in Kansas. Since 1977, numerous reports of Pythium blight on golf courses in the eastern one-third of Kansas have been made. Typical of the disease, aerial mycella were most often observed in association with low, wet spots, areas of poor above-ground air circulation, and stands of turf with a thick thatch layer or lush vegetative growth, all contributing to a humid micro-environment.

3. Pathogens:

Pythium aphanidermatum (Edson) Fitzpatrick (with cardinal temperatures of 10-32-45 C (23,73)) is considered to be the primary Pythium blight pathogen of turf in the south. Less common, but not insignificant, are P. myriotylum Drechsler, also a southern specie as evidenced by cardinal temperatures of 10- 34 to 37 -43 C (67,73); P. ultimum Trow, a cool weather pathogen with cardinal temperatures of 1-28-37 C (43,76); P. graminicola, a low temperature foliage blighter which also has potential to cause a foliage blight and root decline at high temperatures (90); P. vanterpooli, a cool weather (13-18 C) foliage blighter; and P. irregulare, reportedly causing infection at 10 C (34,90). P. arrhenomanes and P. debaryanum have also been reported to cause Pythium blight in the northeastern region of the U.S. (43).

B. Survival, Germination, and Growth of Pythium

1. Survival:

Pythium is thought to survive mainly as oospores in the soil (12,

42,98,101,108) but sporangia and zoospores have also been implicated as survival structures (2,100). Oospores of P. aphanidermatum are produced in colonized host tissue and in soil adjacent to the host (98) apparently because normal oospore (and some sporangia) formation depends upon the presence of sterols (4,36,37). However, Pythium may survive as resting structures for long periods of time in soil devoid of a colonizable substrate (101).

2. Germination:

The majority of free oospores of P. aphanidermatum require an exogenous nutrient source for germination while those embedded in host tissue remain constitutively dormant until microbial degradation of host tissue occurs (12,102). Germination of P. aphanidermatum oospores is also favored by asparagine-amended soils (98,99), calcium (102), high temperatures of 25-37 C (1,106), and high soil moisture levels (1,4,98) but may occur at matric water potentials of -15 bars (99,109). Reports of optimum soil reaction for germination have ranged from a pH of 6.2 (63) to 7.5 (1). Oospores may germinate directly (4,98,99) or indirectly (21,22) producing zoospores in a vesicle in water (37). Subsequent saprophytic growth of P. aphanidermatum in soil has been observed when an adequate food base was available (64).

3. Growth:

Hyphal ingress of the host may be directly through cuticle or epidermis of the leaves or stems, trichomes, stipules or anywhere on the root (54,55). Indirect penetration may be through wounds and natural openings (54). Although adequate information regarding Pythium zoospore attraction to and accumulation on roots is available (55,85,

86, 107), little has been done to study comparable responses of Pythium zoospores to the above-ground plant parts (54). Kim et al. (54) reported necessary times of 30 min and 10 hr at 24 C for penetration of bean leaves by P. aphanidermatum zoospores and mycellal fragments, respectively. Freeman (26) demonstrated that the time required for P. aphanidermatum to cause 50% of maximum damage on ryegrass varied inversely with an increase in temperature. Penetration may be a function of both mechanical pressure and enymatic activity on the host (14,54,55). Cellulolytic and pectinolytic enzymes are responsible for advance breakdown of the host tissue (118).

Aerial hyphae, which occur primarily in a humid environment, developed in as little as 18 hr after inoculation of bean plants with mycelial fragments (54). The hyphae emerged from areas of coiled or twisted mycelium located within the plant. Four days after inoculation, these coiled masses developed into oospores (54). Aerial hyphae which did not originate form knotted mycelium were constricted at the cell walls and cuticle, resulting in dense cytoplasm within the hyphae. In the presence of water, these hyphae liberated zoospores (54).

4. Zoospores:

It is known that oospores (21,53,54), sporangia (53,54), and aerial mycelium (53,54) will liberate zoospores in water. However, zoospores have poor invasive capabilities, especially in soils of heterogeneous pore sizes (97). Thus, zoospores would either need to be at the soil surface to be carried in a film of water, or be contiguous to the host roots in order to cause infection (97). Zoospore production in the rhizosphere is unlikely since saturation of soil with sterile distilled water (SDW) or tap water (TW) containing bean seeds,

exudate, or nutrients caused oospores and sporangia to germinate directly (98). Thirty percent of the oospores and 90% of the sporangia liberated zoospores, however, when soil was saturated with SDW or TW alone (98). Kim et al. (53,54) suggested that infection by zoospores occurs at or near the soil line or on lower leaves and stems of the plant as zoospores are splashed by rain.

P. aphanidermatum zoospores have not been shown to colonize soil. However, Royle (40) observed motile zoospores in non-sterile soil three hours after infestation and Stanghellini and Burr (98) found the same conditions after 96 hr. Although they possess little or no capability to colonize field soil, P. aphanidermatum zoospores were recovered 7 days later from the site of inoculation into non-sterile soil (64). Under experimental conditions, zoospores have been shown to be an effective source of inoculum (53,54,55,60,74,85,95,97,117). In the presence of water, zoospores possess the advantage of motility which enables them to aggregate at points favorable for infection (40). Whereas a single P. aphanidermatum zoospore caused plasmolysis of only one to three root cells of creeping bentgrass, 10 or fewer produced a minor invasion and 100 or more effected substantial necrosis of the cells (55).

Kim et al. (53,54) described the accumulation of P. aphanidermatum zoospores around sockets and bases of trichomes, at stomata, between epidermal cells, and at wounds of bean leaves and stems. Royle and Hickman (85) noted an accumulation of zoospores of P. aphanidermatum in the region of elongation and in wounds of pea roots while Kraft et al. (55) observed the same aggregation in wounds but with a preference for the region of maturation of creeping bentgrass roots. Kraft et al. suggested that turfgrass and monocotyledonous plants generally attract

zoospores to the region of maturation while zoospores accumulate at the regions of differentiation and elongation in dicotyledonous plants. Comparison of the rapidity of infection also suggested zoospores are "much more effective in initiating infection" than mycelium (53,54).

5. Types of Inoculum:

Some researchers have utilized Pythium zoospores as inoculum when simulating infection by Pythium (53,54,55,60,74,85,95,97,117). However, many prefer to inoculate with minced or cut agar slabs or mycelial mats (17,23,27,29,30,31,46,60,61,77,106), infested soil or autoclaved seeds (28,69,91,106), or oospore suspensions (62,74). Zoospores and oospores are easily quantified (App. E) but the latter are subject to variable germination percentages (1,4,25,92,96,102). Inoculum carried in agar or seeds is also influenced by the food base. Lune and Hine (64) demonstrated the effect of such a variation in the amount of food material on the growth of P. aphanidermatum. After 24 hr in field soil, the fungus had grown 50 mm from a 15 mm-diameter disc of LBA but only 30 mm from a 5 mm-diameter disc under the same conditions. Hence, the rapidity of spread of P. aphanidermatum was altered by the amount of food base available and the greater amount of inoculum in the larger disc.

C. The Effects of Water Stress on the Plant and Pathogen

1. Plant Water Stress:

Beard defined drought stress as a "prolonged water stress that limits or prevents turfgrass growth" (8). It may be accompanied by high or low temperatures and, during prolonged dry periods, will

induce wilting and/or symptoms of nutrient deficiency (8). Dormancy may ensue as a means of survival for the plant growing points. Physiological and morphological changes in the plants occur in response to water stress and are generally indistinguishable from those induced by high temperature stress. Thus, the two stresses have often been studied as a unit. High temperature and decreasing moisture availability also influence the soil and the behavior of the pathogens with within that soil.

To understand the processes which occur as a result of water stress in a plant, one should be aware of the movement of water along a continuum and its role at the cellular level. The forces which maintain water in the cell are solute or osmotic potential (Ψs); matric potential (Ψm), where cellular colloids are involved; and turgor potential (Ψp) which puts an additional restriction on cell water movement. The sum of Ψs and Ψm is termed the composition potential (Ψc). A change to a more negative value of any one of these terms implies a decrease in that potential. Thus, the water potential (Ψw) of a cell may be described as:

$$\Psi w = \Psi c + \Psi p = (\Psi s + \Psi m) + \Psi p$$

Water uptake depends upon the formation of a gradient of water potentials from the soil, through the plant, to the air. The water which is transpired through the stomata is replaced by water immediately below in the intercellular spaces. As this replacement water moves to the stomatal cavity, its strong cohesive bonds allow it to pull adjacent water up from the column of water in the xylem. A gradient is formed, with the highest water tension at the leaf surface and the lowest at the root surface.

When a plant is water stressed, the water may (a) undergo extensive binding to cellular proteins (103), (b) move out of the cells to be transpired for cooling purposes, or (c) function in the transport of assimilates and hydrolytic products to the plant sinks. As the protoplasm and cell wall lose water the Ψ_p of the cell decreases. Concommitant solute accumulation functions in maintaining a gradient between the soil-root interface and the stomata-atmosphere interfaces (81). Such a gradient allows water uptake even at low Ψ_p (81). During normal drying conditions in the soil, a plant will respond to decreasing water supply and Ψ_p by closing its stomata earlier each day (20). If the stress becomes severe enough, the stomata will remain closed during the entire day, setting the scene for possible high temperature kill of the leaf. A gradual drought stress will also induce many morphological changes in the plant. These may include an increase in root depth, root:shoot ratio, cuticle thickness, and cell wall thickness (8). Simultaneously, there may be a decrease in numbers of leaves and tillers, total leaf area, leaf thickness, and cell size (8).

During severe water stress, photosynthesis, protein synthesis, and starch accumulation may be inhibited (5) and degradation of cell components may occur. Proteolysis results in an increase in peptides, free amino acids (especially proline), and amides (5,9).

2. Water Stress and Pythium Blight:

Any drought stress of plants in the field will usually affect the Pythium populations in that soil. Stanghellini (99) explored the effects by subjecting artificially and naturally infested soil (5×10^4 oospores/g soil) to -0.01, -0.1, -1.0, and -15.0 bars matric water potential. When the soil was brought to saturation with SDW oospores

did not germinate. However, when the SDW was amended with asparagine over 90% germination occurred even in soils which had previously been maintained at -1.0 and -15.0 bars. Thus, water-stressed oospores of P. aphanidermatum have a high potential (90%) for germination when supplied with exogenous nutrients in the soil. Cyclic wetting and drying of infested soils has been reported to cause a decrease (11) in soil Pythium populations. Mycelial, zoospore, and sporangial germination and growth also respond to soil moisture. Luna and Hine (64) found that only 3-5 hr of exposure of an infested air-dried soil to moisture was necessary to activate hyphal growth.

The influence of soil moisture on the severity of Pythium blight and several other turf diseases - Rhizoctonia brown patch of Seaside creeping bentgrass (10), Fusarium blight of Merion Kentucky bluegrass (18), Corticium red thread of Pennlawn and Rainier creeping red fescue (79), Sclerotinia dollar spot of common Kentucky bluegrass (19), and Pythium blight of Highland bentgrass (77) - has been explored. In each of the above studies, plants were maintained in pots in the greenhouse and subjected to varying cycles of irrigation as shown below.

Treatment I - Soil held at field capacity (FC)

Treatment II - FC→1/2AW*→FC→1/2AW→FC→etc.

Treatment III - FC→PWP*→FC→PWP→FC→etc.

*AW= available water; PWP= permanent wilting point

Pythium blight and dollar spot were most severe on plants that had been extracted to PWP during their life cycles while Corticium red thread on Rainier creeping red fescue was less severe at PWP than at FC. The severity of Corticium red thread of Pennlawn red fescue,

Rhizoctonia brown patch, and Fusarium blight showed no difference under any of the irrigation cycles.

In the case of Pythium blight incited by P. ultimum Trow, Moore et al. (77) found the percentage nitrogen to be greatest in those plants which had been under the PWP-FC cycle. The severity of Pythium blight of Highland bentgrass was greatest when plants had received high or low levels of calcium (77) and high nitrogen levels(77). With regard to Pythium blight, similar tests should be expanded to include the primary southern pathogen, P. aphanidermatum, and a greater number of turfgrass genera and cultivars.

D. Measuring and Monitoring Plant Water

The measurements of plant and soil moisture levels and their activities have been reviewed in detail by Barrs (7), Gardner (33), and Taylor (105). The following is a brief summary of the components of plant and soil water potentials and various methods with which they can be measured.

