The effects of osmotically-induced water stress and stripe smut (Ustilago striiformis) or flag smut (Urocystis agropyri) on Kentucky bluegrass (Poa pratensis 'Merion')

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THE EFFECTS OF OSMOTICALLY-INDUCED WATER STRESS AND STRIPE SMUT (USTILAGO STRIIFORMIS) OR FLAG SMUT (UROCYSTIS AGROPYRI) ON KENTUCKY BLUEGRASS (POA PRATENSIS 'MERION')

Iowa State University

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The effects of osmotically-induced water stress and stripe smut (*Ustilago striiformis*) or flag smut (*Urocystis agropyri*) on Kentucky bluegrass (*Poa pratensis* 'Merion')

by

Jeffery Lynn Nus

A Dissertation Submitted to the Graduate Faculty in Partial Fulfillment of the Requirements for the Degree of DOCTOR OF PHILOSOPHY

Major: Horticulture

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I. INTRODUCTION

Water is an essential component of plant life and comprises approximately 85 to 90% of the total fresh weight in physiologically active herbaceous plants. Many physiological functions of the plant are impaired when the water content falls much below this level (Turner and Kramer, 1980).

Water shortage is more limiting to crop production in the world than any other single factor (Kozlowski, 1968a). Over one-third of the world's cropland receives less than 30 inches of precipitation per year and crop yields are periodically reduced by drought (Townley-Smith and Hurd, 1979). Drought is a major limiting factor of yields even in tropical regions (Wein et al., 1979). Kramer (1980) claims worldwide losses in yield from water stress probably exceed the losses from all other causes combined.

It is important to differentiate between the terms drought and water stress to avoid confusion when studying the effects of water deficits on plants. Drought is a meteorological event defined as the absence of rainfall for a period of time long enough to cause depletion of soil moisture and damage to plants (May and Milthorpe, 1962). The length of time without rain that is necessary to cause injury depends on the kind of plants, the water holding characteristics of the soil in which they are growing, and
the atmospheric conditions that affect the rate of evaporation and transpiration (Kramer, 1980).

Levitt (1972) describes a biological stress as "any environmental factor capable of inducing a potentially injurious strain in living organisms". Crafts (1939) suggested that the term water stress be defined as the state a plant enters when the water potential crosses the zero mark and becomes negative. Although plant water stress always accompanies drought, it may occur in the absence of drought, either because of excessive transpiration or because water absorption is inhibited by cold soil, an excess of salt in the soil solution, deficient aeration, or injury to root systems (Turner and Kramer, 1980).

The degree of dehydration without permanent injury varies widely, depending on the process under consideration, the stage of development, the duration of stress, and the kind of plant (Hsiao, 1973; Begg and Turner, 1976). Because of the overwhelming importance of water for plant growth and development, several books (Kozlowski 1978; 1976a; 1972; 1968a; 1968b; 1964; Kramer 1969; 1949; Lange et al., 1976; Levitt, 1972; Maximov, 1929; Mussell and Staples, 1979; Paleg and Aspinall, 1982; Rutter and Whitehead, 1963; Slatyer, 1967; Slavik, 1974; Turner and Kramer, 1980) and reviews (Boyer and McPherson, 1975; Gates, 1964; Henckel, 1964; Hsiao, 1973; Ilgin, 1957; Kramer, 1963; Macklon and Weatherley, 1965; Shaw and Laing, 1966; Slatyer, 1957;
Vaadia et al., 1961) have been written. Mechanisms for drought resistance have been reviewed by Ilgin (1957), Levitt (1972), and Paleg and Aspinall (1982).

Plant disease is another limiting factor in crop production in addition to shortages of water. Disease is a cause of lowered efficiency or final breakdown in the plants growth and function (Wood, 1953). The relationship between plant disease and environment is complex. Environmental conditions may predispose potential hosts to invasion by pathogens (Schoeneweiss, 1975b, 1978), affect the growth, distribution, and survival of pathogens (Cook, 1973; Griffin, 1978; Cook and Duniway, 1981), and modify the expression of the disease syndrome (Talboys, 1968). Temperature and moisture are the limiting factors for most plant diseases (Miller, 1953), and either can be decisive in the initiation, development, and spread of disease. The disease becomes serious if both are constantly favorable.

Symptoms of plant disease may include disease-induced water deficits in the host, i.e., the drought syndrome (Talboys, 1968). Disease-induced host water deficits may arise in several ways. Vascular wilt pathogens induce water deficits by producing vascular occlusions, toxic metabolites, host degradation products, or host reaction mechanisms (Dimond, 1970). Disease-induced water deficits also may result from loss of membrane integrity.
from the action of toxins or enzymes, or the disruption
Pathogen-induced reduction in root growth and subsequent
decrease in host root-shoot ratio may lead to host water
deficits (Murphy, 1935; Bever, 1937). Finally, dramatic
increases in water loss may accompany infection. Some
pathogens inhibit stomatal function directly (Farrell et
al., 1969; Cruikshank and Rider, 1961; Ayres, 1972).
Dramatic increases in non-stomatal water loss also may
accompany epidermal rupturing caused by sporulation of
rust and smut pathogens (Johnston and Miller, 1934; 1940;
Ayres, 1978).

_Ustilago striiformis_ and _Urocystis agropyri_ are the
causal organisms for stripe smut and flag smut, respectively.
They are serious pathogens in several grass species in-
cluding _Poa pratensis_ (Couch, 1971). There are no cultivars
of _P. pratensis_ that are very resistant to either pathogen
(Kreitlow and Juska, 1959). All _P. pratensis_ cultivars are
to some degree tolerant (Wingard, 1953) of _U. striiformis_
and _U. agropyri_. Under conditions of plentiful moisture
and cool temperatures, these pathogens grow systemically
throughout the aerial parts of the host (Hodges, 1970b;
Hodges and Britton, 1970) and infected tillers and rhizomes
are produced from infected plants (Hodges and Britton, 1969).
Under conditions of limited moisture or high temperatures
(Hodges, 1970c; Kreitlow, 1943), however, infected plants exhibit greater mortality rates than healthy plants. 'Merion' *P. pratensis* exhibits severe damage from infection by either organism (Kreitlow and Juska, 1959).

This research was initiated to investigate the effects of systemic infection by *Ustilago striiformis* and *Urocystis agropyri* on total plant growth, leaf and root growth, growth of tillers and rhizomes, water relations, water use efficiency, osmotic adjustment, and cell wall elasticity of 'Merion' *P. pratensis* under conditions of increasing osmotically-induced water stress.
II. REVIEW OF LITERATURE

A. Water Deficits

1. Application

The most common method of investigating the effects of drought on plants is to withhold irrigation until plants wilt or until a predetermined soil moisture content is reached (Duniway, 1977; Edmunds, 1964; Moore et al., 1963; Couch and Bloom, 1960; Ghaffar and Erwin, 1969; Hobbs et al., 1963; Cykler, 1946). Often these drying cycles range from field capacity to permanent wilting point, or a percentage of field capacity (Cowan, 1965; Ashton, 1956). Field capacity is the water content of a soil profile when, after it has been thoroughly wetted, the rate of drainage from the profile is negligible (Baver et al., 1972). Water potentials of a uniform soil profile at field capacity approach -0.1 bars for sandy soils and -0.3 bars for loams and silt loams (Papendick and Campbell, 1981). Permanent wilting point is the soil water content at the time leaves of indicator plants become wilted and do not recover turgor overnight (Papendick and Campbell, 1981). Permanent wilting point corresponds to a soil water potential of -15 bars (Crafts, 1968). Problems encountered in controlling soil moisture within field capacity to permanent wilting percentage were summarized by Couch et al. (1967).
Interpretation of water deficits based on alternate wetting and drying of soil is subject to criticism since such regular cycles are different than water depletion patterns under field conditions (Schoeneweiss, 1975a). Water stress in nature is more often associated with prolonged drought, where soil water potentials remain low or decrease for a long time and exert more constant stress on plants than wide fluctuations between field capacity and some lower value. Therefore, techniques in which water stress levels can be held relatively constant should provide more valid information on water stress than other methods reported.

Much evidence has accumulated showing that the metabolism and growth of plants in drying soil are influenced by even mild water deficits in plant tissues and that such deficits usually occur long before drying soil approaches the permanent wilting percentage (Kozlowski 1958; 1964). This has led researchers to develop techniques that enabled them to expose plants to relatively constant levels of water stress. Miller and Burke (1974) devised a technique for creating constant water stress which utilized ceramic plates under suction as one side of a soil chamber. They were able to maintain constant matric potentials over a four week period.

Several workers have used various osmotica in aqueous environments in efforts to maintain constant levels
of water stress (Mizrahi et al., 1970; Darbyshire, 1971). These osmoticums include NaCl (Itai et al., 1968; Vaadia and Itai, 1968), mannitol (Itai et al., 1968; Vaadia and Itai, 1968; Plaut and Ordin, 1964), and polyethylene glycol (Lawlor, 1979; Williams and Shaykewich, 1969; Michel and Kaufmann, 1973; Steuter et al., 1981; Lagerwerff et al., 1961; Ayres, 1977; Jarvis and Jarvis, 1965). However, both NaCl and mannitol can be taken up by the tissue (Trip et al., 1964; Kozinka and Klenovska, 1965; Groenewegen and Wilis, 1960; Greenway and Leahy, 1970) and produce some unwanted side effects (Jackson, 1965; Lawlor, 1970). This has led workers to compare the use of mannitol versus polyethylene glycol for use as osmoticums (Williams and Shaykewich, 1969; Nechiporenko and Rybalova, 1980a; 1980b; Michel, 1970; 1971). Most have found that polyethylene glycols of higher molecular weight (greater than 3000 daltons) generally do not penetrate the tissue (Jarvis and Jarvis, 1965; Michel, 1971) nor do they cause injury in most systems (Jarvis and Jarvis, 1965). Some workers have found side effects from using polyethylene glycols (Kaufmann and Eckard, 1971; Lawlor, 1970; Michel, 1971; Ruf et al., 1967; Mexal et al., 1975), so although widely used, the applicability of polyethylene glycols as osmoticum depends on the systems and plants you are using (Hsiao, 1973; Michel and Kaufmann, 1973).
2. Measurement

The only way to determine whether a plant is being subjected to water stress, according to Kramer (1963), is to measure the water deficit in the plant itself. Methods for measuring water deficits of plants and soils have been reviewed by Barrs (1968), Slavik (1974), Rawlins (1976), and Boyer (1969). Plant water deficits are primarily described by two basic parameters: water content of the tissue and the energy status of the water contained therein (Slatyer and Taylor, 1960). According to Barrs (1968), determination of both components is necessary to describe any water deficit completely.

a. Water content Water content, calculated on either a dry or a fresh weight basis, is an unsatisfactory measure of water stress, but water content as a percentage of water content at saturation is useful (Turner and Kramer, 1980). The two most accepted expressions of water content relative to saturation are relative water content (RWC) and water saturation deficit (WSD) (Barrs, 1968). RWC is the water content (as a percentage) relative to the water content of the same tissue at full turgor (Barrs, 1968; Turner and Kramer, 1980). Leaf water contents have been calculated for whole leaves (Slavik, 1963b), leaf discs (Begg et al., 1964; Jarvis and Slatyer, 1966) or leaf segments of long narrow leaves such as the needles of
conifers (Jarvis and Jarvis, 1963) or the blades of grasses (Rychnovska and Bartos, 1962).

A major shortcoming of RWC or WSD as measurement of plant water status is that they are rather insensitive indicators when water deficits are not severe (Hsiao, 1973). Slavik (1963b) also has shown that leaf water content may vary markedly within the area of one leaf blade. Data such as this have led other workers (Kramer and Brix, 1965; Weatherley, 1965) to conclude that whole organ or plant water content does not provide a satisfactory measurement of water stress.

Because size of plant organs is a function of water content (Anderson and Kerr, 1943; Kozlowski, 1964), several indirect methods of estimating water content have been used (Barrs, 1968). These include leaf thickness (Gardner and Ehlig, 1965; Meidner, 1952), stem and trunk diameters (Kozlowski and Winget, 1964; Kramer and Kozlowski, 1960), and size determination by beta ray gauging (Yamada et al., 1961; Mederski, 1961; 1964).

b. Water potential The physiological response of living cells to water stress is closely related to the free energy of the water in the cells (Gates, 1964; Papendick and Campbell, 1981). Water potential is a fundamental concept widely accepted for quantifying the energy state of water in soil, plants, and microorganisms (Hsiao, 1973). Water potential is the difference between the chemical poten-
tial of the water in a particular system and that of pure free water at the same temperature, divided by the partial molal volume of water (Slatyer, 1967). Since pure free water is usually assigned zero water potential, the potential energy of water in these systems is almost always negative. Water flow is spontaneous from high to low potentials and the availability of water for physiological processes decreases as the potential is lowered (Papendick and Campbell, 1981). According to Kramer (1969), "the degree of water stress (in plants) is probably best expressed in terms of water potential, because this appears to be the most closely related to the physiological and biochemical processes which control growth".