1. Plant Water Potential:

Plant water potential (Ψ_w) can be monitored with either liquid or vapor phase techniques. Liquid phase measurements are primarily based on the isopiestic method (7) which involves contact of the sample with the solution until an equilibrium is reached. The changes are determined by noting differences in cell size, volume, or weight, in leaf thickness, length, or curvature, or in density, refractive index, or uptake of the isopiestic solution.

Vapor phase methods take advantage of vaporization of the isopiestic solution and do not allow contact of the sample and the

solution. Measurements include changes in the volume or weight of the solution due to vaporization and changes in the weight of the sample. The vapor pressure osmometer (65) in which the rate of evaporation of a hanging droplet is monitored and the thermocouple psychrometer (84) are two of the most common methods used to measure plant water potential. Refined osmometers and thermistor psychrometers are also available.

2. Plant Solute Potential:

Plant solute potential (Ψs) can be determined by refractive index of plant sap, wet and simple bulb psychrometers, pressure bombs, and freezing point, with the freezing point method being the most widely used. On a smaller scale, changes in the apparent free space within the leaves and limiting plasmolysis have been used.

3. Plant Turgor Potential:

Turgor potential (Ψp) has most often been estimated by subtracting the solute potential from the total water potential of the plant. However, this may produce a negative turgor potential value which generally is not an acceptable possibility among researchers. Direct measurements of turgor potential can be obtained using transducers, by determining compression of the gas formed when a capillary tube with one closed end is inserted into a cell, by noting changes in the volume of a mercury-filled plant cell under pressure, or by applying micromanometers and the aphid-stylet technique developed by Kennedy and Mittler (52).

E. Monitoring and Controlling Soil Water

1. Soil Water Content:

Few methods of measuring soil water content and matric potential are available. The most satisfactory method of quantifying soil water content has been the gravimetric analysis in which the soil is oven-dried at 105 C to a constant weight (33). Gypsum blocks have also been used to calculate soil water content but in actuality have only provided a measure of the matric potential. The necessity of calibration and the shift of the calibrated reference point from one drying cycle to the next limits the use of these blocks. Other methods include the use of neutron probes and gamma ray absorption.

2. Soil Water Potential:

Soil water potential (Ψsoil) may be computed with a soil psychrometer or by analyzing its components. Solute potential (Ψs) is often calculated from soil solution concentrations or measured by thermocouple psychrometers, while the soil matric potential (Ψm) may be measured with tensiometers, gypsum blocks, or pressure membrane plates. The thermocouple psychrometer (84) will also provide a measure of the sum of the matric and solute potentials of the soil. Tensiometers (56) are most commonly used in the field to determine soil matric potential. However, their range only extends from zero to -2/3 bars. At lower potentials, the possibility of air entering the fluid chamber through the membrane increases the risk of shifted readings. Gypsum blocks (56) provide readings in the range of -0.5 to-15.0 bars soil matric potential and are not toosensitive to salt since they are buffered by the calcium sulfate in the block (56). Calibration to

determine soil matric potential using gypsum blocks can become quite involved and the calibration curve may shift from drying cycle to the next. A moisture release curve determined with a pressure membrane apparatus may be used in conjunction with gravimetric analysis (33) of soil moisture to determine the soil matric potential This method is easy to use and does not necessitate burying electrodes or other equipment in the field.

3. Controlling Water Availability in the Root Medium:

Unfortunately, water levels which are used to induce drought stress under field or greenhouse conditions are often difficult to control, thus necessitating the use of osmotic solutions. In the past, such solutions have included salts (NaCl and KNO_3) (45,94), mannitol (94,104), sucrose (94), and polyethylene glycols (44,45,50,58,59,87). The use of salts in osmotic solutions has steadily declined due to their absorption by the plants (45,57). Mannitol in high concentrations has been shown to be toxic to roots (94) and, like sucrose, supports unwanted algal and fungal growth (87).

Polyethylene glycols (PEG; also Carbowax or Poly-G) are highly favored in osmotic solutions because they are available in various molecular weights and in liquid, powder, or flake form, are soluble in water or organic solvents, will not support mold growth, are not hydrolyzed by microbes, have low mammalian toxicity, and are relatively non-absorbable and non-toxic to plants (59,70). PEG reduces the osmotic or solute potential of a solution with each additional increment of PEG or decrease in temperature (72).

Plants grown in the field and subjected to a natural water stress

adjust differently than those which are water stressed by osmotic solutions. Plants undergoing water stress in the soil respond to a decrease in pressure or matric potential whereas PEG-stressed plants are subjected to changes in the osmotic potential. Slatyer (94) observed two phases of adjustment to osmotically-induced water stress in plants. The first consisted of dehydration and the second was an accumulation of solutes in the cells. Janes (45) noted the same types of adjustment for pepper and bean plants grown in NaCl and PEG solutions. After dehydration, plants grown in NaCl and PEG accumulated Na^+ and Cl^- ions and soluble carbohydrates, respectively, within the leaves. Kaufmann and Edkard (50) determined that plants grown in low molecular weight PEG (PEG 400) made osmotic adjustments similar to those described by Janes. Plants grown in PEG 6000 solutions, however, maintained a constant root osmotic potential and instead adjusted the pressure potential of the xylem to produce a potential gradient. Osonubi and Davies (81) suggested that the initial lowering of the osmotic potential in the cell creates a larger water potential gradient between the soil and root which leads to increased uptake of water by the roots. Lawlor(58) showed that as the osmotic potential of a solution is decreased, plants will respond with varying rates of decrease in the osmotic potential and/or pressure potential. The magnitude of the response depends upon the type of plants, the growing conditions, and the size or rate of the decrease in the osmotic potential of the solution. Variations are also evident within different organs of one plant (88).

The rate with which PEG-imposed stress is applied has much bearing on the reactions and recuperation of the plant. Gradual

decreases in root medium osmotic potential such as -0.25 bars/day (87), -0.5 bars/day (88), and -1.0 bars/day (45) resulted in gradual physiological adjustments of the plants within 24 hr after treatment (45). In contrast, rapid changes in osmotic potential of the root medium (e.g. -0.5 to -10.0 bars/day (45)) resulted in poor adjustment, injured plants, and low survival rates (45,88). Such abrupt changes in soil osmotic potential are rarely encountered in nature (58).

The possibility of polyethylene glycol toxicity on plants has been explored (50,87). Lawlor (59) found that commercial PEG's contained impurities such as aluminum, magnesium, iron, chloride, and sulfate but that the levels of these inorganics were non-toxic to the plants. Reid (83) also found concentrations of iron, manganese, copper, zinc, calcium, magnesium and potassium to be below the reference standards which were 60.0, 6.0, 0.2, 0.4, 20.0, 1.2, and 10.0 ug/g PEG 4000, respectively and therefore posed no toxicity problems. However, phosphorous concentrations were higher than those usually found in soil solutions even at high water potentials (83). Lawlor (59) also concluded that PEG's of molecular weights less than 1000 were generally not absorbed by the plants unless the roots had been damaged. Once inside the plant, low MW PEG's were found to accumulate in highest amounts in the leaves as water was transpired (88).

The availability of oxygen must also be considered when using PEG with plant systems. Mexal et al. (71) concluded that O_2 solubility was similar for both PEG 4000 and 6000 up to a 15% (w/v) concentration. Above that point, PEG 6000 contained less dissolved O_2 than PEG 4000 at the same osmotic potential. For both, the O_2 concentration was inversely proportional to the PEG concentration. Mexal suggested that

many of the toxic effects observed by researchers may have been due to decreased O_2 availability in the PEG solution rather than impurities as alluded to above.

II. MATERIALS AND METHODS

A. A Survey for the Presence of Pythium Blight Pathogens in Kansas

In August, 1978, a survey was conducted in Kansas to determine which Pythium species were causing Pythium blight of turfgrass and to secure a native isolate of the fungus for use in subsequent studies. The survey consisted of (a) the collection of soil and tissue samples from golf courses in Kansas and (b) the identification of pathogenic Pythium species in those samples. Thirteen golf courses were selected in the eastern half of Kansas, where the disease was most common. Included were five golf courses in the northeastern region (four in Johnson Co., one in Shawnee Co.), three in the south central area (Sedgwick Co.), one in central Kansas (Saline Co.), and four in the extreme southeastern area of the state (Labette Co. and Cherokee Co.). An attempt was made to select both high and low maintenance golf courses and cover a range of turfgrass types which included five predominantly bluegrass courses, six bermudagrass courses, and two buffalograss courses. Varied numbers of samples were selected from sites which had a history of Pythium blight or areas in which Pythium blight would likely have occurred if the pathogen was present. Previously reported cases of suspected Pythium blight on golf courses and home lawns in Kansas indicated that the pathogen was well adapted to hot, humid conditions.

Soil and plant samples were collected with a 2.5 cm-diameter soil probe then placed in transparent plastic bags and stored in a styrofoam box until taken to the laboratory that same day. All samples were moistened with sterile distilled water (SDW) and stored in folded plastic bags in the greenhouse for 5 days at 30-37 C to induce

Pythiaceous growth. Leaf, stem, and root tissues were then separated, surface sterilized in a 10% clorox solution for 15 sec and plated onto a medium selective for Pythium species SA-PBNC (90, App. D). Three pieces of tissue were placed on each plate and two plates were used per sample. Plates were incubated in darkness for 48-72 hr at 32 C. The accompanying soil samples were also tested for the presence of Pythium. A small amount of soil (approximately 0.25 cc) and two drops of sterile de-ionized water (SDIW) were mixed in a 1 cm-diameter well which had previously been punched into the center of an SA-PBNC agar plate. Two plates per sample were then incubated in darkness for 78-96 hr at 32 C. Pythiaceous growth (typified by coenocytic hyphae and rapid growth) which appeared on the agar after the appropriate incubation time was removed by hyphal tip subculture and plated onto amended PDA (App. D) to aid in obtaining clean cultures and to observe the type and rapidity of growth. A second aid to identification was the development of oospores and antheridia on Schmitthenner's medium (113, App. D) and the production of sporangia and zoospores from tissue as described by Waterhouse (113). Isolates were keyed to species using Matthew's "Studies on the Genus Pythium" (66) and Waterhouse's "Key to Pythium Pringsheim" (113) and "The Genus Pythium Pringsheim" (114).

To evaluate the pathogenicity of the isolates, 21-28 day old Pennfine perennial ryegrass growing in 8 cm pots was inoculated with 1 cm plugs of 24-hr old mycelium of the isolates grown on V-8 agar (App. D). The grass was misted twice daily with SDIW and incubated in closed plastic bags in the greenhouse for 3-5 days at temperatures

of 30-37 C. A pot of non-inoculated plants and one pot of turf inoculated with the Florida isolate (FTCC550) of P. aphanidermatum (courtesy of T.E. Freeman, Gainesville, FL) were also included in the test for controls. Each isolate was observed for its ability to cause disease and the similarity to typical Pythium blight. The pathogen was then reisolated in pure culture.

B. A Greenhouse Study of the Severity of Pythium Blight of Drought Stressed Turfgrass

1. Experimental Design

The experiment was conducted as a completely randomized designed, factorial experiment. The three factors involved were (a) type of inoculation (inoculated vs. non-inoculated), (b) water stress level (drought stressed vs non-drought stressed, or dry vs wet), and (c) time. The experimental unit consisted of one pot of Pennfine perennial ryegrass which was replicated three times within the treatment. The four treatments in the design were "WI" (inoculated plants kept well watered throughout the entire experiment), "WC" (control or non-inoculated plants kept well watered throughout the entire experiment), "DI" (inoculated plants subjected to drought stress immediately prior to inoculation), and "DC" (control or non-inoculated plants subjected to drought stress). This experiment was repeated a total of five times at 2-3 week intervals.

2. Turfgrass Growth and Maintenance:

Pennfine perennial ryegrass was seeded in 10-cm plastic pots and maintained in the greenhouse over a seven to nine week period. Four hundred seeds were planted 0.3 cm deep in each pot of 825 g

(oven dry weight) soil. The soil was a mixture of topsoil: sand: peatmoss (1:1:1, v/v/v), contained 213 pounds nitrogen per acre, 200+ pounds phosphorous per acre, 500+ pounds potassium per acre, and had a pH of 6.8. Routine maintenance during the growth period included weekly mowing at a 5 cm height, two fertilizations with Peter's General Purpose Fertilizer (20-20-20) at recommended strength, a 16 hr photoperiod utilizing supplemental lighting, an average daytime temperature of 20-27 C and night temperatures of 7-13 C.