The components of plant water potential are osmotic potential arising from the presence of solutes, especially in the vacuole; turgor pressure, the hydrostatic pressure developed in the cell in response to osmotic pressure and the tensile strength of the cell wall; and the matric potential arising from the imbibitional forces of colloids in the cell and forces of capillarity in the cell wall (Slatyer and Taylor, 1960). All components of plant water potential are normally negative except pressure potential (Barrs, 1968).

Matric potential in many species does not become significant numerically until much of the tissue water
(.g., 50%) is lost (Boyer, 1967; Wiebe, 1966). So unless the tissue is badly dehydrated, the components of water potential of concern are pressure and osmotic potentials (Hsiao, 1973). As tissue water decreases, changes in total water potential, pressure potential, and osmotic potential are described by the classical Hofler diagram (Salisbury and Ross, 1978). Water potential is related to RWC of the same tissue, the moisture release curve, though the relationship is dependent on species and stages of growth (Connor and Tunstall, 1968; Millar et al., 1968; Gardner and Ehlig, 1965; Knipling, 1967; Sanchez-Diaz and Kramer, 1972).

Mild stress is considered to represent a lowering of plant water potential by a few bars or a lowering of RWC of 8 to 10 percentage points below values in well-watered plants under mild evaporative demand. Moderate stress represents a lowering of water potential by more than a few bars but less than 12 to 15 bars, or a lowering of RWC by more than 10 but less than 20 percentage points. Stress would be considered severe if water potential is lowered more than 15 bars, or RWC more than 20 percentage points (Hsiao, 1973). It is important to note that water stress is not restricted to times of drought. Even in well-watered soils, regular diurnal development of internal water stresses in plants is shown by afternoon decreases in RWC and water potential of plant tissues (Kozlowski, 1968c).
Scholander et al. (1965) noted that xylem streams are usually under stress throughout the major growth period of the plant.

Numerous methods are available for measuring water potential (Black et al., 1965; Brown and Van Haveren, 1972; Barrs, 1968; Slavik, 1974). The techniques are based on the isopiestic principle which dictates that if a sample has the same chemical potential as a surrounding medium, no net change of water will take place (Barrs, 1968). Techniques based on this principle can be divided into two groups: liquid phase and vapor phase.

Liquid phase methods involve immersing parallel samples in a range of concentrations of a non-penetrating solute in order to find the solution in which no net exchange of water occurs between sample and solution (Barrs, 1968). This can be detected by measuring properties of the samples such as the size of cells or cell groups (Ernest, 1931), length or thickness of tissue strips (Crafts et al., 1949), or cylinders (Lyon, 1936; 1940), weight (Ashby and Wolf, 1947), or volume (Currier, 1943) of size standardized samples. Sample water potential has also been determined by measuring properties of the solution before and after sample immersion. These solution properties include density (Kozlowski, 1964), refractive index (Ashby and Wolf, 1947), and volume (Van Overbeek, 1942). After immersion of samples, solutions which give no change
in preceding sample or solution properties are said to be isopiestic with the tissue sample, and the osmotic potential of the isopiestic solution is assumed to be equal to the water potential of the sample (Barrs, 1968). Errors may arise in liquid phase isopiestic water potential determination by injection of intercellular spaces (Ashby and Wolf, 1947), infiltration of plasmolyzed cells (Slatyer, 1958), and solute penetration (Slatyer, 1966).

Vapor phase isopiestic techniques are based on the fact that relative vapor pressure above a solution at temperature equilibrium in a closed container becomes equal to the solution's osmotic potential (Slavik, 1974; Barrs, 1968). Samples can be exposed to a range of known water potential values without being immersed in the solutions and isopiestic solutions are found by finding the solution which gives no change in sample weight (Slatyer, 1958) or solution volume (Barrs, 1968) or weight (Thut, 1938). Because vapor phase isopiestic water potential determination does not require tissue immersion, it circumvents a major source of error inherent in liquid phase isopiestic techniques, namely, solute penetration (Slatyer, 1966).

It follows from the isopiestic principle that if the water in a sample is allowed to equilibrate with water vapor in air in a closed system, the water potential in the liquid and vapor phases will be equal (Rawlins, 1976). Water potential of a sample can be computed if one is able
to measure the vapor pressure of the closed atmosphere surrounding that sample at equilibrium (Brown and Van Haveren, 1972).

A psychrometer is an instrument with wet and dry temperature sensors for measuring the amount of moisture in the air. Thermocouple psychrometry for the measurement of water potentials depends on two thermoelectric effects: the Seebeck and Peltier effects. A thermocouple is formed when wires of two different metals are joined together. If both ends of the wires are joined together to form a closed circuit, an electrical current will flow through the wires if the two junctions are at different temperatures (Brown and Van Haveren, 1972). This current is called the Seebeck effect. The difference in voltage between the two junctions is a function of their temperature difference. By adding a small power source to the circuit, one junction will cool significantly below ambient temperature, while the other junction will become warmer. This is called the Peltier effect.

Psychrometric methods are based on measuring the temperature or rate of evaporation from a water droplet or wet surface (Rawlins, 1976). Spanner (1951) was the first to use thermocouple psychrometry to determine water potentials of plant samples. By using a thermocouple suspended in a closed chamber, Spanner (1951) used Peltier cooling to lower the temperature of the thermocouple below the
dewpoint of the atmosphere, and condensation of water resulted on the cooled junction. When the current was shut off, there was an immediate tendency for the condensed water to evaporate back into the enclosed surroundings. Evaporative cooling depresses the temperature of the thermocouple as a function of evaporation rate, which is a function of the vapor pressure which is a function of the water potential of the sample. By knowing the ambient temperature and temperature depression caused by evaporative cooling, Spanner was able to calculate the vapor pressure of the atmosphere and water potential of the sample. Although it is possible to calculate vapor pressure and hence water potential in this way (Rawlins, 1966; Peck, 1963; 1960; Scotter, 1972), in practice, instruments are calibrated empirically using salt solutions (Rawlins, 1976).

Richards and Ogata (1958) built a modified version of Spanner's design in 1958. With this version, water is placed into a loop or ring surrounding the thermocouple to keep the junction wet. That eliminated the need for Peltier cooling to form a drop of condensation and the larger drop of water on the wet loop yields a steady output for a longer time than Spanner's original psychrometer.

Sources of error in psychrometric water potential determination are heat of respiration (Barrs, 1968; Boyer, 1969), and temperature and vapor pressure gradients (Boyer,
1969; Rawlins, 1976). For these reasons, early workers using psychrometry made measurements in carefully controlled constant temperature baths (Papendick and Campbell, 1981). It has been found that water baths without temperature control, or enclosures constructed of materials having high thermal conductivities eliminates most error (Campbell and Campbell, 1974; Neuman and Thurtell, 1972). Since Spanner's psychrometer was built, many variations have appeared including the use of thermistors (Brady et al., 1951; Muller and Stolten, 1953). Thermistors are more rugged and have greater responses to temperature changes that thermocouples (Rawlins, 1976).

An important advancement of thermocouple psychrometry has been the use of dew-point depression rather than wet-bulb depression (Neumann and Thurtell, 1972). The dew-point measurement is preferable to wet-bulb because 1) the dew-point temperature measures vapor pressure rather than relative humidity or relative vapor pressure, 2) at the dew point, no net water is exchanged at the wet junction which permits measurements to be made without disturbing the vapor equilibrium in the chamber, 3) the relationship of dew-point temperature to water potential is less dependent upon the ambient temperature than is that of wet-bulb temperature, and 4) psychrometric measurements are influenced by the wetting characteristics of the
junctio, whereas the dew-point technique should be relatively independent of these factors (Rawlins, 1976).

Campbell et al. (1973 advanced the dew-point hygrometer further by utilizing electronic switching that allowed alternate cooling and measuring of the thermocouple temperature. This circuitry maintains the thermocouple junction precisely at the dew-point. It is this design that many commercial dew-point hygrometers utilize (Wescor, Inc., Logan, Utah).

Scholander et al. (1964) used a pressure technique for determination of xylem water potential of excised leafy twigs. This technique involves sealing an excised twig or leaf into a closed chamber and pressurizing it until the xylem sap just begins to exude from the cut surface of the stem or petiole. This technique has certain advantages for measuring plant water deficits. It can be used for field use and since the pressure bomb requires only a short time to obtain a reading, the water status measured is close to that in the intact plant (Tyree and Hammel, 1972). However, because it measures xylem water potential instead of total leaf water potential, relationships between these two values must be established before the pressure bomb technique can be used with meaningful data (Barrs, 1968; Boyer, 1966; Kaufmann, 1968a). In most cases, however, pressure bomb readings compare favorably with those made by thermocouple psychrometers (Boyer, 1966;
Kaufmann, 1968a). The technique provides accurate, stable estimates of xylem water potential (Schoeneweiss, 1975b) and gives the best field estimates of plant water potential (Hodges and Lorio, 1971).

3. Effects on plants
   a. Growth. Many workers have shown that plant growth is reduced as water stress increases (Gates, 1968). This has been well-established for native plants (Petrie and Wood, 1938a; 1938b; Wood, 1932) and cultivated plants such as potato (Cyciler, 1946), wheat (Lehane and Staple, 1962), barley (Williams and Shafter, 1955; Aspinall et al., 1964), rye (Williams and Shafter, 1955), sorghum (Whiteman and Wilson, 1965), flax (Tiver, 1942), sugar beets (Morton and Watson, 1948), corn (Peters, 1960; Acevedo et al., 1971), tomato (Gates 1955a; 1955b), cotton (Boyer, 1965), tobacco (Petrie and Arthur, 1943), sunflower (Boyer, 1968), soybean (Boyer, 1970), and turf-grasses (Burton et al., 1957; Doss et al., 1962; Welton and Wilson, 1931).

   The level of stress that results in reduction in growth depends on the conditions under which the plants are grown (Begg, 1980). Plants grown in growth chambers are generally more sensitive to water stress than field-grown plants (Begg, 1980). However, response and tolerance of any plant tissue to reduction in leaf water potential is complex because it involves a number of physiological and
metabolic processes. As a consequence, growth, as an integrated process, can serve as a measure of plant tolerance under stress (Blum, 1979).

Growth is the irreversible enlargement of cells (Hsiao, 1973), and involves cell division and enlargement (Salisbury and Ross, 1978). Of these two processes, cell division appears less sensitive to water stress than does cell enlargement (Taylor and Ratliff, 1969a; 1969b; Vaadia et al., 1961; Acevedo et al., 1971; Green and Cummins, 1974; Wenkert et al., 1978a). According to Hsiao (1973), cell expansion is one of the plant processes most sensitive to water stress, if not the most sensitive of all. Growth of some plants responds to changes in water potential of just fractions of a bar (Cook and Papendick, 1972; 1978). Such sensitive growth retardation responses with increasing water deficits have been cited as a favorable adaptation which increases water use efficiency (Passioura, 1972; Begg and Turner, 1976; Blum and Naveh, 1976). Growth is retarded relative to the degree of tolerance of the tissues or the ability of the whole plant system to avoid drought as moisture stress increases (Blum, 1979). Tissues from different parts of the plant differ in their sensitivity to water stress (Hsiao, 1973). Therefore, it may be useful to consider leaf growth, root growth, and growth of the apical meristem separately.
Leaf growth is known to be very sensitive to water deficits (Boyer, 1970; Hsiao et al., 1976; Acevedo et al., 1971; Begg, 1980). Since leaf growth is generally more sensitive to water stress than stomatal conduction and carbon dioxide assimilation, leaf growth can be reduced by water deficits too small to cause a reduction in stomatal aperture and photosynthesis (Acevedo et al., 1971; Boyer, 1970; Fischer and Hagan, 1965; Hsiao and Acevedo, 1974; Boyer and McPherson, 1975). In some species, leaf enlargement is so sensitive to water stress that it may be largely confined to the night (Boyer, 1968).

Low water potentials may cause the loss of existing leaf area if desiccation is prolonged and severe. The loss is caused by a hastened form of leaf senescence, which is particularly rapid in grasses (Boyer, 1976). Also in grasses, leaf rolling or folding is a common response to stress and results in a marked reduction in effective leaf area and a more vertical leaf orientation (Begg, 1980). This is frequently cited in the literature as an adaptive mechanism, which, in addition to reducing the effective leaf area and the energy load on the plant, may markedly reduce transpiration (Parker, 1968) and increase water use efficiency (Johns, 1978). Other leaf morphological changes in grasses in response to increasing water deficits include decreased leaf number (Olmsted, 1941), area (Grant, 1938;
Weaver and Himmel, 1930), and width (Grant, 1938); leaf thickness generally increases (Roberts and Lage, 1965).

It is a common observation that the root-shoot ratio of plants increases with water stress (Larcher, 1975; Begg and Turner, 1976; Turner and Kramer, 1980; Beard, 1973; Cheesman et al., 1965; Pearson, 1966; Davidson, 1969; El Nadi et al., 1969; Hoffman et al., 1971). Increased root-shoot ratios arise from a greater decrease in growth of shoots compared to the growth of roots (Beard, 1973; Turner, 1979). In some cases, however, increased root-shoot ratios arise, at least in part, to absolute increases in root growth under moderate water stress (Cheesman et al., 1965; Hsiao and Acevedo, 1974; Bennett and Doss, 1960; Clements, 1964; Doss et al., 1960).

According to Begg (1980), the effect is likely to occur at levels of water stress sufficient to reduce shoot growth but not carbon dioxide assimilation. The increase in available assimilates then permits osmotic adjustment and additional root growth.

Greater root growth may be reflected by greater root density or rooting depth. Both characteristics are important adaptations in that they allow the plant to extract more soil water and maintain higher plant water potentials (Turner, 1979). This has important significance in drought resistance because plant water stress generally decreases
as the extent of the root system increases (Donald, 1963; Bertrand, 1966; Weatherley, 1965; Wadleigh et al., 1966). One selection criterion for crop production under moisture stress is a high root-shoot ratio (Kaul, 1967). Merion Kentucky bluegrass has good drought resistance that is partially attributed to a deep root system (Funk and Engel, 1963; Hagan, 1954).

Growth of the apical meristem is extremely sensitive to water stress (Husain and Aspinall, 1970; Barlow et al., 1977). However, the apex exhibits drought survivability better than most plant tissues (Barlow et al., 1977). This is especially true for members of the Gramineae for two reasons: 1) the apex is protected from transpirational losses by its protective location within mature leaf sheaths and 2) the apex is not connected to the stem by functional xylem vessels. Consequently, the water content of the apex changes little during stress.

During periods of water stress, translocation of solutes continues (Wardlaw, 1967). Translocated organic substances are either oxidized to carbon dioxide or accumulate mainly as sugars and free amino acids (Barlow et al., 1980). Accumulation of these substances, along with potassium (Munns et al., 1979) allows the apex to osmotically adjust and maintain turgor at water potentials low enough to kill leaf tissue (Barlow et al., 1977; Munns et al., 1979).
b. Water relations  Water potential is the sum of osmotic, pressure, and matric potentials. It is well-established that plant tissue water potentials decrease as water deficits become more severe (Kaufmann, 1981). Although most researchers have disregarded matric potentials in characterizing tissue water relations, there is evidence that matric potentials become increasingly important as water deficits become severe (Boyer, 1967; Wiebe, 1966; Shepard, 1975). Although water potential gradients in the soil-plant-atmosphere continuum determine water movement (Salisbury and Ross, 1978), osmotic and turgor potentials have more relevance to physiological consequence to increased water deficits (Hsiao, 1973). To fully understand what is occurring in plants subject to water deficits, it is necessary to measure both water and osmotic potentials and to calculate turgor (Kramer, 1980).

The relationship between water, osmotic, and pressure potentials as they change with increasing water deficits is described by the classic Hofler diagram (Salisbury and Ross, 1978). As the tissue loses water from fully hydrated conditions, the most apparent consequence is a loss in turgor until plasmolysis is reached where turgor becomes zero. There have been several reports in the literature of negative turgor, however, these have been discounted in herbaceous plants because the methods used to
determine osmotic potential allowed for apoplastic dilution (Tyree, 1976; Kramer, 1980), or matric potentials were disregarded (Shepard, 1975).

c. Water loss Although the major resistance to water flow occurs in the roots (Kramer, 1969; Boyer, 1971; Wind, 1955), control of water loss from a plant is exercised mainly by regulation of stomatal aperture (Brouwer, 1965; Slatyer and Lake, 1966). Several reviews on stomatal control of water loss have been published (Fischer and Turner, 1978; Begg and Turner, 1976; Jones et al., 1982; Burrows and Miltonorpe, 1976; Raschke, 1975; Cowan, 1978; Turner, 1970; Hall et al., 1976) and will not be discussed here.

Because plant water loss is so dependent upon stomatal aperture, stomatal aperture can also reflect plant water status (Denmead and Shaw, 1962; Barrs, 1968). Stomatal aperture has been used in the field as a criterion for irrigation of crops (Alvim, 1966; Shimshi, 1964). Kramer and Brix (1965) have stated that stomatal aperture does not provide a quantitative basis for comparing water deficits in different kinds of plants because stomatal aperture is affected by many other environmental factors. These factors include light flux density (Turner, 1970; 1975; McCree, 1974; Kanemasu and Tanner, 1969b), leaf water status (Troughton, 1969; Wilson, 1975; Kanemasu and Tanner,
1969a; Biscoe, 1972), humidity (Meidner and Mansfield, 1968; Slavik, 1973), temperature (Goltz and Tanner, 1972; Gifford and Musgrave, 1973; Moss, 1963; Drake et al., 1970), wind (Davies et al., 1974), mineral nutrient supply (Allaway and Milthorpe, 1976), carbon dioxide concentration (Burrows and Milthorpe, 1976), and other gases such as oxygen (Heath and Orchard, 1956; Zelitch, 1965), and various air pollutants (Biscoe et al., 1973; Duggar and Ting, 1970). However, according to Barrs (1968), if the researcher restricts the study to one kind of plant, stomatal aperture can be used to give comparative estimates of water deficits.

Most data clearly demonstrate a threshold level of water potential or RWC above which leaf conductance or resistance remains constant (Cowan and Milthorpe, 1968; Ehlig and Gardner, 1964; Hsiao, 1973). When leaf water potentials or RWC fall below a critical value, this leads to a series of partial stomatal closures and reopenings during the day (Ludlow, 1980). The overall effect is a decline in stomatal conductance in a linear or curvilinear manner until it approaches zero or cuticular conductance (Wein et al., 1979). This indicates that stomata are rather insensitive to mild water stress (Hsiao, 1973). In some species, however, stomata are sensitive to mild water stress showing closure at relatively high water poten-
tials and no plateau in stomatal response to increasing water stress (El-Sharkawy and Hesketh, 1964; Boyer, 1970; Fischer et al., 1970; Millar et al., 1971; Raschke, 1975; Stalfelt, 1955; 1961).

Methods for measuring stomatal apertures and their limitations have been discussed by several authors (Slatyer, 1967; 1958; Barrs, 1968; Kramer, 1969). There are four main techniques to ascertain stomatal aperture: epidermal peeling and fixing (Lloyd, 1908; Gale and Poljakoff-Mayber, 1965; MacDowall, 1963), surface impressions (Sampson, 1961; Zelitch, 1961; Macklon and Weatherley, 1965), infiltration (Heath, 1959; Dale, 1958; Parker, 1963), and porometry (Alvim, 1966; Wallihan, 1964). Of these techniques, porometry is the most widely used for its advantage in expediency and no damage to the leaf being tested (Barrs, 1968).

There are basically two types of porometers: pressure-drop viscous flow type (Alvim, 1966) and diffusional type (Wallihan, 1964). Diffusional type porometers are most popular because they can estimate stomatal aperture on either side of the leaf and are equally effective with leaves having stomata on one or both sides (Barrs, 1968). Diffusion porometers are of two designs: transient and steady state. Transient diffusion porometers depend on measuring the rate of change of water vapor in a small
chamber attached temporarily to the leaf (Kanemasu et al., 1969; Van Bavel et al., 1965). The air in the chamber is dried and the time taken for the humidity to change over a set range, the transit time, is recorded (Monteith and Bull, 1970). The diffusion resistance of the sample is determined from a calibration curve relating transit time to known resistance obtained using calibration plates (Monteith and Bull, 1970; Kanemasu et al., 1969). In a steady state diffusion porometer, water loss from a leaf is determined by maintaining a constant vapor density in a cuvette that is in contact with the transpiring leaf (Bingham and Coyne, 1977). This is achieved by pumping dry air into the cuvette at an appropriate measured rate to obtain a balance (at a predetermined humidity) between the flux of water transpired by the leaf and the moist air out of the cuvette (Beardsell et al., 1972).

Expanding leaves are often accompanied by changing stomatal behavior reaching their highest values for stomatal conductance when the leaves are about 75% of their full size (Burrows and Milthorpe, 1976). Also, there is a rapid increase in the maximum stomatal resistance in the later stages of leaf senescence (Burrows and Milthorpe, 1976). Because of this, sampling precautions for porometer use include establishing a standard position on the leaf (Alvim, 1966), and using leaves of
similar age and position (Barrs, 1968). In addition, readings should be carried out as quickly as possible with minimal handling and utilizing a large number of samples to overcome variation (Barrs, 1968). Stomatal resistances are derived from the difference between the water lost in light and darkness, because it is assumed that stomatal close in the dark and that the cuticular resistance is not affected by illumination (Ayres, 1978).

Transpiration is the sum of stomatal and non-stomatal water loss (Crafts, 1968). However, stomatal closure is the main cause for transpiration decline as water stress develops (Hsiao, 1973) and, therefore, control of stomatal aperture is very important in drought avoidance (Townley-Smith and Hurd, 1979). Plants show a range of stomatal sensitivity to water deficits (Turner, 1979), as shown by plotting leaf diffusive resistance or conductance against leaf water potential. The absolute values of leaf water potential that induce stomatal closure depend on many factors including leaf position on the plant (Turner, 1974; Millar and Denmead, 1976), leaf age (Frank et al., 1973), growth conditions (Jordan and Ritchie, 1971; Beadle et al., 1973), and stress history of the plant (Stocker, 1960; McCree, 1974; Brown et al., 1976; Turner et al., 1978). These factors help to explain the observation that under field conditions, stomata of
several plant species remain open at lower leaf water potentials than those found to induce closure under controlled environment conditions (Davies, 1977; Jordan and Ritchie, 1971). Once threshold water status for stomatal closure is reached, leaf diffusive resistance increases with further drops in leaf water potential (Jordan and Ritchie, 1971; Kanemasu and Tanner, 1969a) or relative water content (Duniway and Durbin, 1971b; Troughton, 1969).

Stomates close when bulk leaf turgor pressure is near zero (Turner, 1974; Turner and Jones, 1980). However, changes in stomatal aperture involve changes in the turgor relations of the guard cells and subsidiary cells (Turner, 1974; Meidner and Edwards, 1975), and several researchers have demonstrated only a loose relationship between bulk leaf turgor and turgor of the guard cells (Turner et al., 1978; Beadle et al., 1978; Turner, 1975; Jones and Rawson, 1979; Brown et al., 1976; Simmelsgaard, 1976). It follows then that if the plant is able to osmotically adjust to increasing water stress and thereby maintain bulk leaf turgor as leaf water potentials decrease, osmotic adjustment can provide an explanation for stomatal adjustment in response to water stress (Turner, 1979). This prompted Kaufmann (1981) to reason that an assessment of drought avoidance capabilities of a plant must include more than a study of the depth and
extent of its root systems and the effectiveness of the cuticle and stomata in restricting water loss. Osmotic adjustment should be evaluated as well. Factors that affect stomatal adjustment include plant maturity and leaf age (Frank et al., 1973; Morgan, 1977a), rate of stress development (Jones and Rawson, 1979), and genotype (Blum, 1979; Henzell et al., 1976; Ackerson et al., 1980).

Even if the stomatal apparatus is sensitive to water deficits and the leaf water potential at which stomata close is high, full benefit is gained only if the cuticular resistance is also high and stomatal closure is complete (Turner, 1979). The cuticle of leaves of many crop plants are appreciably permeable, and cuticular transpiration continues after stomatal closure has occurred (Szabo and Buchholtz, 1961; Crafts and Foy, 1962; Darlington and Circulis, 1963; Yamada et al., 1964; Bukovac and Norris, 1967). Conversely, it is well-established that when prolonged water deficits prevail, there is an increase in the epidermal waxes of leaves which can decrease cuticular transpiration (Skoss, 1955; Blum, 1975).

When considering sites for resistance to transpiration, possibilities have been raised that non-stomatal factors other than cuticular resistance also play a role in cur-tailing plant water loss (Crafts, 1968). The terms "mesophyll" or "wall resistance" have been used to describe
this type of diffusion resistance. However, it is generally agreed that stomatal resistance is by far the greatest resistance to water loss (Hsiao, 1973), and mesophyll or wall resistance is negligible until water deficits become quite severe (Fischer, 1968; Slatyer, 1967).

Expressing dry matter or crop yield as a function of water use has proven to be a useful selection criterion for drought tolerant germplasm (Townley-Smith and Hurd, 1979). This is called water use efficiency, and is a direct measure of the plant's water economy. Various strategies for maximizing water use efficiency have been discussed (Stone, 1975; Pierre et al., 1966).

d. Osmotic adjustment and cell wall elasticity

Lawlor (1979) states that although the terms water potential has been commonly associated with water stress effects on metabolism, the controlling factors may not be tissue water potential per se, but rather osmotic or turgor potential. Turgor pressure is the driving force for cell expansion (Hsiao, 1973). Therefore, in order for growth and turgor-mediated processes such as stomatal conductance to be maintained as water deficits increase and plant water potentials decrease, the plant must be able to lower its osmotic potential to maintain turgor (Turner and Jones, 1980).