3. Stress, Inoculation, Incubation, and Rating

A gradual drought stress was applied by withholding water from six pots of turfgrass for 4-6 days prior to inoculation. The other six pots of turfgrass were kept well watered at 2-day intervals and were not allowed to dry out within the 4-6 day period prior to inoculation.

The pathogen used for inoculation was Pythium aphanidermatum (Edson) Fitz. (Isolate 10-2f) which was collected from fairway #2 on the Wichita State University golf course, Wichita, KS in 1978. Zoospores were produced according to the "V-8" method outlined by Kraft et al (55) with the following modifications: used 12 g agar/liter, ten 1-cm plugs were used per plate, flooded plates were refrigerated for 20 min at 8 C, 4 hr prior to zoospore harvest (App. E). The harvested zoospore suspension was diluted to 5000 zoospores per ml with SDIW and used for inoculation within 90 min of harvest. This isolate generally produced 40,000-60,000 zoospores per ml with this "V-8" method.

At inoculation, the turfgrass in all 12 pots was mowed to a 5 cm height, cores of soil (13 mm diameter, 4 cm deep) were removed from the center of each pot and soil moisture determined gravimetrically (33). All pots were then flooded with distilled water (DW) 30 min prior to inoculation and allowed to drain. Plants were also misted with SDIW in a plastic spray bottle immediately prior to inoculation to provide high humidity and a film of water necessary for zoospore movement. The turfgrass treatments "WI" and "DI" was inoculated by dripping 1 ml of the diluted zoospore suspension through a 1 ml pipette onto the foliage in each pot. Plants were then incubated in individual plastic bags in darkness for 48 hr at 35 C. All bags were opened and the plants misted with SDIW twice daily to maintain a humid environment. Age of the plants was calculated as the number of days from germination to inoculation. The majority of the plants were 50-60 days old and at the five-leaf stage at inoculation.

After 48 hr, pots were removed from the bags. Disease ratings were made immediately by using a ruler to determine the area blighted which was easily distinguished by the aerial mycellum and bleached leaf tissue. The rating was calculated for each of the 3 pots in the treatment as:

$$PB = \text{percent blight} = \frac{\text{blighted area of turfgrass}}{\text{total area of turfgrass}} \times 100$$

Ratings from all five experiments were organized into one large data set and subjected to an analysis of variance and a Duncan's Multiple Range test for significance at $P = 0.05$ using the SAS Computer system (6).

Re-isolation of the fungus from the plants was accomplished by removing leaf and crown tissue of blighted plants, surface sterilizing them in a 10% clorox solution, plating them onto SA-PBNC, incubating the plates at 35 C, and observing for growth of P. aphanidermatum in 2-3 days.

C. A Study of the Severity of Pythium Blight of Turfgrass Subjected to Controlled Water Stress in a Hydroponic System

1. Experimental Design:

The experiment was designed and analyzed as a split block design replicated three times over time with the main plots being inoculated vs non-inoculated. The experimental unit consisted of three pots of turfgrass. Disease ratings from the three pots were combined to give a mean disease rating for the unit. There were four units or treatments in each of the two mainplots. A water stress level (0, -10, -12, and -15 bars osmotic potential) was randomly assigned to each unit giving a total of four treatments in the "inoculated" mainplot (0inoc, -10inoc, -12inoc, -15inoc) and four treatments in the "non-inoculated" mainplot (0chk, -10chk, -12chk, -15chk). These two mainplots comprised the block which was replicated a total of three times over time.

2. Turfgrass Growth and Maintenance:

Pennfine perennial ryegrass was grown in a hydroponic triple-cup (T-C) system used by Saladini (90). Plants were grown and maintained in a Labline growth chamber with a 24-19 C day-night temperature, respectively, and a 16 hr photoperiod. Westinghouse cool white fluorescent tubes provided approximately 2580 ft-c. (27,750 lux) to the turfgrass plants. Plants were seeded at a rate

of 250/cup and germinated in 3-5 days. After approximately one week, the roots had reached the distilled water below. At that time, the water was replaced with a modified balanced Hoagland's solution (82, 110) which was changed weekly. The phosphorous content of the solution was reduced from the recommended 1 ml/L of molar solution to 0.2 ml/L molar solution and the pH of the final solution was adjusted to 5.1-5.2 with .001 M H_2SO_4. Similar experiments growing wheat and corn in Hoagland's solution indicated that a lower rate of phosphorous resulted in better root and shoot growth and tied up less of the available iron than did the higher rate (82). During germination and the first week of growth, plants were watered three to four times each day with distilled water and once each day thereafter with deionized water. The growth medium was aerated for 4-6 hr daily by bubbling air through the solution with a system of tubing and an air pump. All plants were hand clipped to a 5 cm shoot height once each week and immediately before the application of water stress. Although ideal root growth was attained 21 days after germination, the plants were allowed to grow beyond the 21 day period to provide more leaf tissue.

3. Induction of Water Stress:

Polyethylene glycol 6000 (MW 6000-7500; J.T. Baker Chem. Co., Phillipsburg, NJ) was used to induce a controlled water stress in the turfgrass. A gradual, stepwise stress was applied by changing the osmotic potential of the PEG 6000 solution in which the roots were immersed by -2 bars at various time intervals (Tables 1A, 1B).

Table 1A. Sequence of Osmotic Potentials Used to Induce a Gradual Water Stress of Hydroponically-Grown Turfgrass at 24 C (72)

Time Length at Specified Osmotic Potential (hr)	Final Moisture Potential Desired	Osmotic Sequence (bars)[1]			
		0	-10	-12	-15
3		-	-	-	-2
3		-	-	-2	-4
6		0	-2	-4	-6
12		0	-4	-6	-8
6		0	-6	-8	-10
6		0	-8	-10	-12
48		0	-10	-12	-15

[1] Example: The plants which were ultimately subjected to a -15 bar osmotic potential were initially placed in a -2 bar solution for 3 hr, then placed in a -4 bar solution for 3 hr, then placed in a -6 bar solution for 6 hr, etc. A "0" represents zero osmotic potential achieved with distilled water.

Table 1B. Concentration of PEG 6000 Required to Obtain Desired Osmotic Potentials in Solution (72)

Osmotic Potential (bars)	Concentration of PEG 6000 in Solution at 24 C (g PEG 6000/kg distilled water)
0	0 (DW only)
-2	118
-4	176
-6	221
-8	260
-10	293
-12	324
-15	365

The final stresses of 0, -10, -12, and -15 bars were maintained for 48 hr to induce stress symptoms and insure the concommitant physiological changes produced by water stress (45). No nutrients were used in the PEG 6000 solutions. Light intensity and photoperiod were the same as that during routine plant maintenance as described earlier. The temperature was maintained at 24 C during the entire 88 hr stress period and aeration was continuous during the stress period. The plants were 4-5 weeks old at the time of applied stress.

4. Inoculation, Incubation, and Rating:

Immediately prior to inoculation, the osmotic solutions in all cups were poured off the roots, rinsed with tap water, and the water replaced with fresh DW. Foliage was also misted with SDIW to provide a humid environment and facilitate zoospore movement. Three cups of plants at each stress level (0inoc, -10inoc, -12inoc, -15inoc) were then inoculated by dripping 1 ml of a zoospore suspension (isolate 10-2f) containing 5000 zoospores per ml over the plants in each cup. The small volume of water allowed the suspension to remain on the leaves rather than draining into the PEG 6000 solution below. All 24 cups were sealed in a large plastic bag and incubated in darkness for 48 hr at 35 C. The bag was opened and the plants misted twice daily with SDIW from a plastic spray bottle. After 48 hr incubation, the plants were removed, all DW was replaced with Hoagland's solution (as described earlier), and the plants were returned to normal growing conditions in the growth chamber for a two week period to allow renewed growth from surviving plants.

Pythium blight ratings were made after the two week period of regrowth. The first rating scheme was based on a count of the number of plants with renewed growth. Due to approximately 90% germination, the total number of plants per cup averaged 225. A second rating scheme consisted of weighing the plants after having dried them to a constant weight at 115 C. The results of both ratings ("number of plants alive" and "weight of plants") were subjected to an analysis of variance and Duncan's Multiple Range test for significance at P = 0.05 using the SAS system (6). The test provided a comparison of the severity of Pythium blight on turfgrass plants which had been subjected to four degrees of water stress.

D. A Field Study of the Severity of Pythium Blight of Drought Stressed and Non-Drought Stressed Turfgrass

1. Experimental Design:

On April 8, 1978, turfgrass plots were established at the Rocky Ford Research Station, Manhattan, KS for the purpose of evaluating the severity of Pythium blight on drought stressed vs non-drought stressed turfgrass under field conditions. The experiment was designed and analyzed as a randomized complete block in a split plot design.

The experimental unit consisted of a 3' x 3' plot of turfgrass which was replicated four times throughout the mainplot. There were 24 different cultivars of turfgrass per row and four rows per mainplot. The cultivars were grouped by species (Table 2) and these specie groups were randomized within each row. There were two mainplots in the design, the "wet" plot -- in which the

Table 2. Turfgrass Cultivars Used in Field Studies of Pythium Blight of Drought Stressed and Non-Drought Stressed Turfgrass

Cool Season Grasses

A. Creeping Bentgrass - Agrostis palustris Huds

1. Penncross
2. Seaside

B. Kentucky Bluegrass - Poa pratensis L

1. Adelphi
2. Baron
3. Fylking
4. Glade
5. Majestic
6. Merion
7. Park
8. Rugby
9. Sydsport
10. Touchdown

C. Perennial Ryegrass - Lolium perenne L

1. Citation
2. Derby
3. Diplomat
4. Manhattan
5. Pennfine

D. Red Fescue - Festuca rubra L

1. Highlight*
2. Jamestown*
3. Koket*
4. Polar*

E. Tall Fescue - Festuca arundinacea Schreb.

1. Kentucky 31

Warm Season Grasses

A. Bermudagrass - Cynodon dactylon (L.) Pers.

1. Midiron

B. Zoysiagrass - Zoysia japonica Steud.

2. Meyer

*Eliminated from experiment

turf was not allowed to be drought stressed but instead was kept well watered prior to inoculation, and the "dry" plot -- in which the turf was drought stressed for a 39 day period prior to inoculation. These two mainplots were separated by a 20' strip of Pennfine perennial ryegrass seeded in May, 1978.

2. Turfgrass Growth and Maintenance:

Plots were seeded in April, 1978, and were subsequently grown under high maintenance conditions. Zoysia and bermudagrass were sodded in on June 1, 1978. The stands received four pounds nitrogen/1000 ft^2 (as IBDU) in 1978 and 3# nitrogen (IBDU) in 1979, were mowed weekly at an average height of 5-6 cm (clippings removed), and were kept properly irrigated to promote deep root systems. Herbicides and fungicides were used in an effort to maintain a healthy turfgrass stand (App. B).

During the summer of 1978, all red fescues died from a combination of high temperature stress, dollar spot, and brown patch. Thus, the four red fescue cultivars were eliminated from the experiment, leaving twenty other cultivars per row. The dead areas were reseeded with Pennfine perennial ryegrass in April, 1979.

3. Stress, Inoculation, Incubation, and Rating:

Both mainplots were kept well irrigated throughout the summer and were not allowed to be water stressed until July, 1979. Beginning on July 23, the dry plot received no irrigation and natural rainfall was precluded by covering the dry plot with a 4-mil sheet of white plastic during rainy weather. Neither yellowing nor scorching of the leaves occurred under the tarp since it was used to cover the dry plot only at night or on cool, cloudy days and

during daytime if rain was forecast. The water stress period for the dry plot spanned 39 days (July 17 to August 25). During that period, the turf was mowed three times including one mowing 16 hr prior to inoculation.

Plots were inoculated at 11 AM on August 25, 1979. Preparation for inoculation was as follows: (a) both wet and dry plots were mowed 16 hr prior to inoculation; (b) a 7-10 cm wide trench was cut down the center of each row, dividing the 3' x 3' plots in each row into approximately 1.5' x 3' plots. One of these "half-plots" was inoculated while the other half served as an non-inoculated control. This provided 20 inoculated and 20 non-inoculated observations per row, with four rows in each mainplot (wet and dry). The trench prevented aerial mycelium and zoospores of Pythium from spreading to the non-inoculated half of each plot; (c) soil samples were taken to determine the mean degree of water stress for each cultivar in the dry plot. Three to six soil cores were extracted from each 3' x 3' plot, using a 2.5 cm-diameter soil probe to a depth of 2.5-5.0 cm. The cores were combined for each plot and a 100 cc sample was used to determine soil moisture using the gravimetric method (33). The matric potential of the water in each plot was then calculated with the use of a pressure membrane apparatus (41). Both plots were continuously irrigated for the three hr period prior to inoculation to the point of having standing water in both mainplots.

Plants were inoculated with zoospores of P. aphanidermatum (isolate 10-2f) harvested 90 min prior to inoculation. The zoospore

suspension was prepared as in appendix E and was diluted to a concentration of approximately 910 zoospores/ml with SDIW. Inoculation was made by pouring 100 ml of the suspension over a circular area (approximately 15 cm diameter) in the center of each halfplot to be inoculated. This procedure resulted in an inoculum level of 500 zoospores/cm^2 turf area inoculated. Plots were immediately but briefly wetted with a fine mist and then covered with the white plastic tarps (24' x 80') to maintain high temperatures and a high relative humidity. Temperatures under the tarps ranged from 28-40 C but could be lowered to 28-32 C by periodically misting the tarp with water during sunny parts of the day. The ends of both tarps were loosely opened to allow slight air movement underneath the tarps. During the six day incubation period, the tarps were removed during the heat of the day (1-4 PM) to avoid high temperature injury to the leaves. During that time, all plots were watered to the point of having standing water before being re-tarped.

Ratings of Pythium blight were made six days after inoculation. All cultivars were rated for (a) percentage Pythium blight (as a percentage of the total area in each halfplot); (b) number of blighted spots characterized by water-soaking and/or aerial mycelium; (c) diameter of the blighted spots (cm). Using these values the differences in the wet and dry plots were compared using a test for least significant difference (LSD). The mean rating from the four replications of each cultivar in the dry plot were compared with the mean rating from the four corresponding replications of each in the wet plot. For example, the ratings from the four 'Adelphi'

Kentucky bluegrass wet plots were averaged and the mean was compared to the mean of the four 'Adelphi' dry plots using LSD.

Mean group ratings were also compared. For example, the mean rating from the 10 wet bluegrasses were compared to the mean rating of the 10 dry bluegrasses using LSD.

III. RESULTS

A. A Survey for the Presence of Pythium Blight Pathogens in Kansas

Pythium species were confirmed in six of thirty-six tissue samples (App. C). These included five P. aphanidermatum (1-8, 3-2, 5-8, 10-2f, and 15-3) and one P. splendens (9-5) which were then assayed for pathogenicity as indicated below. Four of the five P. aphanidermatum isolates were collected from sites which either showed typical Pythium blight symptoms or were reported to have had a history of Pythium blight. Fungi from four other tissue samples (1-2, 9-14, 10-2, and 11-1) produced coenocytic hyphae and sporangia and/or conidia but no oospores or antheridia on Schmitt-henner's medium or in grass-water culture (113) and so were not identified to species. The remaining 26 tissue samples yielded neither oomycetes nor other fungi on SA-PBNC.

A combination of tests aided in identification of isolated species of fungi (Table 3). These included (a) sporulation in water culture using V-8 agar plugs, (b) sporulation within Schmitt-henner's medium, (c) relative growth rates on amended-PDA (App. D), and (d) pathogenicity on Pennfine perennial ryegrass.

All five P. aphanidermatum isolates and the check (FTCC550) caused a severe foliage blighting of Pennfine perennial ryegrass after 3 days incubation. Unidentified oomycetes 1-2 and 10-2 produced mild foliage blights while P. splendens, 9-14, and 11-1 caused no perceivable damage to the grass. Each of the six P. aphanidermatum isolates and fungus 1-2 were re-isolated from the tissues which they had blighted. However, P. splendens and the remaining undetermined oomycetes (9-14, 10-2, 11-1) could not

Table 3. Results of Tests Used to Identify Fungi Isolated from Turf Samples

Isolate	Isolate Identification: V-8 Water Culture [1]	Schmitthenner's Medium [2]	Relative Growth Rate [3]	Pathogenicity [4]
1-8	P. aph	P. aph	Rapid	+
3-2	P. aph	P. aph	Rapid	+
5-8	P. aph	P. aph	Rapid	+
10-2f	P. aph	P. aph	Rapid	+
15-3	P. aph	P. aph	Rapid	+
9-5	P. spl	P. spl	Mod	-
1-2	UO	UO	Slow	±
9-14	UO	UO	Mod	-
10-2	UO	UO	Mod	±
11-1	UO	UO	Mod	-

[1] P. aph= P. aphanidermatum; P. spl= P. splendens; UO= Unidentified oomycete.

[2] Identification using (66) and (113); induction of zoosporangia and sexual organ formation in water was according to Waterhouse (113) substituting V-8 agar for CMA: Shcmitthenner's medium was used for direct observation of sporulation within the agar (113, App. D).

[3]Relative growth rate: incubated 24 hr at 31 C on amended PDA (App. D); rapid= 8-10 cm-diameter colony; moderate= 4-8 cm-diameter colony; slow= 1-4 cm-diameter colony.

[4]Pathogenicity: incubated on Pennfine perennial ryegrass, 3-5 days, under high relative humidity and 30-37 C; (+)= severe foliar blighting, (+)= mild foliar blighting; (-)= no foliar blighting.

be re-isolated from the plants on which they were inoculated.

Of the thirty-six soil samples, only two yielded identifiable Pythium species (1-8, 3-2), both P. aphanidermatum. P. aphanidermatum was likewise isolated from their corresponding tissue samples. Both isolates were collected in Johnson Co., northeast Kansas. Neither were tested for pathogenicity nor used in further studies.

Four of the five isolates were collected from greens which, in general, were under extremely high maintenance and received frequent irrigation late in the evening or at night. Such water practices would enhance Pythium blight development.

B. A Greenhouse Study of the Severity of Pythium Blight of Drought Stressed vs Non-Drought Stressed Turfgrass

The experiment was analyzed as a factorial experiment in a completely randomized design. Percent Pythium blight (PB) was compared among the four treatments (WI-wet inoculated, WC-wet check, DI-dry inoculated, DC-dry check) using an analysis of variance and Duncan's Multiple Range test for significance at $P = 0.05$.

The WI plant displayed a significantly greater percent of Pythium blight (73.17%) than the other treatments (Fig. 1, Table 4). Percent Pythium blight on DI plants (28.13%) was only one-half to one-third as great as that of the WI plants. Although the WC and DC plants were not inoculated, aerial mycelia of a contaminating fungus were found on a few stems of the DC plants and were reported as blight.

Of the three factors (type of inoculation, water stress level, and time) there was a highly significant difference ($P = 0.0001$) in "PB" between inoculated and non-inoculated plants, a significant

difference in "PB" (P = 0.0001) between drought stressed and non-drought stressed plants, and significant differences (P = 0.0001) among "PB" in each experiment over time.

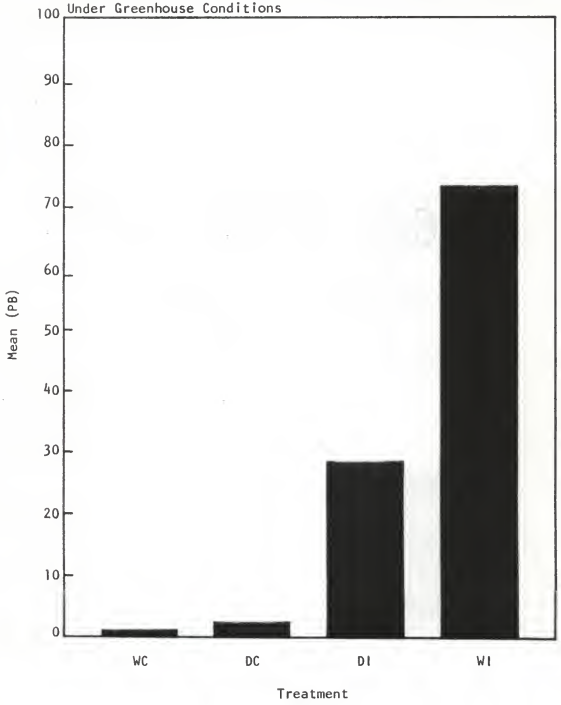
Table 4. A Comparison of the Mean Percentage of Pythium Blight (PB) on Drought Stressed vs Non-Drought Stressed Pennfine Perennial Ryegrass Under Greenhouse Conditions

	Treatment[1]			
	WC	DC	DI	WI
Mean (PB)	0.08a[2]	1.33a	28.13b	73.17c

[1]WC= wet check: non-inoculated plants kept well watered throughout the entire experiment
DC= dry check: non-inoculated plants subjected to drought stress
DI= dry inoculated: inoculated plants subjected to drought stress immediately prior to inoculation
WI= wet inoculated: inoculated plants kept well watered throughout the entire experiment

[2]Means with the same letter are not significantly different at P=0.05 using Duncan's Multiple Range test.

Figure 1. A Comparison of the Percentage of Pythium Blight (PB) of Drought Stressed vs Non-Drought Stressed Turfgrass Under Greenhouse Conditions



C. A Study of the Severity of Pythium Blight of Turfgrass Subjected to Controlled Water Stress in a Hydroponic System

The experiment was analyzed as a split block replicated three times over time. Data from the two rating schemes ("number of plants alive" and "dry weight of plants") were subjected to an analysis of variance and Duncan's Multiple Range (DMR) test for significance at P = 0.05.

1. Inoculated Mainplot:

The DMR test for significance within the inoculated mainplot showed a significant difference among treatments when rated by "dry weight of plants" (Fig. 2, Table 5). The dry weights of plants in treatments -10inoc, -12inoc, and -15inoc (1.94g, 2.00g, 1.78g, respectively) were not significantly different and the dry weights of 0inoc, -10inoc, and -15inoc (1.56g, 1.94g, 1.78g respectively) were not significantly different. Of the inoculated plants, -12inoc (2.00 g) had the greatest mean weight of plants followed by -10inoc (1.94 g), -15inoc (1.78 g), and 0inoc (1.56 g). However, when ratings of "number of plants alive" were tested (Table 6), no significant difference was found (P = 0.05) among treatment means and the order of decreasing "number of plants alive" was -10inoc (48.44), 0inoc (36.56), -12inoc (34.33), and -15inoc (31.67). Thus the data show that the inoculated turfgrass plants which were not water stressed (0inoc) produced less tissue, by weight, during recovery than did those plants which had been water stressed and inoculated.

Table 5. A Comparison of the Mean Dry Weights of the Plants as a Function of Water Stress and Inoculation with Zoospores of P. aphanidermatum

	Mean Dry Weight of Plants (g)			
Osmotic Level	-12	-10	-15	0
inoculated	2.00a[1]	1.94ab	1.78ab	1.56b
non-inoculated	3.13c	3.20c	2.69d	2.58d

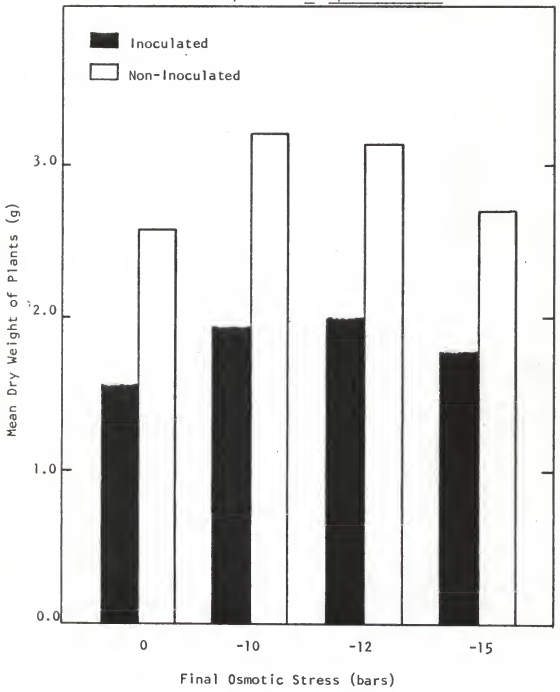
[1]Means with the same letter are not significantly different using Duncan's Multiple Range test for significance at P=0.05.