A lowering of osmotic potential in response to water
deficits can arise from the concentration of osmotic solutes as water is withdrawn from the vacuole and the cell volume decreases, or additionally, from the accumulation of solutes in the cell (Turner and Jones, 1980). Osmotic adjustment refers to the lowering of osmotic potential arising from the net accumulation of solutes in response to water deficits. By actively accumulating organic and inorganic solutes, plants lower their osmotic potentials more than can be attributed to solute concentration due to a decrease in cell volume (Hsiao et al., 1976; Turner and Jones, 1980). Organic compounds that are utilized for osmotic adjustment include sugars, sugar alcohols, free amino acids (especially proline), and carboxylic acids, while inorganic ions include potassium, sodium, and chloride (Borowitzka, 1981).

Earlier literature has demonstrated the ability of plants growing in saline environments to accumulate inorganic ions and thus maintain turgor pressure by lowering their osmotic potentials (Berstein, 1961; Meiri and Poljakoff-Mayber, 1969). There are now several reports in the literature indicating that leaves of higher plants lower their osmotic potentials in response to slowly developing water deficits (Hsiao et al., 1976; Morgan, 1977b; Fereres et al., 1978; Jones and Turner, 1978; Turner et al., 1978; Jones and Rawson, 1979). In addition,
turgor maintenance by osmotic adjustment has been shown to occur in response to the daily changes in water status of leaf tissue (Turner, 1975; Wenkert et al., 1978b).

The degree of osmotic adjustment is measured as the change in osmotic potential at a particular water potential or water content, usually full or zero turgor (Turner and Jones, 1980). However, the degree of osmotic adjustment is limited and depends on the rate of water stress development (Turner and Jones, 1980; Hsiao et al., 1976; Turner et al., 1978; Jones and Turner, 1978) and the plant organ in question (Turner and Jones, 1980). Osmotic adjustment takes place in leaves, hypocotyls, roots, and reproductive organs of several plant species (Turner and Jones, 1980). A greater degree of osmotic adjustment is found in apices compared to expanded leaves.

Several species have been shown to osmotically adjust in response to water deficits. They include sorghum (Fereres et al., 1978), corn (Fereres et al., 1978), sunflower (Jones and Turner, 1980), soybean hypocotyls (Meyer and Boyer, 1972), pea (Greacen and Oh, 1972), wheat (Morgan, 1977b), and rice (Cutler et al., 1980). From these studies, it has become clear that considerable differences exist between species in their capacity for osmotic adjustment. However, even though osmotic adjustment is an important mechanism in the drought tolerance of plants
(Turner, 1979; Jones et al., 1981), not all species or cultivars show evidence of osmotic adjustment (Turner and Jones, 1980).

The pressure-volume technique, originally introduced by Scholander et al., (1964), has been frequently used to measure water, solute, and turgor potentials of a wide variety of tissues (Tyree and Hammel, 1972; Goode and Higgs, 1973; Boyer, 1969; Hellkvist et al., 1974; Richter, 1978; Wenkert et al., 1978b. Pressure-volume curves are plotted as the inverse of the applied pressure (leaf water potential as determined with a pressure bomb) versus the cumulative volume or weight of the expressed sap or residual weight of the tissue (Melkonian et al., 1982). Estimates of osmotic potential are made from the linear region of the curve which is described by the Boyle-Van't Hoff relation, i.e., volume expressed varies linearly with the inverse of the applied pressure (Tyree and Hammel, 1972).

Data can also be plotted as water potential versus relative water content of the tissue (Brown and Tanner, 1983). Plots of such data represent moisture release curves (Jarvis and Jarvis, 1963). Important parameters affecting drought resistance can be derived from water release curves including relative water content at zero turgor, and osmotic potential at full and zero turgor
In tissue with a high osmotic potential, the osmotic potential decreases with tissue dehydration and solute concentration, and the turgor pressure reaches zero at a relative water content of about 92%. In tissue with a low osmotic potential due to osmotic adjustment, the turgor pressure is much higher at all relative water contents and zero turgor is not reached until the relative water content is about 80% (Turner, 1979). Numerous studies indicate that species differences exist, both in the moisture release curve and in relative water content at zero turgor (Turner and Jones, 1980). Age has an effect on this relationship. As the tissue matures, the relative water content becomes higher at given leaf water potentials or at zero turgor (Kassam and Elston, 1974; Knipling, 1967).

The moisture release curve depends not only on leaf osmotic potential, but also on cell wall elasticity (Jones and Turner, 1978; Jones et al., 1981; Weatherley, 1970; Turner, 1974; 1979). Therefore, estimates of cell wall elasticity are important because elasticity influences the relation between water content and water potential (Dainty, 1972; Cheung et al., 1975; 1976; Zimmermann, 1978). Cell wall elasticity is related to cell wall thickness (Turner, 1979; Turner and Jones, 1980). Comparative estimates of cell wall thickness have been made using
turgid weight/dry weight ratios of leaves (Wilson et al., 1980).

Cell wall elasticity has been characterized by volumetric elastic modulus (Jones and Turner, 1980; Gardner and Ehlig, 1965). Estimates of the elastic modulus can be derived from the curvilinear region of the pressure-volume curve where positive turgor is maintained (Melkonian et al., 1982; Tyree and Hammel, 1972; Cutler et al., 1980), or from the relationship between leaf turgor potential and relative water content (Campbell et al., 1979; Melkonian et al., 1982). Although some workers have suggested that dependence of the modulus of elasticity on turgor pressure is inferred from such pressure-volume relations (Hellkvist et al., 1974; Cutler et al., 1980; Steudle et al., 1977; Jones and Turner, 1980), others have shown if estimates are derived from data from a pressure-volume curve of high turgor, then the modulus of elasticity does not depend on turgor or volume (Melkonian et al., 1982; Campbell et al., 1979).

The effect of water deficits on cell wall elasticity are unclear. While water deficits reportedly increase cell wall elasticity in Vicia faba (Kassam and Elston, 1974; Elston et al., 1976), decreases in cell wall elasticity were reported in drought-conditioned sorghum (Jones and Turner, 1978; Jones et al., 1981). No signif-
icant changes in elasticity as a result of drought conditioning was observed in either cotton (Hsiao et al., 1978), rice (Cutler et al., 1980), sunflower (Jones and Turner, 1980), or wheat (Campbell et al., 1979).

B. Disease

1. Effects on plants
   a. Growth  It is axiomatic that pathogens affect normal growth and function of their hosts (Wood, 1953). Pathogens may affect growth of their hosts in several ways. Through alterations of their host's hormone metabolism (Sequiera, 1973), various morphological and developmental changes become evident as a result of infection. Growth of galls, tumors, and nodules are a direct result of pathogen infection (Agrios, 1969). In addition, certain pathogens can induce dwarfing of their hosts (Bewley, 1922; Talboys et al., 1961).

   One pathogen-induced growth response of special interest when investigating the combined effects of infection and water stress is that of reduced root growth. Several pathogens have been shown to reduce root growth of their hosts (Johnston and Miller, 1934; 1940; Duniway and Durbin, 1971a; Amatya and Jones, 1966; Van der Wal and Cowan, 1974; Van der Wal et al., 1975; Gerwitz and Durbin,
1965). Hodges (1977) has shown that infection by *Ustilago striiformis* and, to a greater degree, *Urocystis agropyri* reduce root growth in *Poa pratensis* 'Merion'. Root growth reduction is more pronounced than the reduction in shoot growth (Hodges, 1977). The result is that infected plants exhibit lower root-shoot ratios.

Root-shoot ratios have been used as a selection criterion in breeding programs to improve drought resistance of several crops including wheat (Townley-Smith and Hurd, 1979) and sorghum (Blum, 1979). Plants with higher root-shoot ratios most often are able to maintain higher water status during periods of drought than plants with low root-shoot ratios. Pathogen-induced reductions in root-shoot ratios would likely predispose infected plants to desiccation during periods of limited water supply or high water loss. Hodges (1977) has shown this to be true for *Poa pratensis* 'Merion' infected with *U. striiformis* and *U. agropyri*. Infected plants exhibited greater mortality when irrigation was withheld compared to non-infected, healthy plants.

b. Water economy Plant pathogens have been shown to adversely affect the water economy of their hosts (Ayres, 1978; Talboys, 1968), which can lead to disease-induced water deficits reflected by lower leaf water potentials (Duniway and Durbin, 1971b; Ayres, 1977;
Disease-induced water deficits occur because of decreased stomatal resistance (Duniway and Durbin, 1971a, Ayres, 1976), decreased cuticular resistance (Sempio et al., 1966; Amatya and Jones, 1966; Gerwitz and Durbin, 1965), reduced hydraulic conductivity (Dimond, 1970; Talboys, 1968), and reduced water uptake due to pathogen-induced root growth inhibition (Murphy, 1935; Hodges, 1969; 1970a).

Duniway and Durbin (1971b) found that bean rust totally inhibited the movement of stomata. Other pathogens have been shown to exhibit similar inhibition of stomatal function in potatoes (Farrell et al., 1969), tomato (Rubin and Artsikhovskaya, 1963), barley (Ayres, 1972; Ayres and Jones, 1975), and tobacco (Cruickshank and Rider, 1961). In addition to stomatal inhibition, the pathogen may affect the cuticular resistance of diseased plants by invasion through host epidermis, or rupture of the epidermis to sporulate (Ayres, 1978). This is true for the rust diseases where transpiration is often reduced early in the infection cycle, but increases after the pathogen ruptures the epidermis to release its spores. Rust diseases also reduce leaf area development and dry matter accumulation (Ayres, 1978). This reduction in growth often causes the total amount of water taken up or lost by rusted plants to be less than
that taken up or lost by healthy plants (Johnston and Miller, 1934; 1940; Murphy, 1935; Bever, 1937). Although smut fungi are closely related to the rust, little is known about their effects on host water economy except that they increase transpiration (Ayres, 1978).

Transpiration stream interruption can lead to water deficits in aerial parts of the plant. Various vascular pathogens are known to lower the hydraulic conductivity of their hosts by the production of toxins or gums. Infection also stimulates the hosts to produce occlusions and tyloses (Ayres, 1978). Transpiration stream interruptions may lead to symptom development on leaves which can parallel a drought syndrome (Talboys, 1968).

It is well-documented that many pathogens inhibit root growth of their hosts (Ayres, 1978; Talboys, 1968; Gerwitz and Durbin, 1965; Duniway and Durbin, 1971a, Johnston and Miller, 1934; 1940). Hodges (1970a) has demonstrated decreased root growth of *P. pratensis* infected with *U. striiformis* or *U. agropyri* which contributed to increased mortality of infected plants during periods of water stress compared to healthy control plants.

2. Leaf smuts

*Urocystis agropyri* (flag smut) and *Ustilago striiformis* (stripe smut) are pathogens of a number of grass species including *Poa pratensis* (Kentucky bluegrass)
"Merion" Kentucky bluegrass is one of the most widely planted turfgrasses in the United States, and is very susceptible to both smut organisms (Gaskin, 1965; Kreitlow and Juska, 1959). Primary infection sites include coleoptiles on seedlings and axillary buds on crowns and rhizome nodes of mature plants (Hodges, 1976). Mycelia systemically infects leaf, sheath, and floral parts of the plant (Couch, 1971), and becomes perennially established for the life of the host (Hodges and Britton, 1970). The disease spreads in established grasses by systemic growth within tillers and rhizomes produced by infected plants, and by new primary infections of axillary buds (Hodges and Britton, 1969; 1970).

Invading fungal hyphae cause striking morphological changes in the host (Hodges, 1970a). Infection by $U.\text{agropyri}$ results in overall stunting, reduced root growth, and increased intravaginal branching (Hodges, 1970a). Infection by $U.\text{striiformis}$ has been reported to cause an increase in plant height, increased lamina width, wider lamina to sheath angles, as well as a striping symptom on leaf sheaths and laminas (Hodges, 1970c). Both pathogens produce sori in leaf mesophyll that may extend the entire length of the leaf (Davies, 1922; Kreitlow, 1948; Thirumalchar and Dickson, 1953). As sori mature, sporulation results in epidermal rupturing as teliospores
are released. Epidermal rupturing results in loss of non-stomatal control of water loss and plants become more susceptible to periods of water stress. Hodges (1977) has demonstrated increased mortality rates of *P. pratensis* infected by *U. striiformis* or *U. agropyri* when irrigation was withheld compared to healthy plants. Other researchers have established that infected plants are readily killed by high temperatures and drought (Fischer, 1940; Kreitlow, 1943; Leach et al., 1946).
III. GROWTH OF WATER-STRESSED POA PRATENSIS INFECTED BY UROCYSTIS AGROPYRI OR USTILAGO STRIIFORMIS

A. Abstract

Growth of healthy Poa pratensis 'Merion' or systemically infected by Ustilago striiformis (stripe smut) or Urocystis agropyri (flag smut) was evaluated for total, leaf, and root weights, and root-shoot ratios in nutrient solution and in nutrient solution amended with polyethylene glycol to provide increasing levels of osmotically-induced water stress. Infection by U. striiformis stimulated total and leaf weight, but decreased root weight and root-shoot ratios of P. pratensis grown in nutrient solution. P. pratensis infected by U. agropyri exhibited decreased total, leaf, and root weights and root-shoot ratios.