Table 6. A Comparison of the Mean Number of Plants Alive as a Function of Inoculation with P. aphanidermatum and Water Stress Levels

	Mean Number of Plants Alive			
Osmotic level	-10	0	-12	-15
inoculated	48.44e[1]	36.56e	34.33e	31.67e
non-inoculated	166.44f	191.33f	158.00fg	118.44g

[1]Means with the same letter are not significantly different using Duncan's Multiple Range test for significance at the P=0.05 level.

Figure 2. A Comparison of the Mean Dry Weights of Pennfine Perennial Ryegrass Plants as a Function of Water Stress and Inoculation with Zoospores of *P. aphanidermatum*



2. Non-inoculated Mainplot:

Incubation and various water stress levels produced varying degrees of turfgrass kill in the non-inoculated mainplot (Tables 5,6). When judged by "dry weight of plants," plants subjected to 0 and -15 bars osmotic potential (OP) showed a significantly lower mean weight gain (2.58 g and 2.69 g, respectively) than plants subjected to -10 and -12 bars OP (3.20 g and 3.13 g, respectively). When rated by "number of plants alive," non-inoculated plants subjected to 0, -10, and -12 bars OP showed a significantly greater mean number of plants surviving (191.33, 166.44, and 158.00, respectively) than those stressed at -15 bars OP (118.44). Thus, an appreciable amount of damage due to water stress occurred on plants at an osmotic potential of -15 bars which is considered to be the permanent wilting point of many plants. Lawlor (58) found -12 bars to be the point at which the turgor potential of ryegrass leaf tissue (Lolium perenne L. var. Aberystwyth S.23) approached zero.

D. A Field Study of the Severity of Pythium Blight of Drought Stressed vs Non-Drought Stressed Turfgrasses

Ratings for percentage Pythium blight, number of blighted spots, and spot diameter were taken on August 31, 1979 after removing the tarps from both wet and dry plots.

1. Pythium Blighted Area (PB) as a Percentage of Total Plot Area:

Of the twenty individual cultivars tested, only Penncross creeping bentgrass displayed a significant difference in (PB) between wet and dry plots, using a test for LSD (Table 7).

The percent Pythium blight was greater (P=0.05) in the wet inoculated Penncross plots (1.37%) than in the dry inoculated Penncross plots (0.21%).

When analyzed by species, rather than individual cultivar only the Kentucky bluegrasses showed a significant difference in (PB) between wet and dry plots when tested for LSD (Table 8). The drought stressed bluegrass plots, as a whole, had a significantly greater (2.89%) (PB) than did the non-drought stressed bluegrass plots (0.82).

2. Number of Spots:

In comparing the number of spots in wet and dry plots for individual cultivars, only Penncross creeping bentgrass (P = 0.01), K-31 tall fescue (P = 0.05), and Touchdown (P = 0.09) showed significant differences (Table 7). Drought stressed Touchdown (2.75) and K-31 (1.50) had significantly greater number of spots per plot than the corresponding dry plots (6.75).

When the cultivars were grouped into their respective species (Table 8) only the Kentucky bluegrasses displayed a significantly greater number of spots/plot (P = 0.07) in the dry plots (1.70) than in the wet plots (1.12). Perennial ryegrasses, however, showed a significantly greater number of spots/plot in the wet plots (3.40) than in the dry plots (2.45) at P = 0.09.

3. Spot Diameter:

Ratings of the spot sizes for individual cultivars were not statistically evaluated due to the varying numbers of

Table 7. Mean Percent Pythium Blight (PB) and Mean Number of Blighted Spots per 3' x 3' Plot in each Mainplot

Cultivar	Percent Pythium Blight (PB) WET	DRY	Mean Number Spots/Plot WET	DRY
Kentucky Bluegrass:				
Adelphi	0.79	3.60	1.50	1.25
Baron	1.01	1.51	1.00	1.75
Fylking	1.72	4.92	1.75	2.00
Glade	0.59	2.39	1.00	2.50
Majestic	0.64	0.49	X	X
Merion	1.36	4.92	1.25	3.00
Park	0.71	0.62	1.00	1.25
Rugby	0.39	4.44	1.25	1.25
Touchdown	0.36	2.80	0.50***	2.75***
Sydsport	0.69	1.53	0.50	0.75
K-31 Tall Fescue	0.38	1.03	0.50**	1.50**
Perennial Ryegrass:				
Citation	4.91	2.14	4.75	2.25
Derby	2.73	2.97	2.50	2.50
Diplomat	5.09	6.03	3.25	2.25
Manhattan	3.41	3.40	3.00	1.75
Pennfine	5.30	4.23	3.50	3.50
Creeping Bentgrass:				
Penncross	1.37**	0.21**	2.00*	0.75*
Seaside	1.54	1.00	1.75	1.50
Midiron Bermudagrass	0.00	1.09	0.00	0.50
Meyer Zoysiagrass	0.00	0.02	0.00	0.25

X= spot number not determined due to flooding in plots
* P=0.01
** P=0.05
*** P=0.09

Table 8. Mean Percent Pythium Blight (PB), Mean Number of Spots, and Mean Spot Diameter for Wet (WI) and Dry (DI) Inoculated Plots Grouped by Species

Specie Groups	Mean (PB)		Mean Number Spots/Plot		Mean Spot Diameter (cm)	
	WI	DI	WI	DI	WI	DI
Bluegrass	0.82*	2.89*	1.12***	1.70***	5.85**	7.85**
Tall Fescue	0.38	1.03	0.50**	1.50**	X	X
Perennial Ryegrass	4.29	3.76	3.40***	2.45***	7.62	7.82
Creeping Bentgrass	1.46	0.60	1.87	1.12	5.91	4.62
Bermuda-grass	0.00	1.09	0.00	0.50	X	X
Zoysia-grass	0.00	0.02	0.00	0.25	X	X

X= these species included only one cultivar; see Table 7
* P=0.01
** P=0.05
*** P=0.07 (bluegrasses); P=0.09 (ryegrasses)

spots in each replication. However, spot sizes for the wet and dry plots of the Kentucky bluegrasses, Perennial ryegrasses, and creeping bentgrasses were compared as groups (Table 8). Spot diameters in the bluegrasses, as a whole, which were drought stressed were significantly greater ($P = 0.05$) than those spot diameters for the corresponding wet plots.

IV. DISCUSSION

The fungus causing Pythium blight in Kansas and the effects of drought stress on the severity of Pythium blight of a wide variety of turfgrasses had not been documented prior to this study. Conclusions drawn from this study were:

1. P. aphanidermatum is most likely the primary agent of Pythium blight of turfgrass in Kansas;
2. After being drought stressed, Kentucky bluegrasses, as a whole, were more susceptible to Pythium blight than were comparable Kentucky bluegrasses kept well irrigated. These results supported the observations of Pythium blight in Kansas, in 1977, described at the outset of the paper. At that time, Pythium blight appeared to be more severe on bluegrass homelawns and golf course fairways which had been drought stressed as opposed to those which were kept irrigated. The diameter of blighted spots were significantly greater on the drought stressed than on the non-drought stressed stands. This observation had likewise been made in Kansas in 1977.
3. Pennfine perennial ryegrass and Penncross creeping bentgrass are more susceptible to Pythium blight when not drought stressed. A mild or severe drought stress significantly reduced the severity of Pythium blight of these cultivars over the stands which had been kept well watered prior to the onset of disease.

Although the comparison between (PB) of drought stressed and non-drought stressed perennial ryegrasses showed no significance ($P = 0.05$) in the field experiment. A significantly greater number of spots/plot ($P = 0.09$) was observed in the non-drought stressed (WI) plots than in

the drought stressed (DI) plots. This observation suggested that the four perennial ryegrasses (Citation, Diplomat, Manhattan, and Derby) might give results similar to those of Pennfine if tested under greenhouse conditions (as in RESULTS: part IIIB). Similar greenhouse and growth chamber tests should also be applied to Penncross creeping bentgrass (commonly used on golf greens) which displayed significant differences in Pythium blight severity ($P = 0.01$) when kept well irrigated in field experiments. Moore et al. (77) demonstrated that Highland bentgrass, subjected to numerous wetting and drying cycles was more susceptible to Pythium blight than when maintained at field capacity. However, P. ultimum, rather than P. aphanidermatum, was used in those tests suggesting a difference in cultivar-pathogen interaction.

Although the percentage Pythium blight of drought stressed and non-drought stressed K-31 tall fescue was not significantly different there was a significant difference in the diameter of blighted patches of turf between the two treatments, the larger spots being in the dry plots.

After almost one week of frequent watering and incubation under the plastic tarp, all grasses showed some degree of regrowth. Perennial ryegrasses and tall fescue displayed an extremely lush flush of growth during incubation as exemplified by many grass blades up to 15 cm long. Less re-growth was noted in the Kentucky bluegrass stands. In all cases, the rapid growth was no doubt very succulent. Plant succulence may play a role in susceptibility of turf to Pythium blight. This idea may be supported by the work of Moore et al. (77)

which showed Highland bentgrass to be more susceptible to Pythium blight (P. ultimum) when maintained under high nitrogen levels. Such levels would tend to promote lush shoot growth. The question arises as to which factor (nitrogen level or succulence) is responsible for the increased susceptibility to the disease.

Due to the lack of research information in the literature regarding the effects of drought stress on the host-plant interaction, it is difficult to determine the reason for the difference in Pythium blight severity on drought stressed and non-drought stressed turfgrasses. The degree of susceptibility of the plant is likely a sum of many differences, from whole parts of the plant to the microstructure of the protoplasm, which were brought about by drought stress. In the case of P. aphanidermatum, four possible reasons for the difference in Pythium blight severity on drought stressed and non-drought stressed turfgrasses may be:

1) Orientation or attraction of the fungus to the host was inhibited;

2) the fungus was present on the host but could not efficiently penetrate the host;

3) the pathogen penetrated the host but its spread through the tissue was limited;

4) infection occurred within individual plants but was prevented from spreading to other plants.

These four possibilities will be elaborated to point out the complexity of evaluating the pathogen-plant-environment interactions in this particular case and the need for further research involving the effects of the environment on turfgrass diseases.

A. Attraction of P. aphanidermatum to Turfgrass

P. aphanidermatum oospores are considered the main survival structure in soil (12, 42, 98, 101, 108). In the rhizosphere, these oospores germinate only directly (98) and so zoospores are not likely a primary source of inoculum on the root. Stanghellini (98) also showed that oospore-infested soil which was flooded to over-saturation produced zoospores only in the water above the soil and at the soil line. Unfortunately, a majority of the literature deals with below-ground infection of plant parts by P. aphanidermatum zoospores rather than above-ground infection. Kim et al (53-54) emphasized the importance of zoospores by substantiating the possibility of infection of aerial plant parts. For these reasons and the advantage of quantification of inoculum, zoospores were used for inoculation in the experiments of this thesis.

Turfgrasses, especially those which have been drought stressed, have been shown to liberate considerable amounts of amino acids which may function in attracting P. aphanidermatum zoospores. Chemicals which attract zoospores include glutamic acid, aspartic acid (85), glucose and aminobutyric acid (107). Non-drought stressed perennial ryegrasses have produced considerable quantities of glutamine from leaf tissue (35) while drought stressed perennial ryegrass roots have been shown to liberate significantly larger amounts of amino acids than non-drought stressed roots (49). It seems probable that, after a flooding rain or in standing water, zoospores produced by a soil-borne population of P. aphanidermatum may show greater attraction to perennial ryegrasses which have been drought stressed than to those which

have not been drought stressed because of the large amount of amino acids liberated by the former. This theory should be tested using infested soil and bluegrasses, perennial ryegrasses, and creeping bentgrasses. The attraction of zoospores to glutamine exuded from perennial ryegrass tissue might have been substantiated in our greenhouse and growth chamber experiment; however, the zoospores used in the experiments were purposely inoculated on the turfgrass leaves and had little or no chance to show chemotactic responses other than aggregation.