Total, leaf, and root weights of all three health groups decreased, and root-shoot ratios increased with decreasing osmotic potentials of nutrient solutions. The increases in total and leaf weights of U. striiformis-infected plants compared to healthy plants grown in nutrient solution were lost as nutrient solution osmotic potentials decreased. Healthy plants maintained higher root-shoot ratios than infected plants at all stress levels.
B. Introduction

*Urocystis agropyri* (flag smut) and *Ustilago striiformis* (stripe smut) are pathogens of a number of grass species including *Poa pratensis* (Kentucky bluegrass) (Couch, 1971). 'Merion' Kentucky bluegrass is very susceptible to both pathogens (Gaskin, 1965; Kreitlow and Juska, 1959). Primary infection sites include coleoptiles on seedlings and axillary buds on crown and rhizome nodes of established plants (Hodges, 1976). Mycelium systemically infects leaf, sheath, and floral parts of the plant (Couch, 1971), and becomes perennially established for the life of the host (Hodges and Britton, 1970). The disease spreads in established grasses by systemic growth within tillers and rhizomes produced by infected plants, and by new primary infections of axillary buds (Hodges and Britton, 1969; 1970).

Leaf-smutted *P. pratensis* has higher mortality rates than healthy plants when subjected to water stress (Hodges, 1977). This suggests that the imposition of water stress on leaf-smutted plants enhances the biotic stress induced by the pathogen and increases the mortality rate of diseased plants. It has been proposed that the process of growth may be the most sensitive of all plant processes to water stress (Hsiao, 1973), and that growth, as an
integrated process, can serve as a measure of plant
tolerance under stress (Blum, 1979). This study was
initiated to investigate the effects of progressively
lower nutrient solution osmotic potentials on the growth
of *P. pratensis* 'Merion' systemically infected by *U.
striiformis* or *U. agropyri*.

C. Materials and Methods

A single clone of healthy, *U. striiformis*- or *U.
agropyri*-infected *Poa pratensis* 'Merion' was vegetatively-
propagated from individual shoots and grown in a loam:
peat:perlite (1:1:1) mix under greenhouse conditions.
Individual shoots selected for experiments were removed
from the soil mix, roots were washed, and all but the two
youngest fully-expanded leaves were removed from the
shoot. Individual shoots were placed in specially designed
nutrient culture jars (Fig. 3.1) to provide continuous
aeration of the nutrient solution (Table 3.1). Nutrient
solution was similar to that used by Pellet and Roberts
(1963) except higher rates of nitrogen were used because
Hull et al. (1979) have shown that it favors symptom
development. Experiments were initiated to determine
the effect of infection on growth in nutrient solution
per se, and in nutrient solution with progressively lower
Figure 3.1. Nutrient culture jar used to grow *Poa pratensis*
Table 3.1. List of nutrients, their sources and concentrations used in nutrient solution

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Source</th>
<th>Concentration (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>Ca(NO\textsubscript{3})\textsubscript{2} \cdot 4H\textsubscript{2}O</td>
<td>156 \textsuperscript{a}</td>
</tr>
<tr>
<td></td>
<td>(NH\textsubscript{4})\textsubscript{2}SO\textsubscript{4}</td>
<td></td>
</tr>
<tr>
<td>P</td>
<td>H\textsubscript{3}PO\textsubscript{4}</td>
<td>25</td>
</tr>
<tr>
<td>K</td>
<td>KOH</td>
<td>50 \textsuperscript{a}</td>
</tr>
<tr>
<td>Mg</td>
<td>MgSO\textsubscript{4} \cdot 7H\textsubscript{2}O</td>
<td>19</td>
</tr>
<tr>
<td>Fe</td>
<td>Fe\textsubscript{2}(SO\textsubscript{4})\textsubscript{3}</td>
<td>1.2</td>
</tr>
<tr>
<td>Mn</td>
<td>MnSO\textsubscript{4} \cdot H\textsubscript{2}O</td>
<td>0.25</td>
</tr>
<tr>
<td>B</td>
<td>H\textsubscript{3}BO\textsubscript{3}</td>
<td>0.1</td>
</tr>
<tr>
<td>Zn</td>
<td>ZnSO\textsubscript{4} \cdot 7H\textsubscript{2}O</td>
<td>0.1</td>
</tr>
<tr>
<td>Cu</td>
<td>CuSO\textsubscript{4} \cdot 5H\textsubscript{2}O</td>
<td>0.01</td>
</tr>
<tr>
<td>Mo</td>
<td>MoO\textsubscript{3} \textsuperscript{a}</td>
<td>0.01</td>
</tr>
</tbody>
</table>

\textsuperscript{a}Different from Pellet and Roberts (1963).

Osmotic potentials induced by polyethylene glycol. Healthy (non-infected) control plants were grown under the same conditions.

1. Nutrient solution studies

Seventy-five two-leaf plants were selected for size uniformity from each health groups (i.e., stripe-smutted, flag-smutted, and healthy) for evaluation of
the effect of infection on growth of *P. pratensis* in nutrient solution. Plants were randomized in the growth chamber using a randomized complete block design utilizing 3 blocks and 3 treatments (health groups). Growth chamber conditions included 12-hour photoperiods, 50% relative humidity, 65 (± 5) nE cm⁻² sec⁻¹ light intensity, 23 °C (± 2) constant temperature. Plants were grown in nutrient solution for 4 weeks and the solution was flushed twice per week. Fifteen plants from each treatment (5 from each block) were sampled for total dry weight (dried at 60 °C, 48 hours), leaf dry weight, and root dry weight at 0, 1, 2, 3, and 4 weeks and presented as weekly means for each health group. Root-shoot ratios were calculated from dry weights. Means were bracketed by their standard errors.

2. Water stress studies

Plants evaluated for growth in response to infection and progressively lower osmotic potentials of nutrient solution were preconditioned by growing them in nutrient solution alone for 10 days before applying water stress treatments. Plants were then placed in nutrient solution containing polyethylene glycol (PEG₈₀₀₀) at concentrations of 0, 168, 242, and 292 g/L. These concentrations provided initial osmotic potentials of -0.2, -0.4, -0.6, and -0.8 MPa as measured by a Wescor.
HR-33T dewpoint microvoltmeter using calibrated C-52 sample chambers and the dewpoint mode. Nutrient solutions were flushed twice per week and growth chamber conditions were as previously stated. Plants were grown for 4 weeks under applied water stress after which 12 plants from each treatment combination were sampled for total, leaf, and root dry weights. Root-shoot ratios were calculated. The experiment utilized a 3 by 4 factorial in a randomized complete block design. Treatment factors included 3 health groups (healthy, U. striiformis-, and U. agropyri-infected) and 4 levels of osmotically-induced water stress. Data were presented as means of healthy, U. striiformis-, and U. agropyri-infected plants for total, leaf, and root weight, and root-shoot ratios for -0.2, -0.4, -0.6, and -0.8 MPa initial nutrient solution osmotic potentials. Means were bracketed by their standard errors.

D. Results

1. Nutrient solution effects

*Poa pratensis* infected by *U. striiformis* and grown in nutrient solution increased in total and leaf dry weight, and decreased in root growth and root-shoot ratios compared to healthy plants (Fig. 3.2). Plants
Figure 3.2. Total (A), leaf (B), and root (C) dry weights and root-shoot ratios (D) of healthy (H), *Ustilago striiformis*-infected (stripe smut) (S), and *Urocystis agropyri*-infected (flag smut) (F) *Poa pratensis 'Merion'* shoots grown four weeks in nutrient solution.
infected by *U. agropyri* decreased in total, leaf, and root weight, and in root-shoot ratios compared to healthy plants (Fig. 3.3A-D). No differences were found, however, between root-shoot ratios between flag-smutted and stripe-smutted plants (Fig. 3.2D).

2. Water stress effects

Total, leaf, and root dry weights of all three health groups decreased with decreasing osmotic potentials of nutrient solution (Fig. 3.3A-D). Root-shoot ratios increased as the osmotic potential of the nutrient solution decreased for all health groups (Fig. 3.3D). Total and leaf weight of stripe-smutted plants increased compared to healthy plants in -0.2 MPa nutrient solution, and then decreased as osmotic potential of the nutrient solution decreased (Fig. 3.3A-C). Root dry weights and root-shoot ratios of infected plants were less than that of healthy plants at all nutrient solution osmotic potentials (Fig. 3.3C and 3.3D).

E. Discussion

Systemic infection of 'Merion' *P. pratensis* by *U. striiformis* increased total and leaf dry weight of plants grown in nutrient solution without polyethylene glycol compared to healthy plants, but root growth
Figure 3.3. Total (A), leaf (B), and root (C) dry weights and root-shoot ratios (D) of healthy (H), *Ustilago striiformis*-infected (stripe smut) (S), and *Urocystis agropyri*-infected (flag smut) (F) *Poa pratensis* 'Merion' shoots after four weeks in PEG8000-amended nutrient solution.
is inhibited. Infection by *U. agropyri* inhibits all growth of the central crown. Related morphological changes in *P. pratensis* associated with these pathogens include reduced extravaginal branching and inflorescence production, and increased intravaginal branching and lamina to sheath angles (Hodges, 1969; 1970a). These observations are suggestive of metabolic imbalances induced by the pathogens.

The increase in total and leaf weight of *U. striiformis*-infected plants compared to healthy plants at -0.2 MPa may be related to hormonal imbalances (Sequeira, 1973). Both pathogens decrease the total sugar and amino acid content of infected plants (Hodges and Robinson, 1977; Madsen et al., 1983), but these effects cannot explain the increase in total and dry leaf weights under minimal stress. The decrease in all growth parameters of *U. agropyri*-infected plants, however, may be due to the severe decrease in sugars and amino acids alone. Pathogen-induced dwarfing of hosts has been observed in other plant diseases (Bewley, 1922; Talboys et al., 1961). Decreased root growth of infected plants may be in part due to hormone imbalances that affect assimilate partitioning (Zeevart, 1979), however, one cannot rule out that root growth inhibition is due to assimilate depletion, per se.
The decrease in leaf weight in response to increasing water deficits was disproportionately greater than that of the roots and is in agreement with similar observations (Larcher, 1975; Begg and Turner, 1976; Turner and Begg, 1978; Beard, 1973). The resulting increase in root-shoot ratios is attributed mainly to this differential growth response (Turner, 1979; Beard, 1973), and not to an increase in root growth as has been reported in some cases (Cheesman et al., 1965; Hsiao and Acevedo, 1974; Bennett and Doss, 1969).

Infection of P. pratensis by U. striiformis or U. agropyri sharply reduces root-shoot ratios (Fig. 3.2D) and the effect is persistent as water stress is increased (Fig. 3.3D). High root-shoot ratios are indicative of greater drought resistance and are used as selection criteria for drought resistance (O'Toole and Chang, 1979). The lower root-shoot ratio suggests that drought resistance is decreased by the pathogens and supports the observation that mortality of infected plants increases without irrigation (Hodges, 1977).

If overall growth, as an integrated process, can serve as a measure of plant tolerance under stress (Blum, 1979), then U. striiformis-infected plants grown in nutrient solution can tolerate the amount of stress induced by the pathogen (Fig. 3.3A). Total weight was
not reduced by infection by *U. striiformis* in *P. pratensis* growing in nutrient solution. The detri-
mental combination of the stress imposed by the presence
of the pathogen and osmotically-induced water stress,
however, becomes quickly evident as water stress in-
creases (Fig. 3.3A). Decreases in total weight as a
response to increasing water stress is more severe
in infected plants and may partially explain the ob-
servation that infection by either *U. agropyri* or *U.
striiformis* results in growth reduction of field-grown
grasses (Couch, 1971). This suggests that the pathogens
have decreased the stress tolerance of their hosts,
which when water deficits increase may lead to increased
rates of mortality of leaf-smutted *Poa pratensis* demon-
strated by Hodges (1977).
IV. EFFECT OF WATER STRESS AND SYSTEMIC INFECTION BY 
USTILAGO STRIIFORMIS OR UROCYSTIS AGROPYRI ON THE 
GROWTH OF TILLERS AND RHIZOMES OF POA PRATENSIS

A. Abstract

Water stress and infection of Poa pratensis 'Merion' by Ustilago striiformis (stripe smut) or 
or Urocystis agropyri (flag smut) were evaluated for their effects on tiller and rhizome growth. Plants were grown in nutrient solution and in nutrient solution with increasing amounts of polyethylene glycol to provide increasing levels of osmotically-induced water stress. Stripe smut had no affect on mean total dry weight of rhizomes per shoot, but the mean percentage of rhizome dry weight as a portion of total shoot dry weight decreased compared to healthy plants. Dry weight of tillers produced by stripe-smutted plants in nutrient solution increased and were a greater mean percentage of total shoot dry weight compared to healthy plants. Flag-smutted plants in nutrient solution showed decreased rhizome growth and increased tiller growth compared to healthy plants. The stimulation of tiller growth by stripe-smutted plants was negated with increasing water stress. Flag-smutted plants, however, continued
to show increased tillering at the most severe levels of water stress. Implications of altered hormonal balance in *U. agropyri*-infected plants are discussed.

B. Introduction

*Poa pratensis* (Kentucky bluegrass) vegetatively propagates itself by producing tillers and rhizomes. Rhizome and tiller production is at a maximum in spring, late summer, and fall, and is minimal during periods of environmental stresses including heat, cold, and drought (Beard, 1973).

*Ustilago striiformis* and *Urocystis agropyri* are the causal organisms of stripe and flag smut, respectively. They are serious pathogens of several grass species including *P. pratensis* (Couch, 1971). Invading fungal hyphae can infect leaf, sheath, and floral parts of the host and cause striking morphological changes in the host (Hodges, 1970a; 1969). Because of the directional growth and systemic nature of the pathogens (Hodges and Britton, 1970), tillers and rhizomes from infected plants are almost always infected as they develop from axillary buds of infected plants (Hodges and Britton, 1969). Therefore, such factors as irrigation and nitrogen fertilization that increase the survival of infected
plants and favor tiller and rhizome production can enhance the spread of the disease (Hodges, 1977; Hull et al., 1979).

Physiological control of tillering is subject to auxin and carbohydrate concentrations in the grass plant (Leopold, 1949). Infection by *U. striiformis* or *U. agropyri* may decrease the soluble sugar content in leaves and roots of *P. pratensis* (Hodges and Robinson, 1977; Madsen et al., 1983). Growth responses of *P. pratensis* infected with *U. agropyri* or *U. striiformis* also are suggestive of growth hormone imbalances (Hodges, Hodges, 1970a; 1969).

This study was initiated to determine the effect of infection by *U. striiformis* or *U. agropyri* on tiller and rhizome growth of *P. pratensis* grown in nutrient solution, and to evaluate the combined effects of systemic infection and osmotically-induced water stress on tiller and rhizome growth.

C. Materials and Methods

A single clone of healthy, *U. striiformis*-, or *U. agropyri*-infected *Poa pratensis* was vegetatively-propagated from individual shoots and grown in a loam: peat:perlite (1:1:1) mix under greenhouse conditions.
Individual shoots selected for experiments were removed from the soil mix, roots were washed, and all but the two youngest fully-expanded leaves were stripped from the shoot. Individual shoots were selected for size uniformity for growth chamber experiments.

1. Nutrient solution studies

Seventy-five shoots with two leaves each were taken from each health group (i.e., stripe-smutted, flag-smutted, and healthy) for evaluation of tiller and rhizome growth in nutrient solution. Plants were randomized in the growth chamber using a randomized complete block design utilizing 3 blocks and 3 treatments (health groups). Growth chamber conditions included 12-hour photoperiods, 65 (± 5) nE cm⁻² sec⁻¹ light intensity, 23 C (± 2) constant temperature. Plants were placed individually in specially designed nutrient culture jars (See Chapter III) and grown for 4 weeks. Nutrient solutions were flushed twice per week. At 0, 1, 2, 3, and 4 weeks, 15 plants from each treatment (5 from each block) were sampled for their respective total dry weight (dried at 60 C, 48 hours) of rhizomes and tillers per shoot. Tiller and rhizome percentages were calculated by expressing the tiller and rhizome dry weights as a percentage of the total dry weight of each shoot. Data were plotted as weekly means of healthy, U.
striiformis-, and U. agropyri-infected plants for rhizome dry weight per shoot, rhizome percentage of total shoot dry weight, tiller dry weight per shoot, and tiller percentage of total shoot dry weight. Each mean was bracketed by its standard error.

2. Water stress studies

Plants evaluated for growth in response to infection by U. striiformis and U. agropyri and to osmotically-induced water stress were preconditioned by growing them in nutrient solution without polyethylene glycol (PEG8000) for 10 days before water stress treatments were applied. Plants were selected for size uniformity and placed in specially designed nutrient solution jars (See Chapter III) containing 0, 168, 242, or 292 grams of PEG8000 per liter nutrient solution. This provided initial nutrient solution osmotic potentials of -0.2, -0.4, -0.6, and -0.8 MPa as measured by a Wescor HR-33T dewpoint microvoltmeter using calibrated C-52 sample chambers and the dewpoint mode. Nutrient solutions were flushed twice per week and growth chamber conditions were as previously stated. Plants were grown for four weeks under applied water stress after which 12 plants from each of 12 treatment combinations were sampled for total dry weight per shoot and dry weight of rhizomes and tillers per shoot. Rhizome and tiller percentages were
calculated by dividing rhizome and tiller dry weight per shoot by total dry weight per shoot, respectively, and expressing ratios as percentages. The experiment utilized a 3 by 4 factorial in a randomized complete block design. Treatment factors included 3 health groups (healthy, stripe-smutted, and flag-smutted plants) and 4 levels of osmotically-induced water stress (-0.2, -0.4, -0.6, and -0.8 MPa). Data were plotted as the means of healthy, U. striiformis-, and U. agropyri-infected plants for rhizome and tiller dry weight per shoot and for rhizome and tiller percentage of total shoot dry weight. Each mean was bracketed by its standard error.

D. Results

1. Nutrient solution effects

Infection by U. striiformis had no effect on total dry weight of rhizomes per shoot on P. pratensis grown in nutrient solution (Fig. 4.1A). Rhizome growth of stripe-smutted plants expressed as a mean percentage of total shoot dry weight, however, decreased between the second and third weeks of the study (Fig. 4.1B). Infection by U. striiformis increased the total dry weight of tillers per shoot and their mean percentage of the total dry weight of the shoot (Fig. 4.1D) compared to healthy plants grown
Figure 4.1. Weekly mean dry weights of rhizomes (A) and tillers (C) and their respective mean percentages of the total shoot dry weight (B and D) for healthy (H), *Ustilago striiformis*-infected (stripe smutted) (S), and *Urocystis agropyri*-infected (flag smutted) (F) *Poa pratensis* 'Merion' shoots grown four weeks in nutrient solution.
in nutrient solution.

Infection by *U. agropyri* sharply decreased rhizome growth on plants grown in nutrient solution (Fig. 4.1A and 4.1B). Conversely, flag-smutted plants exhibited a sharp increase in tiller growth compared to healthy plants (Fig. 4.1C and 4.1D).

2. Water stress effects

Progressively decreasing the osmotic potential of the nutrient solution in which healthy, *U. striiformis*-, and *U. agropyri*-infected plants were growing generally decreased the mean dry weight of rhizomes and tillers and their respective percentages of total plant dry weight (Fig. 4.2A-D). Tiller growth associated with both pathogens was greater than that of healthy controls under minimal stress (Fig. 4.2C). Increasing water stress decreased tillering by plants infected by either pathogen, but tillering by flag-smutted plants remained greater than that of controls. Rhizome growth was decreased by both pathogens under minimal water stress, but more severely by flag smut (Fig. 4.2A). Increasing water stress reduced the differential decrease in rhizome growth between healthy and stripe-smutted plants, but flag-smutted plants showed severe decreases in rhizome growth at all levels of water stress (Fig. 4.2A and 4.2B).
Figure 4.2. Mean dry weight of rhizomes (A) and tillers (C) and their respective mean percentages of the total shoot dry weight (B and D) for healthy (H), Ustilago striiformis-infected (stripe-smutted) (S), and Urocystis agropyri-infected (flag-smutted) (F) Poa pratensis 'Merion' shoots after four weeks growth in nutrient solution amended with PEG8000.
This study has shown that systemic infection by *U. agropyri* greatly stimulates the growth of tillers of *Poa pratensis*, but decreases the growth of rhizomes. The same general trend appears for *U. striiformis*-infected plants, but the effect is much less dramatic (Fig. 4.1A-D). This agrees with the observation that *U. agropyri* severely decreases rhizome production of *P. pratensis* grown in pot culture (Hodges, 1970a, 1969). Growth of tillers in grasses uses large reserves of stored carbohydrates (Younger and Nudge, 1968; Sachs and Thimann, 1964). The stimulation of tilering in *U. striiformis* and *U. agropyri*-infected plants may account for the reduction in carbohydrates found in infected plants (Hodges and Robinson, 1977; Madsen et al., 1983).

Tiller and rhizome growth in *P. pratensis* are sensitive indicators of water stress (Fig. 4.2), and decreases in their growth in response to water deficits has been previously observed (Olmsted, 1941; Quarrie and Jones, 1977). Decreased tiller and rhizome growth in response to water stress may be due to the extreme sensitivity of apical meristems (Hsiao, 1973), or environmentally-induced hormonal disturbances. Hormonal disturbances in water stressed plants include increases in abscisic acid
and decreases in cytokinins (Livne and Vaadia, 1972). Increased IAA oxidase activity has also been observed in response to water deficits (Darbyshire, 1971).

Tiller and rhizome growth in grasses depends on development of lateral buds (Beard, 1973), the growth of which may be inhibited by auxin produced in the apical meristem (Snow, 1925; Leopold, 1949; Thimann and Skoog, 1934), or require growth promoters such as gibberellins (Eagles and Wareing, 1964) to stimulate their development. Plant pathogens of the Basidiomycetes often induce hormonal imbalances in their hosts (Brian et al., 1954; Sequiera, 1973). Auxin imbalances induced by \textit{U. agropyri} might promote lateral bud break (Leopold, 1949), and increase tillering, but does not explain the near absence of rhizome production.
V. EFFECT OF WATER STRESS AND INFECTION BY USTILAGO STRIIFORMIS OR UROCYSTIS AGROPYRI ON LEAF TURGOR AND WATER POTENTIALS OF POA PRATENSIS

A. Abstract

Systemic infection by Ustilago striiformis or Urocystis agropyri and water stress were evaluated for their effects on leaf turgor and water potentials of Poa pratensis 'Merion' when grown in nutrient solution. Infection by either pathogen decreased leaf turgor and water potentials during light and dark periods compared to healthy controls. Healthy plants maintained higher leaf turgor and water potentials than infected plants as the nutrient solution osmotic potential was lowered with polyethylene glycol.

Moisture release curves for single leaves from healthy, U. striiformis-, and U. agropyri-infected plants were established to evaluate plants for osmotic adjustment by estimating leaf osmotic potentials and relative water contents at zero turgor as plants were grown in nutrient solution and nutrient solution amended with polyethylene glycol to increase water stress. Turgid weight/dry weight ratios also were calculated for healthy, U. striiformis-, and U. agropyri-infected leaves before
and after water stress treatments.

Water stress lowered the leaf osmotic potentials and relative water contents at zero turgor and decreased the turgid weight/dry weight ratios of healthy and infected leaves. Healthy plants exhibited lower osmotic potentials at zero turgor, however, and smaller turgid weight/dry weight ratios after water stress than infected plants suggesting that infection by either pathogen inhibited osmotic adjustment and an increase in cell wall thickness of *P. pratensis* leaves in response to water stress.

B. Introduction

Several pathogens cause water deficits in their hosts by altering the host's water relations (Duniway, 1973; Ayres, 1977; 1978; Duniway and Durbin, 1971a; Talboys, 1968). Host water deficits can be brought about in a number of ways including accelerated water loss, reduced water uptake, and loss of membrane integrity which may result in the loss of the cell's ability to retain solutes and water (Ayres, 1978). Few studies have considered the combined effects of environmentally-imposed and disease-imposed water stress on plants (Ayres, 1978).
Urocystis agropyri and Ustilago striiformis are pathogens of a number of grass species including Poa pratensis (Couch, 1971). During periods of water stress, mortality of leaf-smutted P. pratensis is higher than that of healthy plants (Hodges, 1977; Kreitlow, 1943). This suggests that infection by these pathogens may impose a direct water stress on the host and/or alter drought resistance of the host. To determine the effects of either environmentally-imposed and/or disease-imposed water stress, it is necessary to measure both water potential and osmotic potential and to calculate turgor potential (Kramer, 1980).

This study was initiated to determine the effect of U. striiformis or U. agropyri on leaf turgor and water potential of P. pratensis grown in nutrient solution and to determine if infection by either organism affects osmotic adjustment or turgid weight/dry weight ratios of leaves as healthy and host plants are subjected to increasing water stress.

C. Materials and Methods

A single clone of healthy, U. striiformis-, or U. agropyri-infected Poa pratensis 'Merion' was vegetatively propagated from individual shoots and grown in a loam:
peat:perlite (1:1:1) mix under greenhouse conditions. Individual shoots selected for experiments were removed from the soil mix, roots were washed, and all but the two youngest fully-expanded leaves were stripped from the shoot. Individual shoots of healthy, *U. striiformis*-, and *U. agropyri*-infected plants were grown in the growth chamber in nutrient solution (see Chapter III) for three weeks, then all but the youngest three fully-expanded leaves were removed and the plants were placed back into the growth chamber in aerated nutrient culture jars (see Chapter III) and held for osmotic and water potential measurements.