B. Penetration of Turfgrass Leaves by P. aphanidermatum Zoospores

Drought stress induces morphological and physiological changes in a turfgrass plant. However, in the short time periods during which the plants were drought stressed in the greenhouse and growth chamber experiments, it was unlikely that the morphology of the plant was altered to an appreciable extent (93) although rolling of the blades was observed in the growth chamber at -15 bars osmotic potential and in the greenhouse stressed plants. In all experiments, plants were flooded within one hr prior to inoculation to simulate a rain following a drought period. However, in all cases, perennial ryegrass leaves were still partially rolled before incubation. Many of the stomata were still covered by the rolled leaf and may likely have been closed due to the loss of turgor pressure in the leaf. Such a combination could have discouraged normal germ tube penetration of stomata of the drought stressed leaves. Leaves of Kentucky bluegrasses folded when drought stressed, still allowing many stomata to be exposed (whether open or closed).

C. Invasion of the Turfgrass Tissue

It is likely that physiological changes in the plant accounted for a major response to drought stress. Within the 88 hr stress period imposed on ryegrass in the growth chamber, chlorotic plants were evident at -10, -12, and -15 bars osmotic potential. Wilting of drought stressed plants occurred both in the field and greenhouse experiments.

The capability of P. aphanidermatum to grow at low water potentials is questionable. Cook (15) stated that a "fungus situated in host parenchyma tissue will be in equilibrium with the water potential of that tissue." If this is so, it might be assumed that P. aphanidermatum can penetrate and grow through leaf tissue at low water potentials. Perennial ryegrass plants in all three experiments of this thesis were extracted to water potentials at or near -15 bars and most likely retained a low water potential during recuperation. Stanghellini and Burr (99), however, demonstrated that infection of alfalfa seeds by P. aphanidermatum in soil occurred at -0.01, -0.1, and -1.0 bars matric potentials but not at -15 bars matric potential. It is probably, then, that P. aphanidermatum hyphae invaded the turgid, non-drought stressed ryegrass much quicker than the partially wilted, drought stressed ryegrass. However, this explanation does not suffice for the drought stressed bluegrasses of the field experiment which showed more severe Pythium blight than those kept well watered.

It is also possible that, under different water stress levels, the host may produce chemicals which enhance or limit the growth of Pythium within the tissue (3). For example, when P. aphanidermatum

was grown on a liquid sucrose basal medium containing proline, (which increases in concentration and may function as a major storage compound in drought stressed turfgrass plants (51)) the increase in proline as a percentage of total nitrogen was greater after four days than was the increase in the percentage of 15 other amino acids (51). The effect of proline on the growth of P. aphanidermatum should be examined in vivo to determine if such high accumulations of the amino acids in drought stressed turfgrass plants affect the growth of the fungus within the host tissue.

D. Limited Spread of the Pathogen to Other Plants

In a typical lawn or turf, Pythium blight may move from plant to plant as aerial mycelium (16). In the experiments of this thesis, aerial mycelia were observed to bridge a gap of at least 1 cm between ryegrass leaves. It spread in this manner on plants in the greenhouse and growth chamber experiments (neither of which had a thatch layer) and in the field experiment. A thatch layer accumulated in the field plots, however, and was noticeably thick (1-3 cm) in the Kentucky bluegrasses, tall fescue, and zoysiagrass. Perennial ryegrass, bermudagrass, and creeping bentgrasses had very thin thatch layers (less than 1 cm). The turf was dense enough to support the spread of aerial mycelia from plant to plant.

V. Summary

Pythium aphanidermatum was found to be the major Pythium blight pathogen on turfgrasses in Kansas.

Greenhouse and growth chamber experiments showed the severity of Pythium blight to be greater on non-drought stressed Pennfine perennial ryegrass than on drought stressed ryegrass. The opposite was found true for Kentucky bluegrasses, as a whole, in the field. To the home-owner or turfgrass superintendent, this suggests that, where Pythium blight is known to occur, Pennfine perennial ryegrass plantings may be less susceptible to the disease if the plants are mildly drought stressed during the hot humid conditions under which the disease normally occurs. Kentucky bluegrasses, in general, should be kept adequately watered and drought stress avoided during Pythium blight weather.

The reactions of Penncross creeping bentgrass to Pythium blight merit particular attention since the many of the golf greens in the U.S. are largely composed of this cultivar. In the field experiment, non-drought stressed Penncross displayed a significantly greater spot diameter and percentage Pythium blight than drought stressed Penncross. This would suggest that the incidence of Pythium blight on a Penncross golf green could be reduced by allowing the grass to become somewhat drought stressed. From a management point of view, however, such a suggestion would be impractical because of the ease and rapidity with which golf greens are injured by high temperature and water stress. The susceptibility of drought stressed and non-drought stressed creeping bentgrasses to Pythium blight should be tested under typical golf green management conditions.

The reasons for the differences in Pythium blight severity on drought stressed and non-drought stressed plants are unknown but are speculated to be a combination of many factors. These might include a difference in (a) attraction of zoospores to the stressed and non-stressed plants based on compounds exuded by the plants, (b) the morphological and physiological changes in drought stressed plants which encourage or discourage P. aphanidermatum penetration, invasion, and pathogenesis within the host. Since zoospores are considered to be an efficient source of plant infection, the study of their behavior on above-ground plant parts should receive much more emphasis in the future.

APPENDICES

Table A. Turfgrass Cultivars Which Have Been Tested for Reactions to *Pythium* Species Which Cause Pythium Blight

GRASS	COMMON OR VARIETAL NAME	DISEASE REACTION	REFERENCES
Warm Season:			
Stenotaphrum secundatum	Common St. Augustinegrass	R	30
	Roselawn St. Augustinegrass	R	30
	Floratine St. Augustinegrass	R	30
	Bitter Blue St. Augustinegrass	R	30
	Florida 32 St. Augustinegrass	R	30
Paspalum notatum	Common bahiagrass	R	30
	Pensacola bahlagrass	R	30
	Argentine bahiagrass	R	30
	Paraguayan bahiagrass	R	30
Eremochloa ophiuroides	Centipedegrass	R	30
Zoysia matrella	Manilagrass	R	30
Zoysia japonica	Meyer Z-52 zoysiagrass	R	30
Z. matrella x *Z. tenuifolia*	Emerald zoysiagrass	R	30
Cynodon dactylon	Common bermudagrass	S	30,39
	Tiflawn bermudagrass	S	30
	Tiffine bermudagrass	S	30
	Tifway bermudagrass	S	30,39
	Tifdwarf bermudagrass	S	
	Everglades bermudagrass	S	30
	Ormond bermudagrass	S	30
	Bayshore bermudagrass	S	30
	Florida 50 bermudagrass	S	30
	Texas 22 bermudagrass	S	30
	Texturf 10 bermudagrass	S	30
	Norne bermudagrass	S	30
C. magennisii	Sunturf bermudagrass	S	30
C. transvaalensis	African bermudagrass	S	30
	Uganda bermudagrass	S	30
C. dactylon x *C. transvaalensis*	Tifgreen bermudagrass	S	30,39
Cool Season:			
Agrostis alba	Redtop	S	30,111
A. palustris	Penncross bentgrass	S	30,80,89,91
	Seaside bentgrass	S	23,30,55,89, 111
	Emerald bentgrass	S	30,89
	Toronto bentgrass	S	80
A. tenuis	Astoria bentgrass	S	30,80,89
	Highland bentgrass	S	30,77,80,89
Festuca arundinacea	Alta fescuegrass	S	30
	Kentucky 31 fescuegrass	S	30,111
F. rubra	Illahee fescuegrass	S	30
	Pennlawn fescuegrass	S	30,80,89
	Creeping red fescuegrass	S	111

GRASS	COMMON OR VARIETAL NAME	DISEASE REACTION	REFERENCES
Cool Season:			
Poa pratensis	Common bluegrass	S	30
	Park bluegrass	S	30
	Delta bluegrass	S	30,80
	New Park bluegrass	S	30
	Merion bluegrass	S	16,80,89
	Windsor bluegrass	S	80,89
	Newport bluegrass	S	80
	Fylking bluegrass	S	80
	Kenblue bluegrass	S	80
P. trivialis	Rough stalk bluegrass	S	30
Lolium multiflorum	Annual ryegrass	S	30,31,60,67
L. perenne	Manhattan ryegrass	S	46,89
	Medalist II ryegrass	S	69
	Linn ryegrass	S	80
	NK 200 ryegrass	S	80
	Pelo ryegrass	S	80
	S-321 ryegrass	S	80

Additional Cultivars Tested in This Thesis

Cool Season:		
Poa pratensis	Adelphi	S
	Baron	S
	Glade	S
	Majestic	S
	Rugby	S
	Sydsport	S
	Touchdown	S
Lolium perenne	Citation	S
	Derby	S
	Diplomat	S
	Pennfine	S
Warm Season:		
Cynodon dactylon	Midiron	S

Table B.

Fungicide and Herbicide Spray Program Used in Field Studies of Pythium Blight (Rocky Ford Research Station, Manhattan, Kansas, 1978-1979)

Date	Fungicides	Date	Fungicide
6/7/78	Tersan 1991	6/10/78	Bromoxynil
6/13	Tersan 1991	7/5	MSMA
7/3	Tersan 1991	7/22	MSMA
7/11	Daconil 2787		
7/15	Tersan 1991	1979	None Used
7/22	Daconil 2787		
7/27	Tersan 1991		
8/3	Daconil 2787		
8/8	Tersan 1991		
8/12	Tersan 1991		
5/14/79	Tersan LSR		
5/24	Fore		
5/31	Daconil 2787		
6/12	Daconil 2787		
6/29	Daconil 2787		
7/18	Daconil 2787 + Tersan 1991		

Table C.

Summary of Survey for Pythium Blight Pathogens in Kansas, 1978

Sample[1]	Location[2]	Pythium Species Isolated[3] from tissue	from soil
1-2	JO	0	-
1-3f	"	-	-
1-4	"	-	-
1-8	"	P. aph	P. aph
2-3	"	-	-
2-7	"	-	-
2-18	"	-	-
3-2	"	P. aph	P. aph
3-7C	"	-	-
3-17C	"	-	-
4-2	"	-	-
4-4	"	-	-
4-7	"	-	-
5-8	SH	P. aph	-
8-w	SE	-	-
8-9	"	-	-
9-2	"	-	-
9-5	"	P. spl	-
9-13	"	-	-
9-14	"	0	-
9-15	"	-	-
10-2	"	0	-
10-2f	"	P. aph	-
11-1	SA	0	-
12-3	LA	-	-
13-1	"	-	-
13-2	"	-	-
13-5	"	-	-
13-5t	"	-	-
13-w	"	-	-
14-1	CH	-	-
14-8	"	-	-
14-9	"	-	-
15-3	MO	P. aph	-
15-4	"	-	-
15-5	"	-	-
FTCC550	FLA	P. aph	

[1]Sample: first numeral represents golf course from which samples were collected; second numeral corresponds to green, tee, or fairway number from which sample was collected; lower case letters "f", "c", "t", "w", represent specific site of sampling as fairway, collar, tee, or low spot on course, respectively; no letter signifies sampling from the green.

[2]Course location: JO=Johnson Co., northeast KS; SH=Shawnee Co., northeast KS; SE=Sedgwick Co., southcentral KS; LA=Labette Co., southeast KS; SA=Saline Co., central KS; CH=Cherokee Co., southeast KS; MO=Montgomery Co., southeast KS; FLA=Florida isolate #FTCC550, courtesy of T.E. Freeman, Gainesville, Fla.

[3]Pythium Species Isolated: (-)=no Pythium or other fungi isolated; (0)= unidentified oomycete isolated; P. aph.=P. aphanidermatum isolated; P. spl.=P. splendens isolated.