Growth chamber conditions included a 12-hour photoperiod, 65 (+5) nE cm\(^{-2}\) sec\(^{-1}\) light intensity, 23 (+2) °C, and 50% relative humidity. Leaf water potentials were determined over a period of 10 days utilizing a PMS pressure bomb. Only the second fully-expanded leaf was used for water potential determinations. Prior to leaf excision, leaf blades were sealed with petroleum jelly to avoid rapid water loss before and during pressurization. A slow pressurization rate (0.01 MPa sec\(^{-1}\)) was used to insure accurate endpoint determination. Osmotic potentials were determined from expressed sap from the same leaf using a Wescor HR-33T
dewpoint microvoltmeter in the dewpoint mode. Calibrated C-52 sample chambers were used with a vapor equilibrium time of 5 minutes. Leaf turgor potentials were calculated as the difference between water and osmotic potentials. Data were plotted as scatter diagrams of water and turgor potentials versus time for healthy, **U. striiformis**-, and **U. agropyri**-infected plants. Each data point represents the mean of three leaves.

Healthy, **U. striiformis**-, and **U. agropyri**-infected plants were measured for prelight (synonymous with predawn) leaf water and turgor potentials under conditions of increasing water stress. Prelight values were taken from 3 hours before to onset of light period. Polyethylene glycol (PEG\textsubscript{8000}) was used to lower the osmotic potential of the nutrient solution at the rate of 0.1 MPa day\textsuperscript{-1} until -0.8 MPa was reached. Solutions were flushed at the end of the light period daily and replaced with progressively more concentrated solutions. Prelight leaf water and osmotic potentials were determined as previously described at nutrient solution osmotic potentials of -0.2, -0.4, -0.6, and -0.8 MPa. Prelight turgor potentials were calculated as the difference between leaf water potentials and osmotic potentials. Data were expressed as prelight leaf water and turgor potentials versus nutrient solution osmotic potential. Each data
Evidence for osmotic adjustment was evaluated by establishing water release curves using a method similar to that described by Brown and Tanner (1983) for the second fully-expanded leaf of healthy, *U. striiformis*-, and *U. agropyri*-infected plants. Curves were established for plants growing in nutrient solution before and after plants were water stressed using PEG$_{8000}$ in the manner previously described. After nutrient solution osmotic potential reached -0.8 MPa (6 days), nutrient culture jars (See Chapter III) were flushed with distilled water and plants were allowed to rehydrate in the dark for at least 6 hours. Before leaf excision for water potential determinations, leaves were sealed with a very thin coat of petroleum jelly. After excision, the leaf was weighed immediately and placed in a PMS pressure bomb and pressurized at the rate of 0.01 MPa sec$^{-1}$. After endpoints were attained, pressure was relieved slowly (approximately 0.03 MPa sec$^{-1}$). The leaf was weighed again, water potential determined, and the process was repeated until leaf water potentials were below -3.5 MPa.

Data were plotted as leaf water potential versus leaf weight. Linear regression was used on the linear
portion of the curve to estimate leaf weight at full turgor (Fig. 5.1A). \( R^2 \) values for these regressions were consistently near 0.99. After curves were established, leaves were oven-dried (60 C, 48 hours) and dry weights recorded. Relative water contents (Barrs, 1968) for each data point were calculated and data were replotted as water potential versus relative water content (the moisture release curve) (Fig. 5.1B). The point of intersection between the linear and curvilinear phases of the moisture release curve was used as the estimate of zero turgor. Values for leaf osmotic potential and relative water content at zero turgor were recorded (Fig. 5.1B).

Eight leaves of healthy, U. striiformis-, and U. agropyri-infected plants were evaluated before and after stress treatments. Turgid weight/dry weight ratios were also calculated for those healthy, U. striiformis-, and U. agropyri-infected leaves of P. pratensis before and after water stress. The ratios were used as comparative estimates of cell wall thickness which affects cell wall elasticity (Wilson et al., 1980).

D. Results

Plants infected with either pathogen and grown in nutrient solution exhibited lower leaf water and turgor
Figure 5.1. Leaf weight versus leaf water potential (A) for a single leaf of healthy P. pratensis 'Merion' grown in nutrient solution to determine leaf weight at full turgor. Data are replotted as relative water content versus leaf water potential to yield the moisture release curve (B) which yields estimates of osmotic potential ($\Pi_p$) and relative water content ($RWC_p$) at zero turgor (plasmolysis).
LEAF WATER POTENTIAL (MPa)

LEAF WEIGHT AT FULL TURGOR

USED AS PLASMINOSIS RWC AT ZERO TURGOR (RWCp)

GASTRIC POTENTIAL AT ZERO TURGOR (Ψp)

POINT OF INTERSECTION USED AS PLASMINOSIS

RWC AT ZERO TURGOR (RWCp)
potentials than healthy plants during both light and dark periods (Fig. 5.2). Healthy plants regained turgor during the dark period rapidly to prelight values of approximately 1.5 MPa. *U. agropyri-* and *U. striiformis-*infected plants regained turgor less rapidly to prelight values of approximately 0.8 MPa and 0.5 MPa, respectively.

Healthy plants subjected to progressively greater water stress were able to regain higher prelight leaf water and turgor potentials than *U. striiformis-* or *U. agropyri*-infected plants (Fig. 5.3). Systemic infection by either pathogen resulted in near equal reductions in prelight leaf water and turgor potentials over the entire range of water stress (Fig. 5.3).

Plants grown in nutrient solution and infected by *U. striiformis* exhibited lower mean osmotic potentials at zero turgor than leaves from healthy plants (Table 5.1). Plants infected by *U. agropyri* exhibited decreased mean relative water content at zero turgor and increased turgid weight/dry weight ratios compared to leaves from healthy plants (Table 5.1). Water stress treatments induced osmotic adjustment of healthy and infected leaves (Table 5.1). Leaves for healthy plants, however, exhibited lower osmotic potentials at zero turgor and lower turgid weight/dry weight ratios than infected plants after water stress treatments. Mean relative water contents of leaves
Figure 5.2. Leaf water and turgor potentials of healthy (○), *U. striiformis*-infected (stripe-smutted) (●), and *U. agropyri*-infected (flag-smutted) (▲) leaves of *Poa pratensis* over a 24-hour cycle from plants growing in a growth chamber in nutrient solution. Each point represents the mean of 3 leaves.
Figure 5.3. Prelight leaf water and turgor potentials versus nutrient solution osmotic potential of the second fully-expanded leaf from healthy (H), *U. striiformis*-infected (stripe-smutted) (S), and *U. agropyri*-infected (flag-smutted) (F) *Poa pratensis* grown in nutrient solution amended with PEG\textsubscript{8000}. Each point represents the mean of 8 leaves and has been bracketed by its standard error.
Table 5.1. Mean osmotic potentials (MPa) and relative water contents (%) at zero turgor and mean turgid weight/dry weight ratios of leaves from healthy, *U. striiformis*-, and *U. agropyri*-infected *Poa pratensis* 'Merion' before and after water stress treatment.

<table>
<thead>
<tr>
<th>Health group</th>
<th>Before Stress Treatment</th>
<th>After Stress Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\pi_p$</td>
<td>RWC$_P$</td>
</tr>
<tr>
<td>Healthy</td>
<td>-1.33</td>
<td>93.1</td>
</tr>
<tr>
<td>Infected by <em>U. striiformis</em></td>
<td>-1.54</td>
<td>91.0</td>
</tr>
<tr>
<td>Infected by <em>U. agropyri</em></td>
<td>-1.40</td>
<td>89.3</td>
</tr>
<tr>
<td>LSD(0.05)</td>
<td>0.15</td>
<td>2.4</td>
</tr>
</tbody>
</table>

*a* Osmotic potentials at zero turgor (plasmolysis).

*b* Relative water content at zero turgor (plasmolysis).

*c* Turgid weight/dry weight ratios.
at zero turgor after water stress treatment were not significantly different between healthy and infected plants (Table 5.1).

E. Discussion

Infection of *P. pratensis* by *U. agropyri* or *U. striiformis* resulted in pathogen-induced water stress. Infected plants exhibited lower leaf water and turgor potentials during the light and less rapid recovery during the dark to prelight levels that were substantially below healthy plants (Fig. 5.2). Pathogen-induced water stress has been demonstrated for other host-pathogen systems (Ayres, 1978; Talboys, 1968; Kozlowski, 1976b; Van der Wal et al., 1975), and can arise from reduced water absorption due to root growth inhibition, reduced water transport due to vascular blockages, loss of cell membrane integrity, or accelerated water loss due to disruption of normal stomatal function or epidermal rupturing (Ayres, 1978; Dimond, 1970; Kozlowski, 1976).

Infection by *U. agropyri* and *U. striiformis* decreases root growth of *P. pratensis* (see Chapter III). Infected plants are subsequently limited in the volume of soil from which they can extract moisture compared to healthy plants. In addition, the hyphae of both
pathogens grow between mesophyll cells and, with the formation of teliospores, rupture mesophyll and epidermal cells (Hodges and Britton, 1970), which may lead to accelerated water loss. The combination of root growth inhibition and epidermal rupturing leading to accelerated water loss may explain the lower leaf water and turgor potentials seen in Figure 5.2.

The ability of cell membranes to retain solutes is reflected, in part, by the degree of osmotic adjustment for turgor maintenance in response to water stress. As plants were subjected to progressively more severe water stress, healthy plants regained higher prelight leaf water and turgor potentials than plants infected with either U. striiformis and U. agropyri (Fig. 5.3). Turgor potential at a given water potential is dependent on the osmotic potential and elasticity of the tissue (Turner, 1979). It seems, therefore, that leaf-smutted plants are less able to adjust osmotically or that tissue elasticity may be adversely affected by infection. Quantitative assessment concerning the degree of osmotic adjustment in healthy versus infected plants, however, cannot be made from data presented in Figure 5.3 because methods for turgor potential determination involving expressed sap are subject to errors by symplastic dilution by apoplastic water and possible differences in
tissue hydration (Brown and Tanner, 1983).

Infection by either *U. striiformis* or *U. agropyri* results in lower leaf water and turgor potentials of *P. pratensis* (Fig. 5.2). This suggests that *P. pratensis* is capable of osmotic adjustment in response to water stress induced by either pathogen as reflected by the lower values for osmotic potential at zero turgor (Table 5.1). Osmotic adjustment of the host also would explain the lower relative water contents at zero turgor exhibited by infected plants.

Healthy and infected *P. pratensis* showed evidence for osmotic adjustment of leaves in response to osmotically-induced water stress (Table 5.1). Infection by either pathogen, however, decreased the degree of osmotic adjustment by *P. pratensis* leaves. Leaves from healthy plants were able to osmotically adjust to lower values than leaves from flag or stripe smutted plants (Table 5.1). Although plants use a number of osmotica to lower their osmotic potentials in response to water stress (Hsiao, 1973; Borowitzka, 1981), soluble sugars are a main component. Hodges and Robinson (1977) and Madsen et al. (1983) have shown that infection by either pathogen results in decreased levels of soluble sugars in *P. pratensis* leaves. This may partially account for the decrease in degree of osmotic adjustment exhibited by
infected plants.

Infection by *U. agropyri* results in a sharp decrease in leaf dry weight compared to healthy or *U. striiformis*-infected plants (see Chapter III). Higher turgid weight/dry weight ratios may be a result of this, and provide evidence for thinner cell walls compared to those of healthy or *U. striiformis*-infected plants (Wilson et al., 1980). Thicker cell walls are positively correlated with greater drought resistance (Turner, 1979).
VI. COMPARATIVE WATER USE BY HEALTHY AND
USTILAGO STRIIFORMIS-INFECTED POA PRATENSIS

A. Abstract

Poa pratensis 'Merion' systemically infected by Ustilago striiformis was evaluated for water economy and stomatal action. Infected plants with leaves having little or no visible sporulation of teliospores and healthy plants did not differ in rate of water use (ml day\(^{-1}\)) or water use efficiency (ml mg\(^{-1}\)). Infected plants with leaves having moderate to heavy sporulation exhibited a sharp decrease in water use efficiency and per day use of water lower than healthy plants or infected plants with leaves having non-sporulating sori. Stomatal closure on infected leaves with non-sporulating sori occurred at lower leaf water potentials than on healthy leaves suggestive of a degree of osmotic adjustment.

B. Introduction

Plant pathogens have been shown to interfere with the water status of their hosts (Ayres, 1978; Talboys, 1968; See Chapter V). Pathogen-induced inhibition of
root growth (Murphy, 1935; Johnston and Miller, 1934; 1940; Hodges, 1969; 1970a; See Chapter III) and vascular blockages (Dimond, 1970) can limit water uptake. Water loss may be accelerated by decreased cuticular resistance (Sempio et al., 1966; Amatya and Jones, 1966; Gerwitz and Durbin, 1965) or inhibition of stomatal movement (Duniway and Durbin, 1971b; Farrell et al., 1969; Ayres and Jones, 1975).