Media Used in These Studies of Pythium Blight

1. Acid PDA: (48)

Difco PDA	39.0 g
distilled water	1.0 L
lactic acid (50%)	2 drops added to each plate after pouring

20 ml per plate

2. Amended PDA: (13)

Difco PDA	39.00 g
K-penicillin	0.06 g
streptomycin sulfate	0.04 g
distilled water	1.00 L

20 ml per plate

3. SA-PBNC: (90)

sucrose	2.40	g
agar	15.00	g
KH_2PO_4	0.15	g
$K_2HPO_4 \cdot 3H_2O$	0.15	g
$MgSO_4 \cdot 7H_2O$	0.10	g
$ZnSO_4 \cdot 7H_2O$	0.0044	g
$FeSO_4 \cdot 7H_2O$	0.001	g
thiamine hydrochloride	0.002	g
ascorbic acid	0.01	g
cholesterol	0.01	g
$MnCl_2 \cdot 4H_2O$	0.00007	g
Benlate (50% ai.)	0.02	g
Terrachlor (PCNB)	0.05	g
distilled water	1.00	L
neomycin sulfate*	0.10	g
chloramphenicol*	0.03	g
L-asparagine*	0.27	g

* added after autoclaving
20 ml per plate

4. Schmitthenner's Medium: (113)

sucrose	2.50	g
asparagine	0.27	g
KH_2PO_4	0.15	g
$K_2HPO_4 \cdot 3H_2O$	0.15	g
$MgSO_4 \cdot 7H_2O$	0.10	g
cholesterol	0.01	g
agar	15.00	g
distilled water	1.00	L

20 ml per plate

5. V-8 Agar: (55)

V-8 juice (cleared for 15 min with low speed centrifugation)	200 ml
$CaCO_3$	2.0 g
agar	12.0 g
distilled water	1.0 L

20 ml per plate

Procedure for Producing Zoospores of P. aphanidermatum

1. Begin with a clean culture of P. aphanidermatum. Clean cultures may be obtained by placing a plug of piece of tissue containing P. aphanidermatum in the center of an empty petri dish and covering the specimen with a slab of agar. After 12 hr incubation at 35 C, superficial pieces of agar containing clean mycelium of P. aphanidermatum may be taken from the surface. Acid PDA and SA-PBNC (App. D) used as agar slabs preclude the growth of bacteria and other contaminants.

2. Place a plug of agar containing a clean culture of P. aphanidermatum in the center of a plate of V-8 agar. Incubate the agar in darkness in a plastic bag for 24 hr at 31 C.

3. Using a #7 cork borer (1 cm-diameter) cut 30 plugs from the mat of mycelium and the V-8 agar. Avoid the center of the plate.

4. Place ten of these plugs in an empty petri dish (100 x 15 mm). Space the plugs evenly within the plate and lay all plugs face up. Cover the plugs with 15 ml sterile de-ionized water (SDIW) and incubate the plates for 48 hr at 31 C in a closed container to prevent excessive water loss.

5. After 48 hr, pour off the old SDIW, rinse once with fresh SDIW, and then cover the plugs with 10 ml SDIW. Re-incubate the plates for 3 hr at 31 C.

6. After 3 hr, refrigerate the plates for 20 min at 8-10 C and then re-incubate for a final 4-6 hr at 31 C.

7. At harvest, pour the zoospore suspension into a flask and quantify

the number of zoospores using a hemacytometer (Bright-Line Hemacytometer, American Optical Corporation, Buffalo, NY). Preparation of zoospores for counting is as follows:

a. Place three to four 1 ml samples from the zoospore suspension in separate tubes or vials. Add one drop lactophenol + cotton blue to each tube (24).

b. After 5 min, withdraw samples from each tube to be placed on the hemacytometer. Be sure to shake samples before withdrawing suspension.

c. Using phase-optics, zoospores may be easily counted by using a 10X eye-piece, 10X objective lens, and a setting of "20" on the phase-optics diaphragm. Zoospores will appear dark blue.

d. The number of zoospores per entire grid multiplied by 1,111 will estimate the number of zoospores/ml of the original suspension.

e. Zoospores may be diluted to a desired concentration by adding SDIW.

f. Zoospores may be encysted by swirling the suspension in a large flask, vigorously, 100-150 times.

The Use of Oospores as a Source of Inoculum on Pennfine Perennial Ryegrass

The use of laboratory grown P. aphanidermatum oospores as a source of inoculum was explored using Florida isolate FTCC550. Oospores were produced by growing P. aphanidermatum in 50 ml of Schmitthenner's synthetic liquid medium in shake culture (1) for 21 days at 31 C. The mycelial mats were rinsed twice with sterile distilled water (SDW) then minced for two 15-sec time periods in a Sorvall Omni-Mix (setting #5), poured onto a 44 um metal screen, and rinsed with 5 ml SDW thereby retaining the mycelium. Measurements of 43 oospores and 43 oogonia produced with this method showed the mean oospore and oogonia diameters to be 20.61 um and 25.32 um, respectively.

The suspension was diluted to 250, 1000, 5000, and 10,000 oospores per ml SDW and 10 ml of each suspension was dripped onto the foliage of seven week old Pennfine perennial ryegrass grown in 8 cm pots. Two pots were inoculated with each suspension and incubated in humid conditions under a plastic tarp in the greenhouse for 6 days at 30-37 C. One ml of each oospore suspension and a suspension of minced but unfiltered P. aphanidermatum containing oospores and mycelia was plated onto SA-PBNC (90, App. D) and incubated for 6 days at 31 C.

After 6 days, Pythium blight symptoms were not observed on any plants. Likewise, the oospore suspension on SA-PBNC showed no evidence of oospore germination or growth. Adams (1), however, obtained up to 71% germination when similarly-produced oospores were subjected to the buried filter method. Since the SA-PBNC plates inoculated with the minced mycelia and oospore suspension were completely covered after 6 days incubation, it was assumed that the percentage germination of oospores produced by the V-8 method (55, App. E) was negligible but that minced mycelia retained the

capacity for growth. As a result of this test, oospore suspensions were not used as a source of inoculum in subsequent experiments in this thesis.

Number of V-8 Plugs Necessary to Produce a Maximum Concentration of *P. aphanidermatum* Zoospores

Although Kraft and Endo's V-8 method (55, App. E) was used to produce zoospores of *P. aphanidermatum*, some modifications were necessary to increase the zoospore concentration with the isolates used in these studies. An experiment was designed to determine the number of agar plugs/flooded dish necessary to produce a maximum concentration of zoospores for isolate FTCC550. Each of the five treatments applied consisted of a different number of agar plugs/flooded dish. The treatments were 5, 10, 20, and 30 plugs/flooded dish and one plate of a 24 hr old *P. aphanidermatum* colony on V-8 agar which was left intact and flooded along with the above four dishes. In order to make full use of the agar, 1 cm plugs were cut in the outer two-thirds of the fungal colony, avoiding the center of the plate where bacterial contamination, if present, would have been the greatest. Kraft and Endo (55) used plugs only from the perimeter of the colony. Isolate FTCC550 was used and zoospres were harvested 6 hr after reflooding. Zoospores from four 1 ml samples from each plate were stained with lactophenol and cotton blue and counted with a hemacytometer (24, App. E). A mean concentration of zoospores was calculated from a total of eight counts taken from the four samples/plate. The experiment was repeated a total of four times.

Using an analysis of variance and a test for least significant difference ($P=0.01$), the data showed that the greatest number of zoospores was produced with ten 1 cm plugs/flooded dish (Table F2). As the amount of agar per dish was increased to 20 and 30 plugs and an entire plate, the concentration of zoospores decreased rapidly. This decrease was most likely due to the inability of the flooding procedure to remove nutrients

from the water after reflooding. Stanghellini (98) showed that in the presence of nutrients in an aqueous environment, sporangia and oospores tended to germinate directly and only produced zoospores in water that was essentially devoid of nutrients.

As a result of this experiment, ten 1 cm plugs from 24 hr old colonies of P. aphanidermatum (grown on V-8 agar) were used to produce zoospores in subsequent experiments.

Table F2. Concentration of P. aphanidermatum Zoospores Produced as a Function of the Number of Agar Plugs/Flooded Dish

Number of Plugs per Flooded Dish	Mean Number of Zoospores Counted per Grid[1]
5	32.00a[2]
10	39.81b
20	15.56c
Entire Agar Slab	1.94d

[1]Concentration of zoospores/ml water can be calculated by multiplying the mean value by 1,111.

[2]LSD= 6.23 and P=0.05; means with the same letter are not significantly different.

Optimum Time Intervals for Harvesting Zoospores of P. aphanidermatum (Isolate FTCC550)

An experiment was designed to determine the optimum time period between reflooding and harvest necessary to produce a maximum number of active zoospores. Kraft and Endo's method (55, App. E) of producing zoospores was used with the following modifications: 12 g agar/L V-8 media was used and ten 1-cm plugs were placed in each flooded dish. Zoospores of isolate FTCC550 were harvested each hour for 6 hr after reflooding and concentrations were determined by staining the suspensions with lactophenol and cotton blue and counting the zoospores with a hemacytometer (24,App. E). Four counts were made per dish. The experiments was performed a total of two times. Data was subjected to an analysis of variance and a test for least significant difference (LSD) at P=0.05.

Statistical analysis showed that the concentration of zoospores harvested 4-6 hr after reflooding was significantly greater than the concentration of those harvested from 1-3 hr after reflooding (Table F3). When harvested 4-6 hr after reflooding, approximately 37,500 - 42,800 zoospores/ml were collected from the suspension. However, zoospores harvested 6 hr after reflooding showed signs of encystment and germination. A slowing-down and encystment of zoospores was more evident 9 hr after reflooding although some zoospores were still swimming 24 hr after reflooding.

As a result of this test, subsequent P. aphanidermatum zoospore harvests were made 4-6 hr after reflooding when Kraft and Endo's method was used. In later tests involving the production of zoospores by the Kansas isolate (10-2f), a cold shock (20 min at 8 C, three hr after flooding) was found necessary to induce zoospore liberation. The Florida isolate (FTCC550) did not require a cold shock.

Table F3. Number of Hours of Reflooding Necessary to Produce a Maximum Zoospore Concentration Using P. aphanidermatum (Isolate FTCC550)

Hours to Harvest	Mean Number of Zoospore per Grid[1]
1	2.25a[2]
2	11.75a
3	21.75b
4	33.63c
5	34.50c
6	38.12c

[1]Concentration of zoospores/ml water can be calculated by multiplying the mean value by 1,111.

[2]LSD= 10.187 at P=0.01; means with the same letter are not significantly different.

A Comparison of the Number of Zoospores Liberated by Four P. aphanidermatum Isolates

In order to test the original hypothesis regarding the severity of Pythium blight on drought stressed turfgrass in Kansas, it was deslrable to secure a Kansas isolate of P. aphanidermatum. Since the fungus was to be used for zoospore production, it was necessary to determine which of the isolates collected produced the greatest concentration of zoospores by the V-8 method (55, App. E).

Five Kansas isolates (1-8, 3-2, 5-8, 10-2f, 15-3) were collected during the survey but only three of them (1-8, 3-2, and 10-2f) were tested for zoospore production since the other two isolates periodically developed abnormal oospores. Three plates of zoospores of isolates 1-8, 3-2, 10-2f, and FTCC550 were produced according to Kraft and Endo's V-8 method (55) with the modifications described in Appendix E. Cultures were not given a cold shock, as was used later for isolate 10-2f, but were harvested 4.5 hr after re-flooding. After staining with lactophenol and cotton blue (24, App. E), four zoospore counts were taken from each of the three plates/isolate. The data were subjected to an analysis of variance and a test for least slgnificant difference at P=0.01. The experiment was performed three times, with the concentration of zoospores of isolates 1-8, 10-2f, and FTCC550 compared in the first two experlments and the concentration of zoospores of isolates 3-2, 10-2f, and FTCC550 compared in the third experiment.

The concentration of zoospore produced by isolate 10-2f was signifi-cantly greater (P=0.01) than that of the other lsolates (1-8, 3-2, and FTCC550) in all three experiments (Table F4). Zoospore production by 10-2f ranged from 5000-65,000 zoospores/ml but generally averaged 40,000 - 45,000 zoospores/ml.

As a result of this test, isolate 10-2f, which was collected from fairway #2 on the Wichita State Univeristy golf course, Wichita, KS in 1978, was used in subsequent studies of Pythium blight of turfgrass in Kansas.

Table F4. A Comparison of the Number of Zoospores Liberated by Four P. aphanidermatum Isolates

	Mean Number of Zoospores per Grid[1]		
Test[2]	I	II	III
Isolate			
1-8	0.83a[3]	20.33b	-
3-2	-	-	7.92b
10-2f	6.17b	35.00c	14.08c
FTCC550	0.92a	5.50a	3.33a

[1] Concentration of zoospores/ml water can be calculated by multiplying the mean value by 1,111.

[2] Test I LSD= 2.06; Test II LSD= 5.05; Test III LSD=3.88; all LSD at P=0.01.

[3] Means (WITHIN EACH TEST) with the same letter are not significantly different at P=0.01.

A Comparison of the Number of P. aphanidermatum Zoospores Liberated at Two Incubation Temperatures

Using Kraft and Endo's V-8 method (55, App. E) of producing zoospores, it was necessary to determine the temperature at which a maximum number of zoospores was liberated. McCarter and Littrell (68) demonstrated a maximum number of zoospores liberated by P. aphanidermatum at temperatures between 28 and 31 C. Thus, 31 C was selected as one incubation temperature under which isolate 10-2f was tested for zoospore-producing capability. The other temperature, 35 C, was selected since it is generally considered to be the optimum growth temperature for P. aphanidermatum. Six plates of zoospores were produced using the V-8 method, with three of the plates incubated at 31 C and three of the plates incubated at 35 C. Isolate 10-2f required a cold shock (20 min at 8 C) in order to liberate zoospores. Four hr after refrigeration, zoospores were collected by combining the three plates of each treatment into one sample, removing four 1 ml aliquots from that sample, staining the suspension with lactophenol and cotton blue (24) and counting the zoospores with a hemacytometer (App. E.) The number of zoospores/ml of harvested suspension was compared for the two treatments to determine the temperature at which the greater number of zoospores was liberated. The experiment was performed twice. Data was subjected to an analysis of variance and a test for least significant difference at P=0.01.

The analysis showed that a significantly greater number of zoospores was liberated when P. aphanidermatum was incubated at 31 C than at 35 C using the V-8 method (Table F5). These results concur with the findings of McCarter and Littrell (68) in which a maximum number of P. aphanidermatum zoospores were liberated at incubation temperatures between 28 and 31 C.

Thus, plates of P. aphanidermatum (10-2f) were incubated at 31 C for

maximum zoospore production in subsequent experiments in this thesis.

Table F5. A Comparison of the Number of P. aphanidermatum Zoospores Liberated at Two Incubation Temperatures

Temperatures	Mean Number of Zoospores per Grid[1]
31 C	25.86a[2]
35 C	7.06b

[1]Concentration of zoospores/ml suspension can be calculated by multiplying the mean value by 1,111.

[2]Means with the same letter are not significantly different at P=0.01.

Concentration of Zoospores Necessary to Produce Pythium Blight Symptoms on Pennfine Perennial Ryegrass Within 48 Hours

Preliminary work was done to determine the number of zoospores necessary to produce discernable Pythium blight symptoms for rating within 48 hr after inoculation. All inoculation was done by dripping a zoospore suspension of P. aphanidermatum (Isolate FTCC550) onto 30 day old Pennfine perennial ryegrass grown in 10 cm plastic pots. Plants were then incubated in the greenhouse in a plastic bag at day temperatures of 35-37 C and night temperatures of 20-25 C. Trial and error, using 1, 5, and 10 ml aliquots containing 100, 1000, 3000, 5000, and 10,000 zoospores/ml showed the combination of 5000 zoospores/ml x 1 ml/pot of turfgrass to be the most effective method of inoculation. The 1 ml aliquot/pot was most desirable because the water remained on the foliage unlike the water of the 5 and 10 ml aliquots which drained onto the soil. At 100 zoospores/ml x 1 ml/pot, symptoms did not appear within 10 days under greenhouse conditions. At 1000 and 3000 zoospores/ml x 1 ml/pot, 5 days incubation were required to produce Pythium blight symptoms. There appeared to be no difference in disease severity after 48 hr incubation between 5000 and 10,000 zoospores/pot (using 1 ml aliquots) and so the former level was preferred to conserve inoculum.

The Effects of Four Types of Dilution-Water on Zoospore Growth

An experiment was designed to evaluate the effects of water from four different sources on the germination and growth of P. aphanidermatum zoospores. The water which was least inhibitory would be used for dilution of zoospore suspensions in later studies. P. aphandermatum zoospores (isolate 10-2f) were produced according to the V-8 method (55) with the modifications listed in Appendix E. Upon harvest, zoospores of three plates were combined and made to encyst by vigorously shaking the 30 ml suspension in a 250 ml flask. Five ml was pipetted into a 25 ml flask, covered and set aside. Of the remaining 25 ml, four 1 ml aliquots were used to prepare the following treatments.

1 ml zoospore suspension + 9 ml sterile tap water (STW)
1 ml zoospore suspension + 9 ml sterile distilled water (SDW)
1 ml zoospore suspension + 9 ml sterile de-ionized water (SDIW)
1 ml zoospore suspension + 9 ml sterile pond water (SPW)

Sterile pond water consisted of 250 ml pond water filtered through a Whatman #1 filter paper, added to 500 ml DW, then autoclaved. All solutions were allowed to stand for 10 min. Four drops from each of the four dilutions were plated onto one half of an agar plate (Schmitthenner's medium (113, App. D)) while four drops of the undiluted suspension were placed on the other half of each plate to serve as a control. The four plates were then incubated in a plastic bag in darkness for 90 min at 35 C. After 90 min, a drop of lactophenol and cotton blue was placed over the original four drops on the plate and the plates were allowed to stand for 10 min. Ratings were made by direct measurements of germ tube length at 100X with a micrometer. Ten zoospores were observed at random in each of the four drops/treatment. Differences in the effect of four water

types on the growth of the zoospores was determined with a test for LSD at P=0.01. The experiment was repeated twice.

Results

The test for LSD showed SDIW to be the least inhibitory to germ tube growth from P. aphanidermatum zoospores. In both tests (Table F7) there was no significant difference in germ tube length between the control dilutions and the dilutions made with SDIW and SDW. Because of the possibility of chemical run-off into the pond from which the pond water was collected, SDIW was selected for use in diluting zoospore suspensions in further studies.

Table F7. The Effects of Four Types of Water Used for Dilution on the Growth of P. aphanidermatum Zoospores

	Mean Germ Tube Length (um)					
Water Type	SDW	STW	SDIW	SPW	Control	LSD
Test I	19.42a[1]	37.76b	53.24c	55.22c	66.16c	13.80
Test II	32.04ab	22.18a	43.88c	36.73bc	38.95bc	11.04

[1] Means with the same letter were not significantly different at P=0.01. Results are given as two separate tests.

The Effects of Pressurization Within and Spraying of Zoospores from a Hand-Pump Pressure Sprayer

In large-scale field studies, it appeared that the spraying of Pythium zoospores onto field plots with a pressurized hand-pump sprayer would facilitate inoculation. A test was devised to determine if pressurization to 35 psi and spraying affected germination and subsequent germ tube growth by zoospores.

P. aphanidermatum zoospores were produced by the V-8 method (55) with slight modifications (App. E). Zoospores were forced to encyst (App. E) immediately after harvest. Five ml were then set aside in a closed 25 ml flask (Treatment 1); the remaining suspension was diluted to a concentration of 5000 zoospores/ml SDIW and five ml of this suspension were set aside in a 25 ml flask (Treatment 2). The remaining suspension was then poured into a Sears 1.5 gallon hand-pumped pressurized spray tank and allowed to stand in the open tank for 10 min after which five ml were extracted and set aside in a 25 ml flask (Treatment 3). The spray tank was then closed, pumped to 35 psi and held at that pressure for 15 min. The suspension was finally sprayed into a one liter flask (Treatment 4).

Four drops from each of the four treatments were placed onto on-quarter of an agar slab of Schmitthenner's medium (113, App. D) and incubated in a moist chamber in an incubator for 90 min at 35 C. After 90 min, each drop was covered with a drop of lactophenol plus cotton blue and allowed to stand five min. Zoospores were observed under the microscope at 200X and germ tube lengths were recorded for ten randomly observed zoospores in each drop. The ten measurements were averaged to determine a mean for each treatment. The experiment was repeated a total of three times.

Results

A test for LSD at P=0.05 showed the following (Table F8):
(a) In two out of three tests, there was no significant reduction in germ tube length after having sprayed the zoospoores through a pressurized sprayer (Treatment 4) when compared to germ tube length of zoospores in a diluted suspension (Treatment 2). Since zoospores would either be used as a spray or a diluted suspension, this comparison was most important.
(b) In two of three tests, there was a significant reduction in germ tube length for Treatment 4 (spraying) when compared to Treatment 1 (no dilution).

In later experiments in which zoospores were sprayed onto Pennfine perennial ryegrass according to the above procedure, production of aerial mycelia was either erratic or did not occur under conditions necessary for Pythium blight. This may have been due to the fact that all sprayed zoospores were encysted and had no chance to aggregate. Kraft et al. (55) stated: "It appears that infection by masses of zoospores is necessary for severe necrosis and subsequent colonization (of the roots)." This may be true for aerial plant parts. Kim (54) sprayed zoospores onto above ground parts of bean plants and noted aggregation since the zoospores had not encysted.

Even though growth of the zoospores appeared to be unaffected by pressurization, it was decided to pour the zoospore suspension onto field plots rather than spraying it on because of the erratic production of aerial mycelia. This observation warrants further study.

Table F8. The Effects of Dilution, Spraying, and Pressurization on the Growth of P. aphanidermatum Zoospores

	Mean Germ Tube Length (um)				
Treatment	1	2	3	4	LSD
Test I	41.17a *	40.43a	33.77b	30.32b	6.39
Test II	55.22a	56.69a	50.78a	53.00a	9.14
Test III	51.03a	41.17b	35.00b	37.96b	9.07

* Means with the same letter (WITHIN EACH TEST) are not significantly different at P=0.05.

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A STUDY OF THE SEVERITY OF PYTHIUM BLIGHT OF DROUGHT STRESSED AND NON-DROUGHT STRESSED TURFGRASSES IN KANSAS

by

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ABSTRACT

During the summer of 1977, an increase in the severity of Pythium blight was noted on Kentucky bluegrass which had been subjected to a drought stress period. The difference in disease severity on drought stressed vs non-drought stressed turfgrass prompted a study of Pythium blight in Kansas. A survey and test at several Kansas golf courses which had reported Pythium blight of turfgrass showed P. aphanidermatum to be the causal agent of the disease in the state. A study was undertaken to compare the severity of the disease on turfgrass which had previously been drought stressed and that which had not been drought stressed.

Under greenhouse conditions, the severity of Pythium blight was significantly greater (P=0.05) on non-drought stressed Pennfine perennial ryegrass (73.17%) than on the same cultivar which was subjected to a drought stress (28.13%) prior to inoculation with P. aphanidermatum zoospores. In a second test, 21-28 day old Pennfine perennial ryegrass was subjected to a controlled water stress in the growth chamber using PEG 6000 which produced water stress levels of 0 (no PEG 6000), -10, -12, and -15 bars osmotic potential (OP). Two weeks after inoculation with P. aphanidermatum zoospores the plant dry weight and the number of plants alive were determined. Plants which were subjected to some degree of water stress (-10, -12, -15 bars OP) showed significantly greater (P=0.05) dry weight (1.94 g, 2.00 g, 1.78 g, respectively) than those which were maintained in distilled water and relatively free of water stress. However, when rated by number of plants alive, no difference was found among plants at the four OP levels.

In a field test, twenty turfgrass cultivars (including 10 Kentucky bluegrasses, five perennial ryegrasses, two creeping bentgrasses, one tall

fescue, one bermudagrass, and one zoysiagrass) were subjected to a 39 day drought stress period while similar plots of the same cultivars were kept well irrigated. Inoculation with P. aphanidermatum zoospores was followed by a six day incubation period and ratings for percentage Pythium blight, number of spots per plot, and spot diameter. Percentage Pythium blight was significantly greater on well irrigated Penncross creeping bentgrass (1.37%) than on drought stressed Penncross (0.21%). Pythium blight severity was also greater on drought stressed Kentucky bluegrasses (as a whole) (2.89%) than on non-drought stressed bluegrasses (as a whole) (0.82%). Drought stress significantly increased the mean number of blighted spots per plot for Touchdown Kentucky bluegrass (2.75), K-31 tall fescue (1.50), and the bluegrasses (as a whole) (1.70) over their non-drought stressed counterparts (0.50, 0.50, 1.12, respectively). Adequate irrigation increased the mean number of blighted spots per plot for Penncross creeping bentgrass (2.00) and the perennial ryegrasses (as a whole) (3.40) over their drought stressed counterparts (0.75, 2.45, respectively). Spot diameter was significantly greater on drought stressed Kentucky bluegrasses (as a whole) (7.85) as compared to non-drought stressed Kentucky bluegrasses (5.85).

These results support the initial observations made in 1977 in which the severity of Pythium blight on Kentucky bluegrass increased after a drought stress period.