Transpiration is the sum of stomatal and non-stomatal water loss (Crafts, 1968). Stomatal resistances are derived from differences between water lost in light and darkness because it is assumed that stomata close in the dark and that cuticular resistance is not affected by illumination (Ayres, 1978). Because stomatal closure is the main cause for transpiration decline as water stress develops, control of stomatal aperture is very important in drought resistance (Hsiao, 1973; Townley-Smith and Hurd, 1979). However, even if the stomatal apparatus is sensitive to water deficits, and the leaf water potential at which stomata close is high, full benefit is gained only if the cuticular resistance is also high and stomatal closure is complete (Turner, 1979).

_Ustilago striiformis_ is the causal organism for stripe smut and is a pathogen of several grass species
including *Poa pratensis* (Couch, 1971). It is a member of the Ustilaginales and produces thick-walled teliospores in sori on leaves, sheaths, and floral parts of infected plants (Couch, 1971). Sporulation of teliospores ruptures the epidermis of the host and may result in rapid water loss in the area of the sorus. In closely related rust diseases, transpiration by infected plants is often reduced early in the infection cycle because of inhibition of stomatal activity (Duniway and Durbin, 1971b; Farrell et al., 1969; Ayres and Jones, 1975). After sporulation, however, water economy is reduced due to epidermal rupturing and a decrease in cuticular resistance (Ayres, 1978). A direct measure of the plant's water economy is water use efficiency and has been used to evaluate drought resistance (Stone, 1975; Pierre et al., 1966; Townley-Smith and Hurd, 1979).

This study was initiated to test the effects of systemic infection by *Ustilago striiformis* on the water use, water use efficiency, leaf diffusive resistance, and stomatal activity of *Poa pratensis* 'Merion'.

C. Materials and Methods

A single clone of healthy or *U. striiformis*-infected *P. pratensis* 'Merion' was vegetatively-propagated from
individual shoots and grown in a loam:peat:perlite (1:1:1) mix under greenhouse conditions. Individual shoots selected for experiments were removed from the soil mix, roots were washed, and all but the two youngest fully-expanded leaves were stripped from the shoot. Individual shoots were then placed in nutrient culture jars (see Chapter III) and grown in a growth chamber with a 12-hour photoperiod (65 ± 5 nE cm⁻² sec⁻¹), 50% relative humidity, and 23 (± 2) C.

1. Water use and efficiency

Plants were stripped of all but the three youngest fully-expanded leaves and carefully selected for size uniformity after three weeks growth in the growth chamber. _U. striiformis_-infected plants were separated into two groups: (1) plants with leaves with immature sori with little or no sporulation of teliospores and without epidermal rupturing, and (2) plant with leaves with mature sori and teliospore sporulation that had ruptured the epidermis. Fifteen healthy plants and _U. striiformis_-infected plants were chosen at random and oven-dried (60 C, 48 hours). Initial mean dry weights (IMDW) were recorded for both healthy and infected plants. An additional fifteen healthy and infected plants were placed individually in aerated nutrient culture jars with 100 ml of nutrient solution (see Chapter III),
and sealed with silicone grease (Dow Corning, Midland, Mich.). Plants were grown two weeks and nutrient solution was flushed twice per week. Total water loss was recorded at each nutrient solution flush which included both water usage by the plant and evaporation from the solution aeration exhaust port (see Chapter III). Evaporation from the aeration exhaust port was determined by running the same experiment at equal aeration rates without plants and the rubber stoppers sealed with silicone grease. Evaporation was recorded, and plant water usage was calculated as total water loss minus evaporation from the aeration exhaust port.

All plants were oven-dried (60 C, 48 hours) at the end of the two week growth period. Initial mean dry weights (IMDW) for healthy and infected plants were subtracted from each healthy or infected plant, respectively. Estimates for dry matter produced during the two week period were thus obtained. Water use efficiency was derived by dividing dry matter produced by plant water usage for each plant (Kramer, 1980).

2. Leaf diffusive resistance

A Li-Cor leaf diffusive resistance meter (Model LI-60) was used to measure diffusive resistance of healthy leaves and leaves with non-sporulating and sporulating sori of plants grown in nutrient solution.
Measurements were taken after greenhouse-grown plants were selected for size uniformity at a fully-expanded two leaf stage and grown in a growth chamber for three weeks. Growth chamber conditions were as previously stated. Data were plotted as diffusive resistance of healthy leaves and leaves with non-sporulating and sporulating sori over a 24-hour period. Each point represents the mean of three measurements.

Leaf diffusive resistance also was recorded as a function of leaf water potential for healthy and infected plants. Twenty 6-cm² plastic pots of healthy and infected plants growing in a 1:1:1 (loam:peat:perlite) mix were taken from the greenhouse and placed in the growth chamber. Plants were watered daily with distilled water and fertilized with nutrient solution at 5 day intervals. After two weeks, pots were allowed to dry and leaf diffusive resistance and water potential measurements were initiated. Leaf diffusive resistance measurements were taken only during the light cycle and only on the second fully-expanded leaf blade. Humidity sensor placement on U. striiformis-infected leaves was restricted to non-sporulated areas of the sori. Leaf blades were then sealed with petroleum jelly and excised. Leaf water potential measurements were obtained using a PMS pressure bomb at a pressurization rate of 0.01 MPa sec⁻¹.
Data were plotted as leaf diffusive resistance versus leaf water potential for healthy leaves and infected leaves with non-sporulated areas of the sori.

D. Results

Leaves of plants infected with *U. striiformis* showing little or no sporulation or epidermal rupturing of sori did not differ in water use from healthy plants (Fig. 6.1A). Infected leaves of plants with moderate to severe epidermal rupturing of sori used less water than leaves of healthy plants or infected leaves with non-sporulating sori (Fig. 6.1A).

Dry matter accumulation (mg day$^{-1}$) of *P. pratensis* decreased as leaf sori matured and epidermal cell rupturing increased (Fig. 6.1B). Leaves of infected plants with immature sori and little or no epidermal damage did not differ in dry matter accumulation from healthy plants, but leaves of infected plants with mature sori and moderate to severe epidermal damage decreased in dry matter accumulation compared to healthy or *U. striiformis*-infected plants with immature sori.

No differences were found in the water use efficiency between healthy plants and infected plants with immature leaf sori (Fig. 6.1C). Infected plants with
Figure 6.1. Water use (A), dry matter production (B), and water use efficiency (C) of healthy and *Ustilago striiformis*-infected *Poa pratensis* 'Merion' leaves with immature (non-sporulating) and mature (sporulating) sori.
mature leaf sori and moderate to severe epidermal damage were less water efficient than healthy plants or infected plants with immature leaf sori.

No differences were observed in leaf diffusive resistance between healthy plants and infected plants with mature or immature leaf sori during the 12-hour photo-period (Fig. 6.2). Leaf diffusive resistance in the dark was affected by infection on leaves with both immature and mature sori (Fig. 6.2). Infected *P. pratensis* with leaves having mature sori exhibited little resistance to water loss in the dark (Fig. 6.2). However, infected leaves with immature sori (non-sporulating) also exhibited slightly less leaf diffusive resistance in the dark than leaves of healthy plants (Fig. 6.2).

Leaf diffusive resistances of healthy leaves exhibited rapid increases at leaf water potentials between -1.2 and -1.7 MPa (Fig. 6.3). Immature portions of sori on infected leaves exhibited sharp increases in leaf diffusive resistances at leaf water potentials of -2.1 to -2.4 MPa.

E. Discussion

The decreased water use per day exhibited by *U. striiformis*-infected plants with mature leaf sori seems
Figure 6.2. Leaf diffusive resistance of healthy (●), and immature (non-sporulated) (○) and mature (sporulated) (▲) sori of *Ustilago striiformis*-infected *Poa pratensis* 'Merion' grown in nutrient solution
LEAF DIFFUSIVE RESISTANCE (sec cm⁻¹)

DARK

LIGHT

- HEALTHY
- STRIPE (NON-SPORULATED)
- STRIPE (SPORULATED)

TIME

2100 2300 0100 0300 0500 0700 0900 1100 1300 1500 1700 1900 2100
Figure 6.3. Leaf diffusive resistance versus leaf water potential of healthy *Poa pratensis* 'Merion' leaves and *Ustilago striiformis*-infected leaves with immature, non-sporulating sori.
inconsistent with the ruptured epidermal cells associated with mature sori (Fig. 6.1A and 6.2). A decrease in water use also has been observed in rusted wheat (Johnston and Miller, 1934; 1940), oats (Murphy, 1935), and barley (Bever, 1937). The dry matter accumulation of infected plants with mature leaf sori was decreased reflecting less cell division and expansion; these responses would decrease water use and partially explain the decrease in water use per day. Infection by *U. striiformis* also results in decreases in leaf water potential in *P. pratensis* leaves (see Chapter V). Such pathogen-induced water stress in *U. striiformis*-infected plants may result in stomatal closure on leaves of infected plants with mature sori. This also could contribute to a decrease in dry matter accumulation due to limited carbon dioxide fixation (Burrows and Milthorpe, 1976).

Water use efficiency is a direct measure of the water economy of the plant (Kramer, 1980). Water use (ml day⁻¹) of infected plants with mature leaf sori decreased compared to healthy plants, but, because dry matter production decreased even more, water use efficiency (mg ml⁻¹) also decreased (Fig. 6.1B and 6.1C). Water use efficiency reflects drought resistance (Townley-Smith and Hurd, 1979). The sharp decreases in
water use efficiency of infected plants with mature leaf sori may partly explain the increase in mortality of diseased plants during periods of limited soil moisture (Hodges, 1977).

Leaf diffusive resistance of infected plants with immature leaf sori and a non-ruptured epidermis was not as great as that of healthy leaves in the dark. Decreased leaf diffusive resistance in the dark may arise from decreased cuticular resistance or incomplete stomatal closure (Sempio et al., 1966; Duniway and Durbin, 1971b). Other pathogens have been shown to directly interfere with stomatal action (Farrell et al., 1969; Ayres, 1972; Cruickshank and Rider, 1961). This also may apply to U. striiformis in P. pratensis. The presence of the pathogen's hyphae or teliospores within the mesophyll and beneath the non-ruptured epidermis may affect normal cuticle formation or function in infected leaves and cause a decrease in cuticular resistance to water loss.

The comparative leaf diffusive resistance of healthy leaves versus immature portions (non-sporulated) of leaf sori on infected plants suggests the stomates associated with immature leaf sori of infected leaves close at lower leaf water potentials than those on healthy leaves. Stomatal conductance is turgor dependent (Hsiao, 1973), and closure occurs when bulk leaf turgor is near zero
(Burrows and Milthorpe, 1976). Stomatal closure at lower leaf water potentials is evidence for pathogen-induced water stress. \textit{P. pratensis} can osmotically adjust to the water stress imposed by \textit{U. striiformis} and maintain turgor and stomatal aperture (See Chapter V) at lower leaf water potentials than healthy plants. Rapid increases in leaf diffusive resistances at lower leaf water potentials in infected plants compared to healthy plants is not evidence for direct pathogen inhibition of stomatal movement. Rather, decreased stomatal sensitivity in \textit{U. striiformis}-infected plants seems to suggest that the host reacts to pathogen-induced water stress by osmotic adjustment.
Growth responses of *Poa pratensis* suggest that infection by either *Ustilago striiformis* or *Urocystis agropyri* disrupts normal hormonal balance of the host, but in different ways. Growth responses of stripe-smutted plants are suggestive of growth promoting actions, whereas, flag smut results in dwarfing of *P. pratensis*, a proliferation of tillering, and virtual elimination of rhizome growth. Because so little is known about hormonal control of tillering versus rhizome growth, the *Poa pratensis-Urocystis agropyri* complex may prove to be an excellent system to study such differential control.

Overall growth as an integrated process can also serve as a measure of the plant's tolerance during water stress. Healthy plants are better able to maintain overall growth as water stress increases which suggests healthy plants possess greater drought tolerance than infected plants. Turgor pressure is the driving force for growth, therefore better maintenance of growth during periods of water stress implies healthy plants have a higher water status than infected plants. This is true for *P. pratensis* infected with *U. agropyri* or *U. striiformis*. 
There are several reasons why healthy plants are able to maintain a higher plant water status and tolerate water stress better than infected plants. Infection by either pathogen greatly decreases root growth of \textit{P. pratensis} which leads to lower root-shoot ratios of infected plants. Infection by either pathogen inhibits the ability of \textit{P. pratensis} leaves to osmotically adjust to water stress. The increase in cell wall thickness in response to water stress of \textit{P. pratensis} leaves as measured by turgid weight/dry weight ratios is inhibited by infection by either pathogen. Thinner cell walls limit the amount of leaf turgor for a given leaf water potential. Lastly, as sori from infected leaves mature and leaf epidermal cells are ruptured, non-stomatal water loss is greatly increased, and water use efficiency is subsequently reduced sharply. The combination of these actions predispose infected plants to desiccation during periods of water stress and results in increased mortality of diseased plants.
VIII. LITERATURE CITED


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